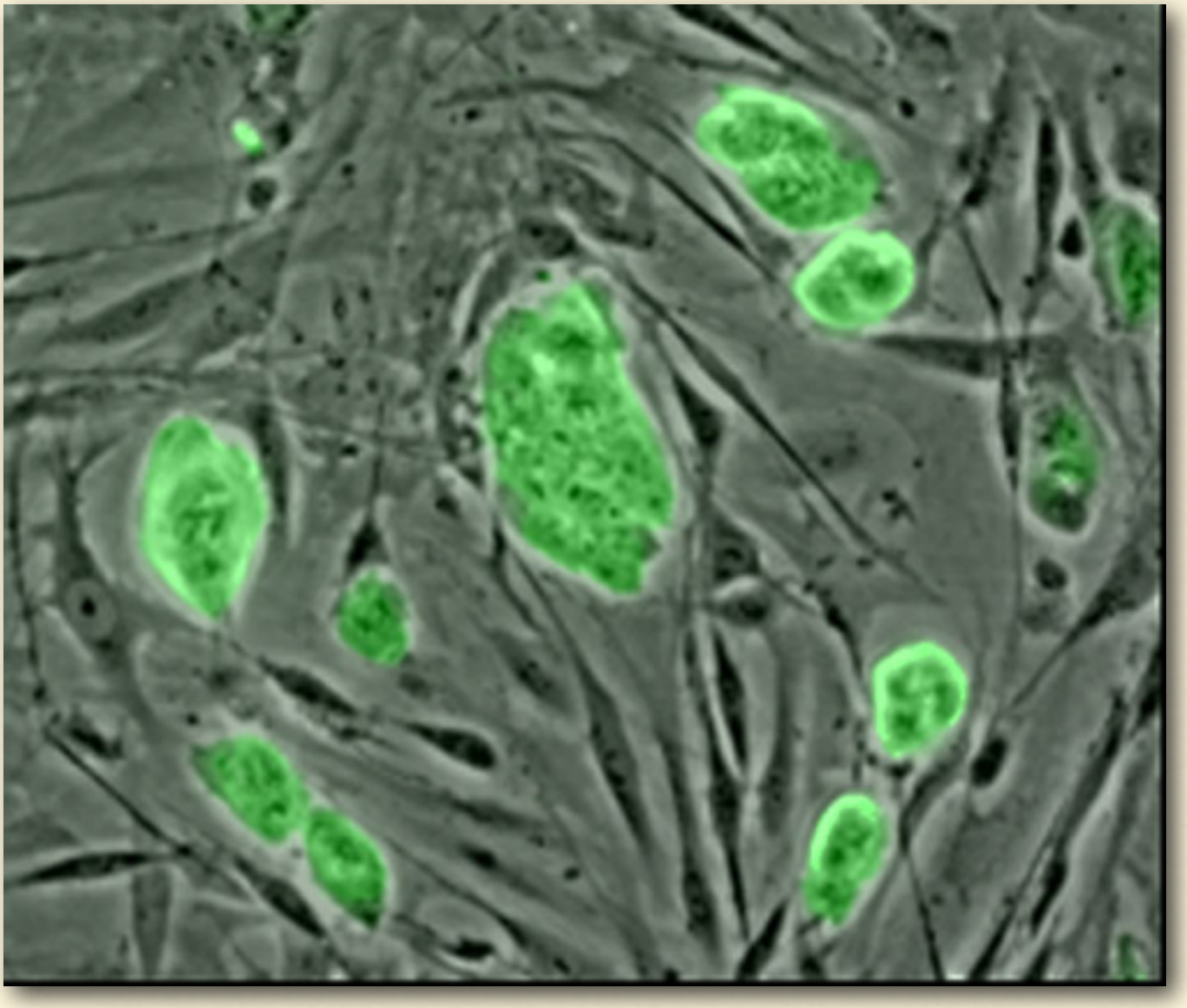
Clones and Stem Cells

Past, Present, and Potential

William Sofer



Dedication

To my wife, my sons, their wives, and my grandsons. How lucky I am.

Preface

My career in scientific research began in the 1960's. I didn't realize it then, but the revolution in molecular biology was in its infancy, and growing rapidly. Biologists were, in the words of Francis Crick, unravelling the "secret of life". We were making stunning advances at a whirlwind pace. The genetic code was being deciphered. Genes were being isolated, purified, and manipulated. Genomes were being sequenced. New drugs were being developed, and advanced therapies tested. Biotechnology companies were surfacing like mushrooms after an autumn storm. Fortunes were being made and lost. Outrageous ways to use the new discoveries in medicine, agriculture, and forensics were being suggested. A few even succeeded. It was an exciting time and I was fortunate to be involved while it was all happening.

Cell biology appears to be at a similar stage. New discoveries follow each other in a seemingly endless parade. Cells can be removed from a diseased individual, grown in culture, and therapeutic agents can be tested on them in a petri dish. Endangered animals can be cloned via nuclear transfer. Organs can be sculptured with three dimensional printers. And cells can be made to change their fate at the whim of a laboratory technician. It's another exciting era. Prospects seem unlimited.

I'm not a cell biologist, only a retired molecular geneticist who spent his entire research career working with fruit flies. I

OLLI

The Osher Lifelong Learning Institutes (OLLI) were established by Bernard Osher in 1977 with the purpose of providing non-credit courses to "seasoned" adults 50 years old and older who are "interested in learning for the joy of learning". There are some 119 OLLI programs at colleges and universities across the United States. The five programs at the University of Texas, with over 1,500 member, are particularly active. I joined OLLI in 2009. My wife and I jumped to the head of a three year waiting list when I volunteered to teach.

became interested in stem cells because I was persuaded to present a seminar on the subject to an audience of senior citizens at the University of Texas in their OLLI program. I had previously given talks to this same group on topics that I had taught to undergraduates at Rutgers University.: genetics and molecular biology. I was reasonably well acquainted with this material and I enjoyed the experience. Teaching older students who are not obsessed with their grades turned out to be very rewarding. It also gave me a chance to retire a kind of debt. I felt an obligation to share some of the insights that I had gained during my career with members of the general public to partially make up for the funds that I had been awarded from the government over many years in support of my research. While delivering my lectures I felt like an itinerant minister, passing along the "truths" of molecular genetics to receptive parishioners.

But presenting a seminar on cloning and stem cells, subjects with which I was much less familiar, turned out to be more of a challenge. It was not helped by the fact that field of stem cell biology is advancing at near light speed. I had to learn about techniques that I had never used, to read papers with which I was totally unfamiliar, and to become acquainted with terms that I never had bothered to learn. In order to organize my thoughts and present a coherent story, I found that writing a book at the same time as I prepared the lectures was the only way that I could deal with the task.

When I finally came to deliver the seminar, I found a responsive audience who were interested in not only the promising new therapies of stem cell research, but the history of the field and the stories of its prominent researchers. At the same time, I also came upon many misconceptions and misunderstandings. Just when I thought I had successfully conveyed an idea, someone would ask a question that indicated that they were missing a critical piece of fundamental information. No wonder. Cloning and stem cells are complex subjects. They are difficult to get a handle on without a foundation in molecular and cell biology. In turn, these subjects aren't particularly accessible either, requiring a grounding in basic chemistry and biology, subjects most of my audience were exposed to in the distant past.

This book represents my attempt to remedy that situation by conveying the excitement of cloning and stem cell research to a wide audience while building on a foundation that starts from basic

principles. I have tried to write a book that builds from more of less ground zero and works its way up to current advances. I've also attempted to keep jargon to a minimum so that readers aren't always turning to a dictionary to look up definitions. Lastly, I've attempted to take advantage of the tools that the iBooks format offers in order to clarify matters using cartoons, animations, three dimensional representations of molecules, and sidebars.

In the course of writing the book, I also found that I also had to confront some non-scientific issues. While new cell biology has incredible promise – biologists are developing tools to offer some extraordinarily useful therapies – some in the public are wary of the power of these techniques. They fear a slippery slope. They claim that the scientific community is "playing God". They bring up deep ethical and moral issues and pose profound questions like "When does life begin?".

These concerns and questions are well worthy of consideration and discussion. I encountered many similar apprehensions when molecular genetics was in its infancy. I'm aware of the potential dangers. I don't want to minimize or make light of them. And, while they're not a major focus of the book, I have tried to address some of the major issues with as little prejudice as I can muster.

It's pretty clear that many of these fears emanate from ignorance. People read about new discoveries and applications but don't understand them. And no wonder. The latest advances are almost magical. To the uninitiated they're based on techniques and insights that the layman has virtually no knowledge of. Cell biologists, like most scientists, don't devote a lot of time trying to explain their findings to lay audiences. It's not necessarily because they're incapable of doing so. Many are quite articulate. However, they're often just too busy writing papers and grant proposals, discussing issues with their already well informed colleagues, and working with their students and post doctoral fellows.

Newspapers and most of the host of modern communication media aren't much help at translating the science that goes on in the laboratory into a comprehensible form. News articles are too brief. Reporters have too little time and space to properly develop a story or to provide the appropriate foundation. Many simply don't have the appropriate scientific education to do the job correctly. Tweets and YouTube videos may help, but

certainly can't possibly do justice to the complexity of the subject.

Thanks goodness for scientists like Paul Knoepfler who writes "The Niche", the Knoepfler Stem Cell Blog, and who is the author of "Stem Cells: An Insider's Guide". His book offers a remarkable overview of the field of stem cells from the perspective of an active research scientist in the field. Similarly, Christopher Fasano and Yosif Ganat host an electronic effort called "The Stem Cell Podcast". It features audio episodes in which they summarize recent important papers and interview leading lights in the stem cell field. These scientists and some others are using the latest tools and social media techniques to take the newest findings that come from cell biology laboratories and make them comprehensible by the lay public.

This book represents my effort to add to that endeavor.

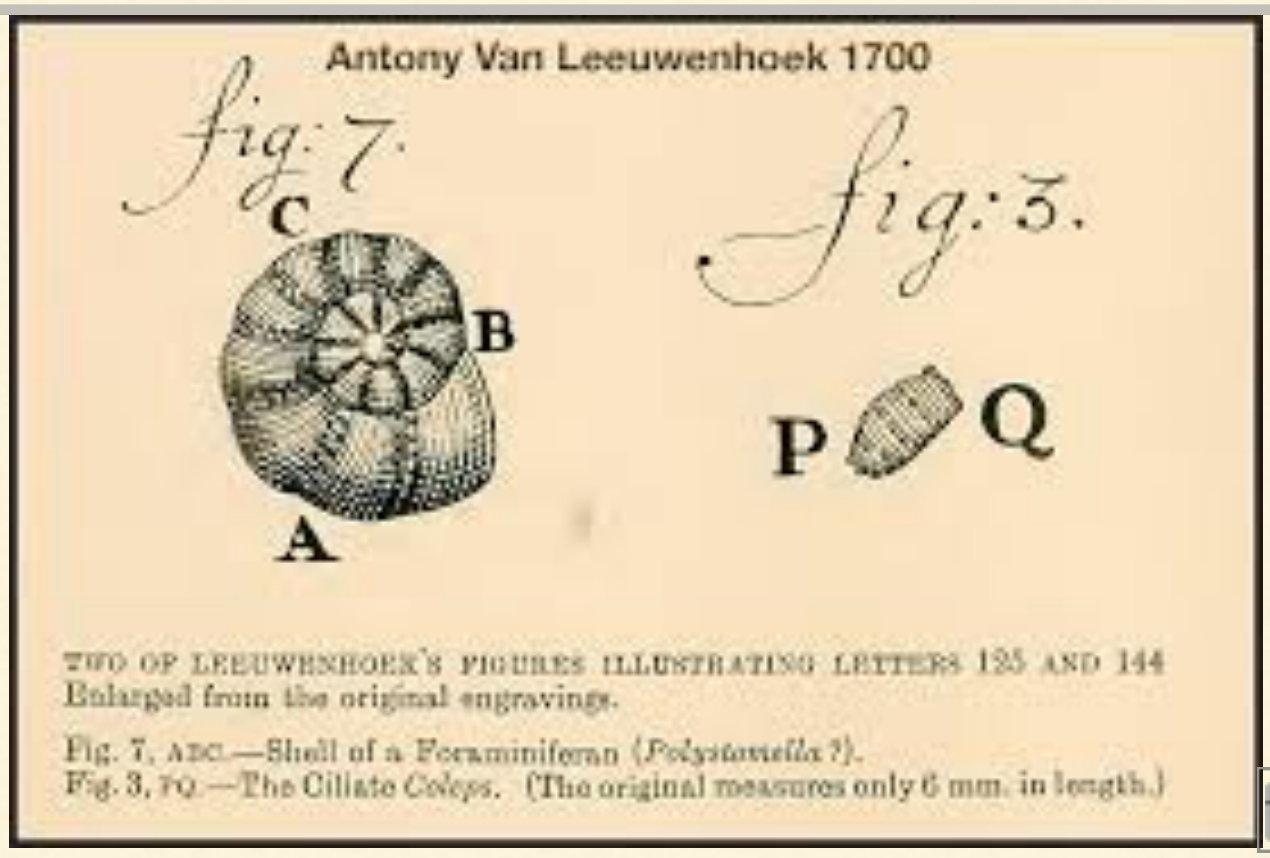
Bill Sofer

December, 2015.

SECTION 1

Cells

This introductory section concerns cells, their discovery, their anatomy, and their behavior. There's also a chapter on molecular biology, and one on cell division.



TWO OF LEEUWENHOEK'S FIGURES ILLUSTRATING LETTERS 125 AND 144
Enlarged from the original engravings.

Fig. 7, ABC.—Shell of a Foraminiferan (*Polystomella*?).
Fig. 3, PQ.—The Ciliate *Coleps*. (The original measures only 6 mm. in length.)

1 Early Microscopy

Most of the major advances in the biological sciences have come as a result of technological innovations. One such breakthrough was the invention of the microscope, a development that resulted in a huge leap in biologists' ability to probe the structure and function of cells. In fact, prior to the use of microscopes, people had no idea that cells even existed.

Leeuwenhoek

Microscopes were first fully exploited for the study of biological specimens by Antony van Leeuwenhoek (Figure 1.1) in the late 16th and early 17th

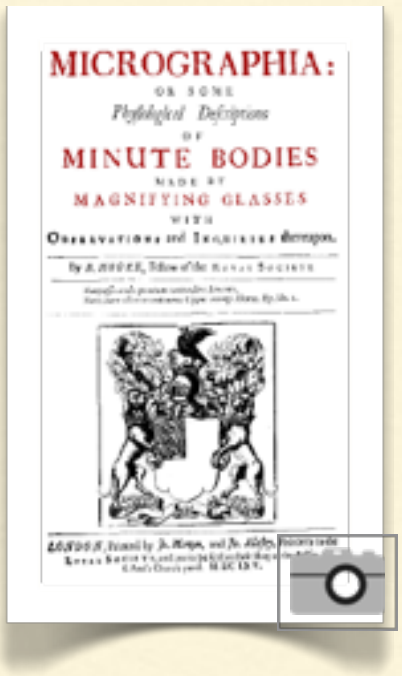
Figure 1.1

Portrait of Antony van Leeuwenhoek (1632-1723).

century. Born in 1632 into a middle class family in Delft, Holland, their fifth child and first son, Leeuwenhoek was sent to Amsterdam to learn to be a cloth merchant at the age of 16. Six years later he returned to Delft and remained there for the rest of his life except for a brief holiday visit to London in 1668. He married in 1654, and had five children, all but one of whom died in infancy. His only surviving child was a daughter, Maria, who was born in 1656. She never married and faithfully looked after her father all of his life. His wife died in 1666 and Antony remarried in 1671. His second wife predeceased him in 1694.

While primarily a tradesman, Leeuwenhoek held many other positions. One lucrative but not particularly onerous one was chamberlain to the Sheriffs of Delft council. Appointed in 1660, his duties were to open and close the chamber for business, to tend the coals, to clean the chamber hall, and, most importantly, to keep to himself whatever he may have overheard during council meetings. For this he was paid somewhere between 300 and 450 florins, about $4,000 in today's money. He was also a licensed town surveyor as well as the municipal "winde-gauger", a person who assayed the wines and spirits that came into the city.

Figure 1.2

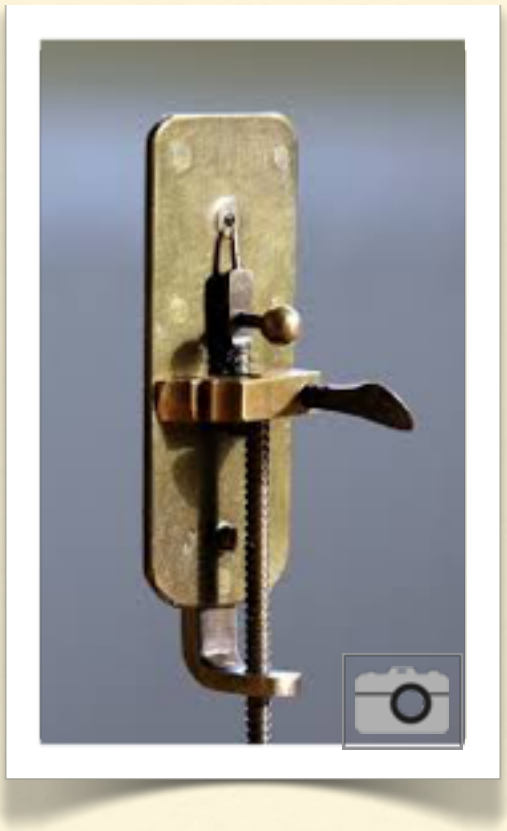


Robert Hooke's book, "Micrographia

Apparently, Leeuwenhoek was a man of considerable energy, ability, and versatility; a solid and well respected citizen. One indication of the high esteem in which he was held was that he was chosen to be the executor of the will of the painter Vermeer. Born in the same year as Leeuwenhoek, Johannes Vermeer had died at the age of 43. He left little money but many paintings to his wife and children. Leeuwenhoek was entrusted by the city council to straighten out his estate.

These offices and duties would seem to have been sufficient to fully occupy the life of anyone, but Leeuwenhoek had an avocation, microscopy, for which he became renowned both in his time and thereafter. No one is certain how he came to acquire this "hobby". Perhaps he was inspired by Robert Hooke's well known

Figure 1.3



A replica of one of Leewenhoek's microscopes

book, "Micrographia" (Figure 1.2). Perhaps he became acquainted with magnifying lenses in the course of examining cloth. For whatever the reason, once he began examining specimens under his primitive but powerful microscopes, it became an obsession. His microscopes (Figure 1.3) were simple in that they made use of a single lens. More sophisticated "compound" microscopes (Figure 1.4), consisting of a tube with a lens at each end had been invented decades earlier. In fact, Hooke used them in his studies. But at the time, they were only capable of magnifying objects some 20 or 30 times. Leeuwenhoek's microscopes were better by a factor of 10 although they were difficult to use.

Figure 1.4

Reproduction of first compound microscope made by Hans and Zacharias Janssen, circa 1590.

Fame

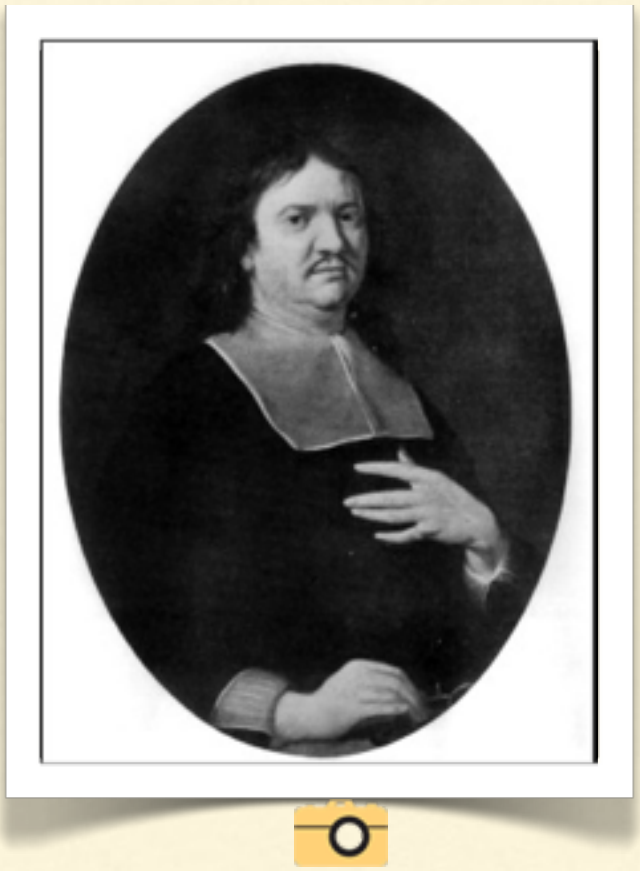
Leeuwenhoek's rose to prominence through his association with the Royal

Figure 1.5

Robert Hooke
(1635 - 1705)
A portrait drawn from a description of Hooke by Rita Greer.

Society of London, an institution founded in the early 1660's with the goal of gathering together a group of distinguished natural philosophers and physicians ("fellows") to discuss and verify the discoveries that were being made throughout the world. The fellows began by meeting weekly to witness experiments and to exchange ideas. Robert Hooke (Figure 1.5), the originator of the word "cell", and an avid user of microscopes, was named first curator of experiments. Henry Oldenburg was the Society's first secretary (Figure 1.6). His job was to correspond with prominent scientists around the world in order to get word of new significant scientific developments.

Figure 1.6



Henry Oldenberg (1618 - 1677), the first secretary of the Royal Society of London

One correspondent was a Dutch physician named Regnier de Graaf, best known today for the eponymous follicle in the ovary. De Graaf wrote a letter to Oldenburg describing some of Leeuwenhoek's results. He told Oldenburg that Leeuwenhoek's microscopes and observations were far superior to those of any of his contemporaries. As evidence, he enclosed a letter from Leeuwenhoek that contained a description of the mouth parts and eye of a bee and a louse.

Oldenburg was impressed. He published a translation of Leeuwenhoek's letter in the Transactions of the Philosophical Society (the oldest journal in continuous publication in the world). From that time until he died, Leeuwenhoek regularly sent reports to the Royal Society. They all had to be translated into English because he only spoke and wrote in Dutch. While a keen observer, Leeuwenhoek was a poor draftsman, and almost all the illustrations in his letters were drawn by a hired artist. He never wrote a book, or for that matter, a scientific paper. His only communications were through his letters. For these, he was elected a Fellow of the Royal Society in 1680, a great honor to this day. Training his simple optical devices - he made hundreds of them, often one for each observation - on specimens obtained from a variety of sources, no material was dirty or bizarre enough to escape Leeuwenhoek's attention. He was the first

to describe red blood cells, sperm, large bacteria, protozoa, and more. He had no formal scientific training or advanced degrees, but his insatiable curiosity made him a first rate scientist. Initially his descriptions of tiny organisms were questioned. Many thought that the presence of invisible creatures in the water and air was bizarre. But ultimately he was vindicated.

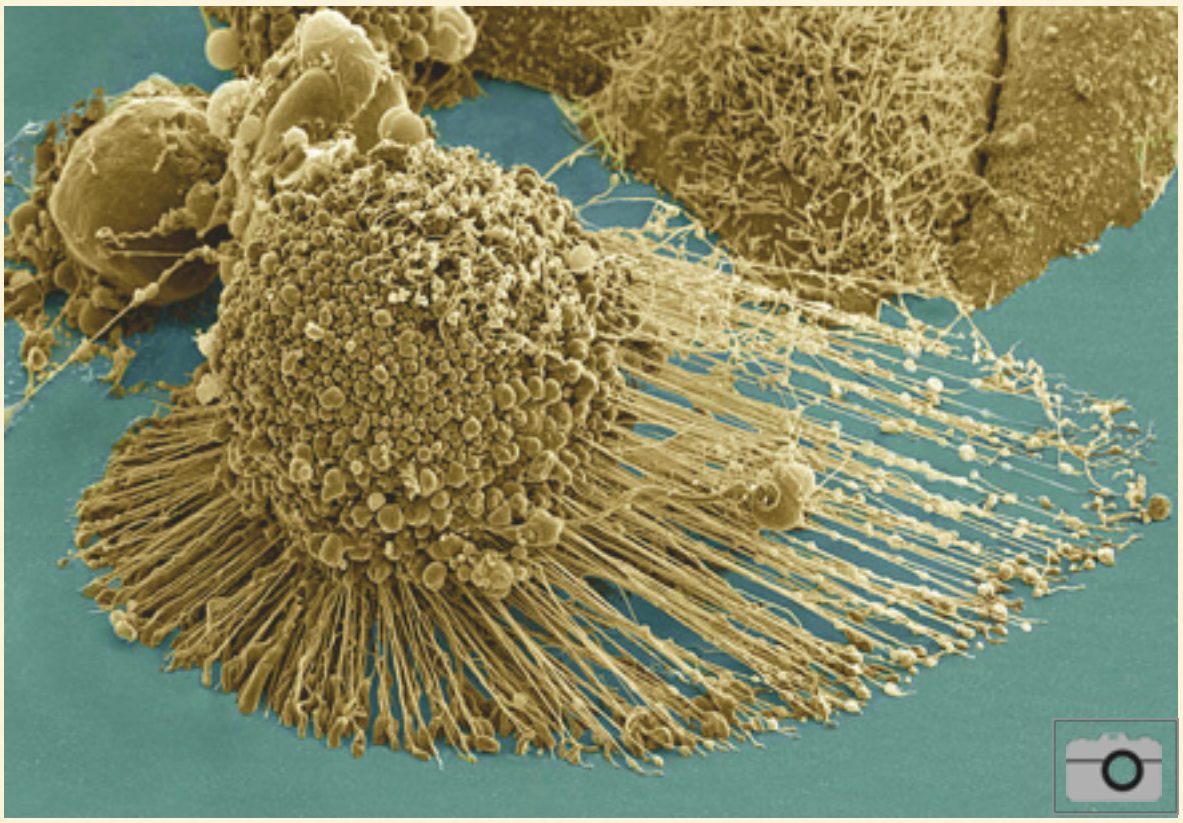
Figure 1.7

Robert Hooke's depiction of cells from cork

After Leeuwenhoek

After Leeuwenhoek's death a long period ensued in which microscopy lost its scientific allure. Apparently, few were able to match Leeuwenhoek's ability to construct suitable instruments or to make accurate observations. Only the development of more powerful and sophisticated instruments in the nineteenth century brought microscopy back as a major tool for biological research.

As microscopy improved and became more widely available, an important principle became apparent: all living things were found to be composed of minute building blocks called "cells", a name that Hooke had given to the empty spaces that he had observed in thin slices of cork (Figure 1.7). A group of brilliant German scientists working with the newly refined microscopes of the time, took this finding further and developed what has become known as the "Cell Theory", a set of principles that underlie present cloning and stem cell biology. I'll discuss the cell theory and describe some of the major players who helped developed it in the next chapter.



2

Cellular Origins Somatic vs. Germ Cells

The Cell Theory *

The cell theory is the foundation upon which all stem cell research rests. It has three parts.

First, that all organisms are composed of one or more cells.

Second, that cells are the fundamental units of function of organisms.

And last, that all cells originate by division from preexisting cells.

As described in Chapter 1, the path leading to these concepts began in the seventeenth century with the work of Leeuwenhoek and other early microscopists. While they led the way, it was to take another 200 years before their contributions gave birth to the cell theory. The scientists who ultimately developed the theory were a collection of European biologists, the best known of whom were a group in Germany who were students or associates of the distinguished anatomist, microscopist, and physiologist, Johannes Peter Müller. * Some of their names, like

Theodor Schwann, Matthias Jakob Schleiden, and Rudolf Virchow, are familiar to us to this day. Others, like Robert Remak, are less well known.

In Berlin from the 1830's to the end of the century, a community of biologists that included the ones noted above began examining a variety of biological materials under the newly improved compound microscopes of the day. They were frequently at odds. They argued vehemently within their tight circle and with other microscopists in other parts of Germany, France, and Great Britain. They attacked each other remorselessly in print. They fought over precedence. Often they failed to acknowledge each other's work. In the end, exactly who was responsible for the discoveries that ensued is difficult to untangle. Sometimes the individual who is generally given credit in textbooks was the one who reached the widest audience or came up with the best catchphrase rather than the individual who made the initial finding.

Figure 2.1

Theodor Schwann (1810 - 1882)

I'm convinced that because science is a social activity, many of its great advances emerge from circumstances like these, where a group of individuals publish new ideas, fiercely debate theories and interpretations, and argue over priorities. Gregor Mendel's almost single-handed discovery of the laws of genetics is an apparent exception to this rule. But his relative isolation had profound consequences: his work was not recognized until 35 years after its publication.

Theodor Schwann *

Schwann (Figure 2.1), along with the botanist Jakob Schleiden, are often cited as the pair that originated the concept that all organisms are composed of cells. The real story is a bit more complicated.

Schwann was born in 1810 in Neuss in what is now extreme western Germany. At the time of his birth, Neuss was part of France, but with the defeat of Napoleon in 1814 it became Prussian. Always deeply religious, Schwann studied theology at first, but after graduation from a Jesuit

college at the age of 18 he decided to pursue medicine and natural history. At the University of Bonn he met Johannes Müller and followed him to Berlin where he became one of his assistants. Schwann had a talent for crafting suitable instruments. Making use of this skill, he made contributions to a variety of disciplines. His fame, however, originated from an apparent chance conversation with Jakob Schleiden over dinner one night in October, 1837.

Figure 2.2

Robert Remak
(1815 - 1865)

They were comparing notes, and Schleiden commented on the presence of nuclei in the plant tissues that he was studying. Schwann, who at the time was analyzing some embryonic frog tissues had an epiphany. The tissues that he had been studying were also full of cells with prominent nuclei. Perhaps, he thought, nuclei played similar roles in both plants and animals. Perhaps they were involved in the generation of new cells. Going further, since cells were common to both plants and animals, perhaps they were present in all biological tissues. Schwann immediately invited Schleiden to look at the specimens that he had been working on. Schleiden agreed that the cells looked more or less the same.

Shortly thereafter, Schwann published a monograph, the second section of which was entitled, "Cells as the Basis of All Tissues in the Animal Body". Apparently, this bold statement caught the eye of his contemporaries and is the basis for Schwann's association with the cell theory. Modern historians who have examined the literature have found that several of Schwann's contemporaries expressed similar ideas at about the same time, but they were not as successful in communicating them to their colleagues. By the way, Schleiden was not mentioned in Schwann's monograph, but he had published an article in 1838 in which he noted the role of cells in plant tissues. Unfortunately he went further and claimed that the cell nucleus is the component that gives birth to the rest of the plant cell by a process akin to crystallization that occurs outside of the cell's boundaries. Schwann was of a similar opinion, although the two

differed in some details. Of course, both men were wrong about how cells originate. The correct answer was to come from another one of Müller's students.

Robert Remak *

The scientist who was most responsible for showing that cells arose by division was Robert Remak (Figure 2.2), someone who is not as well known as either Schleiden or Schwann.

Remak, like Schwann was a physician, a microscopist, and a student of Müller. Unlike Schwann, he was Jewish and a Polish nationalist. Both these characteristics proved to be enormous burdens for a scientist living in Germany. In particular, the antisemitic climate that existed in Berlin at the time weighed heavily against him. Because he refused baptism, he found it difficult to earn a paying position at a university. In fact, at the time that he earned his medical degree Jews were not permitted by law to hold a position at any Prussian university. In order to continue his research, he was forced to earn a living by working as a physician in private practice.

Figure 2.3

Rudolf Virchow
1821 - 1902

Despite these difficulties his scientific life was extraordinarily productive. For example, his doctoral thesis demonstrated that the axon, the long process that carries nervous signals, is a part of the nerve cell, not some independent entity as was previously thought. His contributions to embryology were of an equally high order.

With regard to the cell theory, his microscopic analysis of chicken development convinced him that cells didn't appear via crystallization as Schwann and Schleiden claimed, but rather arose from other cells by division. He published his observations in a paper published in 1852. He voiced his disbelief in Schleiden and Schwann's mechanism of

how cells develop with strong language that was typical of the time:

"For myself, the extracellular creation of animal cells ... is as incredible as the spontaneous creation of organisms" (translation by Kisch).

It's clear from this quote that Remak had formulated the last proposition of the cell theory, that cells only derive from other cells. And they do so by cell division. Later, he even remarked that cancer cells obey this same principle. Yet, Remak's contributions were largely ignored. Instead, credit for this idea often is given to another student of Müller, one whose religion and nationality were a much better fit to the time and place; one who ultimately achieved a high position and fame; and someone who came up with a memorable phrase to express his views.

Rudolf Virchow *

Rudolf Virchow (Figure 2.3) was perhaps the greatest physician of the 19th century. He was the first modern pathologist and the primary spokesman for German biological science and medicine for 50 years. Born in 1821 in Scheivelbein Pomerania near present day Poland, he was an only child. His excellent grades and the help of a well connected family earned him a scholarship to study medicine at the prestigious Friedrich Wilhelm Institute, a military academy in Berlin. After graduating, he took a position in the same institution, studying under Müller. Virchow's main interests were pathology and microscopy, and he came to believe that disease was caused by defects in cells rather than by ill humors (or even tiny creatures) floating in the air. In later life he came to accept that microbes were the source of many disorders, but he acquiesced to this view reluctantly.

Virchow was a man of supreme self confidence, a bit of a martinet, and highly opinionated. He was also a polymath. In addition to being the founder of modern pathology, he spoke ten languages, wrote two books on anthropology, and had a long political career. Politics first entered his life when, as a young 27 year old physician, he was asked to investigate an outbreak of cholera in Silesia among Polish peasants in 1848. His report was not simply a list of required hygienic measures, but an indictment of the government for permitting the range of injustices and lack of opportunities for the locals. As one might anticipate, the report proved very unpopular among government officials and he was dismissed from his position in Berlin and "exiled" to an appointment as the Chair of the Department of Pathological Anatomy at the University of Würzburg. He spent seven years there,

The Sausage Duel

There is a story that has been popular since the 1890's about a duel between Virchow and Bismarck that I will pass along although it has little to do with science. The account goes that Virchow publicly attacked Bismarck's war budget as profligate. His language irritated the Chancellor sufficiently so that Bismarck challenged Virchow to a duel. Since Virchow was the challenged party, he had the choice of weapons. He chose sausages, one uncooked and laced with the Trichenella parasite, and other one well done. The Chancellor was to pick one at random and eat it; Virchow would ingest the other one. The Chancellor declined to accept the conditions and the duel was called off.

Unfortunately, the tale is almost certainly apocryphal. There is no record of the sausage offer in contemporary records, and neither Virchow or Bismarck mention it in their correspondence. Virchow did verbally attack Bismarck, a duel was proposed (by Bismarck), and it was never fought. Moreover, Virchow was interested in parasites and was an outspoken advocate for cooking pork well done. That much is fact. But the reason for

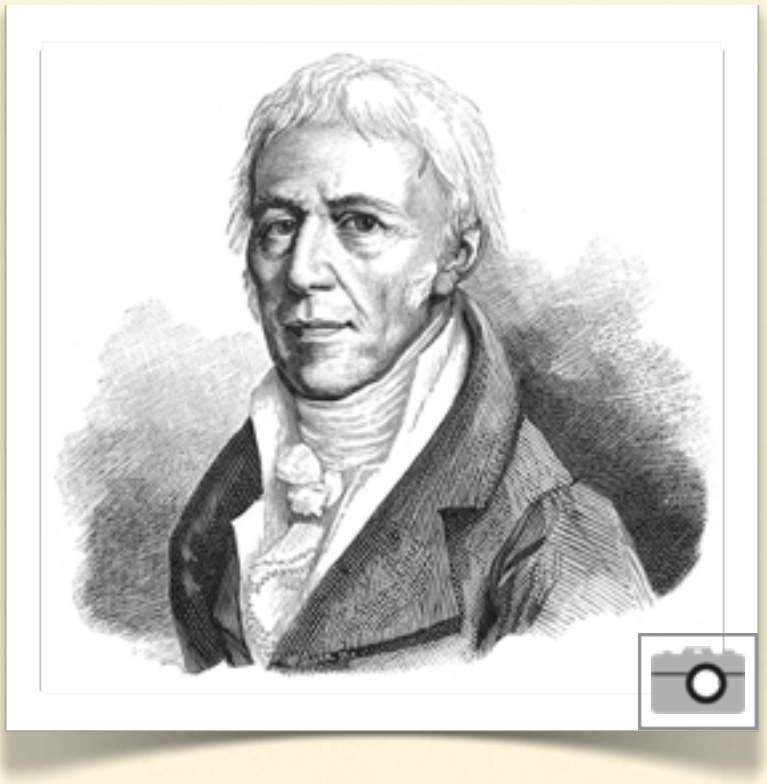
forgoing politics, focussing mainly on research, thereby reinforcing his already formidable scientific reputation.

When he returned to Berlin he continued his superb scientific work but also reentered the political arena. He was elected to the Reichstag. He became chairman of the finance committee, a post he held for 13 years. In this position, he often crossed swords with Otto von Bismarck, the "Iron Chancellor" (see sidebar, "The Sausage Duel"). *

Virchow is best known for the phrase "*Omnis cellula e cellula*", all cells come from other cells. As noted, the discoveries that led to this aphorism originated with Remak. At first Virchow appropriated Remak's work in a book of his own without acknowledging his colleague's contributions. However, perhaps in a fit of remorse, he later credited Remak's role. Subsequently, though a series of highly influential lectures, wielding the weight of his reputation and force of his personality, he managed to convince the scientific community of *Omnis cellula e cellula*. Due to his strong advocacy he is generally given credit for the concept. Remak has been largely forgotten. As Francis Darwin put it,

"... in science the credit goes to the man who convinces the world, not to the man to whom the idea first occurs. Not the man who finds a

Figure 2.4



Jean-Babtiste Lamarck
(1744 - 1829)

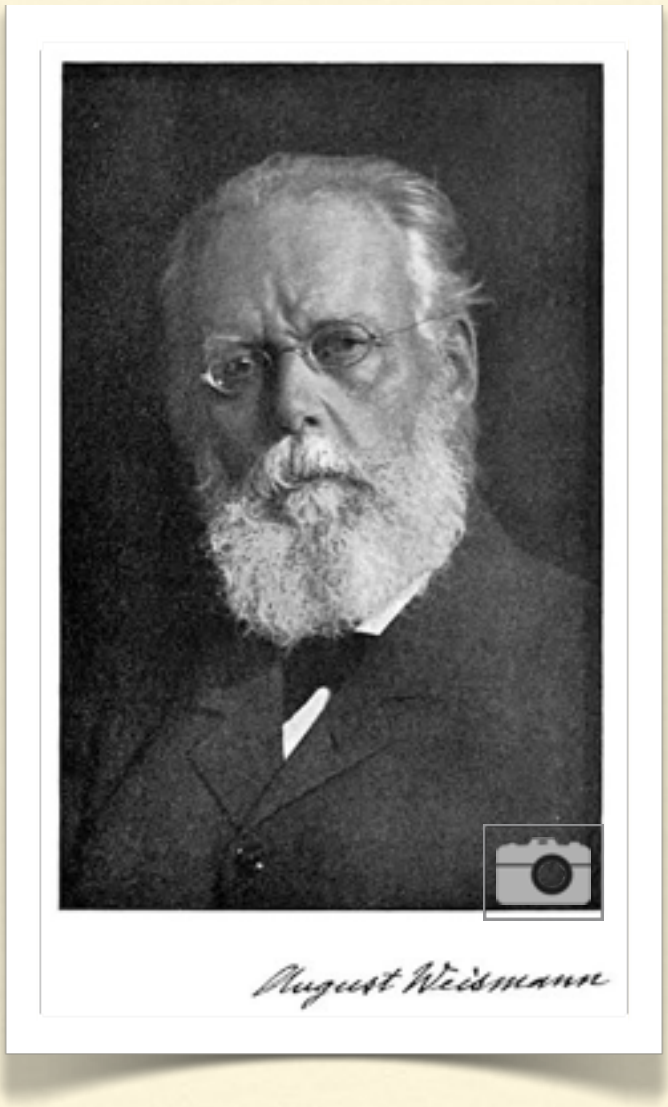
grain of new and precious quality but to him who sows it, reaps it, grinds it and feeds the world on it." *

August Weismann *

In addition to the cell theory, a second concept that is critical to an understanding of stem cells came from another great German scientist of the 19th century, August Weismann. Weismann, born in 1834 into a middle class family, studied medicine and had a successful practice in Frankfurt. During the Franco-Austrian war of 1859 he served as a field doctor. And from 1861 to 1863 he was the private physician to Archduke Stephan of Austria. But from his earliest days he had a fascination with zoology, embryology and evolution, and later published extensively in these areas. In 1863 he gave up his medical career and joined the faculty of the University of Freiburg, eventually becoming the first to hold the Chair in Zoology at that University.

In chapter 8, we'll see that Weismann was responsible for a theory that explained how cells become different from one another. But he is most well known for an idea that arose from his fierce opposition to Lamarck's theory of the inheritance of acquired characteristics.

Figure 2.5



August Weismann
1834 - 1914

Darwin and Lamarck

Was Darwin a Lamarckian? Several passages in "Origin" refer to "use and disuse", a phrase coined by Lamarck. Did Darwin think that these processes added to the pace of evolutionary change? Did he believe that it might augment natural selection? Perhaps. It's a controversial issue.

Another possible explanation is that Darwin thought of use and disuse in the same way as modern evolutionary theorists do, except, of course, that Darwin, wasn't aware of genes or DNA.

Here's the modern interpretation. All genes are subject to mutation. Those that control processes that are unimportant or seldom used will accumulate more mutations over evolutionary time than genes that make a great contribution to survival. That's because when deleterious mutations occur in essential genes (and most mutations are, in fact, deleterious), those genes are not passed on to future generations because the organisms that harbor them are less capable of mating. Mutations in genes that don't matter much don't, by definition, affect the ability of the organism's ability to have offspring. The mutations aren't as readily eliminated and they tend to accumulate.

Jean-Babtiste Lamarck, a Frenchman born more than a century before Charles Darwin published "The Origin of Species", is often credited with being the first to have proposed a comprehensive theory of evolution. He attributed evolutionary changes in organisms to "use and disuse". To cite an often cited example, he believed the necks of giraffes became extended because generations of giraffes had stretched to reach leaves high in trees. Moles, he wrote, lost their eyes because they didn't use them while burrowing underground.

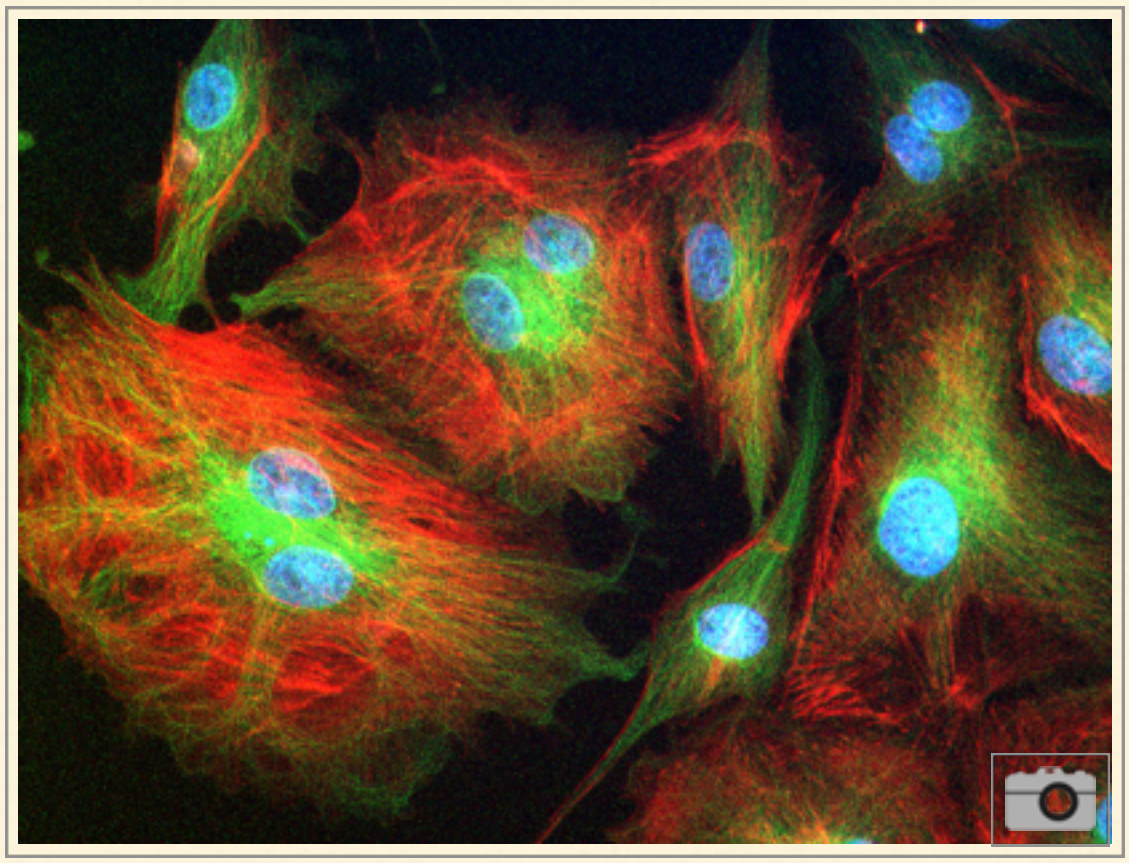
It is not commonly acknowledged, but Charles Darwin resorted to "use and disuse" explanations in later editions of "Origin" in order to provide a mechanism to accelerate the rate of evolutionary change (for a different point of view, see the "Darwin and Lamarck" sidebar). He did so because the physics community in the nineteenth century had incorrectly concluded that the earth wasn't old enough to support the slow pace of evolution that natural selection required. However, if Lamarck was correct, his theory required a mechanism that allowed for changes in parts of the body (like the neck or the eyes) far removed from the testes or ovaries to influence the inheritance of future traits. To account for these influences, Darwin devised a theory

of heredity, called “pangenesis” . It never came to much.

Weismann, a strong supporter of natural selection, felt that Darwin was mistaken in both his theory of pangenesis and his invocation of “use and disuse”. In fact, while not widely recognized for his experimental efforts (he was partially blind), Weismann was driven to carry out one study in which he cut off the tails of 68 mice over 5 generations. He examined more than 900 or so mice in subsequent generations, and found that despite what was done to the parents, the tails of their progeny weren’t lost or even shortened.

As a result of his experimental work and an extensive analysis of the literature, Weismann came to the conclusion that cells of multicellular organisms could be divided into two distinct categories. One type, termed “germ cells”, sperm and eggs in animals, are set aside early in development and are responsible for passing characteristics from one generation to another. The remainder, called “somatic” cells, do not participate in heredity because they are distinct from eggs and sperm and have no influence on them. In Weismann’s view, somatic cells could endure whatever indignities heaped upon them without there being an effect on future generations. The fact that the soma couldn’t alter future traits came to be known as the “Weismann barrier”.

Why is the Weismann barrier relevant to stem cells? Germ cells and their immediate progeny are capable of forming all of the cell types in the body. For most organisms, somatic cells are restricted in this ability. One long term objective of the scientific community has been to increase the developmental capacity of somatic cells; to make them, like germ cells, capable of forming any kind of cell, and to do so in a precise manner at the discretion of the scientist. As we’ll see in later chapters, this feat was partially accomplished only a decade ago.



3 Cell Anatomy

A photomicrograph of a cell taken from a human cheek is shown in Figure 3.1. It's about 10 micrometers across (see "Metric Matters").

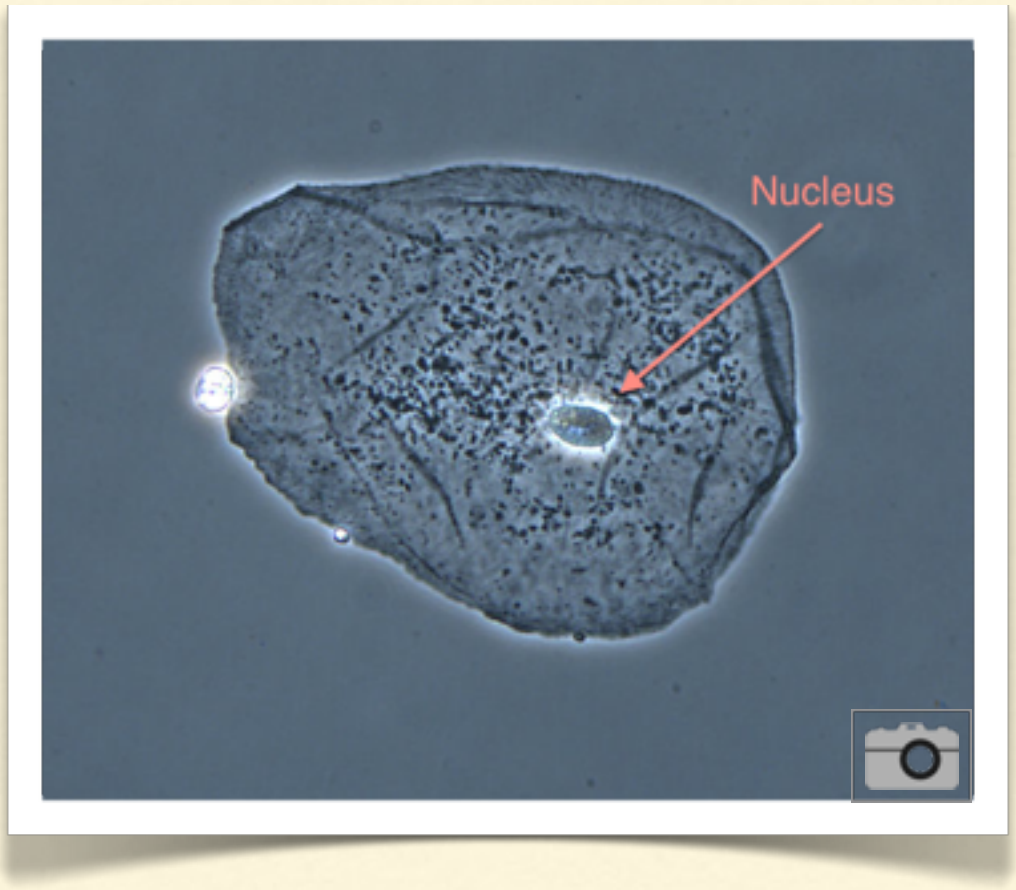
This cheek cell was alive when its photograph was taken. Ordinarily if you were to examine an unstained living cell under a microscope, you would only see a ghostly image, with little contrast and with few discernible features. The structures in the cell pictured in Figure 3.1 are easily visualized because of an

Metric Matters

Here's a brief primer on metric units that are used when measuring very small structures.

A meter is about a yard long. A millimeter is a thousandth of a meter. To put that in perspective, there are approximately 25 millimeters to the inch. A micrometer or micron is a thousandth of a millimeter, a millionth of a meter. If you do the math, this means that there are about 25 thousand micrometers to the inch. A little arithmetic reveals that the cell depicted in Figure 3.1 is therefore about 1/2500 of an

Figure 3.1



A phase contrast image of a living human cheek cell

optical technique called "phase contrast" microscopy invented by Frits Zernike for which he was awarded the Nobel Prize in 1953.

The most prominent feature of the cell pictured in Figure 3.1 is a relatively large membrane-bound ellipsoid body that is visible near its center called the "nucleus", which contains nearly all of the cell's hereditary instructions. I use the word "nearly", because additional genetic material is carried by the mitochondria and, in plant cells, chloroplasts. More about these structures below. Inside the nucleus are the chromosomes, thread-like structures composed of DNA and proteins. It is the DNA that carries the cell's instructions, our genes. The word "**chromosome**" means "colored body". They're so named because they stain deeply with some common dyes. The word "**chromatin**", which we'll encounter later, refers to the entirety of DNA and the proteins bound to it that are present in all the chromosomes. The term "**genome**" refers to the entire complement of genetic material in the nucleus of a cell.

We humans have 46 chromosomes, arranged in 23 pairs. One member of every pair is contributed by each of our parents. The two members of a pair are "**homologs**", and said to be "**homologous**" to one another. Homologous refers to the fact that each

chromosome of a pair lines up with its homolog at meiosis (see chapter 13). The two homologs are generally of approximately the same size and shape, with the same genes arranged in the same order. However, the individual genes on homologous chromosomes often differ from one another. These differences arise from "**mutations**", changes in the sequence of DNA. I'll elaborate on this point in the next chapter.

Outside the nucleus is the "cytoplasm", a complex, protein-rich, jelly that is delimited from the outside world by the cell membrane. In the cytoplasm are several structures, "**organelles**", that are visible under the light microscope. These include mitochondria, which are the major energy producing structures within cells; chloroplasts, which convert sunlight into energy and basic foodstuffs in plants; and the Golgi apparatus, a stack of membranes that plays an important role in the secretion of proteins. Except for chloroplasts, which are found only in plants, these organelles are present in the cells of virtually all higher organisms.

Eukaryotes and Prokaryotes

The nucleus and many of the other organelles that I discuss in this chapter are characteristically found in a group of organisms called "eukaryotes". Animals, plants, and fungi fall into this category, and they are the focus of most of the discussion in this book.

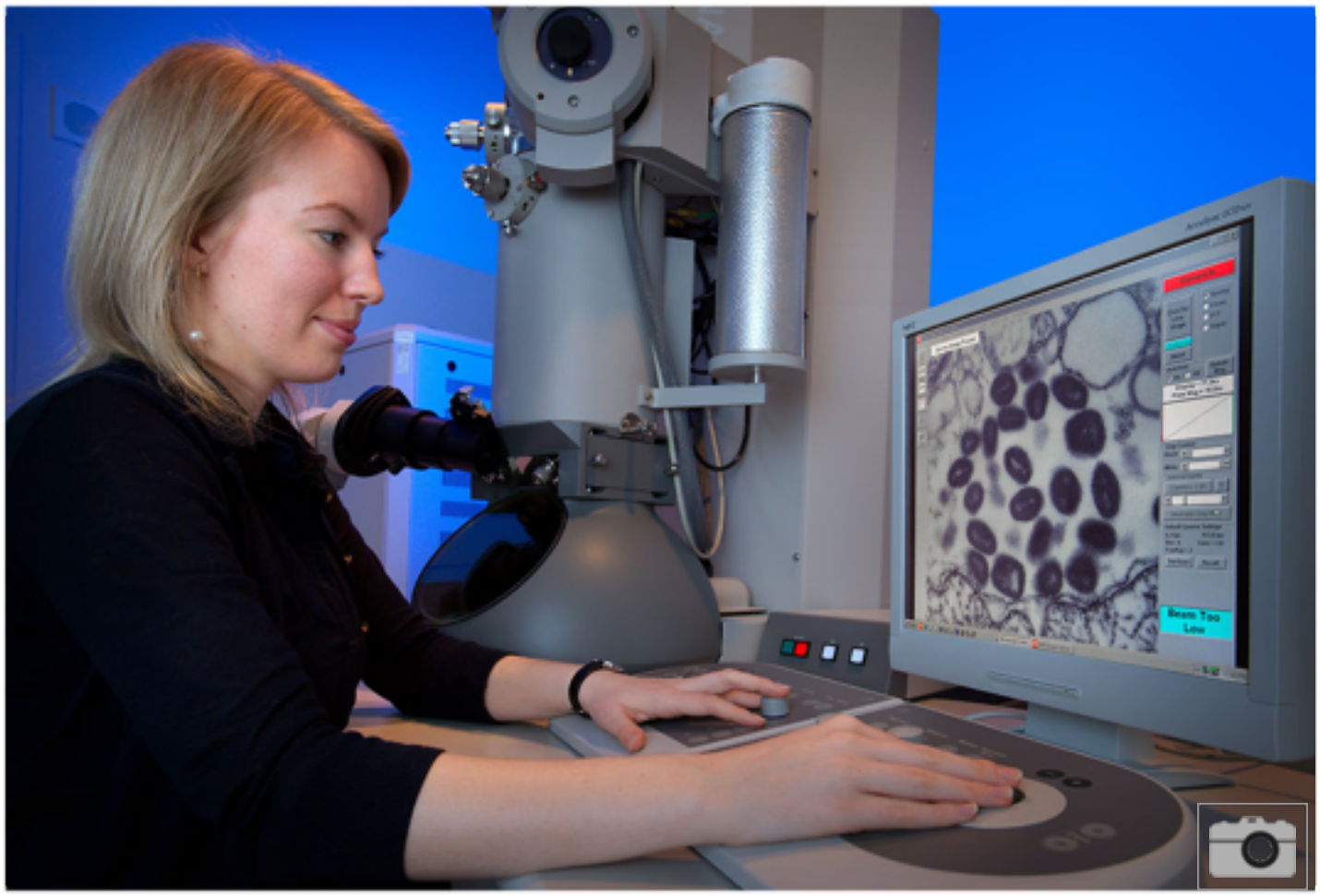
Bacteria and archaea (single cell organisms that look very much like

Electron Microscopy

Despite the development of sophisticated staining and optical techniques, microscopes in the early twentieth century were limited in resolution by the fact that they used light to illuminate their specimens. Green light has a wavelength of about 0.5 microns, and structures that are smaller than this cannot be resolved. When the electron microscope was invented in the early 1930's by Ernst Ruska and Max Knoll, an instrument with much higher resolving power was placed in the biologist's toolbox. Electron microscopes (Fig 3.2) are highly sophisticated and expensive instruments that use a beam of electrons to illuminate specimens. Because electron beams have wavelengths about 10,000 times shorter than light, they enable microscopists to see structures that are invisible under the light microscope.

Once electron microscopes became readily available, additional details were rapidly

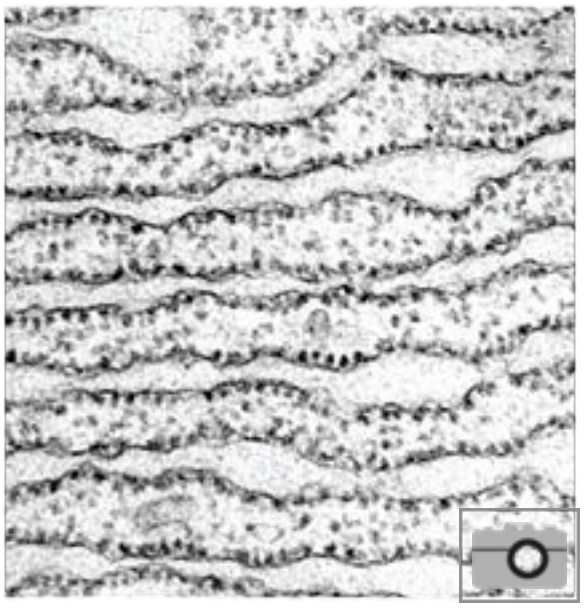
Figure 3.2



Using the electron microscope

revealed about organelles that had already been identified. In addition, a host of previously unknown structures were discovered. For example, when thin sections of the cytoplasm were examined under the electron microscope a complex series of flattened membranes were seen. Taking up more than 10% of the total volume of some cells, this "**endoplasmic reticulum**" or "ER" is often studded with small roughly spherical particles called "**ribosomes**" (Fig 3.3). The ER plays a

Figure 3.3



Endoplasmic reticulum as visualized by electron microscopy

role in exporting proteins from the interior of the cell to the outside world.

The ribosomes that accompany the ER are complexes of RNA and proteins. Sometimes they are found free in the cytoplasm. They are used by all living things to synthesize proteins. Again, I'll discuss ribosomes and protein synthesis is more detail in the next chapter.

Other improvements in microscopy have followed. Two in particular, have made great contributions to our appreciation of the inner workings of cells. The first is called "epifluorescence microscopy" or just plain "fluorescence microscopy". In it the specimen is illuminated with light of a specific wavelength. Certain chemicals can be introduced into the cell that have the ability to absorb the light and reemit it as a different color. Substances that behave this way are termed fluorescent. Using the appropriate filters, the fluorescent

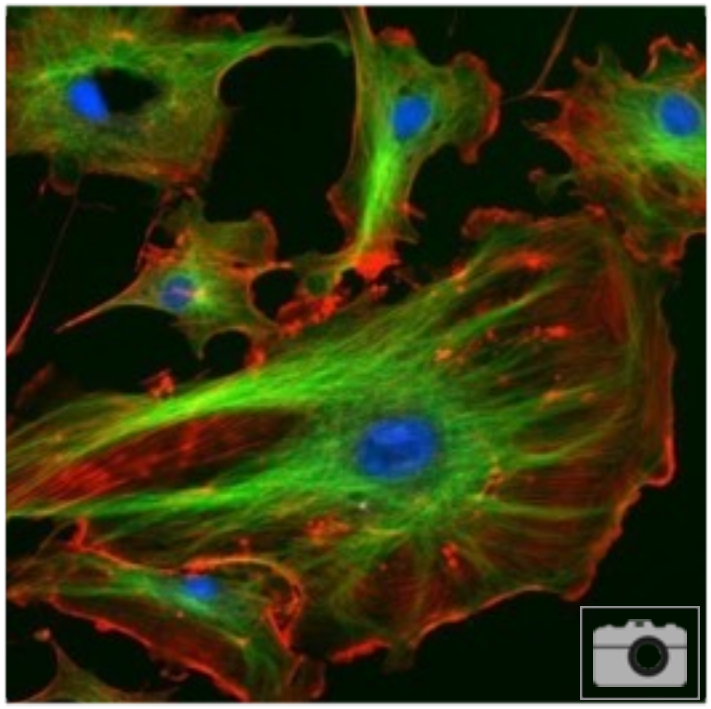
More About Antibodies

Antibodies or immunoglobulins are proteins involved in the immune response of vertebrates. They serve to defend against invading foreign parasites and microbes. Plentiful in the blood of humans, they make up about a fifth of the total protein of plasma.

The body is capable of making literally billions of different antibodies. Secreted by a type of white blood cell called a "lymphocyte", each antibody has a distinct shape that allows it to recognize and bind to a specific substance, called an "antigen".

Scientists take advantage of the specificity and tight binding ability of antibodies and use them as reagents to unambiguously identify specific molecules, often other proteins. They attach a variety of signaling molecules to the antibodies, including fluorescent dyes, so that they can visualize the molecules in which they're interested.

Figure 3.4

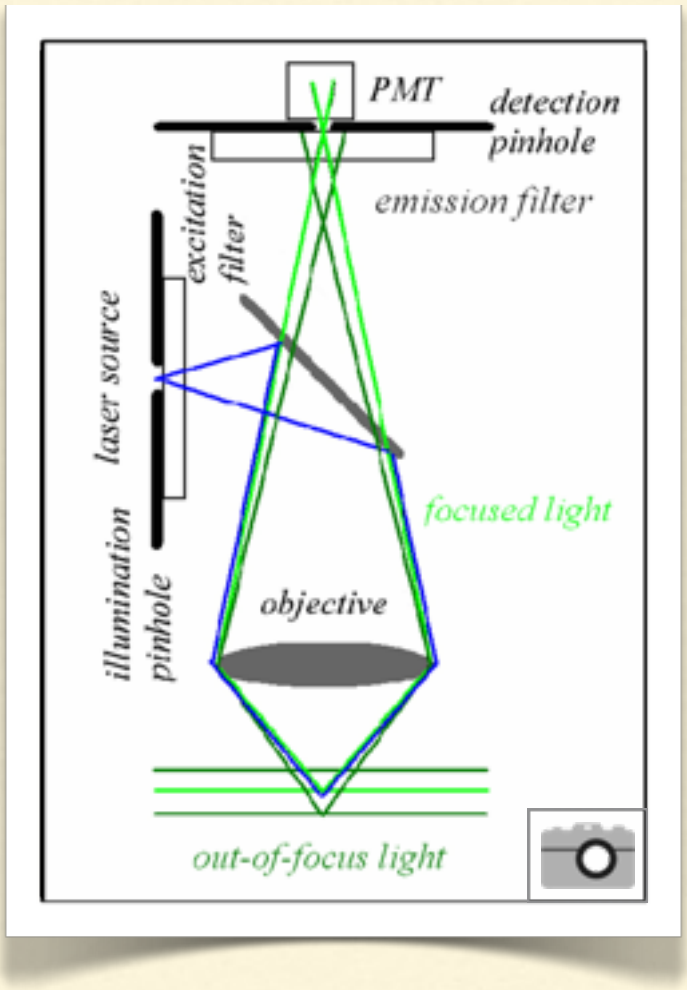


Cells stained with fluorescent dyes. Nuclei fluoresce blue because they bind DAPI. The green and red structures have been stained using specific fluorescent labeled antibodies (see text).

microscope is designed to block the illuminating light and only detect that which is emitted by the specimen.

The micrograph shown in Fig. 3.4 illustrates the power of this technique. A fluorescent dye called DAPI is added to the sample under inspection. It binds strongly to DNA. When illuminated with ultraviolet light, the bound dye emits light of a blue color. By combining fluorescent microscopy with DAPI staining, the microscopist is able to easily visualize nuclei and the chromosomes within them because the dye has bound to DNA.

Fig 3.5



Principle of confocal microscopy
The sample is illuminated with light from a laser (blue). The fluorescent signal is detected by a photomultiplier tube (PMT). A pinhole prevents out-of-focus light from entering the PMT.

To visualize other cellular components a more sophisticated technique that takes an indirect approach is often used. Biologists inject chemicals of interest into rabbits, mice, goats, or horses. These substances are recognized as foreign and cause the animals to produce antibodies, specialized proteins (see “More About Antibodies”) that can recognize the foreign molecules and bind tightly to them.

For example, in order to produce the photo in Fig 3.4 antibodies were raised against two different structural proteins. Each antibody had a different fluorescent dye tethered to it. One emitted green light; the other red. When cells affixed to a slide were bathed in solutions containing these fluorescent labelled antibodies, the proteins that the antibodies recognized and were tightly bound to could be visualized. The fluorescent microscope reveals this light in brilliant color as shown in Figure 3.4. The structures stained green, called “microtubules”, have had antibodies directed at the protein tubulin bound to them. Microtubules act to help transport materials from one place in the cell to another. They also function during cell division, driving the chromosomes apart at the appropriate time. The red staining

structures, visible because they have labelled antibodies directed against the protein actin bound to them, are called "actin filaments". They function in cellular locomotion and to help the cell assume an appropriate shape. (There is a third class of structural proteins commonly found in cells and not shown in the figure. They're called "intermediate filaments". They provide mechanical strength and structural integrity to the cell).

A second technique, called "confocal microscopy" makes use of fluorescence emissions too (Fig 3.5). Cells are scanned with a minute spot of laser light that traverses the sample line by line. The fluorescent signal emanating from the sample is processed in such a way that light from the out-of-focus areas is discarded. The result is an image that is not only sharper than that afforded by epifluorescence microscopy, but also one that represents a thin section through the specimen. Confocal microscopes can thereby make successive slices through a sample without having to actually physically cut into it.

These and other similar techniques have enabled biologists to get a remarkably detailed look at the inner components of cells. They reveal labyrinthine structures composed of a host of constituents. Of course, even the most powerful microscopes can only unveil a small fraction of a cell's complexity. For a still more intimate and detailed view, scientists turned to analysis by the techniques of biochemistry and molecular biology. I'll discuss some of the fundamentals of these disciplines in the next chapter.

The three dimensional molecular images in this and other chapters were prepared using the UCSF Chimera package. Chimera is developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIGMS P41-GM103311).

4 Molecular Biology Overview

It's helpful to have a rudimentary understanding of molecular biology in order to fully appreciate the latest developments in cloning and stem cell biology. For those readers who have never learned the basic concepts of this subject or who are in need of a refresher, I'll try to provide just enough background so that later portions of the book are more comprehensible. Rather than bog you down with a wealth of minutiae, jargon, and extraneous matters, I'll attempt to present a brief overview of the field, omitting many of its fascinating details, passing over its innumerable exceptions, and only providing a glimpse at its major features. For those who want a slightly deeper immersion in the subject, some additional information is provided in Slideshow 4.1, later in the chapter.

Polymers

Molecular biology has at its core a well established, but infrequently publicized, fundamental principle:

All of the processes of life are performed by a class of molecules called "polymers".

Polymers provide a storehouse for life's "software", the instructions that manage the entirety of operations in living things. Moreover, the machinery, the hardware, that carries out these instructions is also made of polymers. In short, polymers are the substances that are responsible for life on this planet.

What are polymers? They have two defining characteristics. First, they're repetitive molecules. That is, they consist of many instances of a small chemical, a **monomer**, forged together into long molecular chains. Second, in a given polymer, the monomers are linked to one another by similar or identical bonds.

3D Molecule 1

3D model of cellulose
Carbon atoms are gray, oxygens are red, and hydrogens are white. This fragment of seven monomers only represents a small portion of a cellulose molecule.

Both artificial and natural polymers exist. A three dimensional representation of a small section of a natural polymer (cellulose) is shown in "3D Molecule 1". I show it to illustrate the two properties noted above. Notice the numerous identical hexagon shaped monomers. These are glucose molecules composed of rings of five carbon atoms and one oxygen atom, as well as some attached oxygen and hydrogens. Note too that each glucose monomer is tethered to its neighbor in the same way. Polymers like cellulose may have chains that are many thousands of units long.

Artificial polymers are found almost everywhere in the modern world. Many of the manmade fibers that comprise our clothing, such as nylon, rayon, and polyester are polymers. Moreover, all plastics are polymers, including such familiar synthetic substances as polyethylene, polypropylene, polystyrene, and teflon.

Plastics were invented in the late nineteenth century and polymeric fibers somewhat later, but it appears that Nature scooped mankind by billions of years. Cellulose, which we've already considered, is a natural polymer that's been around for eons. It makes up the bulk of all plant material and is the most abundant biologically derived substance on earth. Starch and glycogen are polymers too. Chitin, the stuff of insect, crab, and shrimp exterior skeletons, is also a commonly encountered polymer. It's composed of multiple copies of a monomer whose structure is similar to the glucose units shown in 3D Molecule 1.

Mixed Polymers

All of the polymers that I've mentioned so far, both natural and artificial, consist of a single kind of monomer repeated many times. But matters get more interesting when a polymer is composed of more than one kind of monomer. Such molecules are called "mixed polymers" (more properly "mixed substituent polymers"). Of course, in order to meet the second part of the definition of polymers, the different monomers in mixed polymers must be similar enough so that they can be joined together by the same kind of bonds.

There are three principle kinds of natural mixed polymers in organisms: **DNA**, **RNA**, and **proteins**. They constitute the major molecules of life. First, I'll describe their structure and illustrate what they look like using three dimensional models. Later in the chapter, I'll discuss the functions they serve.

Proteins

Let's begin with proteins. They are comprised of 20 different monomers that belong to a group of chemicals called "**amino acids**". (One representative amino acid, valine, is shown in 3D Molecule 2). Valine, and any of the other 19 amino acids may be linked to one another in chains that vary in length from about a dozen to many thousands of amino acids.

3D Molecule 2
Valine

Valine is one of 20 amino acids found in proteins. The carbon atoms are shown as dark gray spheres. Oxygen atoms are red, nitrogen blue, and hydrogen atoms are white. You can rotate this image to get a clearer idea of what this amino acid looks like.

Sizes

The holder of the record for the longest human protein is "titin", a protein that consists of a chain of about 30,000 amino acids. However, more typical proteins may be 450 - 500 monomers in length.

Short chains of amino acids exist too. Polymers of up to about 50 amino acids

The amino acids in proteins are bound together by strong links that keep the monomers from exchanging positions in the chain. And while the monomers in proteins can be arranged in any order, every different protein has a specific arrangement, a fixed sequence of amino acids that doesn't change throughout its lifetime. As we'll see, the sequence of a protein determines its function. In fact, a second fundamental principle of molecular biology is:

The sequence of their monomers determines the role of individual DNA, RNA, and protein molecules.

There are at many tens of thousands of different proteins in humans, no one knows exactly how many, each with a characteristic sequence of amino acids. And, in accord with the second principle of molecular biology, each protein performs a function that is dictated by its specific sequence.

3D Molecule 3
Human Myoglobin

Myoglobin is a relatively small protein comprised of 153 amino acids. It is shown here in a simplified format. Each colored sphere represents not atoms, but a different amino acid. The amino acids are not drawn to scale.Notice the convoluted shape of this protein.

Gallery 4.1 Myoglobin - Different Views

Human Myoglobin
Ball and stick model. The atoms in the amino acid are shown. Notice how difficult it is to make out the overall structure.

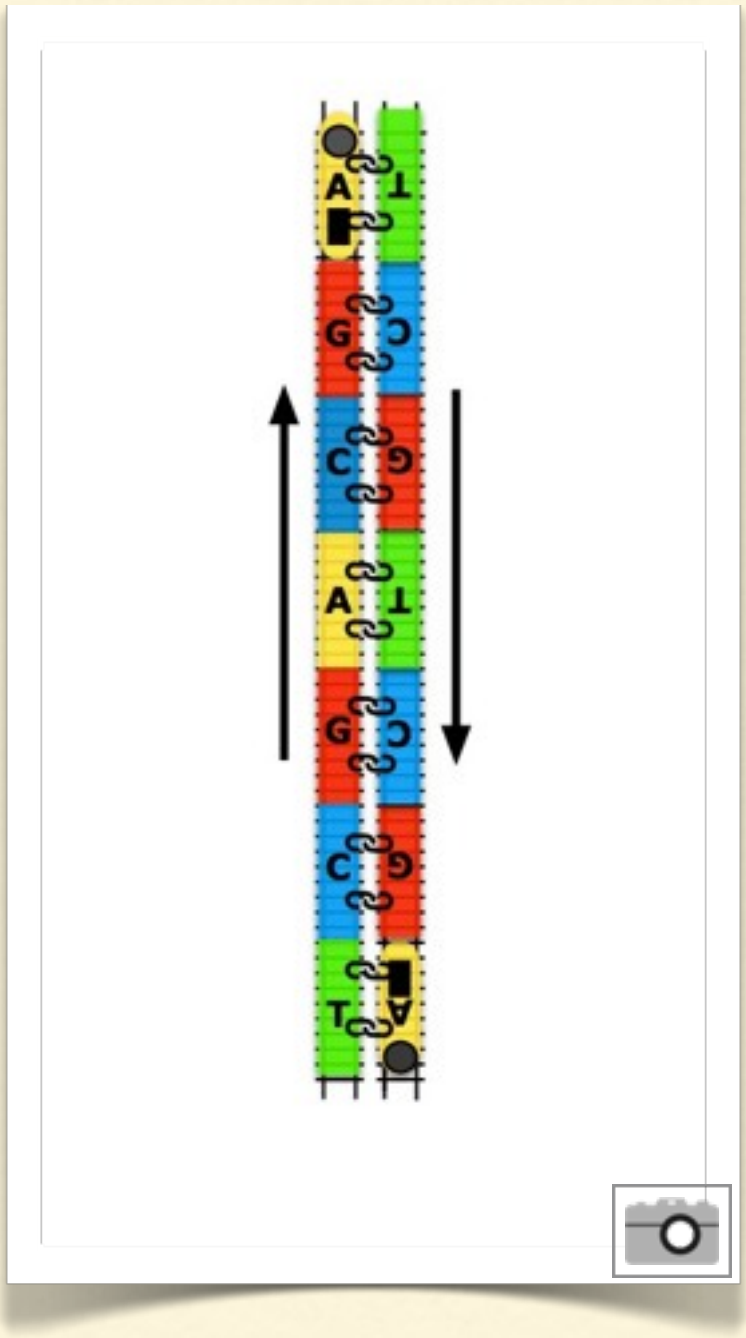
There's a further complication: some proteins, in fact most, consist of more than one chain of amino acids. These often bind loosely to one another and act cooperatively so that the composite structure can perform its intended function.

As illustrated in Gallery 4.1, proteins have a complex shape. In recent years, the computer has proved invaluable in giving the scientific community a more comprehensible picture of the three dimensional structure of many proteins. Gallery 4.1 shows five different views of the relatively simple protein myoglobin as drawn by a computer program. Compare view number 2, which traces the path of the amino acid chain through space, with view number 4, which depicts the surface of the protein in all its irregular splendor.

3D Molecule 4
Nucleotide

Nucleotides are the monomers of DNA and RNA. This one is "adenine" or "A". DNA and RNA nucleotides differ slightly in structure.

Figure 4.1



Each "train" represents one chain of DNA. The two trains are linked to one another through bonds that go from a car on one track to a corresponding car opposite to it. Notice that "A" cars pair with "T""s and "G" cars pair with "C's". Notice too, that the two trains face in opposite directions, as indicated by the arrows.

3D Molecule 5
Simplified Model of Double-Stranded DNA

The bases between the two polymer strands are shown as flat blue slabs.

(Fig 4.1). The trains represent individual polymer molecules. Neither train is going anywhere because each car is attached to one on the opposite track directly across from it. DNA in this form is "double stranded" (Fig. 4.1).

The four monomers in DNA can be arranged in any order. But the bonds between chains (the trains in Fig. 4.1) force one chain to carry a sequence that is dictated by the other. That is, each car can have one, and only one, kind of car opposite it on the parallel track.

For example, if there's an "A" on the car of one DNA "train" the matching car on the other track must be a "T". Similarly, "G"'s are matched with "C"'s. This phenomenon

DNA

Next let's consider DNA. It's a polymer consisting of four kinds of monomers, called "nucleotides" or more commonly "bases" that are usually designated "A", "T", "C", and "G". In most organisms under most circumstances DNA takes the form of two polymers closely intertwined with one another. A good way to visualize the structure of DNA is to ignore its familiar twisted depiction (3D Molecule 5), and instead think of it as two trains located on separate parallel tracks a few inches apart

3D Molecule 6 RNA

tRNA - An example of an RNA molecule. This RNA consists of a single polymer chain that folds upon itself.

is called "**base pairing**", a characteristic of DNA and its relative RNA (Fig 4.1).

DNA chains are typically several orders of magnitude longer than those of proteins. In humans, the longest DNA chain consists of nearly 250 million pairs of bases. Other human DNA molecules vary in size from 240 million base pairs to 46 million. Keep in mind that each of these molecules has a fixed arrangement of the four bases. The sequence of the 3 billion or so nucleotides that constitute the entirety of the human genome was worked out in the early years of the twenty first century. This feat, which cost billions of dollars, represents one of the great accomplishments of science.

RNA

Lastly, there's RNA. Chemically, RNA is similar to DNA, consisting of chains with four monomers: "A", "C", "G" and "U". Three of the bases are the same as DNA. The fourth, "U", substitutes for "T".

Typically, RNA, unlike DNA, comes as a single chain (3D Molecule 6). DNA and RNA are so similar that they can base pair with one another. "C" and "G" on RNA form pairs, respectively, with "G" and "C" on DNA. "A" and "U" on RNA pair with "T" and "A" on DNA. RNA is typically much smaller than DNA. In humans, RNA molecules range widely in size from about a dozen up to a 100,000 or so bases in length.

How It All Fits Together

OK. Now that you've met DNA, RNA, and proteins, life's three major players, it's time to examine what they do and how they do it. Again, let's begin with proteins.

In essence, proteins do nearly everything that goes on in cells. Some act as structural elements – beams, cables, rods, and so forth. They shape the cell, help it move, and form internal cableways along which molecular cargo is transported from place to place. Other kinds of proteins fulfill a more active role. For example, there's a group of proteins that reside in the cell membrane and act as sensors that allow cells to monitor what is going on in the outside world. They recognize neighboring chemicals in their surroundings and signal cells to change their behavior depending on external circumstances. Other membrane bound proteins act as portals to keep bad stuff from getting into cells and as disposal systems to throw things away.

There's another group of proteins, called transcription factors, that bind to DNA and mechanically regulate the flow of information. We'll discuss these in much greater detail in a Chapter 23.

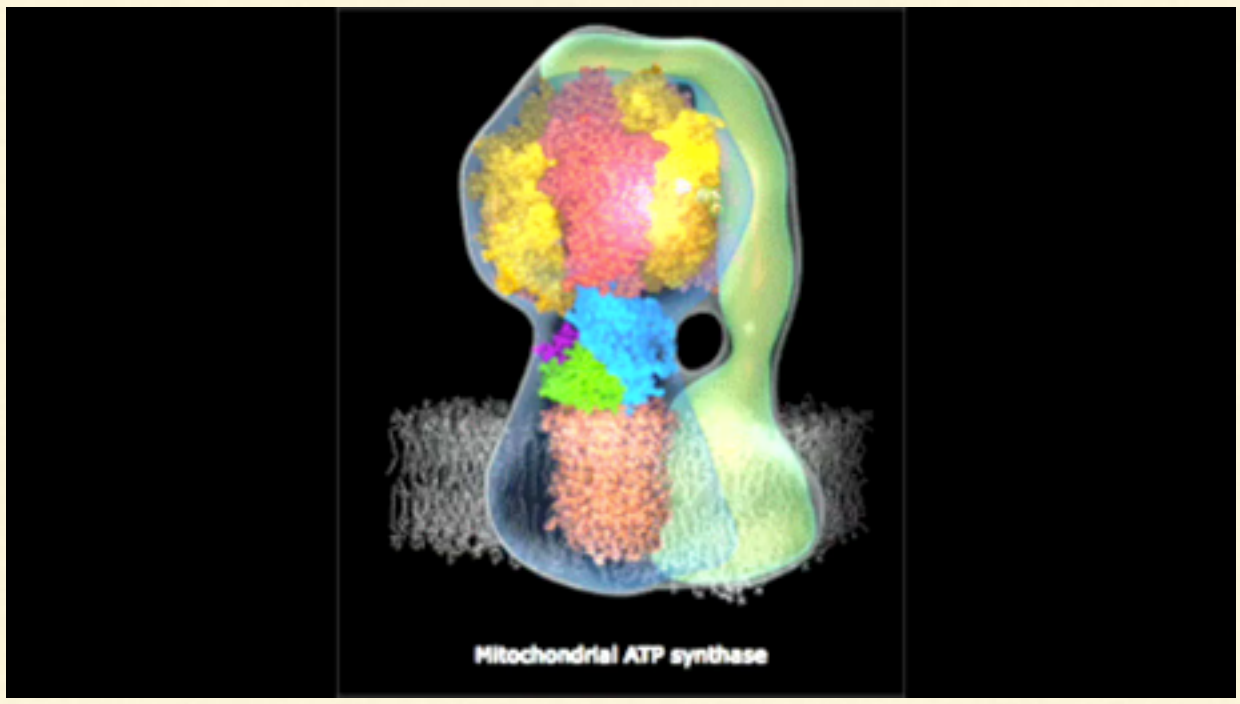
Perhaps the most well known class of proteins, enzymes, serve as machines that perform the chemical reactions that are characteristic of living things. Proteins do all these things and more. To repeat, virtually all the work that goes on inside a cell is conducted by proteins.

How do proteins perform their function? I've indicated that some act as tiny machines. A particularly striking example of one such protein is shown in Movie 4.1. This protein, an enzyme, is called ATP synthase. It's found in almost all living things because it performs a nearly universally required function. Its a turbine. As its parts rotate, energy is generated. How it accomplishes this feat is beyond the scope of this book. I introduce it here in an effort to try to convince you that proteins really function as miniature machines. Calling them machines is not a metaphor, nor an analogy. They're really mechanical devices. (If you want to learn more about ATP synthase, see the article by David Goodsell). *

But how are such tiny, sophisticated, and complex devices fashioned inside of cells? The amazing answer, discovered in the 1950's is this:

The arrangement of amino acids in each protein (its sequence) causes the chain of amino acids to fold autonomously into a working device.

Movie 4.1 ATP Synthase



ATP Synthase in action. As parts of the molecule rotate, energy is generated. Adapted from a video by John Walker, Mitochondrial Biology Unit ©Medical Research Council

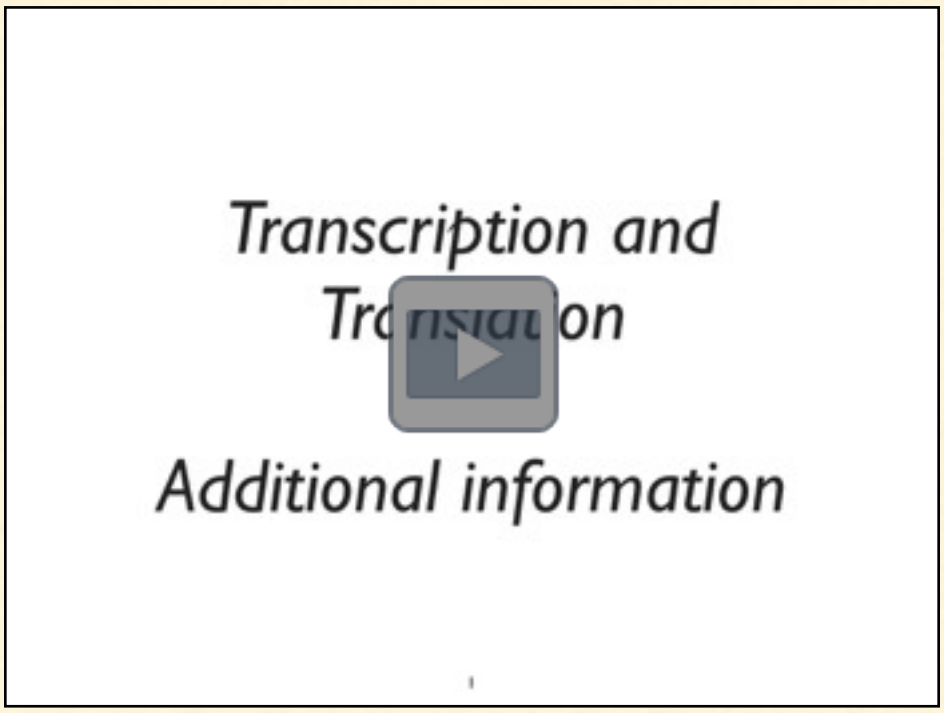
It's an astonishing feat. It's as if a chain of connected car parts assembled themselves to form an automobile on their own. It seems bizarre and counterintuitive, but there's more than a half century of evidence that that's the way proteins achieve their ultimate three dimensional shape, and thereby their function.

Different arrangements of amino acids cause proteins to fold into distinct structures. In general, each protein performs only a single task, one that is dictated by its structure. Proteins are specialists. That's why many tens of thousands of different ones are required for organisms to function.

What about DNA? DNA, in contrast to proteins doesn't actually perform any work. It acts as a storehouse of instructions in the form of "genes". These instructions are encoded in its sequence of nucleotides. It is the arrangement of the monomers in a gene that dictates the sequence of amino acids in a protein.

But DNA doesn't perform this task directly. It acts through an intermediary. The DNA of a gene gets copied into RNA, and it is the sequence of

Slideshow 4.1



A summary of how DNA, RNA, and proteins are related.

Figure 4.2
Charles Darwin

Charles Darwin (1809 - 1882)
This painting is the earliest known picture of Darwin (age seven).

nucleotides in this RNA copy that gets "translated" into the sequence of amino acids in a protein (See Slideshow 4.1 for more details).

To recapitulate, in order to make a cellular machine, perhaps an enzyme, a sequence of DNA, a gene, gets copied into a similar sequence of RNA. The sequence of nucleotides of RNA is used to specify the sequence of amino acids in a protein. The protein then folds into a working machine autonomously and spontaneously. Since proteins do nearly everything inside cells, this scheme accounts for nearly all the activities of life.

The last paragraph is a summary of the secret of life. However, I've failed to mention one key step in this scenario. I've said that source of information for specifying protein sequences originates in DNA, and that proteins do nearly everything in cells. But I haven't mentioned where the sequence of nucleotides in DNA originates. It's the DNA sequence that is at the root of the whole process. It is what ultimately provides the information for making the machinery of life. From where does the DNA sequence come?

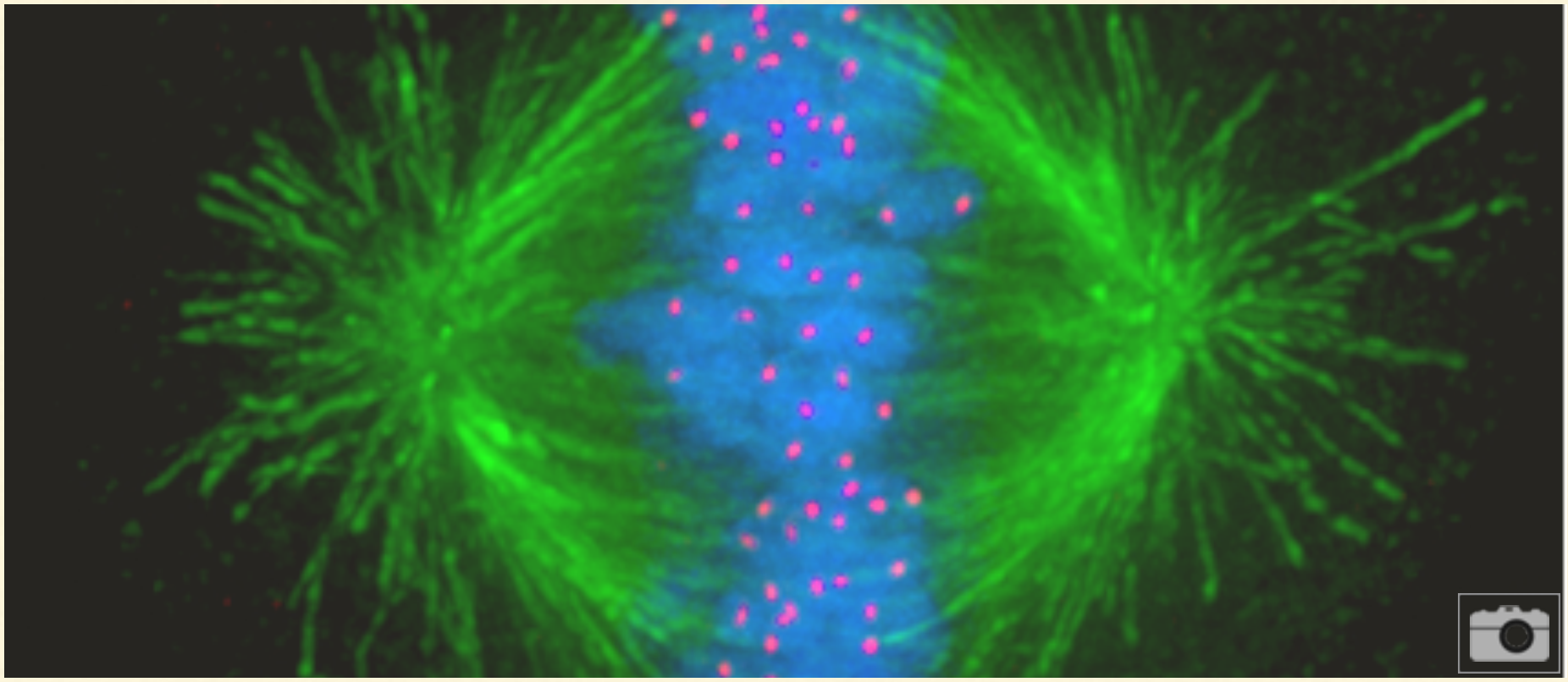
The answer to this question was worked out in the middle of the nineteenth century by Charles Darwin (Figure 4.2).

Without any knowledge of DNA or RNA, and with little understanding of proteins, he accumulated a massive amount of evidence in favor of the hypothesis that evolutionary change occurs by natural selection. The advances in our understanding of molecular biology in the latter half of the twentieth century strongly supported his ideas. In modern terms, DNA sequences are passed from one generation to the next. These sequences carry random changes, mutations. A few of these changed sequences specify favorable proteins, ones that help the organism reproduce. Others do not. The ones that are the most advantageous are passed on to the next generation because the organisms that carry them are the most successful at mating. In contrast, those DNA sequences that specify defective proteins are eventually lost. In other words, the sequence of DNA is derived and modified by trial and error over eons; by what he called "natural selection". The errors tend to be lost in succeeding generations; the successes retained and passed on. It's a process that was started billions of years ago and is ongoing.

Darwin's theory explains how DNA sequences are refined and modified. It doesn't offer an explanation of how the original DNA sequence arose. The

scientific community has some ideas about that one, but the issue remains unresolved.

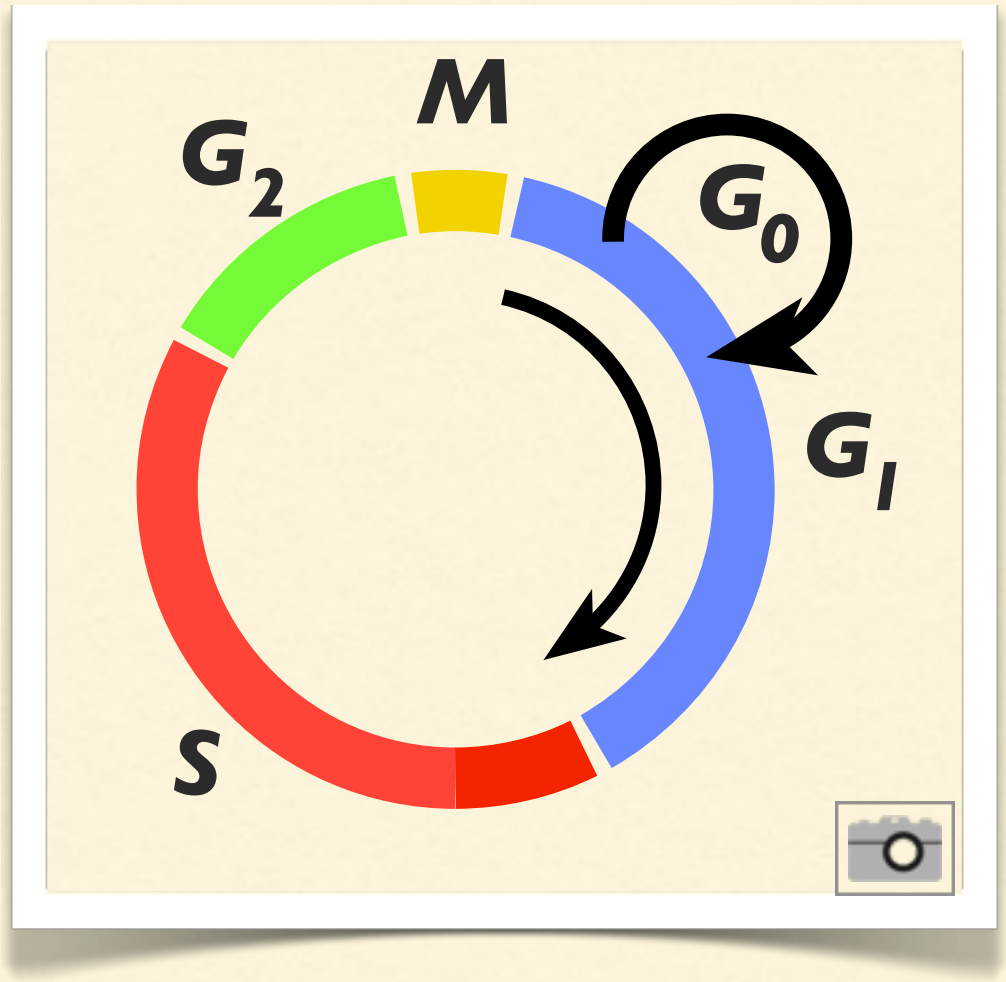
Of course, there's much more to molecular biology than the brief outline I've presented here. I'll add some additional details to these fundamentals in later chapters as the subject warrants. Meanwhile, the next chapter continues our discussion of cell biology, tackling the topic of cell division.



5

Cell Division

Figure 5.1
The Cell Cycle



M = Mitosis, G1 = Gap 1, G2 = Gap 2, S = Synthesis, Go = Gap zero

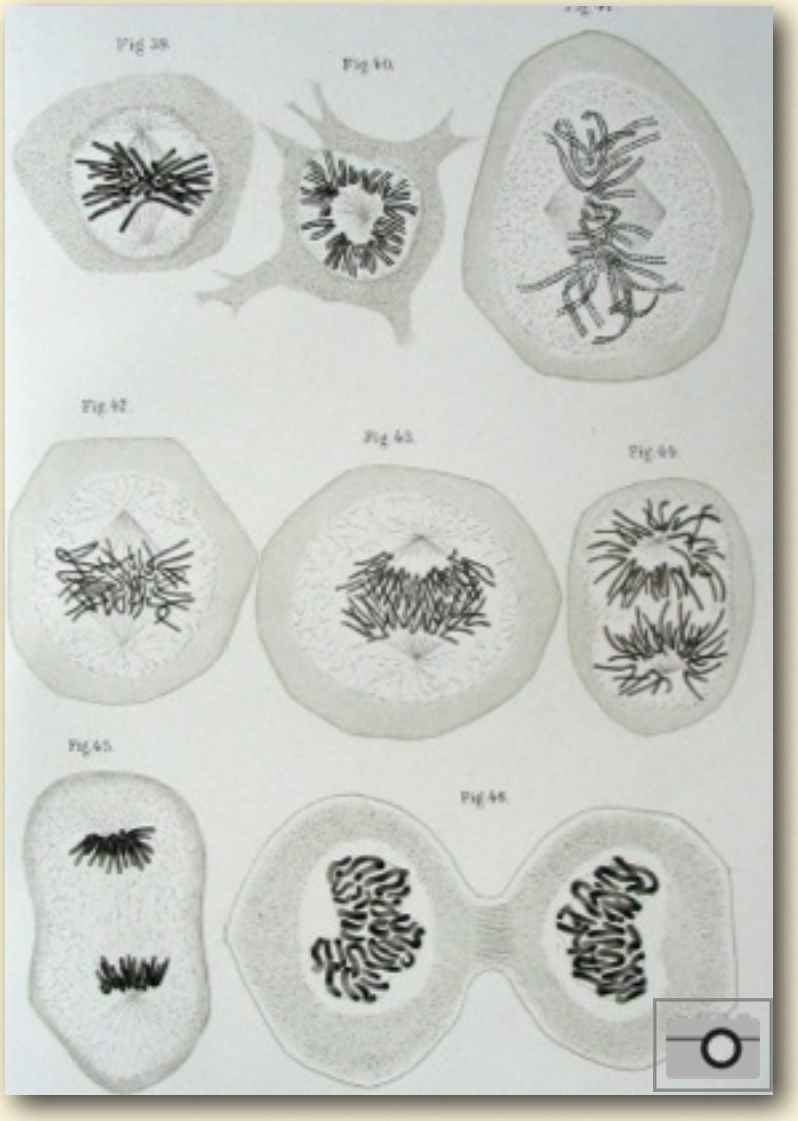
Cell Division *

Before a cell of a multicellular organism can reproduce to form two "daughters" it must engage in an intricate biochemical maneuver called the "cell cycle". A simplified diagram of this process is illustrated in Figure 5.1. I show one cycle, after which a single cell will have divided into two daughters in which the cycle will begin again. It takes about 24 hours for a typical mammalian cell to complete one cycle, although there is great variation among different cell types. The length of each of the colored arcs in Figure 5.1 is roughly proportional to the fraction of the time spent at each stage.

Mitosis

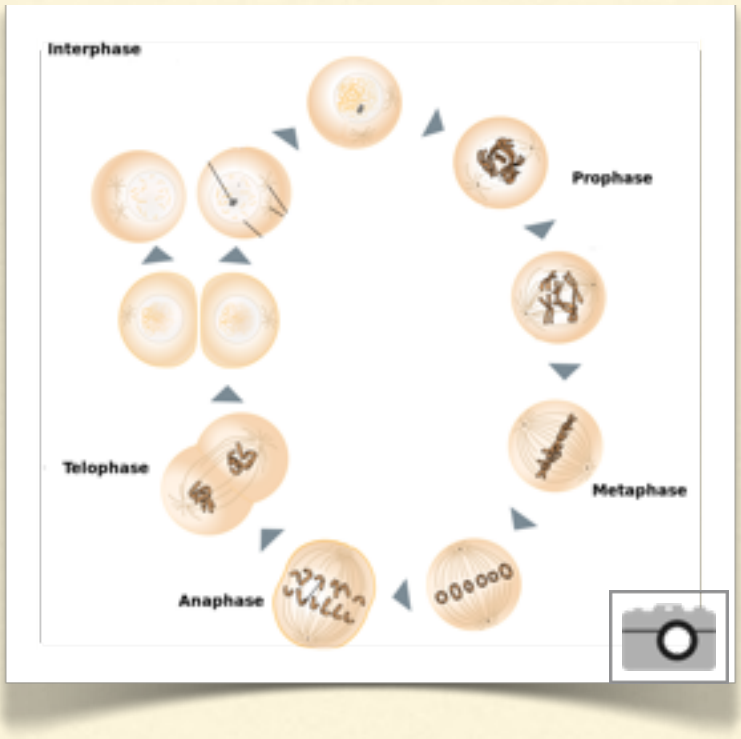
You'll notice that the physical division of the nucleus and its contents, the stage called "mitosis" or "M" (marked in yellow in Figure 5.1), occupies only a short portion of the total sequence.

Figure 5.2



The stages of mitosis as depicted by Walther Flemming in 1882

Figure 5.3



The various stages of mitosis

Mitosis wasn't recognized until well after the cell theory was proposed. Robert Remak, who asserted that cells divide by splitting in two, felt that the nucleus also simply cleaved in half when a new cell was born. Some years later, his more influential colleague, Rudolph Virchow, seconded this view, and it came to be widely accepted. However, as microscopy improved, it became evident that nuclear division involved a more intricate process.

Walther Flemming

Walther Flemming (Figure 5.4) was able to

Figure 5.4

Walther Flemming (1843-1905)

observe this more complicated picture and coined the term "mitosis" for it although he was certainly not the first to describe the phenomenon. Flemming, writing in the last quarter of the nineteenth century, was critical of Remak's and Virchow's views. He accurately illustrated the individual stages of mitosis, recognized the longitudinal splitting of the chromosomes, developed sophisticated staining and fixation techniques for the study of division, and established the universality of mitosis by extending his observations to a great variety of both animals and plants. An example of one the beautiful drawings that accompanied his book, "The Substance of Cells, the Nucleus, and Cell Divisionl", is shown in Figure 5.2.

Today, we recognize several distinct phases of mitosis. Generations of students have memorized their names: "prophase", "metaphase", "anaphase", "telophase",and "cytokinesis" (Figure 5.3), although there are probably more important terms to remember. Cytokinesis, the actual division of the cell (in contrast to the division of the nucleus) is not strictly speaking considered a part of mitosis, but that seems like an artificial distinction.

Interphase

The cell spends the bulk of its time before and after mitosis in "interphase", shown as blue, red, and green arcs in Figure 5.1.

During mitosis, the transcription of genes (See Chapter 4) is relatively quiescent. This reflects the fact that the chromosomes are tightly coiled, a condition that restricts the rate of RNA expression. In interphase, activity picks up as the DNA unwinds and the cell resumes growth. Interphase is divided into three stages (Figure 5.1):

G_1 - The "Gap 1" or "G_1" stage begins after mitosis and continues until the cell starts to replicate its DNA. During this period, sometimes referred to as the growth phase, the cell increases in size as it synthesizes its constituents. Often, when a cell differentiates (becomes specialized) it will leave the cycle at G_1 and

cease dividing, either temporarily or permanently. This so-called "resting phase" is called Gap 0 or G_0. Most of the cells in an adult human are in G_0. Under special circumstances a cell can renter the cycle, leave G_0 and begin DNA synthesis.

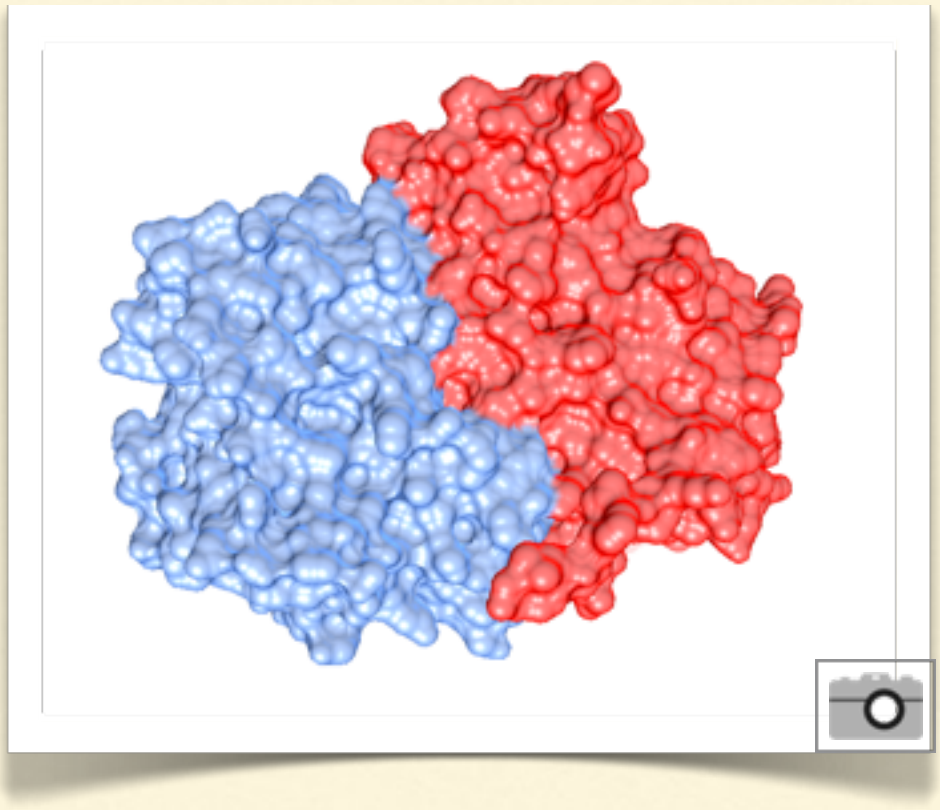
S - One function of the cell cycle is to exactly duplicate the genome and then allocate the duplicated copies precisely to the two daughter cells. The fraction of the time that the cell spends actually duplicating its DNA is called "S" (for "synthesis"). In most mammals, the S phase last about 10-12 hours, consuming about half of the total cycle.

G_2 - The period after DNA replication terminates is termed "Gap 2" or "G_2". During this phase the cell is preparing for mitosis, synthesizing the apparatus that is required to move the chromosomes apart as well as the other components that help cleave the cell in two.

Cell Cycle Control

The cell cycle is regulated by a complex interplay among many proteins. Our familiarity with these players and the processes they control have come primarily from the genetic analysis of cell division in two species of yeast and the biochemical study of actively dividing frog, clam, and sea urchin eggs. Remarkably, in each of these organisms, as well as in humans, the constituents of the cell cycle are quite similar. In fact, in many cases scientists have even been able to successfully substitute yeast proteins for their human counterparts and vice versa. Such exceptional conservation of the machinery that controls cell division indicates its antiquity and great evolutionary importance.

Figure 5.5



A cyclin (blue) and a CDK (red) in intimate contact. CDK's require such binding in order to perform their enzymatic function.

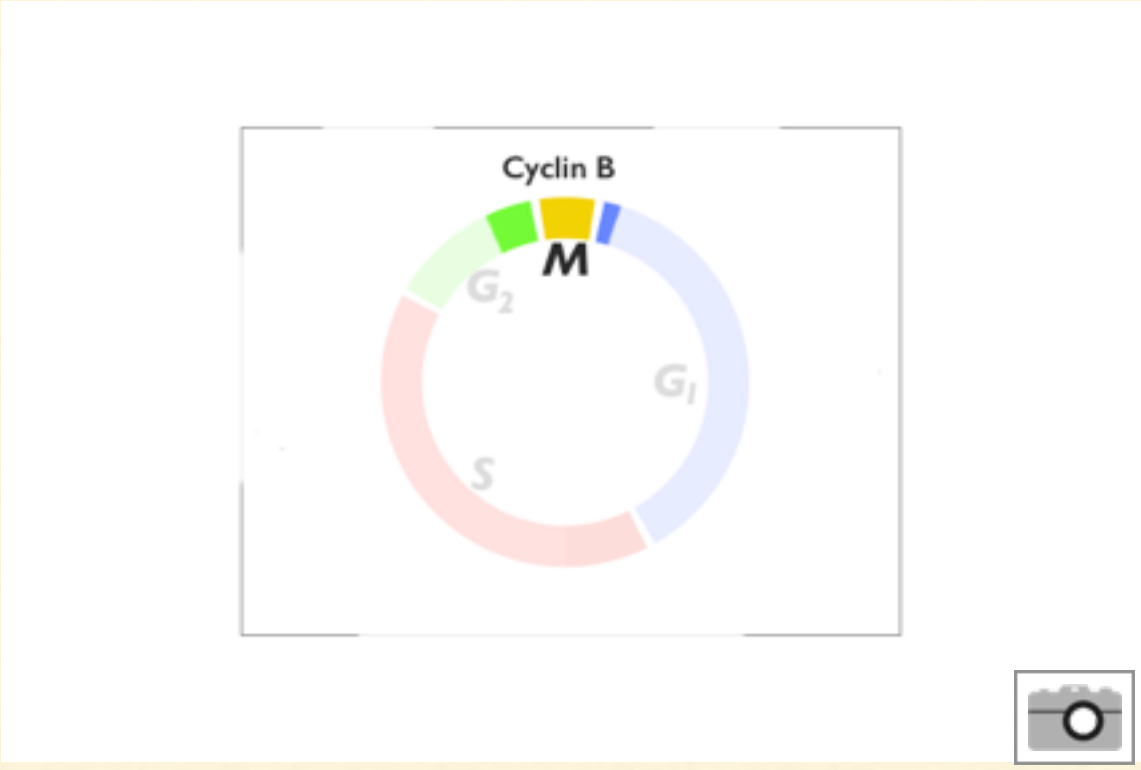
The major players in the cell cycle are two sets of proteins that interact with one another. The first are enzymes with the imposing names of "cyclin dependent kinases" or CDK's. "Kinase" is a term for an enzyme that transfers a phosphate

group from one substance to another. These particular kinases are, as their name implies, dependent for their catalytic activity on a second group of regulatory proteins called "cyclins" that themselves are devoid of enzyme activity. The cyclins bind to the CDK's

cyclins (there are multiple ones in humans) vary in amounts during the cell cycle (as shown in Movie 5.1) while the CDK's (there are many of these too) concentrations remain unchanged.

Once bound in a tight embrace, the cyclin dependent kinases/cyclins add charged

Movie 5.1



CDK4 and CDK6 in association with cyclin D are active during G1. Cyclin E together with CDK2 controls the transition from G1 into S. Cyclin A along with CDK2 takes the cell through S, and the same cyclin this time in association with CDK2 is important for making the S to G2 transition. To pass through mitosis, the cell uses the cyclin B/CDK1 complex. The figure only show the appearance of the various cyclins at different phases during the cell cycle. The CDK's tend to remain steady throughout.

and by affixing to them, cause them to "turn on" (Figure 5.5). That is, without the attachment of a cyclin, the CDK's don't exhibit enzyme activity. The cyclins act as a switch, regulating the activity of the CDK's by virtue of their being yoked together. In general, the

phosphate groups on to other proteins. One complex can modify many proteins. Once tagged with a phosphate, the targeted proteins may either become enzymatically active, or, in some cases may lose the enzyme activity that they were already expressing. By switching on the

Apoptosis

Quite different from accidental cell death by extreme chemical or physical mistreatment, apoptosis or programmed cell death is the orderly self destruction of cells to promote some purpose in a multicellular organism.

Some examples. The size of organs like the liver or pancreas is controlled by the production of new cells via mitosis and the destruction of already existing ones by apoptosis. It is the balance between these two processes that is critical. Apoptosis also plays an important role in sculpturing organs during development. For example, the digits of mammalian hands and feet are formed by precise cell death in an initial paddle-like appendage during embryogenesis. In another example, the tail of a tadpole is eliminated during frog metamorphosis by apoptosis. Finally, as we have seen, apoptosis ensures that aberrant cells are killed before they can do harm by multiplying out of control.

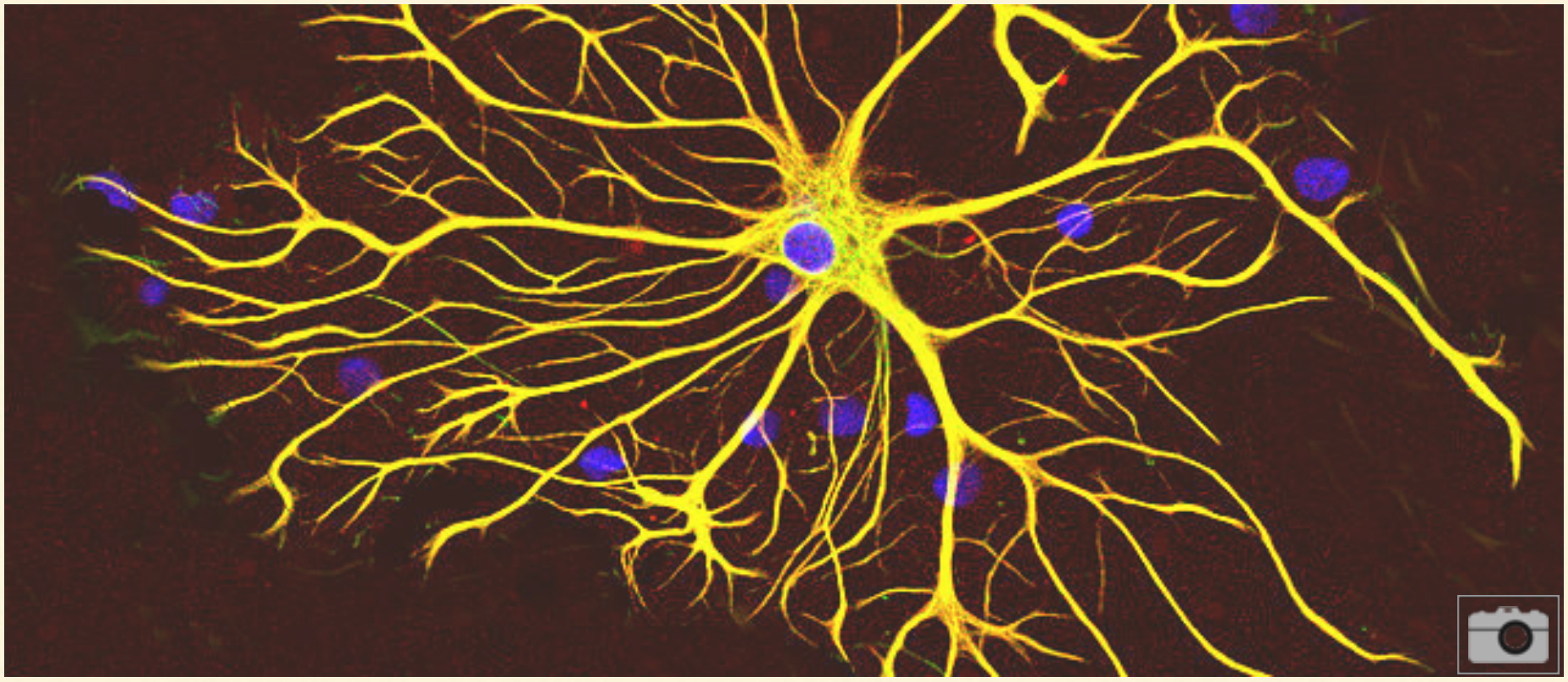
enzymatic activity of proteins that are involved in the various processes of cell division, and turning off the activity of others that may be inhibiting a given process, the various steps of the cell cycle can be precisely regulated.

Checkpoints

As you might imagine, Nature has gone to great lengths to ensure that the cell cycle works as it should. If things go wrong, the consequences can be dire. For example, if cells divide faster then they can be eliminated, a tumor may develop. In addition, abnormally rapid cell division may give rise to other problems. Cells may not get a full genome equivalent because a chromosome hasn't separated from its sister. Or they may not get a full complement of genes because DNA synthesis has gone awry. In any of these eventualities, the affected cells may behave aberrantly with unknown consequences. To try to avoid these problems, Nature has placed checkpoints at a number of stages in the cycle. These ensure that one step doesn't proceed until all has gone well in a preceding one. The checkpoints act by actively interfering with cell division, halting the process until whatever has gone wrong has been repaired. If such repairs don't occur, the cell may undergo apoptosis, programmed cell death (see side bar, "Apoptosis"). Apparently, Nature is

willing to sacrifice some cells that may be damaged in some way, rather than have them go on to reproduce aberrant offspring.

A summary of the mechanism of control of the cell cycle in mammals is shown in Movie 5.1. Note the presence of multiple CDK's and cyclins. This is characteristic of mammalian cells.



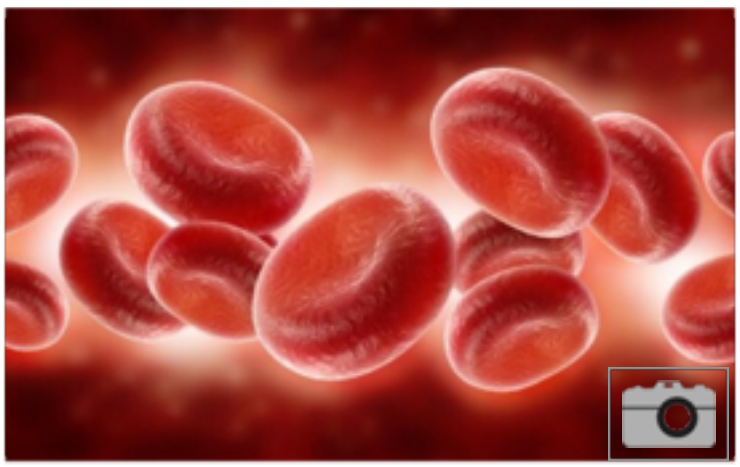
6

Cellular Types and Origins

Some organisms, like amoebae and yeast, are composed of a single cell. Others, like sponges, pine trees, jellyfish, worms, insects, and vertebrates, are made up of millions, billions, or even trillions of cells. We humans have more than 30 trillion red cells in our blood alone and an estimated 100 trillion cells in total. Some creatures have even more. But regardless of how many cells an organism ends up with, most multicellular creatures start out as a single fertilized egg – a **zygote** – that divides many times to produce a myriad of daughter cells, many of which differ from one another in appearance, function, and composition. As we'll see beginning in the next few chapters, there is strong evidence that each of these daughters (with some interesting exceptions) carries the same instructions – the same genes, no matter how much they look different from one another. That's because, as I've intimated, at every cell division the DNA duplicates more or less exactly and mitosis ensures that each gets an exact copy of the genetic information that was bequeathed to the zygote at fertilization.

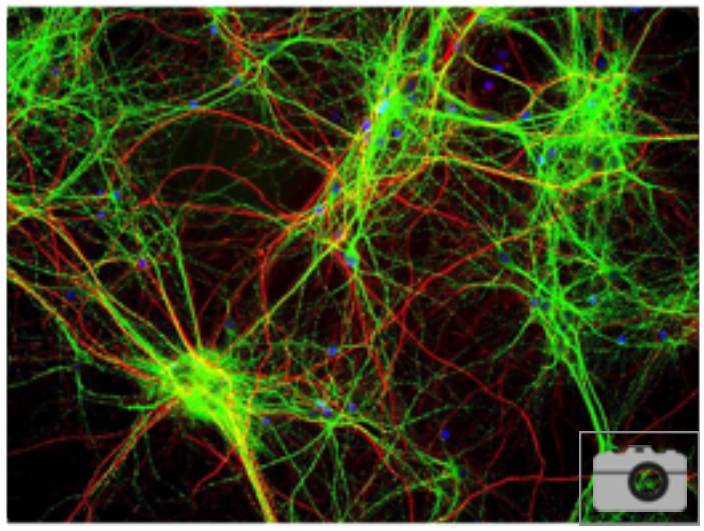
Of course, changes in the sequence of DNA can and do occur during the lifetime of an organism. In almost all cases, these changes represent largely random mistakes due to a variety of causes. For example, at each round of DNA replication, errors can occur in the copying process. Furthermore, environmental insults such as gamma radiation, UV radiation, and tobacco smoke can wreck havoc with DNA, increasing the number of errors many fold. But, as a general rule, Nature attempts to pass on unchanged DNA from one cell generation to the next even if it doesn't always succeed.

Figure 6.1



Mammalian red blood cells.

Figure 6.2



Nerve cells
Some nerve-specific proteins are stained red and green. Nuclei are stained blue.

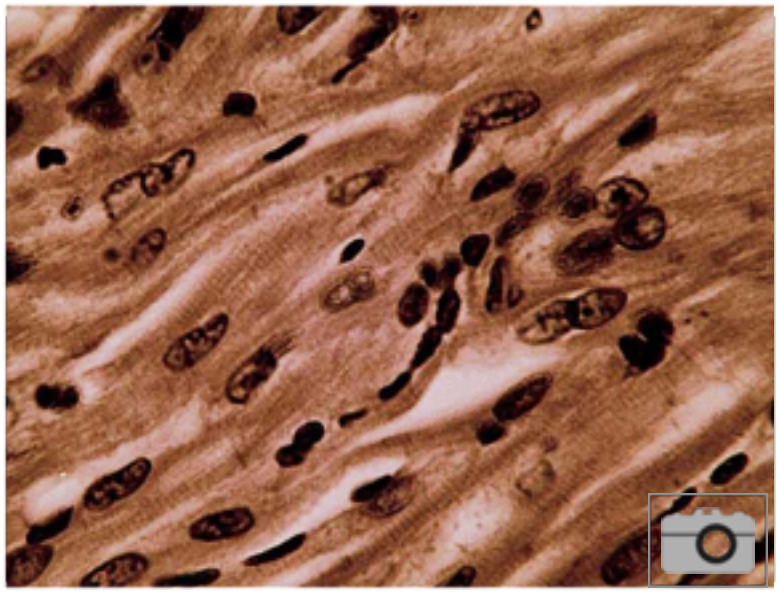
Differentiation and Determination

During development, cells come to look and act differently as they assume specialized functions. For example, some blood cells become oval and biconcave, lose their nuclei, and fill with hemoglobin, a red colored protein that binds oxygen (Figure 6.1). Similarly, nerve cells typically become elongated and accumulate proteins that help in the transmission of electrical signals (Figure 6.2). Muscle cells (Figure 6.3) fill with a variety of contractile proteins, enabling them to move limbs, the heart, and other organs.

All in all, there are scores (over 200) of different cell types in the human body. Each has a specific and particular complement of proteins and other molecules that give them a distinctive appearance and behavior. They acquire these traits through two processes: "**determination**" and "**differentiation**".

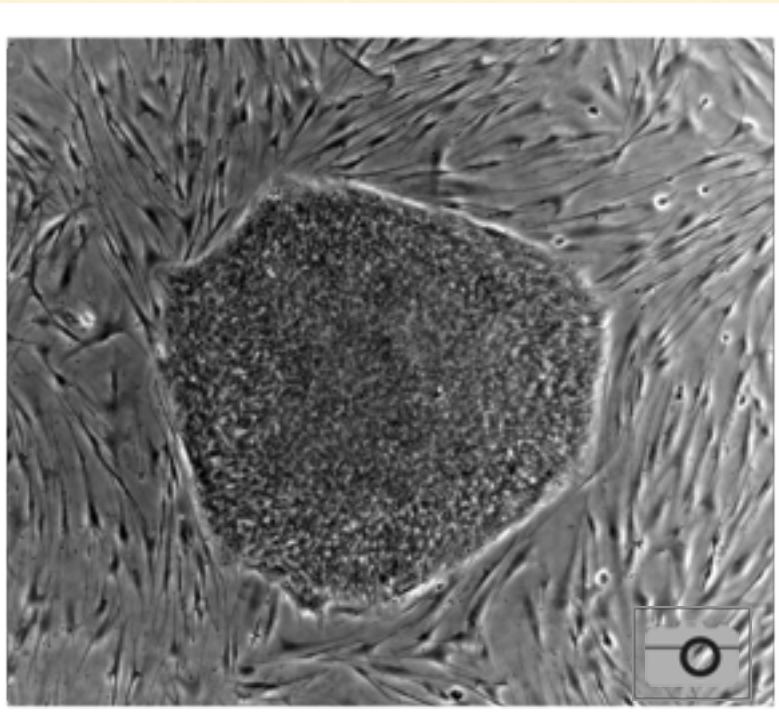
Cells are said to be determined when their developmental potential becomes restricted. The ultimate undetermined cell is, of course, the zygote. It will give rise to all the cell types in the body and is said to be "**totipotent**". Cells are differentiated when they actually acquire the accoutrements characteristic of a specific cell type. But differentiation seems to exact a price. For most cells differentiation diminishes a cell's ability to multiply. Differentiated cells leave the typical cell cycle described previously and remain in Go, sometimes for the life of the organism. Keep in mind, most cells in adult humans are differentiated. If they do retain the ability to multiply, their progeny are also differentiated. That is, most differentiated cells are no longer capable of giving birth to cells different than themselves. Despite the fact that they carry the same genetic instructions as a fertilized egg, they can only give rise to their own kind.

Figure 6.3



Light microscope photograph of a section of dog cardiac muscle

Figure 6.4



Human embryonic stem cells on a layer of mouse cells. The mouse cells serve as a "feeder" layer, supplying the stem cells with nutrients and growth factors.

Stem Cells

But not all cells become fixed in their fate. A relatively small number retain both the ability for continued division and the potential to form several or many types of cells. These are called "**stem cells**". They come in several different varieties. Some can form nearly all the cell types of the body and are said

to be "**pluripotent**" (from the Latin "pluri" meaning more than one, and "potent", indicating potential). Cells obtained from very early embryos are examples of this kind of stem cell. Cells from later developmental stages have a more limited repertoire. They are said to be "multipotent". Fetal and adult cells fall into this category. For example, there are stem cells in the bone marrow that are capable of giving rise to both red blood cells, which carry oxygen, and white b;ood cells that are involved in fighting off invading microbes.They don't, however, seem to be able to form muscle or nerve cells. Still other kinds of stem cells have been created in the laboratory. Embryonic stem cells and induced pluripotent stem cells fall into this category. I'll have much more to say about these laboratory creations in later chapters.

Stem cells, whether natural or not, offer the prospect for remediating or even curing many maladies. In fact one kind has been widely used for therapy for decades. The stem cells that accompany bone marrow transplants (and are the active players in the procedure) have been used to replace blood forming cells that have been damaged or killed as a result of radiation, chemical treatment, or disease. These transplants have proved remarkably successful.

Stem cells are the major focus of the second half of this book. Some of the questions that I"ll address are: Where do they come from? Can differentiated cells be transformed into stem cells? If so, how? To what specific uses can stem cells be put? What ethical and practical difficulties face their use?

Generation and manipulation of stem cells and their use in therapy is a relatively recent development. There's much to be learned, but the promise of stem cells is extraordinary. The prospect of the replacement of worn out organs, a deeper understanding of disease, more efficient ways of searching for effective pharmaceuticals, even the extension of life, all these appear to be potential uses for stem cells.

Cloning

However, before entering the complex world of stem cells, I'm going to devote several chapters to a different but related subject: cloning. I'll discuss its history, its ultimate achievements, its prospects, and the work that led to the creation of the sheep Dolly and her relatives.

What is a clone? It's any individual organism – a plant, animal, or microbe – that arises by means other than through sex. For example, plants grown from cuttings and via grafting are clones. The

Figure 6.5

Farmer Inserting a Graft in a Tree (Jean-François Millet, 1865)

cloning of plants is a common agricultural practice that has been going on for many hundreds of years (Figure 6.5). There doesn't seem to be controversy over agricultural cloning since most of the fruits that we get in the supermarket arise in this way.

Surprisingly, clones of animals, even humans, are also rather commonplace. Identical human twins and triplets, for example, are clones although we don't often refer to them in that way. They arise from the splitting of an embryo at the two cell stage (see chapter 7) into two individuals with identical, or near identical, genetic endowments.

Some organisms reproduce solely by asexual means and thereby generate clones. There are many species of plants that naturally multiply this way. By contrast, animals that reproduce only asexually are relatively rare, and don't seem to persist for long, at least on an evolutionary time scale.

You might wonder what advantage sex has over asexual reproduction. What's the evolutionary advantage of having two sexes one of which, the male, usually takes little part in raising the next generation and yet utilizes resources that could otherwise be available to the mother? The answer seems to be that Nature favors diversity. As we'll see in Chapter 13, sexual reproduction produces offspring that differ from both parents. Apparently, this heterogeneity allows a sexually reproducing species to endure changing conditions.

But why discuss cloning at all? What's the relationship between cloning and stem cells? As we'll see, scientists have been attempting to clone animals for over 100 years. In the course of their investigations their efforts have yielded a wealth of information that is critical to our understanding of how stem cells arise, and what causes them to differentiate into a particular cell type.

For example, investigators in the early twentieth century examined the question of when in development cells became determined and lose their totipotency. These early studies led directly to the question of what restricts the developmental potential of cells as development proceeds and whether it could be reversed. Does differentiation involve a loss of genetic material? Are genes irretrievably modified during the course of development? What are the molecules that direct a cell along a particular developmental pathway?

Trying to answer these profound questions that first arose as a result of early attempts at animal cloning stimulated an enormous research effort that is still ongoing all over the world. Much progress has been made; and, of course, much more needs to be done. However, what has been discovered has been crucial to our understanding of how to manipulate stem cells so that they can differentiate into the cell types we'll need for therapy and other purposes.

SECTION 2

Embryology and Cloning

The next few chapters deal with embryology. A basic knowledge of how organisms develop is crucial to an appreciation of the experiments on cloning described in the second part of this section.

7

Embryology

In the initial half dozen chapters I've skimmed over a diverse set of subjects including microscopy, the development of the cell theory, cellular anatomy, molecular biology, the cell cycle, and differentiation. All of this in the way of providing some background to help better understand cloning and stem cells. Losing patience? This chapter presents one more topic with the same aim.

Embryology is the discipline that studies the succession of events that occur beginning with fertilization up to the birth of an organism. The field of embryology is relatively young because much of the steps of development must be examined under the microscope, and, as I've noted, microscopy fell out of favor for about 100 years after Leeuwenhoek's contributions.

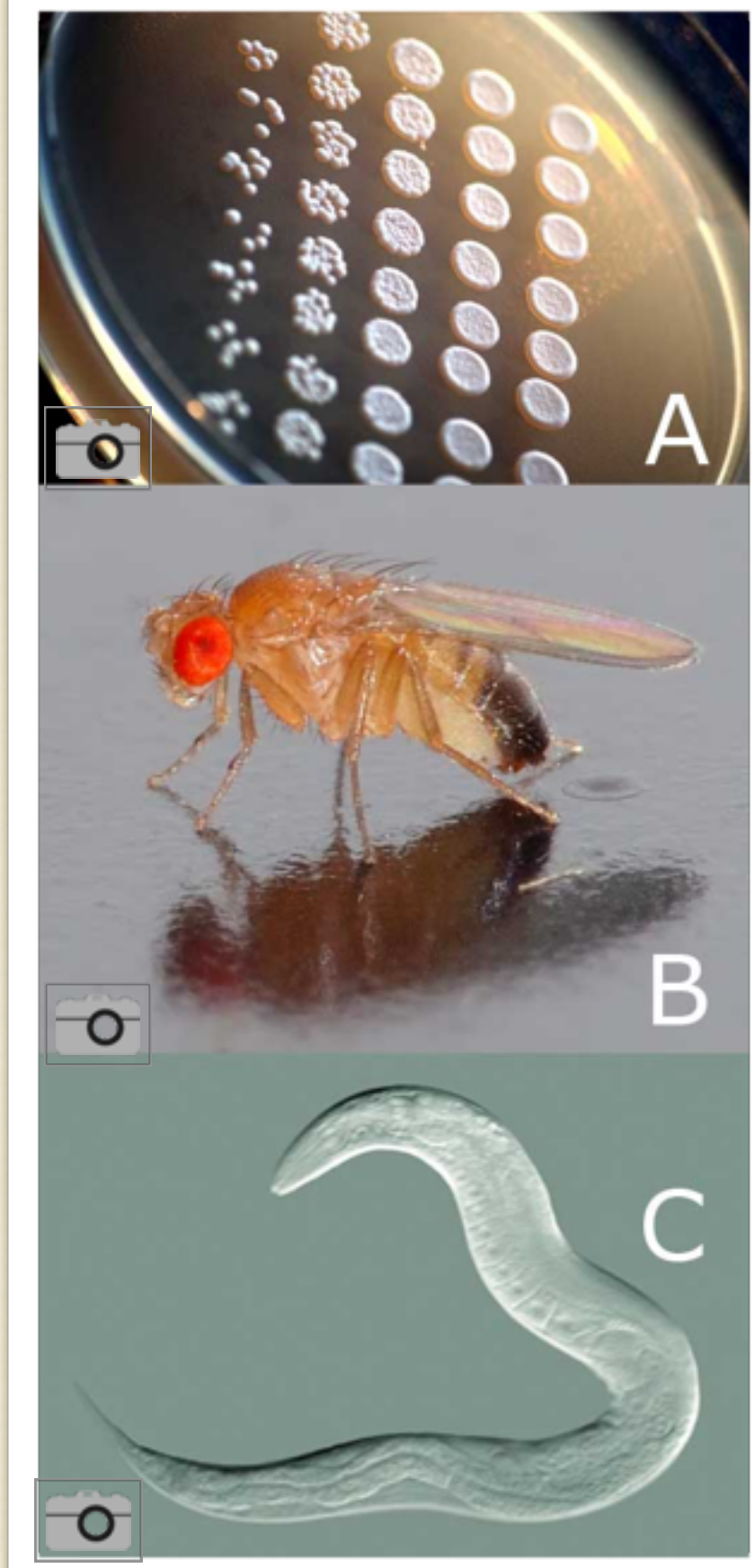
When microscopes began to be improved in the nineteenth century, early embryologists simply described what they saw: a succession of changes in the embryo, including a series of regular cell divisions, complex cellular movements

and interactions, and the formation of new cell types and structures.

However, while these early embryologists made many significant observations, they had little success in understanding the mechanisms behind the events taking place. Later, beginning in the latter part of the 19th century, a small group of European scientist began to manipulate embryos in an effort to understand the processes that were at work during development. This new field was called experimental embryology. Still later, in the middle half of the twentieth century, researchers used biochemical methods to gain insight into how embryos develop. Even more recently, geneticists and molecular biologists have applied their sophisticated tools to achieve the same ends.

Modern embryologists, now more often called "developmental biologists", have used all of these techniques to study a great variety of organisms. It's proved valuable to take a comparative approach in an effort to figure out how different organisms have solved the problems of development. But mammalian embryos, which are of obvious interest, have been late to the party because they they are more difficult to work with than most. Because fertilization is internal in

Figure 7.1



Three Common Model Organisms
A. Baker's Yeast (S. cerevisiae)
B. Fruit Fly (Drosophila melanogaster)
C. Nematode Worm (C. elegans)

Baker's Yeast

Unexpectedly, yeast has been particularly valuable in aiding our understanding of development although it is a microscopic one celled organism and doesn't undergo embryogenesis (Figure 7.1A). Nevertheless, many genes first identified in yeast have shown themselves to be critical players in the development of humans and most other organisms.

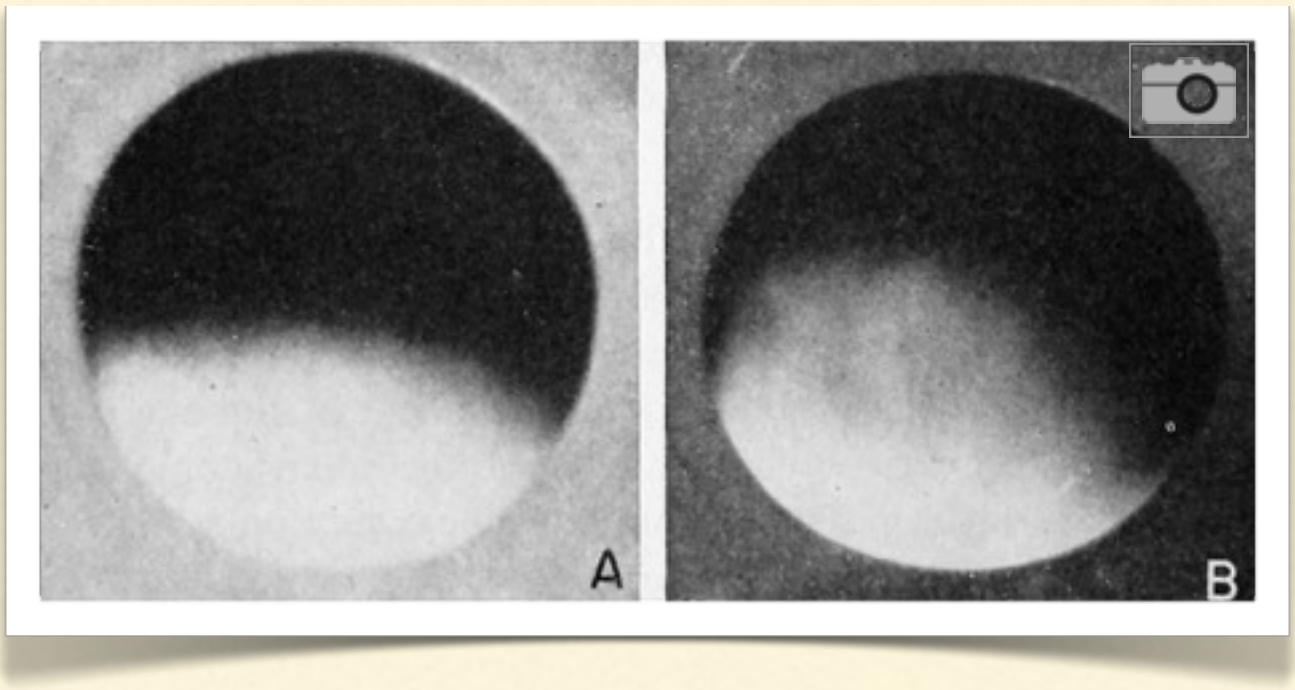
mammals, and subsequent development occurs deep within the mother's body, embryos are not readily accessible. While it is possible for eggs to be removed from mammalian mothers and fertilized in a dish thereby allowing the embryo to be examined at early stages, this has been a relatively recent technological advance and ultimately the embryos have to be returned to the womb to continue developing. Another disadvantage in working with mammals is that they produce relatively few offspring, thereby limiting genetic analysis. Moreover, because mammalian eggs are so small and so few in number, its been difficult to apply traditional biochemical methods to their analysis.

Animals that release their eggs in large quantities into the environment where embryogenesis can be easily analyzed are much more suitable for studying both the biochemical and microscopic events of early development. An additional advantage of working with accessible embryos is that it has allowed embryologists to tinker with the development process. By moving cells from one location to another, removing cells entirely at various stages, and mixing cells and tissues from different developmental stages, experimental embryologists have gained many insights into the mechanism of how development works.

There's yet a another reason for studying so-called "lower" forms, one that was not fully appreciated by the first generations of embryologists. Because organisms like fruit flies and nematode worms allow for genetic analysis that is well nigh impossible in mammals, studies of the genes in these "model organisms" (Figure 7.1) has been a principle source of information about similar genes that regulate early development in humans.

For these reasons, I'll begin our discussion of embryology by describing early development in frogs, animals which deposit their eggs externally. These creatures have played an important role in

Figure 7.2



A) Unfertilized frog egg
B} Fertilized frog egg. Note the gray crescent

the development of cloning, as I'll discuss beginning in the next chapter.

Early frog development

The jelly coated eggs of the common leopard frog, *Rana (Lithobates) pipiens*, are released by females into the water in the spring. The male clings to the female and releases his sperm as the eggs are deposited. Within the jelly the eggs rotate such that the darker half (Figure 7.2A), the "animal hemisphere" faces upward. The lighter colored half, the "vegetal hemisphere", lies south of it.

Upon sperm entry, the cytoplasm rotates about 30°, leaving a less dark, crescent shaped area behind (Figure 7.2B). This "gray crescent" forms opposite to the point where the sperm entered the egg. Another event that occurs at about this time is the completion of meiosis and the extrusion of

Animal and Vegetal

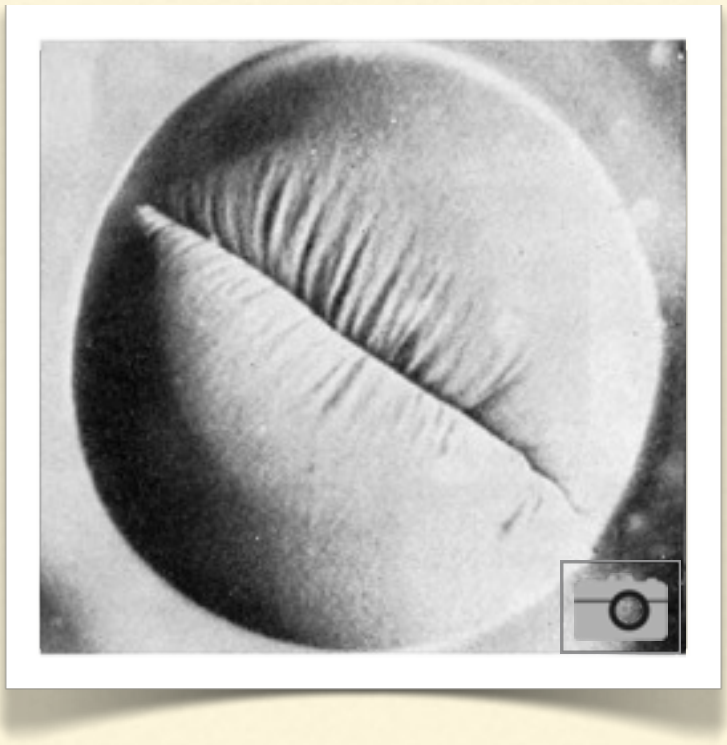
Beginning students of embryology often mistakenly call the lower half of the egg the "vegetable" hemisphere, which sometimes draws derision from their instructors. That's not fair. It's an easy error to make since the term "vegetal" in common usage refers to plants.

The origin of the terms "vegetal" and "animal" has been attributed to Richard Remak whom we've already encountered in Chapter 2. It isn't entirely clear why he chose this nomenclature. One idea is

the second polar body. I'll consider the process of meiosis later in the book.

A few hours after fertilization, the frog egg (more properly called an embryo now) begins a series of divisions that results in the formation of smaller and smaller cells. As shown in Figure 7.3 the embryo is first cleaved by a furrow that begins forming at the top of the animal hemisphere. This

Figure 7.3

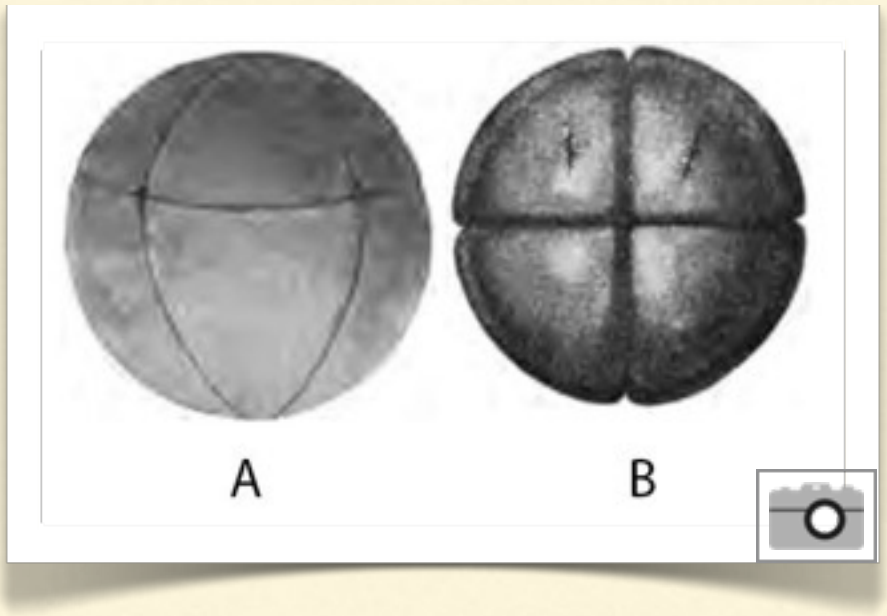


Two celled frog embryo

fissure works its way longitudinally down through the cell, ultimately dividing it in two.

Shortly before completion of the first division, another cleavage begins, at right angles to the first, again progressing from the animal to the vegetal hemisphere. A third division soon occurs at right angles to the first two, this time bisecting the embryo a little above its equator (Figure 7.4). At this point, eight cells have formed.

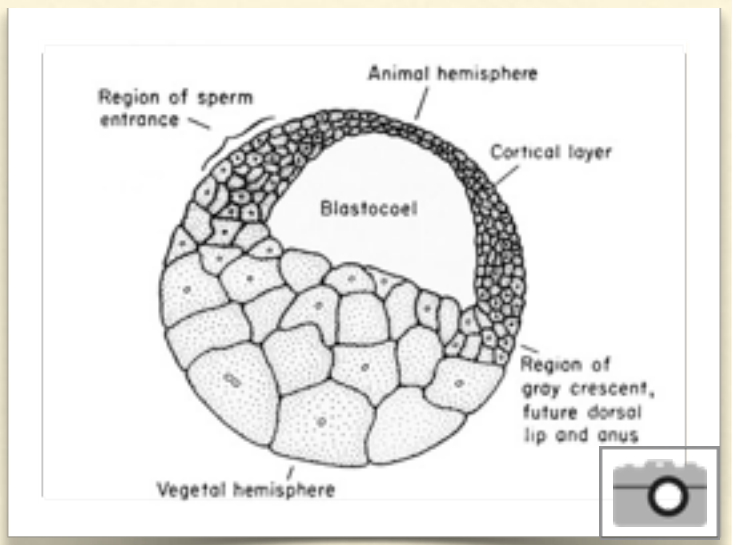
Figure 7.4



A. Eight cell frog embryo (lateral view)
B. Eight cell frog embryo (top view)

As you can see, not all the cells are the same size. The ones in the animal hemisphere are much smaller than those in the vegetal.

Figure 7.5

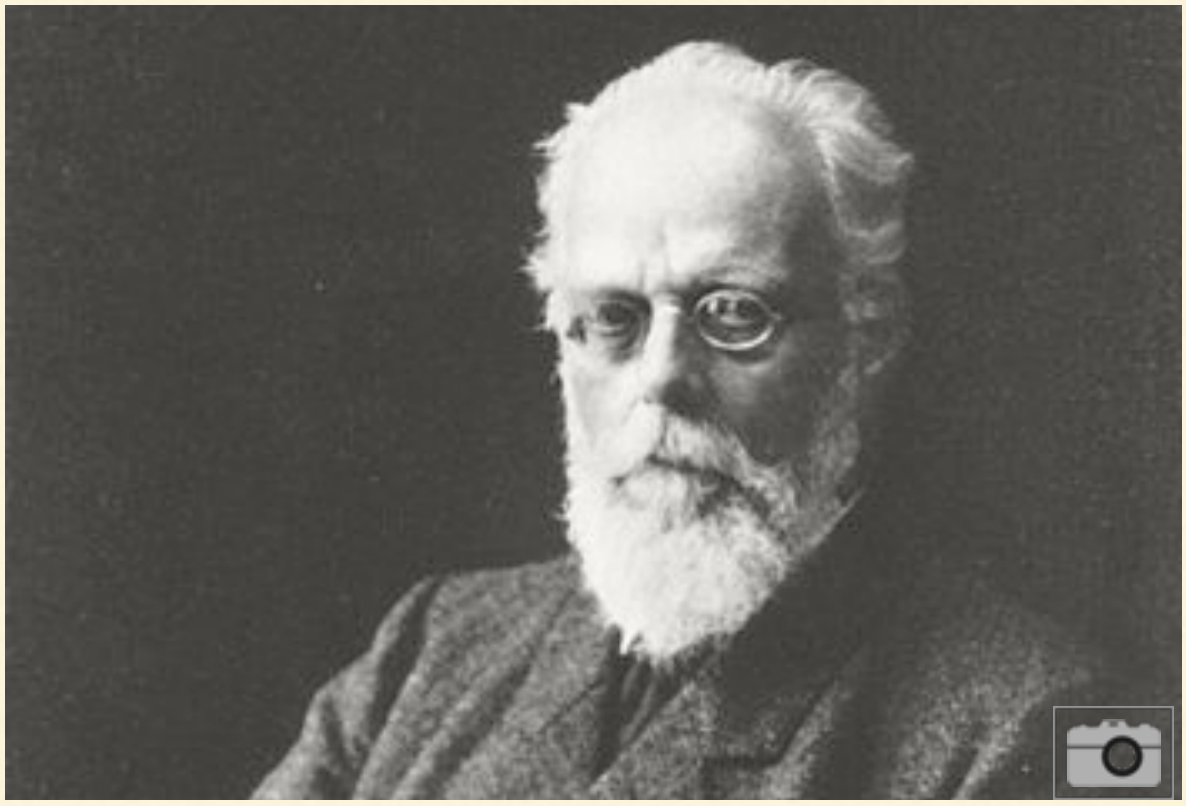


Frog embryo at the blastula stage

More cleavages ensue until after a while the embryo becomes divided into a large number of cells. A cross section at this stage (Figure 7.5) shows two prominent features. First, the cells in the animal hemisphere continue to be much smaller than those in the vegetal. Second, a distinct cavity (filled with fluid) forms within the embryo. Thomas Hunt Morgan, the Nobel Prize winning biologist who began his scientific career as an embryologist before he became interested in genetics, called this the "segmentation cavity", but it is now more commonly given the name "**blastocoele**". Embryos at this stage of development are called "**blastulae**" (singular "**blastula**").

All blastula cells look more or less alike. Of course some are bigger than others, but there are no specific cell types to be seen. No muscle, nerves, skin, or bones. Following the formation of the blastula, the embryo engages in a complex series of movements of cells, a process called "**gastrulation**", that result in the beginning of cell differentiation and tissue formation. These events are fascinating but are beyond the scope of this book.

As I noted earlier, the focus for much of the 19th century was on trying to get an accurate description of these and subsequent events. Near the beginning of the 20th century attention turned to experiments. Can each of first two cells formed after the first cell division each form a complete embryo? Or does one form left left and the other the right side? What is the developmental potential for the cells of the blastula? Are they determined? If so, what types of cells can they become? These questions began to be investigated as the 19th century came to a close and are the subject of the next chapter.



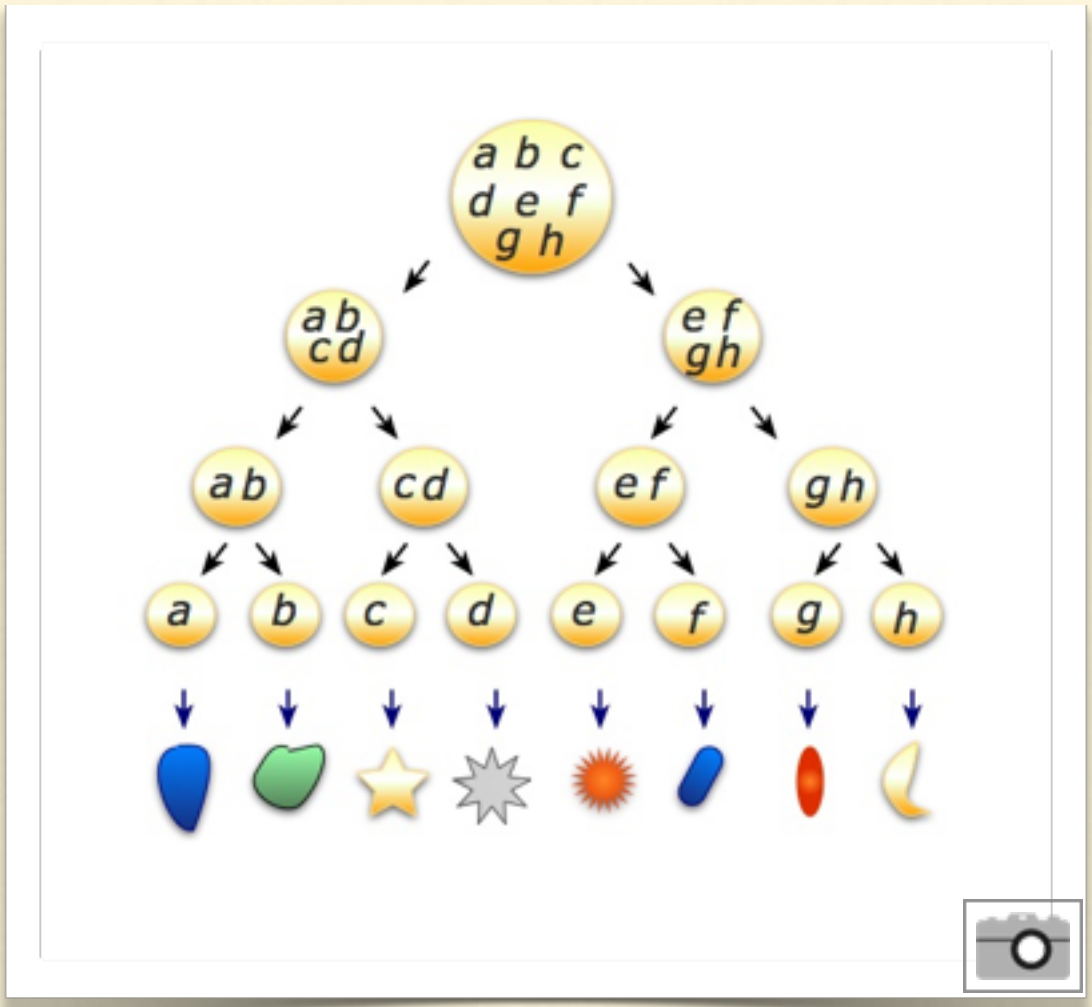
8

Weissmann and Roux

In the late 19th century August Weismann, whom we've already encountered in Chapter 2 in connection with the distinction between somatic and germ cells, proposed a mechanism to account for how cells become different from one another. His brilliant idea was that the genetic information that cells carry changes during development. According to Weismann, when a cell divides some genetic determinants (entities that we now call genes) are parceled out differentially to the daughter cells. In the first division, for example, Weismann might have predicted that the information for the left half of the embryo went into one cell, while the directions to make a right side went into the other. In subsequent divisions, each cell would get a more restricted set of genes until individual cells were left with instructions to construct only one particular cell type. Having been handed down only enough information to direct it towards one destination, that cell would then differentiate accordingly. The only cells that would escape this progressive loss of genetic information would be those that form the eggs or sperm – the germ cells. In accord with

Weismann's concept of the independence of germ cells, they couldn't lose genes because their function is to to pass a full complement on to the next generation so that a complete individual can be formed.

Figure 8.1



Simplified Illustration of the Sorting Out of Genes During Development as Hypothesized by August Weismann

A simplified diagram (Figure 8.1) may help clarify Weismann's theory. The letters "a" through "h" represent genes or sets of genes; determinants in Weismann's terms. At the first division one daughter would get determinants a, b, c, and d, and the other e, f, g, and h. When these cells divide, they also distribute their determinants so that the next generation gets fewer than its parents. One gets a and b; another, c an d; the third, e and f; and the last, g and h. Finally, after one more division, the eight cells that are formed are left with only a single determinant and they assume the fate dictated by these genes.

Figure 8.2

Wilhelm Roux (1850 - 1924)

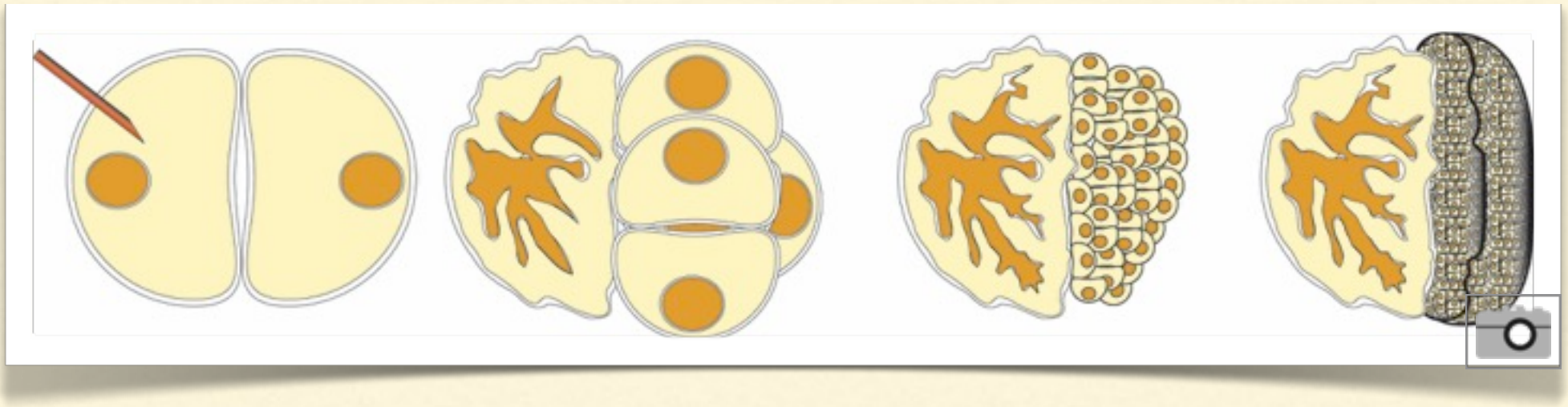
Weismann's book, "The Germ Plasm: A Theory of Heredity" describing his hypothesis was published in 1893. It was widely read and discussed in the scientific community.

Wilhelm Roux *

Weismann's theory may have originated in part from an investigation begun in 1888

by Wilhelm Roux (Figure 8.2) - a study that

Figure 8.3



Roux' Experiment

many believe launched the field of experimental embryology. Roux, the son of a fencing instructor, was born in Jena, Germany in 1850. After serving in the military in the Franco-Prussian war, he trained in medicine with help from a home study course. He obtained his medical degree in 1878 after studying with several prominent scientists including Rudolph Virchow. He ended up abandoning medicine and devoting most of his life to basic research.

Roux joined the faculty at the Anatomical Institute in Breslau in 1879, rising to the position of Director of the Institute of Embryology 10 years later. In 1895 he became Director of the Anatomical Institute at the University of Halle. Aside from his pioneering experiments, his most lasting achievement was the establishment of journal called "Archive for Developmental Mechanics" (colloquially called Roux' Archives") that is still being published, albeit with a different title ("Development Genes and Evolution") .

Roux' Experiment

Roux' seminal experiment was conceptually very simple. He wanted to know what happened if one of the two blastomeres from an early embryo was destroyed. What would the remaining cell do? What tissues would it form? Accordingly, he took a fine needle, heated it against a hot brass sphere, and inserted it into one of the two cells of a frog embryo (Figure 8.3). A zone of coagulation formed around the spot where the needle entered indicating that the cell was indeed severely injured and incapable of division. The crucial question then became: "What was the developmental fate of the uninjured sister cell?

Roux found that the undamaged cell developed into what he thought was a half embryo (Figure 8.3); either a right or left half (he couldn't distinguish between the two). It appeared to Roux that all the instructions necessary to form differentiated cells was being divided into two parts right at the beginning of development, in accord with the scheme shown in Figure 8.1.

Roux did several other experiments after his classical study of 1888. He reported the effect of electrical current on embryogenesis (in his hands, there was none), and he tried disaggregating and reaggregating cleavage cells. None of these had the impact of his initial experiments. By 1894, he had completed his career as an experimentalist and turned instead to promoting his journal and writing long books reviewing his and other's results.

Meanwhile, a young investigator had repeated Roux' 1888 experiment but with a completely different outcome. We'll take up his story in the next chapter.

9 Driesch

Just three years after Roux' work with frog embryos, another biologist initiated a second experiment similar in intent and design. The experimenter was another distinguished German scientist. This time, the embryos came from a different animal. Most importantly, the results contradicted those of Roux.

Hans Driesch *

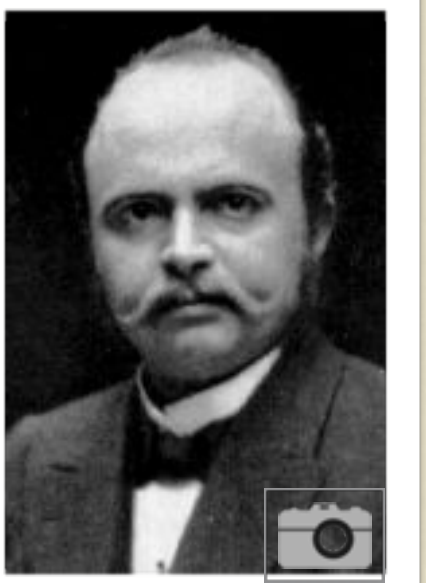
Hans Adolf Eduard Driesch (Figure 9.1), born in 1867, was the only son of a wealthy merchant who had died when Hans was two years old. Stimulated by his mother's interest in zoology (their home housed a mini zoo, a collection of exotic birds, fish, salamanders, alligators, lizards and snakes), he showed an early interest in science and studied with August Weismann for two semesters at the University of Freiburg. He earned a doctorate in 1889 under Ernst Haeckel, an early champion of Darwinism in Germany. His doctoral thesis took a combined descriptive and experimental approach to the study of early development of coelenterates (jellyfish). He continued to work on marine

invertebrates at the famous Naples Zoological Station, a marine laboratory in Italy, which was to become a mecca for the new science of experimental embryology.

Driesch's most well known experiment was carried out with sea urchin embryos, exclusively marine organisms (Figure 9.2) that mostly live in relatively shallow water, move slowly, don't have sharp spines, and are plentiful. These characteristics make them easily collectable. There are species that live in deep water and have long sharp poisonous spines. For good reasons, they are generally avoided by researchers.

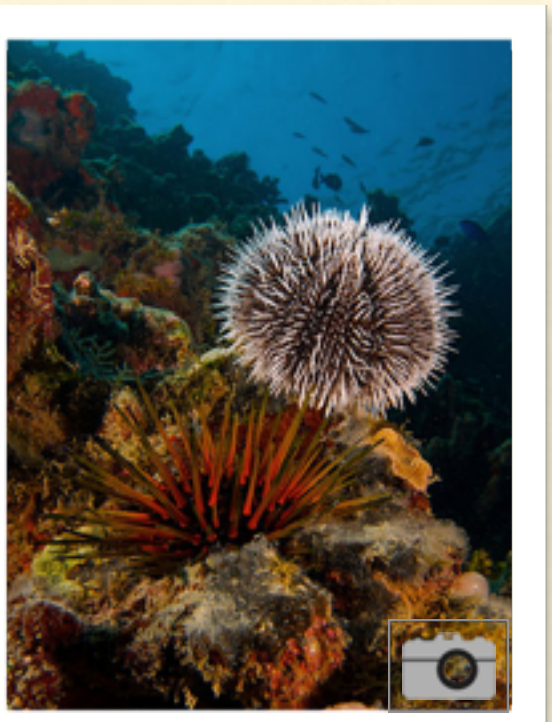
Sea urchin sexes are separate but are indistinguishable externally in nearly all species. However, you can tell males from females by administering a mild electrical shock or injecting a few milliliters of a solution of potassium chloride. These treatments don't seem to harm the animals, but they do cause the release of sperm or eggs, thereby revealing the sex of the animal. Fertilization can be achieved in a beaker by simply mixing the sperm and eggs in sea water.

Figure 9.1



Hans Driesch (1867 - 1941)

Figure 9.2



Adult sea urchins

The early development of the sea urchin is somewhat similar to that of frogs, although the sea urchin embryo is only about 0.1 mm in diameter (Figure 9.3). The first three divisions cleave the egg along similar lines. That is, the first two divisions occur along the animal/vegetal axis while the third division splits the embryo roughly along the equator. Unlike frog eggs, the third cleavage occurs almost exactly in the midline, producing eight cells that are more or less equal in size. Subsequent cleavages eventually result in a blastula with a fluid filled cavity reminiscent of the same stage of frog embryogenesis. However, unlike in the frog, the cavity in

the sea urchin blastula is more or less centrally located and takes up a larger volume of the embryo. (Figure 9.3e. The figure comes from a lecture Driesch presented in 1907 in Scotland. He was said to have spoken excellent English and, in fact, was a visiting professor at the University of Wisconsin for a time).

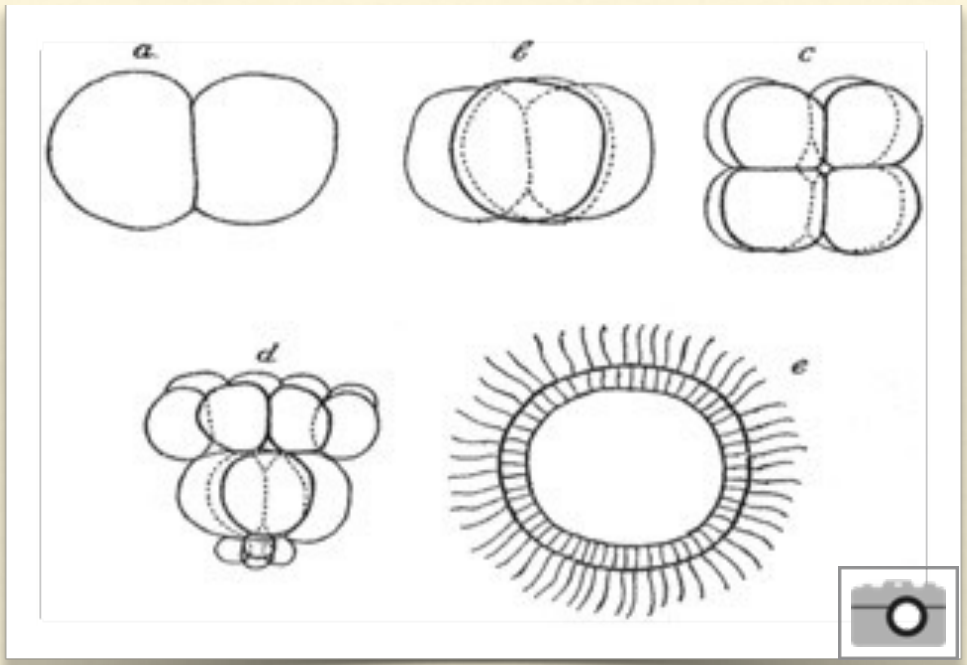
Driesch's Experiment

Driesch set out to replicate Roux' studies. In his words: "... I was interested in repeating Roux's experiment on material which would be resistant, easily obtainable, and readily observable; all three of these conditions are most satisfactorily fulfilled by [sea urchins]."

However, because of their small size, Driesch wasn't able to replicate Roux' experiments exactly. He couldn't kill one of the two initial cells with a hot needle. He decided to take another tack. By violently shaking embryos at the two cell stage, he succeeded in separating the two initial cells from one another. Like Roux, he wondered what would happen next.

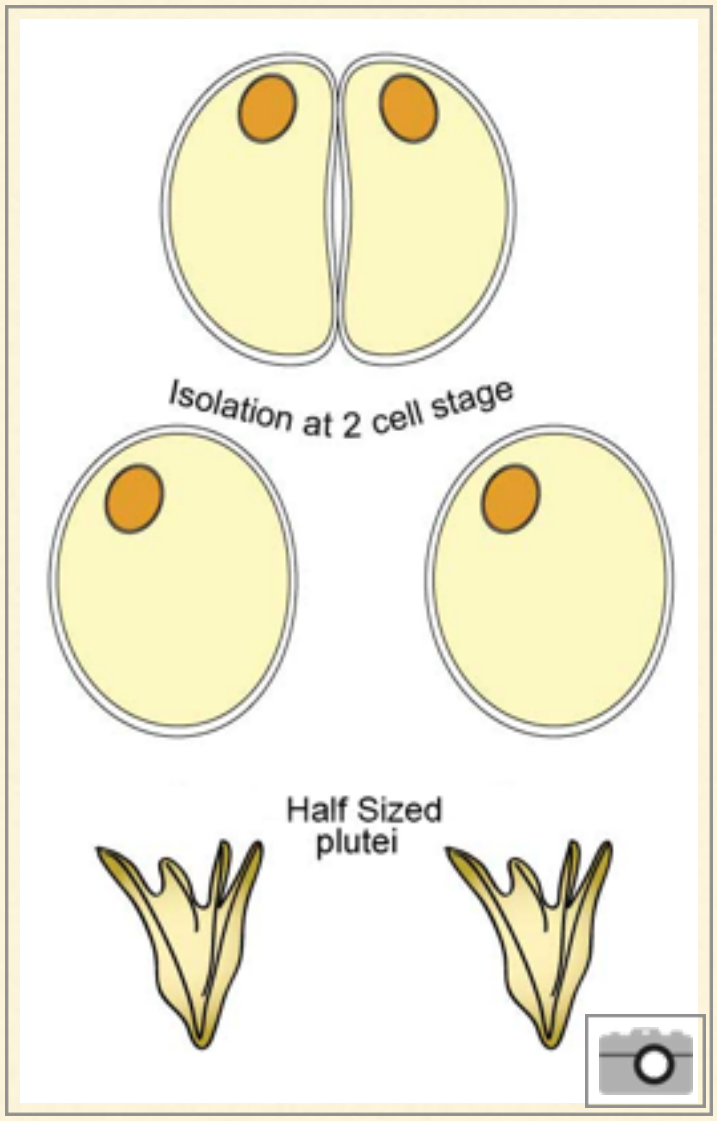
For the next few divisions, these "half-embryos" behaved as if they were right of left halves. Driesch

Figure 9.3



Early development of sea urchin embryo
a. Two cell stage
b. Four cell stage
c. Eight cell stage
d. Sixteen cell stage
e. Blastula

Figure 9.4



Driesch Experiment

wrote that he went to bed expecting to see a half blastula the next day, although he found it difficult to imagine exactly what it would look like. “Instead, the next morning I found in their respective dishes typical ... blastulae of half size.” (Figure 9.4).

These results directly contradicted those of Roux. It appeared that each of the initial two cells of the embryo was “totipotent” just like the zygote itself, and capable of forming an entire complete, albeit smaller, organism, not just the left or right half.

To be clear, as shown in Figure 9.4, Driesch only grew his embryos to the larval stage in these experiments. I did my doctoral thesis on sea urchin embryos and learned that it is very difficult to grow mature sea urchins in the laboratory. Driesch didn’t attempt to take the larvae any further then the pluteus larval stage. He thought that was far enough to prove his point.

Driesch then asked, “... up to what stage are blastomeres still able to produce a complete small [larva]?” He was able to isolate single cells from the four cell stage and succeeded in obtaining whole (but quarter sized) larvae from them. To my knowledge, he did not take these isolation experiments any further.

Driesch was puzzled by these results. The prevailing view in his time (and to this day) was that organisms were complex mechanical devices that obey the laws of chemistry and physics. Driesch couldn’t conceive of a machine that when divided in half formed two complete miniature copies of itself, each of which continued to work perfectly well. Quite puzzled, he tried altering embryos in other ways. In one experiment, he combined blastomeres from more than one embryo. The result was a giant, but otherwise normal larva.

From these and similar studies, he concluded that living organisms followed laws that were somehow beyond that of the physical sciences. This concept, is known as “vitalism”. Driesch ceased experimental work and became a philosopher and its most prominent proponent.

Despite Driesch’s problems with their interpretation, the data he obtained in his sea urchin experiments were very clear. There appeared to be no restriction in developmental fate during the formation of the first four cells of sea urchins. But what about the apparent conflict with Roux’s work? Some years later, other embryologists repeated Roux’ frog experiments, this time isolating the two blastomeres instead of destroying one. The

isolated halves each developed into a complete, although smaller, larva, just as in sea urchins.

That leaves a nagging question: If there is indeed no restriction in the ability of isolated cells from the first two divisions to produce a whole organism, what was the explanation for Roux' original results? In retrospect, it appears that Roux thought that he was seeing a left of right half embryo because of the distortion caused by the presence of the dead cell next to the unoperated one. And in fact, a more careful reading of Roux' 1888 paper confirms this idea. Roux actually noted that the undamaged cell was eventually capable of going on to form tissues from the other half of the embryo, but, perhaps because he was so committed to one particular interpretation, he chose to overlook these results.

Driesch's experiments naturally lead to other questions: If each of the four cells of an early embryo can form a complete larva, how about the cells at the next division? Or the next? When, if ever, do developmental restrictions start to take place? As it turns out, subsequent investigators found that totipotency, at least as measured by the ability of an isolated cell to form a complete organism, seemed to stop at the four cell stage. That is, none of first eight cells of a sea urchin embryo can go on to form a normal larva. Was that because the cell had irretrievably lost some genetic information at this point in development as Weismann had postulated? Or was it due to the fact that the information was all there and other factors were at play? Additional experiments were needed to clarify this issue and they were performed by another German scientist of considerable repute, the first embryologist to garner a Nobel Prize.

10 Spemann

Hans Spemann *

Hans Spemann was born in Stuttgart, Germany in 1869. He studied medicine but, like Roux, decided to pursue a career in science. He earned his doctoral degree in 1895. He was a skilled microsurgeon who developed ingenious techniques and instruments to help him transplant, excise, and otherwise modify salamander embryos in an effort to learn more about how they developed. For his many outstanding contributions, he was awarded the Nobel Prize in 1935, the first embryologist to receive that honor.

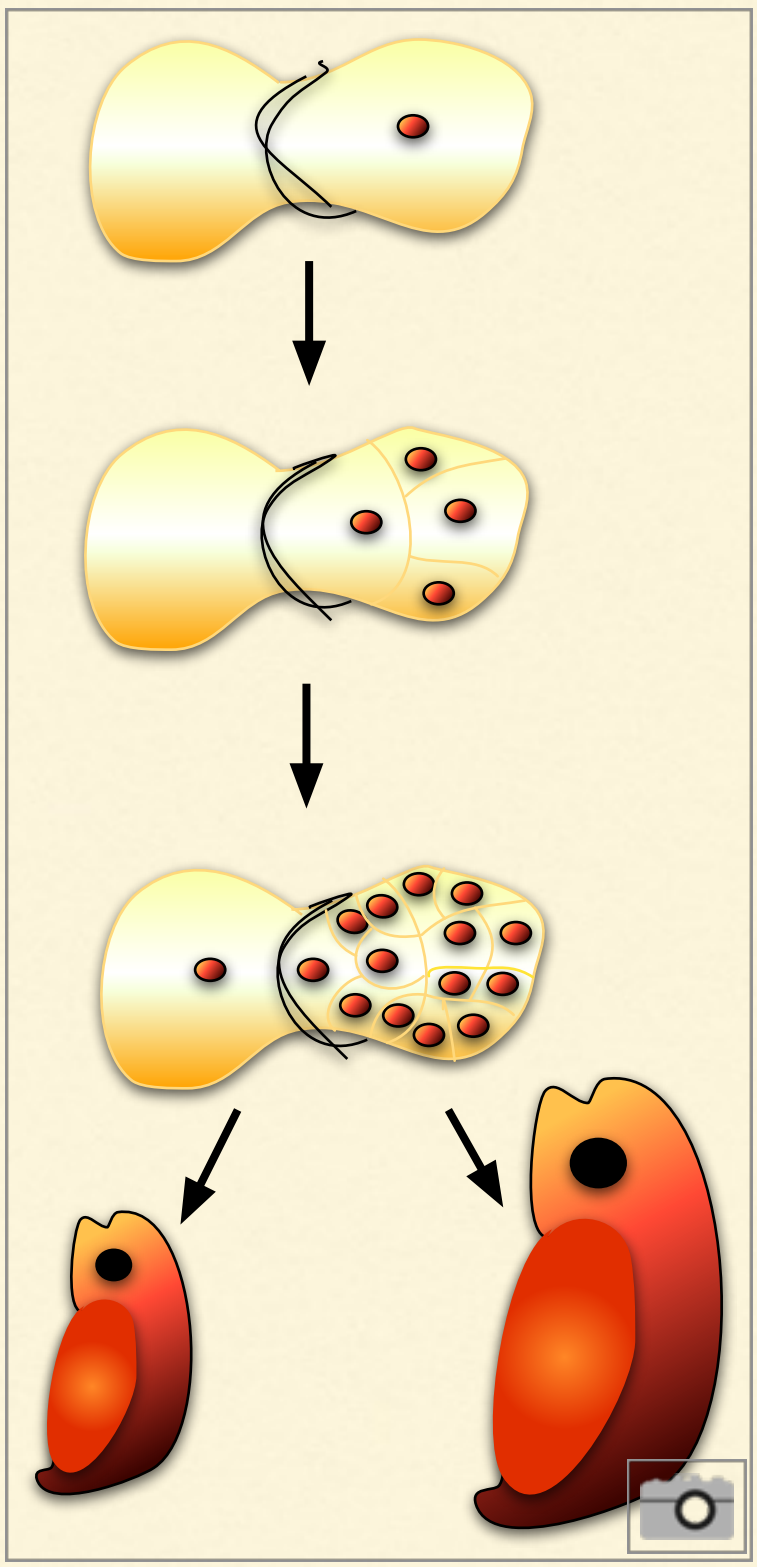
Figure 10.1

Hans Spemann (1869 - 1941)

One of Spemann's experiments that is most relevant to our present discussion, took place in 1914. Spemann, using a

hair plucked from the head of his baby daughter, drew a noose around a newly fertilized salamander egg along its north-south axis, thereby forcing the embryo to assume a dumbbell shape (Figure 10.2). The nucleus of the zygote was pushed to one side of the ligature. The half in which the nucleus came to rest began to divide into smaller blastomeres. The other half didn't. After some time, 16 cells formed on the nucleated side. At that point Spemann loosened the constriction slightly, allowing a single nucleus from one of the newly formed cells to slide into the enucleated half. With a newly resident nucleus, it too began to divide. By tightening the hair again and separating the two halves, Spemann was able to produce two twin embryos, clones, one or which began life with the nucleus that came from one of the cells of a sixteen cell embryo.

Figure 10.2



Using a fine strand of his daughter's hair, Spemann constricted an embryo, forcing the nucleus to one side. See text for additional details.

How would this formally enucleated embryo develop? Spemann envisioned two possibilities. If genetic information in the nucleus had been lost, the embryo formed under the influence of the nucleus that moved into the enucleated half would develop abnormally. If all 16 nuclei were equivalent in their genetic potential, the late developing half would form a complete larvae. And it did!

Spemann's results seem to contradict those of the previous chapter. Recall that Driesch found that isolated single cells from an eight cell embryo, as well as those from later stages, were incapable of forming a complete individual. How was it

then that a nucleus from a 16 cell embryo could promote normal development?

This question is a critical one. If it occurred to you, give yourself a pat on the back. I'll postpone answering it now, but will return to it when I discuss transplant experiments using nuclei from later developmental stages.

Sometime after being awarded the Nobel Prize, Spemann realized that the constriction studies that he had done might be extended, but with an elegant twist. In his book, "Embryonic Development and Induction", he proposed what he called a "fantastical experiment". Imagine if you could remove the nucleus from a newly fertilized amphibian egg and replace it with the nucleus from a fully differentiated cell, say a skin cell or a cell from the liver. "This experiment might possibly show that even nuclei of differentiated cells can initiate normal development in the egg protoplasm. Therefore, though it seems an anticipation of exact knowledge to say that every single cell possesses the whole apparatus of potencies, ... yet this opinion may be right."

While Spemann speculated that a nucleus from a differentiated cell would give rise to a whole organism, that's just one possible outcome of such a nuclear transfer experiment. Another exciting possibility might be that the differentiated nucleus would reprogram the egg into which it was introduced so that it could begin forming tissue reminiscent of the nucleus' origin. For example, if the nucleus of a liver cell were to be transferred into a zygote, perhaps the egg would form a mass of liver tissue.

The experiment was very cleverly conceived by Spemann, because either normal or abnormal development would be a fascinating outcome. But because the technology of the time wasn't up to the task, he never actually performed it. However, in 1953, twelve years after his death, two American scientists did.

11

Briggs and King*

Cloning by Nuclear Transfer

Robert Briggs and Thomas King were working together in a laboratory in Philadelphia, Pennsylvania when they carried out the experiment that Spemann had proposed decades earlier. Briggs was born in Massachusetts in 1911. Upon the death of his mother when he was two years old, he was raised by his grandparents in a small town in New Hampshire. Inspired by a high school science teacher, Briggs became interested in the biological sciences. But, because it was the midst of the great depression, he took the financially conservative course of beginning college as a business major. However, the siren call of the sciences drew him back to biology. He graduated from Boston University in 1934 with a degree in biology and soon thereafter enrolled at Harvard for graduate study. He earned a Ph.D. degree in 1938 while studying metabolism in frog embryos. For four years, he served as a fellow in the Department of Zoology at McGill University where he investigated tumors in frogs. In 1942 he joined the Lankenau Hospital Research Institute (now the Fox Chase Cancer Center) in

Philadelphia. He was to carry out research on amphibian embryos for the remainder of his life.

His collaborator, Thomas King, was recruited by Briggs because of King's expertise in microsurgery. King was a New Yorker, some 10 years younger than Briggs. He too suffered the death of a parent as a child, and was raised by an aunt in New Jersey. He attended Fordham University as an undergraduate and served as a teaching fellow at New York University. He was still a graduate student when Briggs contacted him.

In actuality, neither Briggs nor King were aware that Spemann had suggested, 20 years previously, the possibility of transplanting a nucleus into an enucleated egg. Briggs had a long term interest in the role of genes in development, and had had extensive discussions about the subject with Jack Schultz, a colleague at the Lankemau Hospital. Schultz, a scientific gadfly, was the one who suggested the nuclear transfer experiment and Briggs became excited about the idea. He applied for funds from the National Cancer Institute so that he could hire someone who could do the microscopic manipulations. At first, the grant request for a modest amount of money was turned down, but Briggs reapplied and eventually

Figure 11.1

Rana pipiens
Common leopard frog

was awarded funds to recruit King, who had taken a course in micro manipulation.

Briggs and King began their collaboration in 1950. They published their ground breaking paper in 1952. Conceptually, the experiment that they carried out was very similar to the one first proposed by Spemann (Figure 11.2). The idea was to remove the nucleus from a frog egg – the common leopard frog, *Rana pipiens* (Figure 11.1) – and replace it with the nucleus from a cell further along in development.

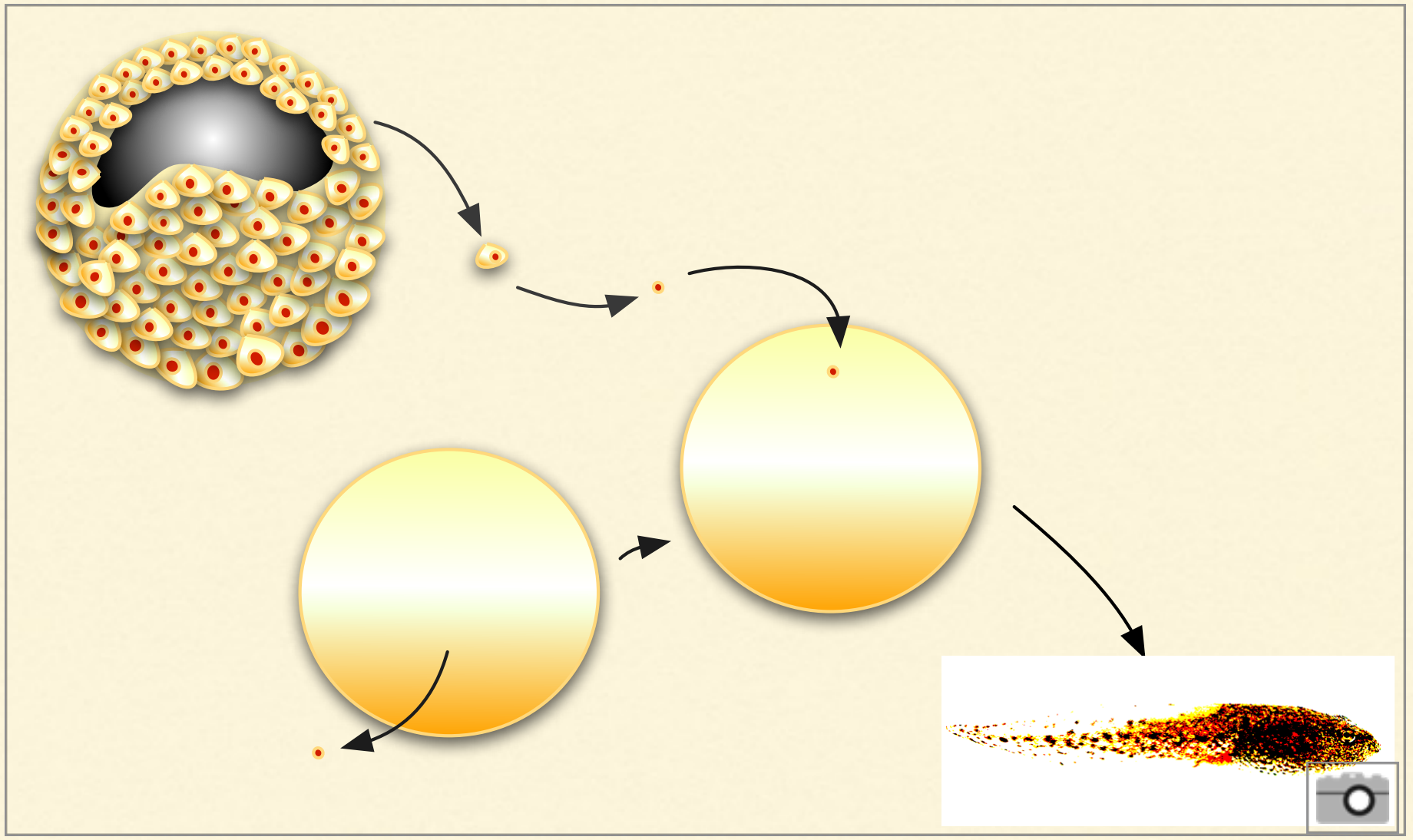
The experiment proved difficult to do. In particular, they found that when the donor nucleus was exposed to the water in which the eggs were developing, it seemed to be damaged and no longer supported development. Briggs and King hit upon

the idea of using a micro pipet just a little bit smaller than the cell that contained the donor nucleus. In this way, when they sucked up the donor cell into the pipet it broke the cell membrane, but the nucleus remained surrounded by cytoplasm and thus protected from exposure to the external medium. They were then able to inject both the nucleus and the small amount of surrounding cytoplasm into the egg (Figure 11.3).

They described their technique in a classic paper published in 1952.

"The transplantation of nuclei is carried out in the following steps: First the recipient egg is pricked with a clean glass needle. This activates the egg and causes it to rotate so that the animal pole is uppermost and the egg nucleus can be taken out with a glass needle ….The outer jelly coats are then removed and the egg is placed in a depression in a wax-bottomed dish ….A blastula or early gastrula …, placed in the same dish, is then opened up and one of the subsurface animal pole cells is dissected free from its neighbors. The cell is now drawn up into the mouth of a thin-walled glass micropipette, the lumen of which is

Figure 11.2



Nuclear transfer in frogs. See text.

somewhat smaller than the diameter of the cell. … The tip contains the column of solution drawn up with the cell. Provided the needle is really clean the movements of the column can be controlled accurately. Now, as the cell is drawn up into the needle it is compressed and distorted in such a way as to break the cell surface without dispersing the cell contents. The needle is then inserted into the enucleated egg and the broken cell is injected, thus liberating the nucleus within the egg."

After several months of perfecting this technique, they were able to get it to work a little less then half the time. That is, when injected with a nucleus from a blastula cell, 40% of the enucleated eggs were capable of forming an embryo. A lesser amount even developed to the tadpole stage. These first studies conclusively showed that nuclei removed from blastula cells (and even somewhat later stages) were fully capable of supporting normal development, a stunning advance over Spemann's ligature experiments. Moreover, since a single blastula embryo consists of hundreds of cells, hundreds of clones could be produced from one individual. Briggs and King had succeeded in developing a method for mass production of clones.

These initial studies convincingly demonstrated that early embryonic nuclei retained all the information necessary to prime development. But the nuclei were obtained from cells at the blastula stage of development. And cells at this stage are not differentiated. What would happen if a fully differentiated cell nucleus were transplanted?

When Briggs and King went on to transfer nuclei from cells at later stages, they had less and less success: as development of the donors proceeded. And nuclei from differentiated cells were abject failures. They were completely unable to prime normal development when injected into enucleated eggs.

Why this lack of success? Like most failed experiments, Briggs and King's results may be interpreted in distinctly different ways. One possibility is that the donor nuclei were irreversibly altered during differentiation and thereby were incapable of priming complete development. They might even have lost genetic information as Weismann had suggested. Of course, irreversible changes don't necessarily have to mean missing genes. But that possibility certainly suggested itself.

Another possibility is that differentiation is in fact reversible, but that differentiated cell nuclei have more difficulty in changing course as they progress through development. If you think of

differentiation as a kind of programming, perhaps the reprogramming necessary to become a totipotent nucleus isn't as easily achieved after it has undergone differentiation.

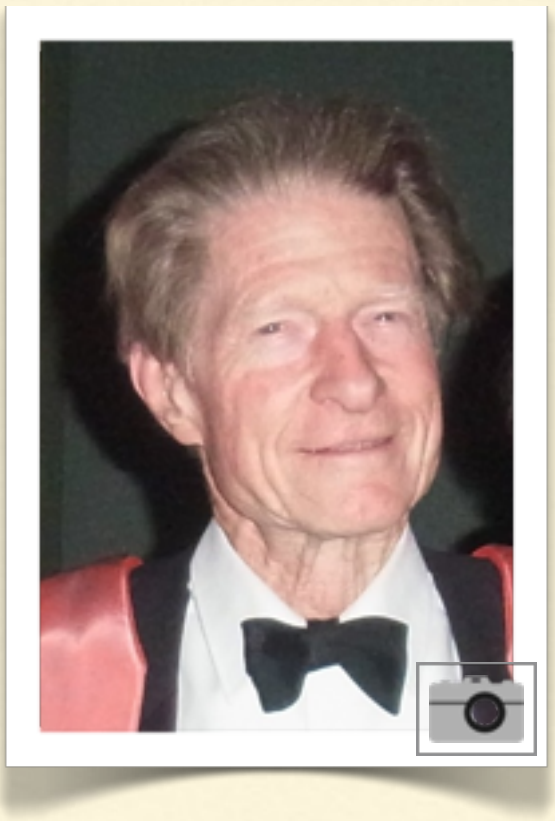
In addition, there are some relatively trivial explanations for Briggs and King's failures. It might be that there were technical issues with transferring more developmentally advanced nuclei. Perhaps, for example, they didn't support normal development because they were more readily injured during transplantation. Or perhaps nuclei were simply more difficult to remove intact from differentiated cells.

As is often the case, more experiments seemed to be required. Soon another scientist took up the task of repeating Briggs and King's work in an effort to resolve the problems that Briggs and King experienced.

12 Gurdon

John Gurdon, an English embryologist, repeated Briggs and King's experiments as a graduate student at Oxford. Gurdon was born in Hampshire England. His parents sent him to Eton, a prestigious boy's school, where he proved to be a poor student. His biology teacher, a Mr. Gaddum, sent home a report that read in part, "I believe he has ideas about becoming a Scientist; on his present showing this is quite ridiculous, if he can't learn simple Biological facts he would have no chance of doing the work of a Specialist, and it would be sheer waste of time, both on his part, and of those who have to teach him." Gurdon, who

Figure 12.1



John Gurdon (1933 -)

was to receive the Nobel Prize in Medicine in 2012, had the report framed and it hangs in his office. In an interview with Nick Collins, science correspondent for "The Daily Telegraph" of London, Gurdon remarked, " When you have problems like an experiment doesn't work, which often happens, it's nice to remind yourself that perhaps after all you are not so good at this job and the schoolmaster may have been right."

Figure 12.2

Xenopus laevis

Xenopus laevis

Gurdon's choice of an experimental organism was critical to the success of his studies. *Xenopus laevis*, the South African clawed toad, which, by the way, is an aquatic frog, not a toad, has several advantages as an experimental organism. For one thing, it lays eggs all the year round unlike its American cousin, *Rana pipiens*. In addition, it reaches maturity in less than a year, much faster than *Rana*. And most important of all, a mutant version of *Xenopus* was available at Oxford, where Gurdon was working. As Gurdon has remarked, "...we had the huge advantage of a genetic marker, which was not available in other species. This was important, because if you transplant a nucleus and get a normal animal out if it, you really want to prove beyond doubt that the animal has come from the transplanted nucleus and not from the resident egg nucleus which, occasionally, might not have been removed."

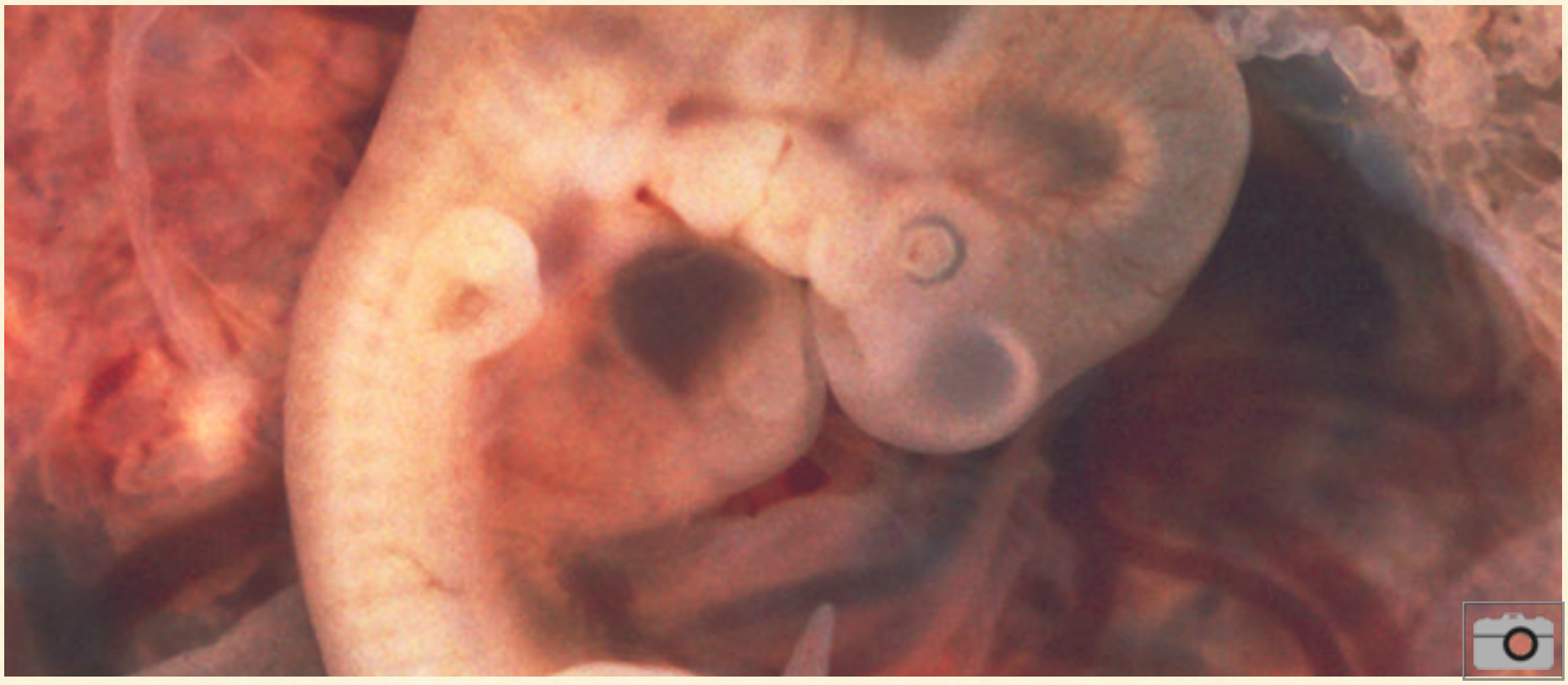
There were a few procedural differences between Gurdon's and Briggs and King's experiments, but in essence, their methods were the same. Remarkably, their results were similar too. In both studies, the ability of a transplanted nucleus to elicit development decreased with the age of the cell from which the nucleus was derived. Gurdon had very few successes.

However, Gurdon interpreted his experiments differently. He reasoned, as I noted in the last chapter, that abnormal development could be due to all kinds of

circumstances. The fragility of the donor nuclei (or their host cells) could increase with age, or the stage of division in which the cells found themselves could have adversely affected the ability of the nuclei to sustain normal development. The important outcome in Gurdon's view wasn't the abnormals, the failures, but the few normal embryos and tadpoles that he produced. The fact that any nuclei at all could sustain normal development must mean, Gurdon reasoned, that these embryonic nuclei hadn't irreversibly lost developmental instructions. And of course, he was right. Development doesn't proceed as Weismann had thought. Differentiation doesn't work by the parcelling out of genes.

In time Gurdon was able to transfer nuclei from fully differentiated intestinal cells and insert them into *Xenopus* eggs and produce a few tadpoles. The process wasn't very efficient but he had successfully carried out Spemann's dream experiment. He had generated frogs from differentiated cell nuclei. Gurdon's results were a breakthrough in our understanding of development.

However, besides their theoretical value, there were other implications. When you take a nucleus from a fully differentiated cell and transplant it into an egg, you produce a clone of the frog from which the nucleus was obtained. Clones produced from adult cells was now shown to be feasible. If it worked in frogs, the public wondered, could cloning of mammals – even humans – be far behind? Could I take the nucleus from one of my cells and produce another me?



13

Mammalian Oogenesis and Early Development

In order to appreciate some of the difficulties that scientists encountered while attempting to clone mice, sheep, primates, and similar animals, it's worthwhile to take a moment to examine how mammalian eggs are formed, as well as the events that occur after they are fertilized. I'll focus on human eggs and embryos in the following discussion for three reasons. First, because human gametogenesis and development are more or less representative of mammals in general. Second, because I'll be discussing human cloning in a future chapter. And third, because most people's understanding of these processes, if they've thought about them at all, are somewhat limited.

When the molecules that control the processes of gametogenesis (how sperm and eggs arise) and early development were compared across species by molecular biologists, the many similarities among all organisms were striking. These parallels have enabled the scientific community to learn an enormous amount from the study of "model organisms", creatures like fruit flies and frogs that

have unique experimental advantages for the analysis of genes and proteins that regulate development in the laboratory.

However, at the macroscopic level, gametogenesis and the events following fertilization are quite different amongst different creatures. Humans, in particular exhibi t some special features indicative of the fact that development takes place internally. For example, women have tiny eggs, about two hundredth's of an inch in diameter (Figure 13.1, 13.2). While they're among the largest cells in the body, human ova are just barely visible with the naked eye. Their small size compared to many of our vertebrate cousins like fish, amphibians, and birds probably reflects the fact that the nutrients required by the developing human embryo don't have to be packaged within the cell. They are provided by our mothers – another debt we owe to mom.

Figure 13.1

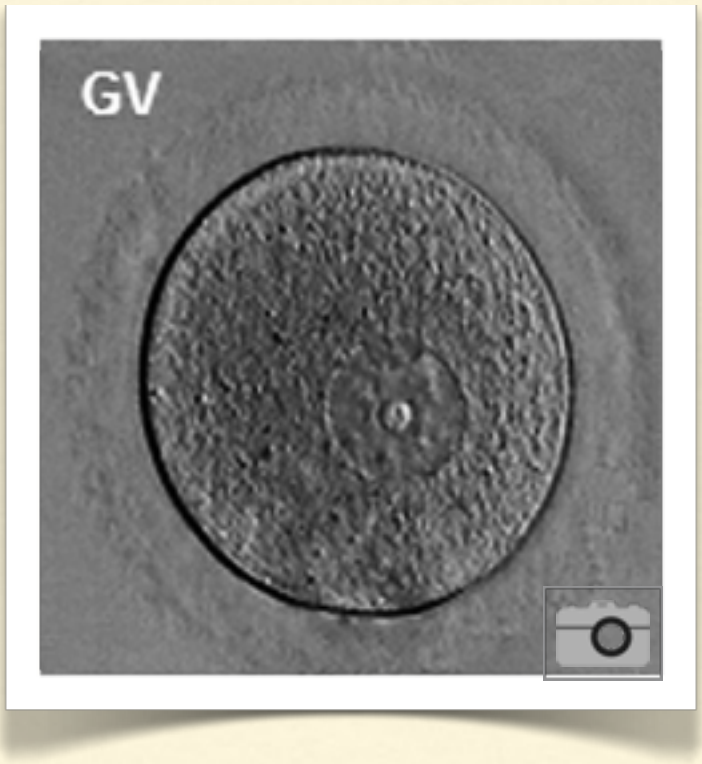
A drawing of a human ovum.

Oogenesis

A particularly distinguishing feature of human development is seen during oogenesis – the process whereby eggs are formed. Unlike sperm, which are produced continually after puberty in males, it is thought that all the eggs that a woman will bear in a lifetime are present before birth.

Oogonia, early cells that are destined to become eggs, divide rapidly in embryos, resulting in several million or so daughters by the seventh month after fertilization. For an unknown reason, all but a few hundred of these die before a woman reaches puberty. The remainder, now called primary oocytes, begin the process of **meiosis** and replicate their DNA. (In textbook descriptions, meiosis is

Figure 13.2



A photography of a human oocyte.

often described as the process that takes a single cell with two copies of each chromosome, to four equivalent cells, each of which carries a single copy. I'll have more to say about meiosis later on).

Oocytes now contain twice the genetic material of a normal diploid cell. At this juncture, something remarkable happens: the oocyte stops its progression through meiosis. This pause lasts at least a decade, and may continue for many years more.

Figure 13.3

The human chromosome set stained with dyes that are specific for each chromosome

Time passes. After puberty, meiosis resumes in some cells on a monthly basis. A few oocytes undergo one unequal cellular division with half of the chromosomes moving into each cell. A large cell (the secondary oocyte) persists; another, a small one, the "polar body", is discarded. At this stage, the secondary oocyte carries half the DNA of the primary oocyte, and almost all of its cytoplasm. The secondary oocyte resumes meiosis, entering the second meiotic cycle, but again, instead of completing the second meiotic division meiosis comes to a halt for a second time. Because they haven't completed the second meiotic division, secondary oocytes carry two sets of chromosomes, the same number as they began the second meiotic cycle with. These secondary oocytes are now ready for ovulation despite the fact that they haven't completed meiosis. They are released from the follicles of the ovary and travel down the fallopian tube hoping to meet some accommodating sperm. If that happens, fertilization may ensue.

Fertilization

Fertilization in humans accomplishes two things. First, it "activates" the egg, causing it to complete meiosis and to loose the machinery that allows the embryo to begin division. Completion of meiosis results in the loss of one chromosome set into a

second polar body. The newly fertilized egg thereby carries a haploid nucleus. (Remember, it started out with twice the genetic material carried by a diploid cell and underwent two divisions). Second, it introduces the father's haploid set of chromosomes into the egg. Most biologists, myself included, were under the impression that the newly entered sperm nucleus, and the egg nucleus that has just finished meiosis, immediately fuse together to form a single diploid nucleus, but that is not the case. The chromosomes in the two nuclei remain separate and each replicates so that there is now twice as much DNA in total as there is in a diploid cell. It is at this point that the chromosomes condense and mitosis begins and a two cell embryo forms.

Meiosis

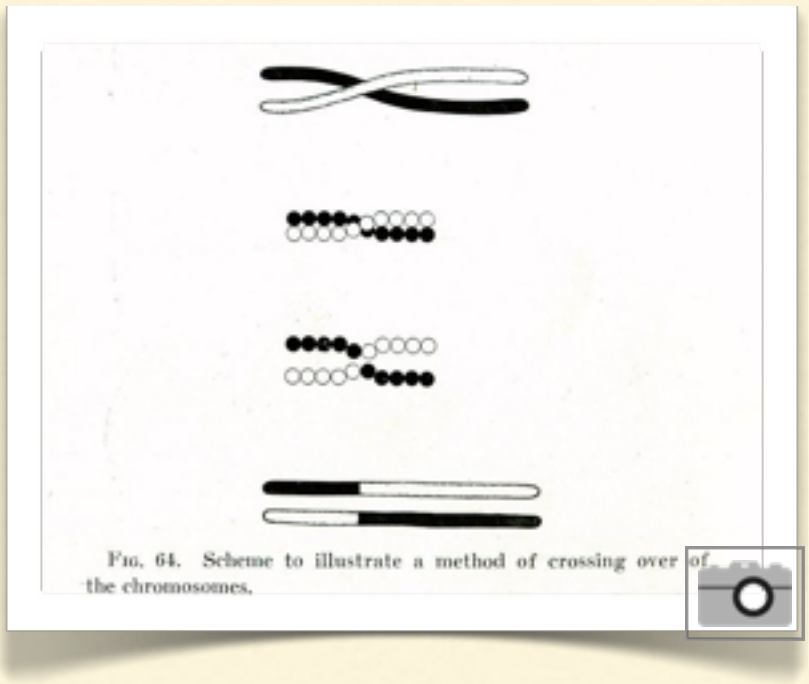
Before discussing what happens next, I'll take a brief detour and say a bit more about meiosis. Meiosis achieves two primary objectives, one of which, the reduction in chromosome number, has already been discussed. It's second function, under-appreciated by many, is to mix up the genetic material. Humans, for example, have 46 chromosomes, half of which we get from each parent. The maternal and paternal sets look very much alike. Every member in a set has a "homologous" partner, indicating that each has a near twin from the opposite sex with a similar size and appearance. Each member of a homologous pair carries a specific set of genes arranged more of less in the same order. But because our mothers and fathers are generally not closely related, the sequence of some of their homologous genes may differ. The chromosomes are therefore similar, not identical. Hence the word "homolog", not "identilog".

What does this have to do with mixing up the genetic material? In meiosis, as we've seen, the oogonia are diploid cells, with a set of chromosomes that come from each parent. During the first meiotic division, each homologous pair lines up next to its corresponding homolog. When the chromosomes move into the two daughter cells, the maternal or paternal member of each pair may move into one cell or the other. The choice is random. Consequently, the daughter cell will carry some combination of both maternal and paternal chromosomes. For that reason, the chances are that no two eggs will be genetically identical. Similarly, each sperm or egg cell will likely carry a different combination of maternal and paternal chromosomes.

But that's just part of the story. When the homologous chromosomes line up and pair

with one another, an exchange takes place between parts of homologs. As a result, new chromosomes form with genetic material from both parents intermixed. Accordingly, after this stage of meiosis, there are no longer pure maternal or paternal chromosomes. The process of gamete formation has made a mishmash of heredity, separating genes derived from mothers and fathers even on the same chromosome. This phenomenon was discovered by Thomas Hunt Morgan and his students. Figure 13.4, which comes from Morgan's textbook, illustrates this phenomenon.

Figure 13.4



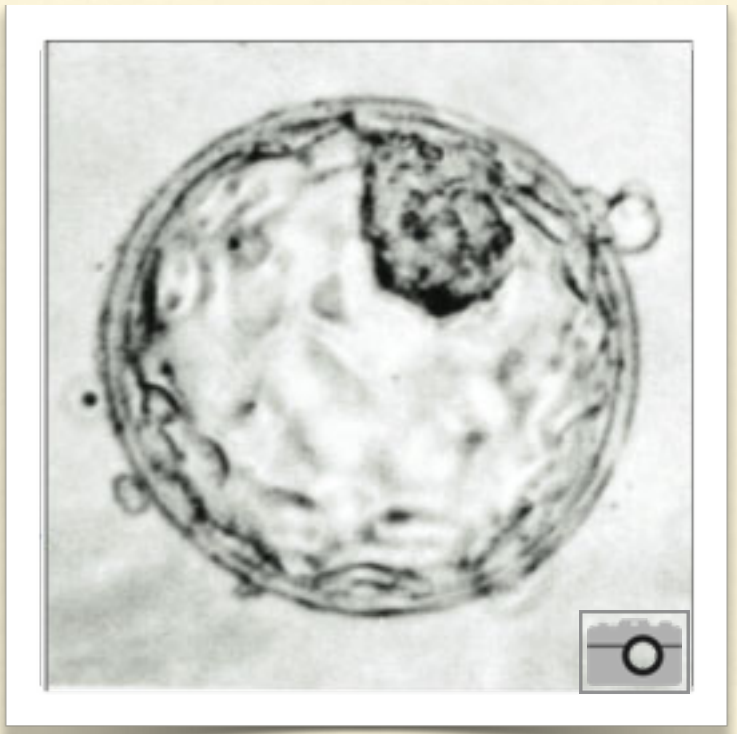
Recombination, as depicted by Thomas Hunt Morgan in 1916.

Early Cleavage

Back to early mammalian development. As shown in Figure 13.4, human ova are covered by a membrane, called the zona pellucida, and a mass of cells. This layer must be traversed by sperm in order for fertilization to occur. It is not shed until the embryo "hatches", some five days after fertilization.

The very first cleavages in human embryos are not as regular as those in sea urchins, but the general picture is the same. However, at about the 16-32 cell stage a distinct difference between the two organisms occurs. In mammals, a group of cells separates from the others. They huddle together, continue to divide, and form the so called "inner cell mass" or ICM. The cells of the ICM are destined to form the embryo proper.

Figure 13.5



Human embryo at the blastocyst stage. The inner cell mass is near the top right of the figure.

Embryos at this stage are called "blastocysts" (Figure 13.4).

Because mammalian embryos must get their nutrients from their mothers, they have to have some means of allowing the embryo to attain close contact with the mother's blood. The cells that do this, the **trophoblast**, form a thin ring around a central cavity. They will eventually attach to the mother's uterus, forming the embryonic part of the placenta.

After the blastocyst stage, the cells of the embryo move around, interact with each other in complex ways, and differentiate. Tissues and organs form, and ultimately a new individual will emerge some nine months after conception.

14

Cloning Mammals

Cloning Beginnings

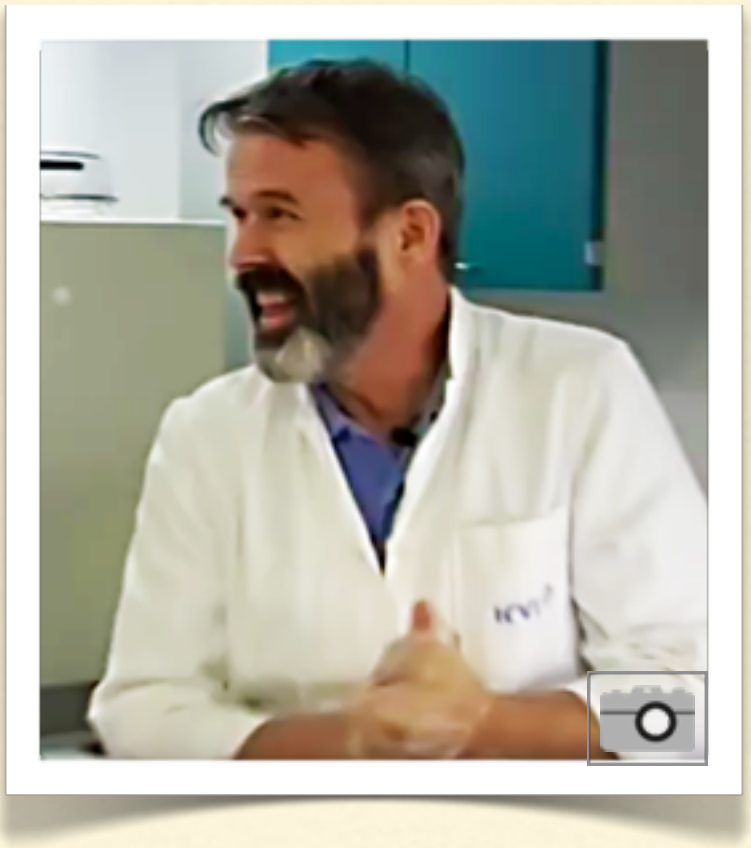
The first purposely generated mammalian clones – identical twins – were produced by splitting two cell sheep embryos in half. This feat was accomplished in England almost 100 years after Driesch's similar feat with sea urchin embryos in 1979 by Steen Willadsen. A Danish born reproductive physiologist, Willadsen is a scientist who is variously described as mysterious, grandiose, dominating, inventive, and extroverted. He's also been called an iconoclast, a genius, and a visionary. His work directly led to the cloning of Dolly. In fact, Ian Wilmut has said that if he - Wilmut - is the "father" of Dolly (a description that he doesn't believe gives sufficient credit to his team), then Willadsen is her "mother".

Steen Willadsen

Willadsen, born in 1943 in Copenhagen, was the youngest of three children. They lived with his divorced mother and extended family. He was a gifted student who decided to become a veterinarian. But the routines of veterinary work

bored him. He turned to research and earned a PhD in reproductive physiology at the Royal Veterinary College in Copenhagen. After obtaining his degree, he searched for a position in which he could pursue his broad ambitions. Nothing in Denmark appealed to him. Finally an opportunity opened in Great Britain, at the Agricultural Research Council in Cambridge (ARC). His was the only application and, not surprisingly, he got the job.

Figure 14.1



Steen Willadsen (1943 -)

There was little pressure to publish at the ARC. And little in the way of direction. He found himself in the fortunate position of being free to work on whatever came to mind. Using sheep as his subjects because they were cheap and readily available at the ARC, he decided to duplicate Driesch's experiment, but with mammals.

But he soon ran into a problem. As noted previously, the mammalian egg is covered by a coat, the *zona pellucida*, which the sperm has to penetrate to effect fertilization. Only after several days post fertilization does the embryo "hatch" from this covering. In sheep, but not in mice and some other mammals, if the *zona pellucida* is removed prematurely or even damaged, the embryo will not develop. Willadsen needed to remove the *zona* in order to separate the blastomeres. How do could he do that and yet enable the embryos to develop?

Willadsen came up with an ingenuous solution. He guessed that he could remove the *zona*, separate the two blastomeres, and then enclose the naked half embryos into an empty *zona*. Subsequently he would cover the embryos with agar, the clear jellylike substance used in culture plates to grow bacteria and cells. The strategy worked. He was able to house the embryos in agar cylinders, transfer the cylinders to recipients ewes, allow the embryos to develop for a bit, remove the embryos, remove the agar, and transfer the now naked embryos to another set of receptive

ewes to continue their development. Out of 66 original embryos separated at the two cell stage, he was able to recover 5 pairs of identical twins. He had succeeded in cloning a mammal.

Willadsen published his discoveries in a paper in the prestigious journal "Nature. In it, he suggested that the agar embedding method would prove useful in other kinds of experiments. It was a prophetic statement. He followed up on his initial studies by bringing each of the first four blastomeres to term. Unlike sea urchins, even single blastomeres at the eight cell stage could be persuaded to become lambs. Not satisfied with cloning sheep, he used the same procedure to clone other animals, including cows, pigs, goats, and horses. Then, apparently bored by cloning only one kind of animal at a time, he tried mixing blastomeres from different species. He succeeded in creating a chimera of a goat and a sheep, a "geep".

(A short aside. Gina Kalota tells of a party that Willadsen attended just before he left England to take a job in Texas with Grenada Genetics in which he was served

Figure 14.1

A geep, a mosaic animal consisting of cells from a sheep and a goat.

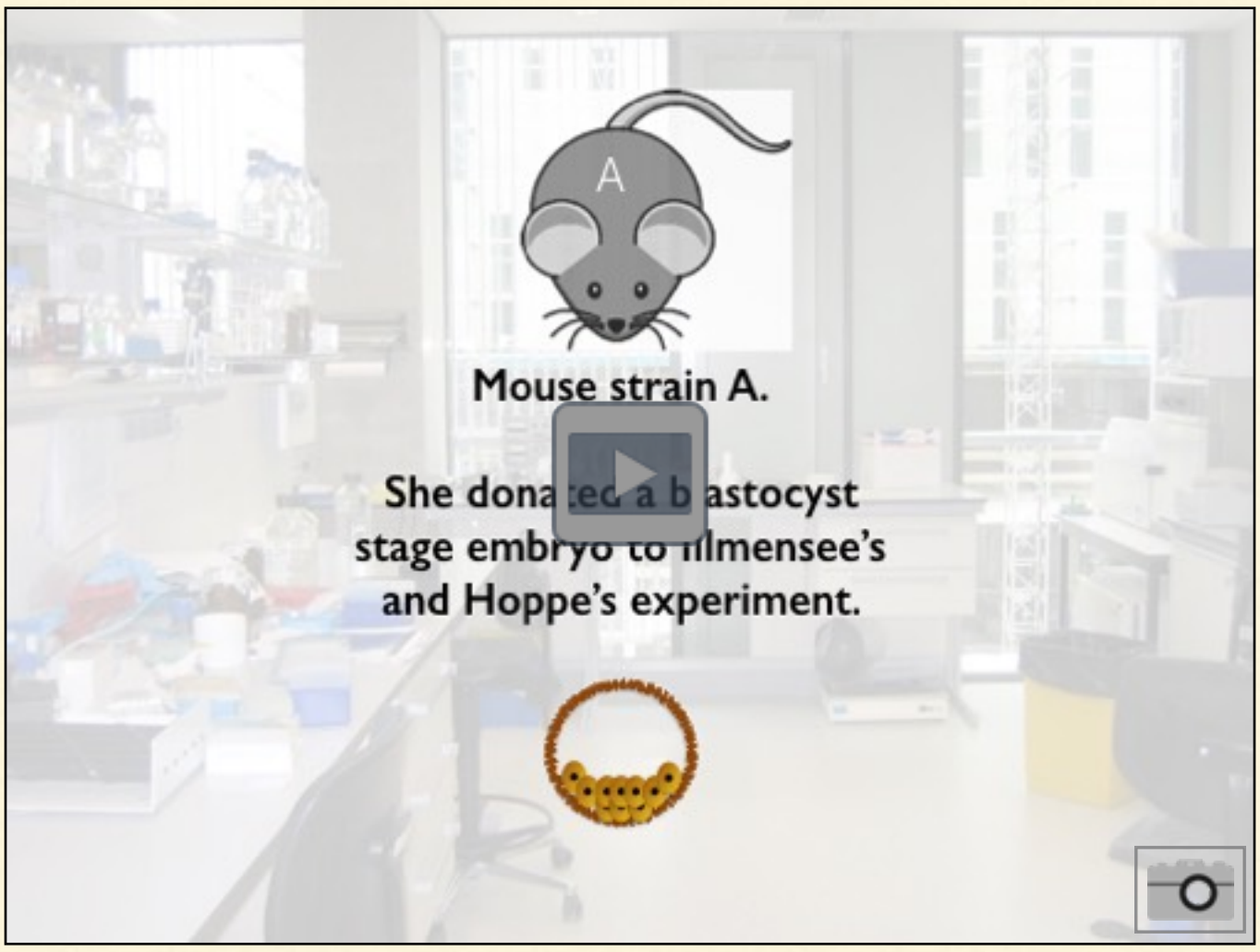
a roasted geep. Willadsen says that it wasn't particularly good, tasting more like mutton than lamb.)

Willadsen had cloned sheep by separating blastomeres. What he hadn't done was to produce clones by nuclear transfer, as Briggs, King, and Gurdon had reported. But two others had. Or at least said that they had. And not with sheep. With mice.

Cloned Mice?

In 1981, Karl Illmensee and Peter Hoppe published a bombshell paper in which they claimed to have produced three mice by transferring nuclei from inner cell mass cells into enucleated eggs (See Slideshow 14.1). Their technique was similar to that of Gurdon's, except, given that mice have much smaller eggs than frogs, it took considerable skill to carry out the procedure. Illmensee seemed up to the task. He had a reputation as a accomplished micro surgeon, expertise gained from his doctoral thesis work with Drosophila embryos. After obtaining his PhD, he turned his attention to mice and had produced some impressive papers. But his and Hoppe's 1981 paper was a step up. It seemed historic.

Slideshow 14.1 The Illmensee/Hoppe Experiment



Illmensee and Hoppe's experiments seemed to have been carefully conceived and of high quality. They developed some innovative techniques and seemed to have carried out appropriate controls. One new idea was to treat their recipient eggs with a substance derived from fungi that "softened" or relaxed the cytoplasm allowing for easier penetration of their micro pipets. As a control, they injected nuclei from both the inner cell mass and from the trophoblast. The trophoblast doesn't give rise to tissues in the embryo proper and nuclei from them was thereby less likely to prime normal development (they didn't). As a further check the used several strains of mice so that they could be sure that the nuclei that they were injecting were the true source of the genes required for subsequent development. And, when their experiments ultimately produced healthy (and long lived) mice, they crossed them to mice conceived via the normal route and found that subsequent generations continued to pass on traits from the original donated nuclei.

Illmensee's and Hoppe's studies seemed like a revolutionary breakthrough. Other researchers rushed to repeat their work. They were not successful. Perhaps, some suggested, the procedure didn't work because other scientists lacked the technical proficiency required. But attempts to repeat these experiments failed even when done by other members of Illmensee's laboratory.

Soon, there were suggestions of fraud. When pressed, Illmensee was reluctant to allow outsiders to visit his laboratory to confirm what he had done. An investigation by an international commission was initiated by the University of Geneva, Illmensee's employer. While no one found that he had fabricated his results, the commission found evidence of sloppy record keeping. Under a cloud, Illmensee resigned from the University of Geneva in 1985 and Hoppe retired in the same year.

In the end, Davor Solter and James McGrath at the Wistar Institute in Philadelphia, two prominent and highly respected reproductive physiologists, took on the task of carefully and methodically determining if Illmensee and Hoppe really had cloned mice by the techniques that they claimed to have used. They issued their report in the prestigious journal Science in 1984. Their article was entitled "Inability of mouse blastomere nuclei transferred to enucleated zygotes to support development in vitro". The title says it all.

Solter and McGrath's results were unambiguous. Nuclei from cells at the two

stage when injected into eggs could indeed prime development, but this ability seemed to be lost rapidly as the donor embryo divided and matured. Indeed, Solter and McGrath found that nuclei from eight cell stage embryos were completely unable to promote development even to the blastocyst stage. And that was certainly not true for more advanced nuclei from the inner cell mass as Illmensee and Hoppe claimed. Solter and McGrath concluded: "... cloning mammals by simple nuclear transfer was impossible". However, to some, the word "impossible" is a call to action. Steen Willadsen was one such scientist who thought that Solter and McGrath might not be correct.

15

Cloning Sheep

Some five years after Illmensee and Hoppe's paper and just two years after Solter and McGrath's decree that cloning mammals via nuclear transfer was impossible, Steen Willadsen, working with sheep, and Neal First with cattle, succeeded in transferring a nucleus from an early embryonic cell into an enucleated egg, an experiment that produced live lambs in one case and calves in the other. These were the first successful transplants of a nucleus into an enucleated egg in mammals that unequivocally resulted in live births (discounting Illmensee and Hoppe's results). We'll focus on Willadsen whom we've already met. What was his secret? How had he achieved the "impossible"?

Willadsen

Willadsen used two different methods to introduce nuclei from eight or 16 cell embryos into enucleated, unfertilized eggs. I use the word "introduce" because neither method used injection to get the desired nucleus into the egg. Instead, both methods fused the smaller embryonic cells with the larger egg cell. In one procedure, Willadsen used a

virus to achieve fusion. The other method – much more effective – was to use an electric shock. In both cases, Willadsen used the technique of agar protection mentioned in the last chapter to protect the embryos. However, the major difference between his technique and Illmensee and Hoppe's, the one that probably was responsible for his success, was to introduce the donor nuclei into enucleated **unfertilized** eggs. In 1986, in a paper in the journal "Nature", Willadsen reported the birth of three lambs, produced via transfer of nuclei into enucleated eggs. The "impossible" was achieved. Some time later, Willadsen joined a Texas company and succeeded in producing adult animals after using cell nuclei from 128 cell embryos as donors.

Figure 15.1

Roslin Institute, Edinburgh

Ian Wilmut

At this point the creators of Dolly and her predecessors enter the story. Dolly was cloned in Scotland at the Roslin Institute, part of the University of Edinburgh. Her foster parents were Ian Wilmut and Keith Campbell, two Englishmen originally from the Midlands who had moved to Scotland to further their careers. Wilmut is the older of the two (born in 1944) and is more well known. His original ambition was to purse a career in agriculture, even though he was raised in the city, but he recognized that a farmer must also be a businessman, and wasn't interested in running a business. While an undergraduate, he won a scholarship to work as a summer intern at a research laboratory that studied pigs. Although a pig research facility would seem like an unlikely place to have an epiphany, his tenure there convinced him that scientific research was his calling. After earning a PhD from the University of Cambridge where he helped develop methods for freezing pig sperm, he

remained at Cambridge and became the first person to successfully freeze and thaw a calf embryo, and grow it to term. He named the calf "Frostie" and the publicity of this achievement gave him a taste of the incredible media storm that was to rain down several decades later when Dolly came along. He left the ARC in Cambridge the year that Willadsen arrived. In fact, Willadsen inherited Wilmut's fellowship and lab bench.

Wilmut moved to the Roslin Institute in Scotland in 1973. He ran into trouble soon after he arrived. The first problem was that the facilities were inadequate. He wasted almost a decade getting his laboratory up and running. Then a larger issue arose: molecular genetics – the DNA revolution – was entering the mainstream of science. A good many research laboratories were switching to this hot new area. An edict was passed down by the agency that funded his studies: change your research focus or leave. Wilmut protested vehemently. He was a trained reproductive biologist and embryologist and had already achieved many successes. Why should he switch to a new field in which he was a neophyte? Because scientists value their independence greatly, he was particularly resentful about being told in which direction to take his studies. He considered moving elsewhere, but, ultimately, primarily because he didn't want to move his family, he reluctantly went along. In the end, it turned out well. He came to recognize the incredible power of being able to directly manipulate DNA molecules and he became an enthusiastic proponent of genetic engineering and molecular biology.

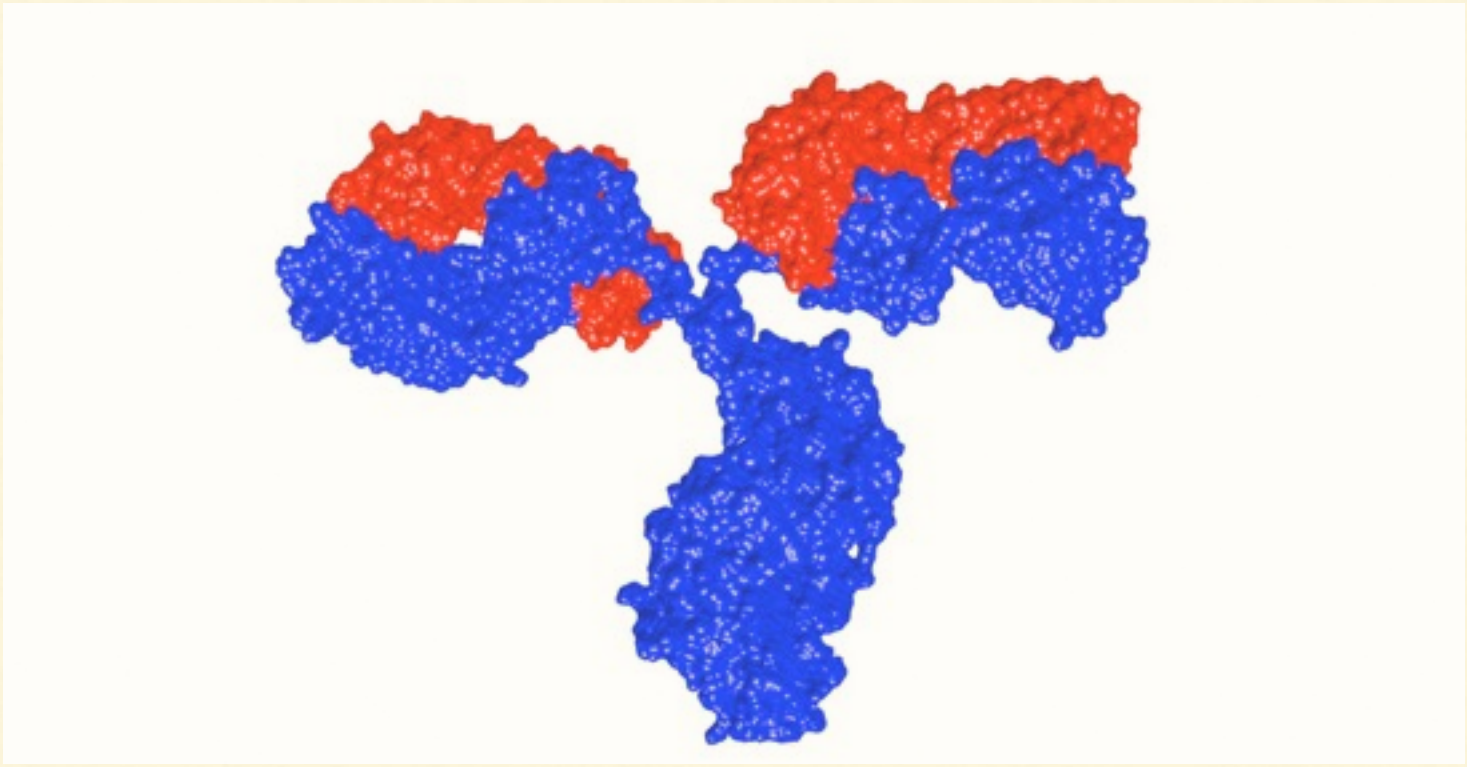
Figure 15.2

Ian Wilmut and Dolly

One particularly exciting tool that DNA technology offered was the possibility of being able to transfer genes directly into embryos and have them expressed in adults. Previously, when an improvement in an animal or plant was needed, the desired change had to be carefully selected over many generations. Occasionally, if a farmer was extremely lucky, a mutation would appear that would cause an a particularly advantageous way, but such desirable mutations are extremely rare. And they appear by chance.

With the advent of recombinant DNA, farmers were no longer limited by the slow process of selective breeding or the lucky emergence of favorable mutants. They could, at least in theory, place a desirable gene into an organism in one step. Moreover, the gene could come from any sort of organism. They could put a gene from a goat or fish or even a bacterium into a sheep if the foreign gene promised a useful function.

A specific version of this idea took shape at Roslin. Someone asked: "Why not place a human gene into a sheep and have the sheep synthesize the corresponding human protein?" For example, a gene for a human blood clotting protein could be introduced into a sheep. Such proteins are difficult to purify from humans and are in great demand given the incidence of blood clotting disorders. Scientists had already been using a similar strategy to produce therapeutic proteins in bacteria and yeast. An important extension of this concept would be that the gene could be specifically engineered so that it was expressed in the sheep's mammary gland. The protein would then appear in the sheep's milk where it could be readily harvested. But using sheep to produce human proteins has several advantages that are missing in microbes. To understand these, we'll have to take another brief detour into molecular biology, this time delving more deeply into the chemistry of proteins.



16

Proteins

Most of my previous discussion of proteins (in Chapter Four) concerned their structure. In this chapter I'll emphasize what proteins do and how they're used for therapy.

What are proteins and why are they valuable?

As you may recall, proteins are the incredibly tiny polymeric machines that actually do almost all the work of living organisms. DNA, one of the other "secrets of life", isn't a laborer. It can be thought of as the foreman shouting instructions or a cookbook providing life's recipes. But it actually doesn't **do** anything. Proteins are the molecules that do the heavy lifting, the actual work. Of course, by this I don't mean to disparage DNA. In fact, without DNA, proteins wouldn't be constructed correctly. DNA is the stuff of genes, and genes, segments of DNA that are found on chromosomes, specify the amino acid sequence of proteins. As we'll see, the reason for introducing a gene into an organism is often to have that gene provide the

instructions for making a particular protein.

A three dimensional depiction of a specific proteins, one of these molecular machines, is shown in 3D Molecule 1 and 2. Alcohol dehydrogenase is an enzyme whose role is to detoxify the ethanol in the wine, beer, and stronger drinks that we imbibe. It's one of my favorite proteins. My laboratory studied it in detail for 25 years. The particular version of alcohol dehydrogenase that we worked on came from fruit flies, but the enzyme serves the same function in them as it does in us. That's because fruit flies live on rotting fruit, like grapes and apples, and are regularly exposed to high concentrations of alcohol.

3D Molecule 2

Alcohol dehydrogenase, an enzyme that detoxifies ethanol. This view of the enzyme is a computer generated depiction showing the major regular features of the protein in cartoon form. Compare this view with a surface depiction in 3D Molecule 2.

3D Molecule 1

Alcohol dehydrogenase
This view of the enzyme depicts the surface of the molecule. This enzyme is a dimer, composed of two identical chains, here colored red and blue.

How can protein help treat diseases?

Proteins, as a group, are enormously versatile. There are many tens of thousands of them in any organism, each with a distinct structure, each performing a specific task. Occasionally, organisms are born with a defective gene that in turn specifies the structure of a defective protein. When that occurs, the organism may have problems, the extent of which will depend on the role that the protein plays in the cell.

For example, some fruit flies carry a change in the sequence of bases (a

mutation) in the gene that specifies the structure of alcohol dehydrogenase and therefore lack a functional enzyme. When they are exposed to concentrations of alcohol that encounter in the wild, they die. To remedy this kind of situation in both flies and humans, it may be possible to substitute a working protein that makes up the for loss of function. But that's easier said than done. Proteins are large and fragile molecules. They're difficult to harvest in large amounts, especially from humans.

Let's consider a specific example of a dysfunctional protein and a molecular remedy that was suggested in the last chapter. Blood clotting is a complex process that requires the interplay of a great many proteins. Sometimes, one of the genes that specify one of these proteins carries a defective mutation, resulting in a clotting disorder, like hemophilia. It would seem logical to introduce a non-defective protein into an affected individual in order to correct the problem. But these proteins are present in very small quantitates in human blood, making their isolation, purification, and commercial production laborious, complicated and extremely expensive.

Another challenge is that most proteins are unstable. If clotting proteins were introduced into the blood stream they would soon break down. That would mean that the protein would have to be replenished on a regular basis, thereby increasing the cost of the therapy.

Why not use animal proteins?

In theory you could cut costs by extracting a needed human protein from some other creature, like a pig, sheep, or cow. Remarkably, many (perhaps most) of the proteins in these organisms are interchangeable with the corresponding ones from humans. They have similar structures and perform almost identically. However, if you tried to substitute a bovine clotting protein for its human equivalent, for example, you would run into the problem of immune rejection. Cow proteins, although similar to that of humans, are different enough that the human immune system will recognize them as foreign substances. The consequences can be dire, producing severe immune reactions. At the very least, the foreign protein will be quickly eliminated from the body, thereby negating its efficacy.

When genetic engineering was invented, scientists thought that they could solve this dilemma by synthesizing human proteins in bacteria. It would work this way: they would isolate the appropriate

Protein Modification

Most proteins are enzymatically modified after they are synthesized. Such modifications may affect the stability of the protein, its distribution in the cell, its activity, or all of the above. The alterations may take several forms.

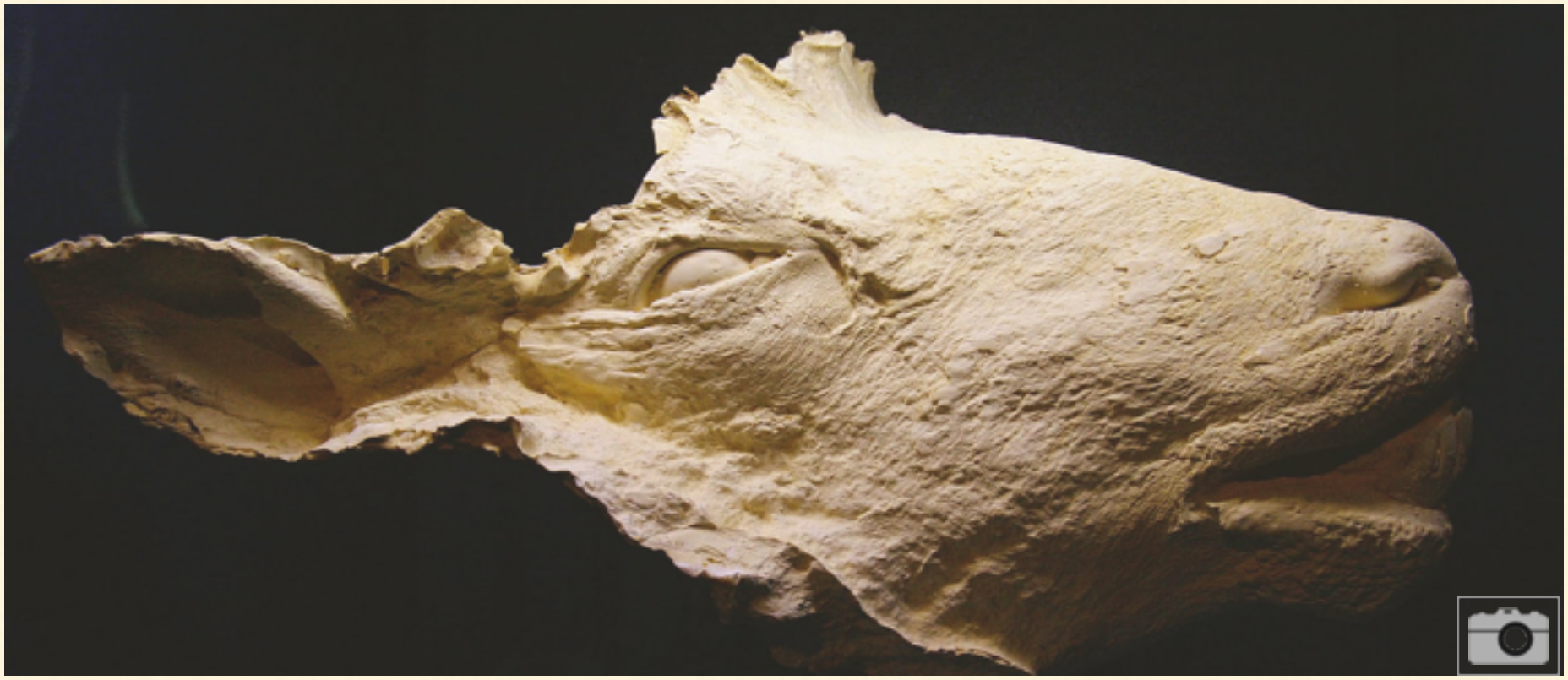
One particularly dramatic example occurs in the hormone insulin. It is synthesized as a single chain of amino acids which is cleaved at various points to produce two chains that link together to form an active molecule. During this process, parts of the original protein are discarded.

human genes, and transfer them to bacteria or yeast. Once integrated into the genome of these microbes the genes would provide the information for the synthesis of exact copies of the human proteins. In this way, the human immune system wouldn't have to cope with a foreign protein. And, since bacteria and yeast can be grown in enormous quantities, it was thought that it would be easy to prepare the human proteins in bulk and at low cost.

Some proteins. including human interferon, have, in fact, been produced commercially by this technique. However, an unanticipated problem soon arose. It turns out that the great majority of human proteins are enzymatically altered after they are synthesized (interferon is one exception), and the enzymes that do this job are not present in microbes. In other words, you can get a microbe to synthesize a protein with exactly the same sequence of amino acids as its human counterpart, but because it isn't properly altered after it is synthesized the resultant molecule often isn't as good as the real deal made in a human. In fact, it might not work at all.

The enzymes that modify human proteins are, however, found in other mammals. Thus the bright idea arose of having a domesticated animal, like a cow, goat, or sheep, supply therapeutic **human** proteins. The concept was to introduce a human gene into, say, a sheep, set up the control of the gene so that the protein it specifies is synthesized in the milk of the recipient, and then harvest the protein from the copious amounts of milk that can be obtained from a ewe. Organisms that carry introduced genes from other species are referred to as "**transgenic**". And the process whereby transgenic animals are harvested for valuable proteins is called "**pharming**". As we'll see, using a transgenic sheep to make lots of a valuable human protein seems like an attractive

commercial idea, but it turned out, like many bright ideas, to have some unanticipated practical issues.



17

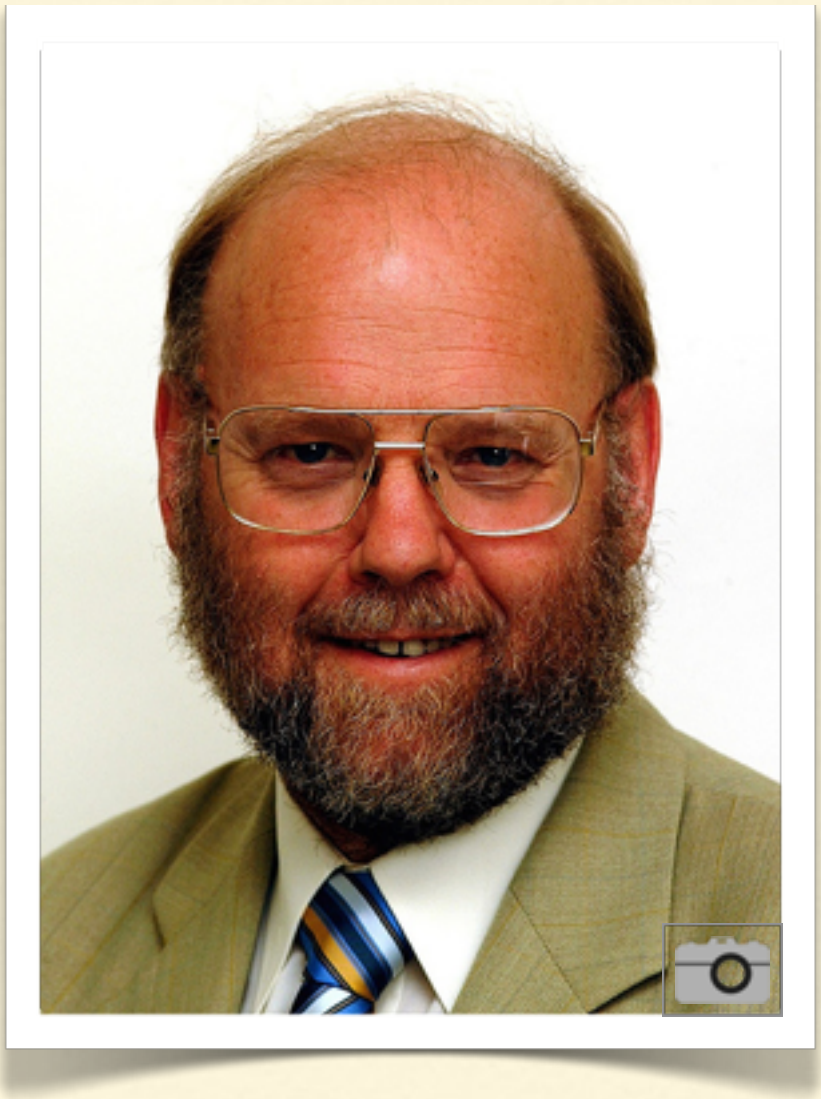
Dolly et al.

This idea of using animals like sheep as transgenic agents to produce human proteins answers a commonly posed question: Why clone animals like sheep? They're large and have a long generation time. Why not use other animals like mice that take less up less space and reproduce rapidly? The answer is that Dolly and her relatives were created, at least partially, for commercial purposes, with the ultimate goal of producing therapeutic proteins. It was an enterprise that seemed to offer huge potential profits. A related question then arises: "What does cloning have to do with the production of transgenic sheep?"

The folks at Roslin, Wilmut's bosses, were the ones who came up with the clever notion of transferring the genes for potential therapeutic proteins into large farm animals and harvesting the proteins specified by that gene from the animal's milk. The genes that are introduced into them are called "**transgenes**". It is possible to introduce a human transgene into a sheep or goat by simply injecting it into fertilized eggs. The transgene can integrate into the sheep

chromosomes and specify the desired protein. In fact, Ian Wilmut was one of the pioneers in this endeavor. The group of scientists he headed was the first to report the production of therapeutic proteins from the milk of transgenic sheep after direct injection of a gene into an egg. Creating a transgenic sheep to make a commercially valuable protein wasn't Wilmut's idea. He was enlisted into the project because of his expertise with working with embryos.

Figure 17.1



Ian Wilmut

But simply injecting human genes into the eggs of sheep is not quite as straightforward as it appears. For one thing, injected DNA doesn't always integrate into the chromosomes of the host. When integration fails to occur, the transgene doesn't partition with the chromosomes during mitotic divisions and gets lost after several cell generations, thereby wasting the embryo that was injected.

For another, if the DNA does actually integrate, it doesn't always do so before the recipient embryo undergoes cell division. If integration occurs later, after several cells have formed, some blastomeres of the injected animal may carry the introduced gene and other may not. The result is a mosaic animal. Recall Weismann's principles concerning the separation of the germ and somatic lines. If the progenitors of a germ cell doesn't receive the transgene, the animal won't pass it on to the next generation.

And there's yet another potential pitfall. Even if the DNA does integrate into the chromosomes of the egg, where it ends up is important. At the time that these experiments were conceived there was no way of determining where the transgene would land in the genome. If it happened to integrate into a gene that was required

for proper functioning of the animal, it might disrupt that gene and cause problems. Possibly major ones.

These difficulties make for major inefficiencies. Many, many embryos must be injected and the embryos or new borns examined for the presence, location, and expression of the injected gene. It became quickly evident that it would be much easier to transfer human genes into the genome of cultured sheep cells (cells grown in a dish), as opposed to eggs. Millions of cells could be independently "**transformed**" (the technical term for the uptake and integration of DNA by a cell or organism) by simply soaking them in a solution of the desired gene. Although DNA wouldn't get into all the cells, in those in which it did, each cell would house the DNA in a different location. Once that had been achieved, scientists could use standard molecular techniques that allow the cultured cells to be quickly checked for the presence, chromosomal position, and expression of the transgene.

It seems easy. But not so fast. The problem is what you do after you're sure that you have identified cells with the transgene in the proper location. How do you get the DNA from the cultured cells into a whole animal?

That's where cloning comes in. The answer is to use Steen Willadsen's technique of transferring nuclei from early stage sheep embryos into enucleated eggs. Of course, Willadsen had only succeeded in obtaining live offspring from nuclei of early embryonic cells, not from cells grown in tissue culture. Would the technique work for tissue culture cells?

As Wilmut tells it, for a long time he, and the bulk of the scientific community, didn't think so. He began to change his mind one day in 1987 in a pub in Dublin. He was attending a scientific meeting. Often the conversation after the scientific talks is more valuable than the presentations themselves. He was socializing with Geoff Mahon, who was an employee of Granada Genetics in College Station Texas. Mahon was a colleague of Steen Willadsen. Mahon told Wilmut that Willadsen had succeeded in using nuclei from 64 cell embryos, essentially inner cell mass cells, to prime full development. Wilmut hadn't heard that before. He immediately recognized that if he could get inner cell mass cells to grow in a petri dish, a human gene could be transferred into them. The appropriate transgene-bearing cells could be identified, and these cells could serve as the source of nuclei for introduction into enucleated eggs.

One complication was that Wilmut knew that inner cell mass cells from sheep hadn't been grown in culture. But mouse cells had. Of course, that didn't guarantee that sheep cell would behave similarly. Still, this approach held promise and Wilmut decided to pursue it.

In order to better learn Willadsen's technique, Wilmut visited him in Calgary Canada, where Willadsen had recently moved. Willadsen generously shared many technical details with Wilmut, and Wilmut has gratefully acknowledged the debt he owes to Willadsen for his help in carrying out successful nuclear transfers.

Figure 17.2

Keith Campbell
(1954 - 2012)

Upon returning to Scotland, Wilmut with the help of a colleague, Lawrence Smith, repeated Willadsen's experiments with nuclei from embryonic cells. In 1989, four sheep were born five months after nuclear transfers. One emanated from an inner cell mass nucleus. Roslin was now a player in the nuclear transfer business. But the cells used for the transfers weren't grown in culture. They were taken directly from the embryo. Then, in 1991 a new postdoctoral fellow, Keith Campbell, joined Wilmut's lab. He was destined to play a big role in the Dolly story.

Keith Campbell

Campbell and Wilmut formed a productive duo of different backgrounds and personalities. Campbell, who spent his early years in Birmingham, England, had had a multifarious career before coming to Roslin. He trained to be a medical technician, but found the actual work intellectually unsatisfying. He went on to earn a BS degree in Microbiology. A job in Yemen as a pathologist followed. A stint working to help eradicate Dutch Elm disease in Sussex followed that. Then he decided that he wanted to be able to develop his own research projects. That required a doctoral

degree. Lacking funds, he studied by night and worked as a research assistant by day. He was unlucky at first. His dissertation director became ill and retired. Without an advisor, he was forced to move to another institution, the University of Sussex, and began an investigation of the cell cycle in yeast. Upon completion of his doctorate, two postdoctoral stints ensued, the last one in Scotland, both involving work with the cell cycle and cell growth. Finally, in 1991, five years after obtaining his PhD, at the relatively ripe old age of 37, he answered an ad for a job at Roslin.

Before Campbell arrived, Wilmut's laboratory was still diligently trying to get inner cell mass cells to grow in culture. As we'll see in Chapter 22, the first cultured inner cell mass cells (called "ES cells") were derived from mouse embryos. However, it was evident early on that sheep ES cells were difficult to come by. With considerable effort they could be grown in culture, but after a while, they began to change shape and differentiate. The scientific consensus at the time was that such differentiated cells were not suitable donors for nuclear transfer to eggs. People thought that they had changed their programming so that they no longer could form all kinds of cells, but instead were dedicated to their new role.

Campbell thought otherwise. His expertise was in the cell cycle. He thought that coordinating the cell cycles of the donor nuclei and recipient oocytes was critical to the success of cloning via nuclear transfer. As a newcomer to Wilmut's laboratory, his views met some resistance among the people already at work there. He was discouraged and almost left. Ultimately, he stayed and persuaded Wilmut and his other colleagues to do a series of experiments to determine whether he was correct.

The Cell Cycle Revisited

We've already discussed the cell cycle in some depth. Recall that after mitosis there is a period before DNA synthesis called G1 and a period after, G2, that precedes a second round of mitosis. What we haven't discussed is how these stages relate to the development of the egg. How is the cell cycle controlled during meiosis?

The answer to that question has been known, at least in part, for some time (the first discoveries date from 1971). An extract obtained from hormonally stimulated frog eggs (the active principle of which was later found to be a complex of two proteins) advanced embryos from G2 into the second meiotic division. The researchers who discovered the proteins called it "maturation promoting factor" or

MPF. Later, it was renamed "mitosis promoting factor" because it promoted mitosis as well as meiosis. MPF was found to cause nuclear membrane breakdown, chromosome condensation, changes in the shape of the cell, and formation of the microtubule network that promotes chromosome movement.

You should not be surprised to learn that MPF is another name for the cyclin/CDK complex that I discussed in chapter 6. (I'll call it MPF in the remaining discussion.) Recall that MPF operates by enzymatically appending phosphate groups to a series of different proteins. When there's a lot of MPF around the events noted above occur, and when MPF is low, they do not.

The meiotic cycle in mammalian eggs is controlled by MPF. When oocytes arrest during metaphase of the second meiotic division (you might want to review Chapter 13), MPF levels are high. This makes sense in that the chromosomes at this stage are condensed, the nuclear membrane has been dissolved, and the cell has rounded up. MPF levels remain high until fertilization. Then they drop precipitously, the egg and sperm nuclear membranes reform, and the chromosomes unravel. MPF doesn't go up again until the zygote is ready to enter mitosis again and form a two cell embryo.

Megan and Morag

Given this information, Campbell tried three different experiments to determine the effect of MPF levels on the success of nuclear transfers with the differentiated cells grown in culture from the inner cell mass. He introduced nuclei from these cells into oocytes that were high in MPF, high in MPF but where the MPF was falling, and low in MPF. We'll disregard exactly how these conditions were obtained because it is a detail that is not necessary for the story. The donor nuclei in all cases were derived from diploid cells in culture, in the Go stage of the cell cycle. A total of 244 nuclear transfers were carried out in 1995. Some pregnancies were detected, and members of the research team, including Campbell and Wilmut took turns sleeping with the sheep to ensure that when the ewes gave birth, there would be someone around to help the lambs. As it turned out that wasn't necessary; all the sheep were born in the afternoon.

A total of five lambs were born. It didn't seem to matter what the MPF level of the recipient was since there was at least one born from each protocol. Three died either at birth or shortly thereafter. The two survivors were named Megan and Morag. They both were introduced to an eager ram, but only Morag got pregnant.

Morag died from a lung infection, but Megan celebrated her 10th birthday in 2005 with a cake. The birth and subsequent growth to adulthood of Morag and Megan conclusively demonstrated that nuclei from cultured sheep ES cells could be used to support development of a mature animal.

However, Campbell and Wilmut knew that the cultured cells that they used were derived from the inner cell mass. Mouse ES cells, similarly derived, appeared to be undifferentiated. But sheep cells showed evidence of differentiation by virtue of some biochemical markers and their shape. However, they certainly weren't fully differentiated like mature liver or skin or nerve cells. To find out whether nuclei from differentiated cells would work required the next experiment: Try the same procedure with nuclei from cultured **adult** cells.

Dolly

Wilmut and Campbell began their experiments that resulted in Dolly the year after they cloned Megan and Morag, who were born in August 1995. By October, Wilmut and Campbell had applied for a grant to do take the next step, to clone a sheep from a differentiated cell. The grant was awarded (for a little less than £500,000). In addition, support came from PPL Therapeutics, a company located on the Roslin campus, founded for the purpose of commercializing the results of Roslin's research efforts.

PPL's goal was to use animals as a source of protein therapeutics. Originally, they thought that they could introduce human genes into chickens and harvest the human proteins from egg whites. Then they learned of Roslin's work with sheep and cows. It was an easy step from chicken eggs to sheep milk.

PPL had on hand cultured udder cells from a six year old Finn Dorset sheep, a breed with a white face. No one knew the whereabouts of the animal or her fate, but she was likely "put down" shortly after her cells had been harvested. These cells had been frozen away for three years in a vial in PPL's freezers. When thawed, the cells grew well. Of course, they weren't originally intended for nuclear transfer experiments. Rather, they were being studied by PPL scientists in order to better understand the molecular basis for the control of milk production.

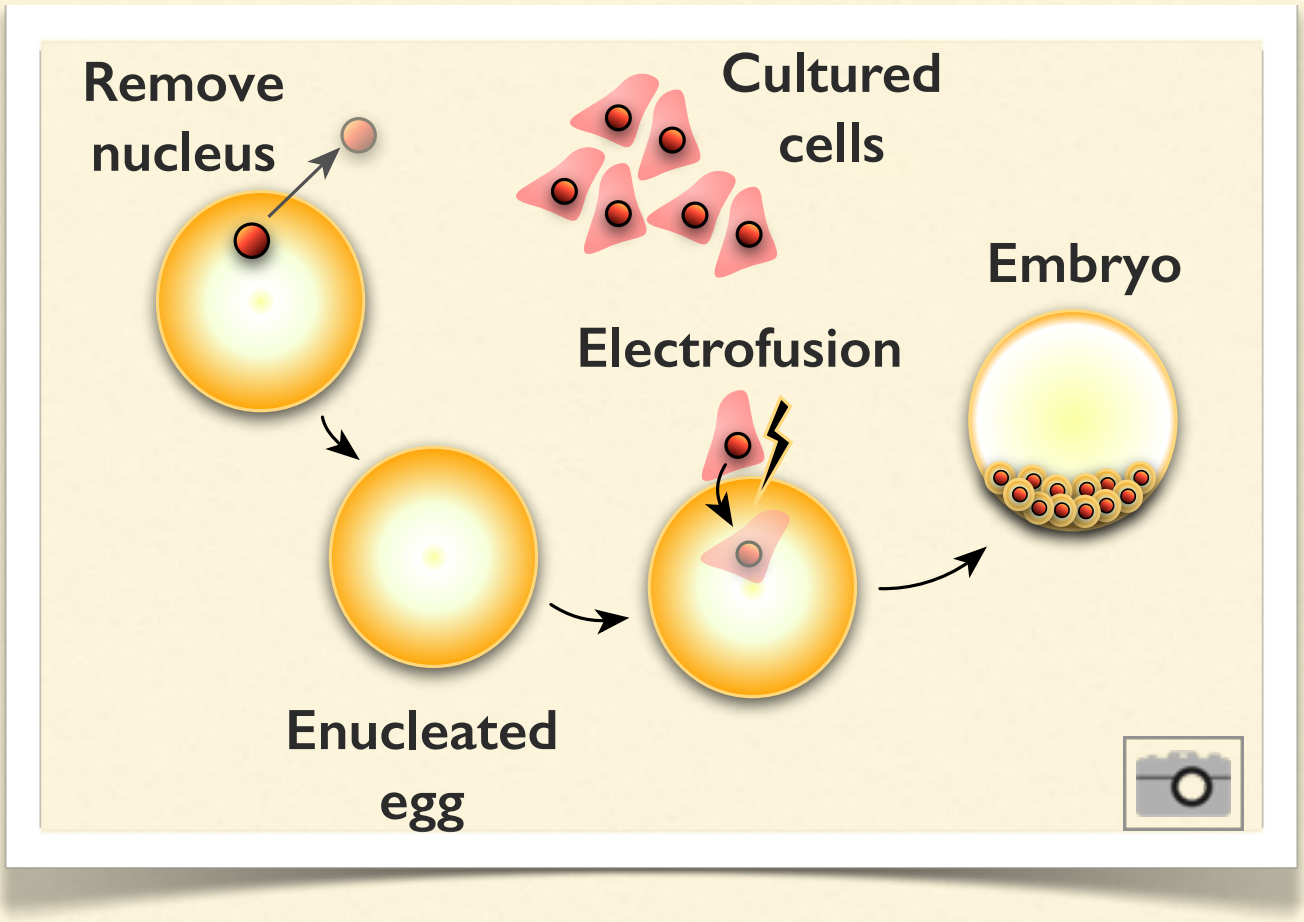
The experiment that lead to Dolly involved many animals, because Wilmut and Campbell wanted to get as much information as possible all at once. There were three parallel studies. They injected nuclei from differentiated mammary cells,

and also from cultured fetal and embryonic cells. All cells were in Go and introduced by electrofusion into oocytes. Out of 385 nuclear transfers from the embryonic cells, four genetically identical lambs were born named Cedric, Cyril, Cecil and Tuppence. Out of 172

"Viable Offspring Derived from Fetal and Adult Mammalian Cells".

The response to this paper (actually, the news was leaked before publication) was extraordinary. The popular press all over the world went wild. Political and religious

Figure 17.3



The method for cloning mammals by nuclear transfer

experiments done with fetal nuclei, two live lambs resulted: Tweed and Taffy. The crucial experiment with mammary cell nuclei involved 277 nuclear transplants and produced only one live birth: Dolly. She was named after the country singer, Dolly Parton. The announcement of her origin and arrival was made in the journal Nature on February 27, 1997. The paper is entitled

leaders criticized the work. Slippery slope arguments were advanced. Some said the whole thing was a fraud. But there were also serious scientific objections. One was that the nucleus which gave rise to Dolly wasn't from a truly differentiated cell. Scientists pointed out that the mammary gland is composed of a variety of cell types, and some may be stem cells. As noted previously, stem cells are capable of

reproducing. They also have the capacity to produce more than one kind of daughter. It would have been less of a feat if Dolly came from a stem cell rather than a fully differentiated one. Wilmut and Campbell acknowledged that this was a possibility in their Nature paper. Some years later, in 1998, other experiments in mice showed unequivocally that a differentiated cell nucleus could indeed support normal development. It appeared that the Roslin team had got it right.

Dolly went on to a long and pampered life. Because of all the attention that she received, she was more friendly to humans than most sheep. There's also a romantic side to the story. She met a ram, fell in love, and bore a normal baby lamb named Bonnie. A year later she had twins, and a year after that triplets. At the age of six, she succumbed to a lung infection, common among sheep that are kept indoors most of the time.

While Dolly was the most famous sheep to come out of Roslin, she had no intrinsic commercial value. Recall that PPL supported the experiments that gave rise to Dolly in the hope that it would lead to a more efficient method of producing transgenic sheep, animals from which a human protein could be purified. Dolly didn't house any transgenes, but Polly, born in 1997, and generated similarly to Dolly (but from a fetal cell), did. She bore a gene for human factor IX, a protein missing from people with a form of hemophilia.

It appeared that a milestone had been reached. A flock of Polly's could supply factor IX protein in quantity. Hemophiliacs could be relieved of the burden of having to get factor IX from humans and PPL would become a financial success. But it was not to be.

PPL is long gone. The story of its demise is behind the scope of this book. In the 17 years from Polly's birth to the present, only two proteins from transformed farm animals has been approved for human use by the FDA. A company called rEVO Biologics markets a recombinant protein from goats that they call ATryn, a genetical engineered form of antithrombin. It is used to treat people with hereditary antithrombin deficiency a disorder of blood clotting. From the NIH: "People with this condition are at higher than average risk for developing abnormal blood clots, particularly a type of clot that occurs in the deep veins of the legs. This type of clot is called a deep vein thrombosis (DVT). Affected individuals also have an increased risk of developing a pulmonary embolism (PE), which is a clot

that travels through the bloodstream and lodges in the lungs. In hereditary antithrombin deficiency, abnormal blood clots usually form only in veins, although they may rarely occur in arteries. About half of people with hereditary antithrombin deficiency will develop at least one abnormal blood clot during their lifetime. These clots usually develop after adolescence."

The other product is called Reconest from Pharming, a Dutch company. Purified from the milk of transgenic rabbits, Reconest it is used to treat hereditary angioedema, a disease caused by a genetic defect that occurs in about 1 in 25,000 people. Symptoms include swelling in the hands, feet, abdomen, and throat.

Getting approval of products designed to go into humans is a long and rough road. Consequently, firms hoping to make a profit from transgenic organisms have turned their attention elsewhere. For example, Nexia Biotechnologies, a Canandian company, after failing to market a protein that dissolved blood clots (tissue plasminogen activator), pursued the clever idea of having goats mass produce spider silk in their milk.

Spider silk is remarkably strong. The company claims that a 3/4" cable made of it, could support a "fully loaded jet". It could be used for lightweight bulletproof vests or biodegradable fishing line. Other uses that have been proposed include improved parachutes and air bags. Unfortunately, the company went bankrupt in 2009. (A German firm, AMSilk, has begun manufacturing a similar product. They made the silk protein in bacteria.) Other firms have recently succeeded in inducing transgenic silkworms (Kuwana et al) to make a mixed form of natural and spider silk. Using silkworms for this purpose bypasses the necessity of forming fibers artificially.

Wilmut and Campbell

The fame that came from Dolly apparently rested easily on Ian Wilmut's head. He found that he had a talent for communicating with the press and with the public at large. He liked talking to general audiences and explaining both what he had done and what the new technology might mean in the future. He wrote two books aimed at laymen detailing his cloning efforts. He garnered many honors including a knighthood (it's Sir Wilmut now), and was made a fellow of the Royal Society of London. He is now retired.

Campbell did less well. Wilmut attributed 66% of the success of Dolly's cloning to Campbell, but Campbell did not seek the

limelight and received much less publicity. In 1997, he left the Roslin Institute and took a top level position in PPL. While at the company, they cloned other transgenic animals including pigs and cows.

A few years later, he left PPL and became Professor of Animal Development at the University of Nottingham. In 2008, he, Wilmut, and Shinya Yamanaka (whom we'll meet later) were awarded the prestigious Shaw Prize for Medicine and Life Sciences.

Campbell died in 2012. His colleagues uniformly remember him as friendly, happy, and outgoing but there must of been a darker side to his his life that was only revealed to the public after his demise. The following is an excerpt from a report in the International Business Times written by Timur Moon which appeared in 2013.

"Professor Keith Campbell, 58, tied a belt around his neck and hanged himself from a ceiling beam in his bedroom.
But the coroner recorded a verdict of death by misadventure and not suicide after hearing evidence from Campbell's wife, Kathryn.
Mrs Campbell, a voluntary worker, told the inquest her husband had begun to behave "irrationally" on 5 October last year, after returning to their home in Ingleby, Derbyshire, from his work at Nottingham University.
Professor Campbell was a "regular" drinker who suffered from hypertension, high blood pressure and a heart condition, she said. He had been drinking that day, and had begun to pick a fight with her, she told the inquest.
At one point he had threatened to cut himself with a kitchen knife, and later he smashed a downstairs window, Mrs Campbell said.
He then told his wife that he was going to throw himself in the river, saying later that he was going to the bedroom and ordering her not to come in until morning.
Mrs Campbell, 46, said she had swept up the broken glass and was taking it outside to the dustbin when she next saw her husband, through the ground-floor bedroom window.
He was hanging from a beam in their bedroom, she said. She rushed in to cut him down, but could not save him. He asphyxiated and would have died almost instantly, the hearing was told.
Robert Hunter, coroner for Derby and South Derbyshire, said Professor Campbell had no intention of dying and was fully expecting his wife to rescue him in time.
Describing it as a "cry for help", he ruled that Professor Campbell did intend to put the belt round his neck, but did so knowing his wife was nearby and "expecting his wife to come into the bedroom to rescue him."
He said there was no other evidence to suggest he was considering suicide, and recorded a verdict of misadventure.
After the hearing, Mrs. Campbell said: "He was a lovely man and a wonderful husband."

Summary

As we have seen, the birth of Dolly established that cells derived from adult tissue could serve as a source of nuclei for successful production of a clone. A few years later, scientists used sheep cells into which human genes were transformed as a source of nuclei. Wilmut and his coworkers engineered a sheep, Polly, that was bred to one another, eventually forming a herd of bioreactors.

It must be kept in mind that the efficiency of cloning via nuclear transfer is very low. Wilmut and his colleagues made more than 270 efforts before they succeeded in getting Dolly. To some extent the inefficiency of the method doesn't matter too much. Once an adult clone has been obtained, its genes may be replicated the old fashioned way (via sex) to produce additional organisms with the same introduced gene. However, it turned out that despite Wilmut's success in obtaining many sheep with an introduced human gene, making pharmaceuticals this way has proved difficult, and for the most part, a commercial failure.

In the end, Wilmut's biggest contribution was to basic science. The fact that a differentiated cell's nucleus could, when placed inside an egg, support the development of a fertile adult animal was strong evidence that differentiation is reversible. No permanent changes to DNA apparently occur to the sequence of the DNA during development. Somehow or other, the cytoplasm of the egg rearranges the directions of the introduced nucleus - it reprograms it – so that it no longer gets to make a mammary gland cell, but instead a whole organism. What establishes the programming in the first place? How does the original cell know how to become a mammary gland cell? How is this programming reversed? I'll discuss these questions at length in a later chapter. But before jumping into scientific issues, let's turn to a controversial ethical question: Could we and should we clone humans?.

18 Human Cloning

Many (but not readers of this book) are surprised to learn that there are already human clones living amongst us. They're called identical or monozygotic twins (Figure 18.1) (some triplets are monozygotic too). They're clones that result from blastomeres separating from one another during very early development, a natural accident of unknown causation, reminiscent of Driesch's sea urchin experiments described in Chapter 9 and Willadsen's work with sheep related in Chapter 15. The outcome of this kind of event is the formation of two genetically identical cells, each of which can go on to give rise to a complete individual.

Most of us don't think of identical twins as clones. Nearly all discussions of human

Twins

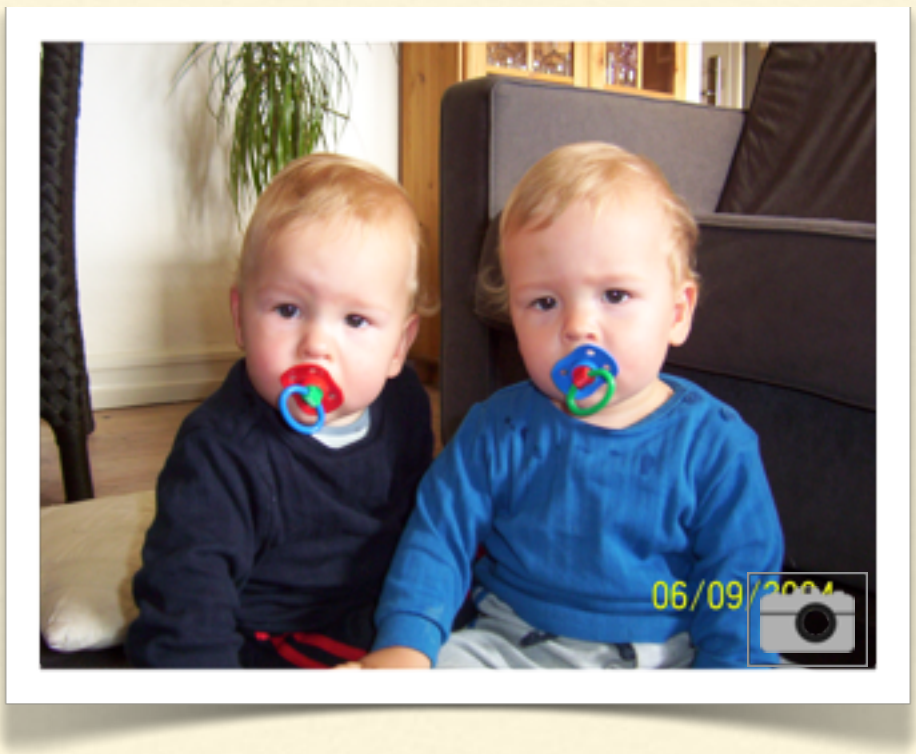
Fraternal twins result from the fertilization of two different eggs by two different sperm. Hence, the two offspring will have the same relationship to one another as any other pair of siblings, except for the fact that they were born at about the same time.

cloning center on artificial cloning via nuclear transfer. Are human clones being produced by this technique? Is this something to be fearful of? What are the ethical issues involved?

As to the first question, to my knowledge, despite some claims to the contrary, no one has provided good evidence that they have generated a baby or even a fetus via nuclear transfer. Nuclear transfer has indeed been used to create human clones, but development has not been allowed to proceed past very early embryonic stages. The goal of these experiments was to generate human stem cells. Embryonic stem cells were produced as we'll discuss in more detail in later chapters.

With regard to the ethical issues raised by the procedure, I've not been trained as an ethicist and have no particular expertise in this area. But there are certain misconceptions that are widely held that are pertinent to any informed discussion of the ethics and morality of the procedure. Recently the television program "Frontline" had several scientists and ethicists discuss some of the issues raised by people objecting to human cloning. Dr. Lee Silver from Princeton University, when asked whether the practice of cloning dismayed him, answered:

Figure 18.1



Identical twins

I am not dismayed by cloning, because I don't think that it's going to be used in all of the outrageous kinds of ways that people have thought up, like the egomaniac, for example, that wants to have a replica of him. Cloning does not achieve immortality.

Put in more stark terms, cloning hundreds of Hitlers as envisioned in Ira Levin's 1976 novel "Boys from Brazil" (later made into a popular movie starring Gregory Peck and Lawrence Olivier) would probably not produce one hundred Fascist dictators.

But why is that? If DNA provides all the instructions for making and maintaining a human being, why won't cloning produce exact copies of individuals, evil or otherwise?

There at least three reasons. First, surprisingly and in apparent contrast to what I wrote in the beginning of this chapter, two cloned humans will **not** have the exact same DNA. That's because errors occur during replication at every cell cycle. And of course many cell divisions are required to go from a zygote to an adult. The exact error rate isn't known, but it is estimated to be between one and ten in a billion. Since humans have about 6 billion bases, on average there will be roughly one error, one mutation, every time a cell replicates. Some of these mistakes will exchange one base for another, a "G" for an "A", for example. Other errors will be more extensive. Sometimes, stretches of many bases may be moved, inverted, deleted, or added.

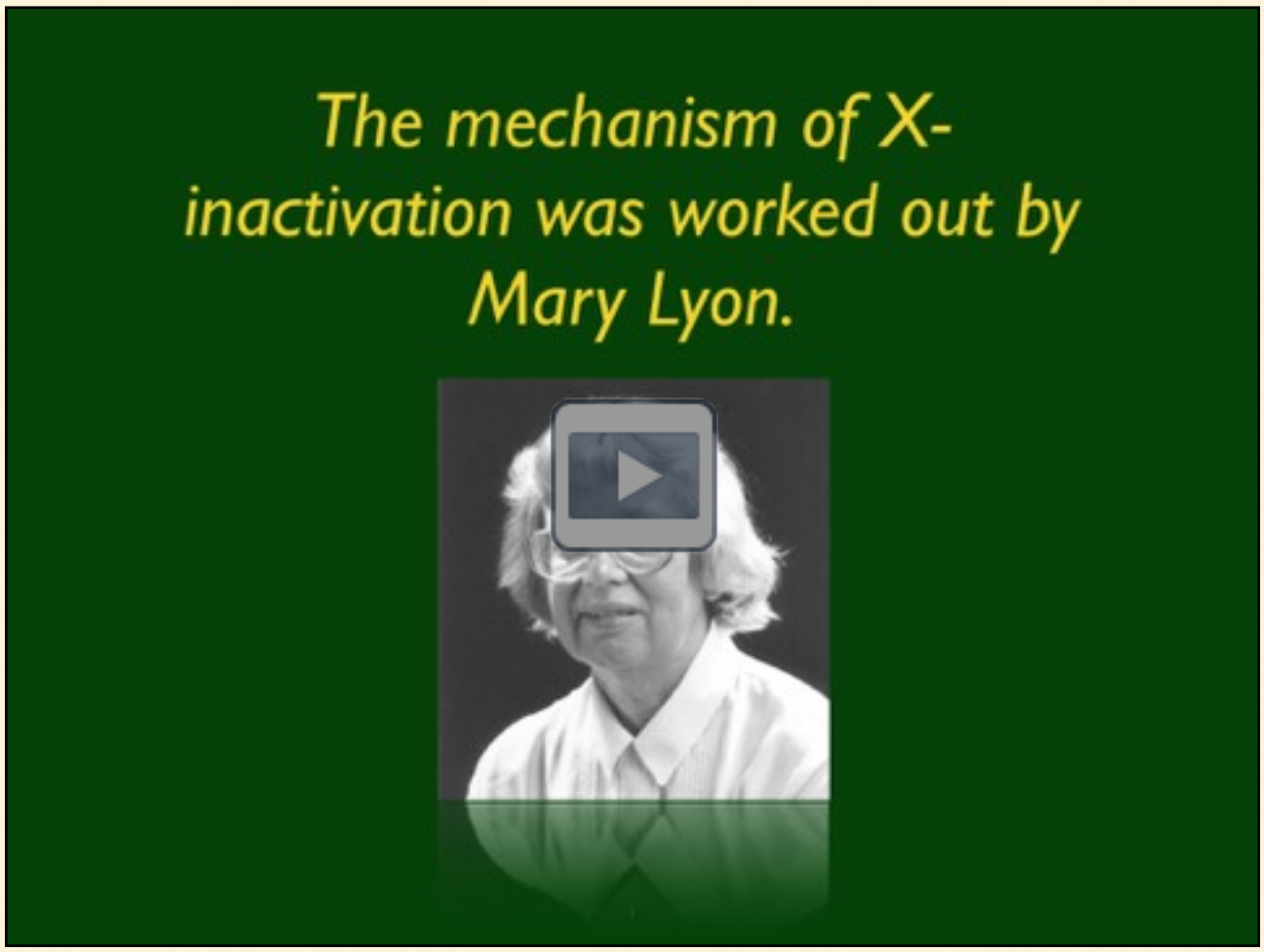
Most errors will be random. But there are "hot spots", sites in the genome that are more prone to replication errors than others. One common mutation involves segments of DNA that are repeated. The replication apparatus apparently has problems dealing with this arrangement and changes in the number of repeats are frequently observed (see sidebar).

Other errors occur in addition to those that happen during replication. That's because DNA is subject to chemical and physical insults. We are, for example, constantly being bombarded by minute subatomic particles that pass though our cells, often wreaking havoc in their wake. Cosmic radiation and radioactive decay are examples that exact a daily toll in DNA damage. And omnipresent chemical agents play a significant role as well. Again, any mutations that occur via this route will be largely random meaning that if one begins with two identical but separate

Copy Number Variants

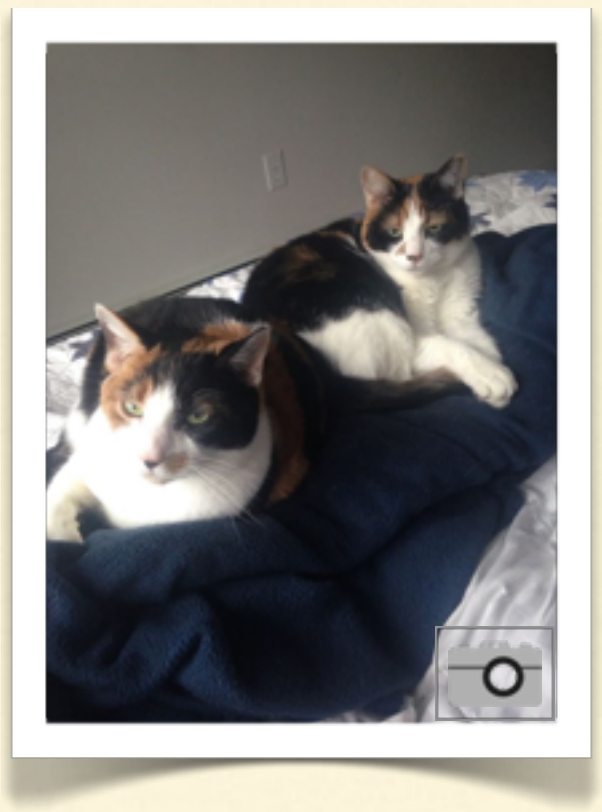
More than 20 neurologic diseases are known to be caused by "stutters" in genes; a series of three bases that are repeated many times, one after the other. Such repeats are normally found in these genes, but in disease states the number of repeats is increased. An example of one such condition is Huntington's disease. It is caused by an abnormal number of repeats of the DNA sequence "CAG" in the Huntingtin gene. In most people, this gene bears a stretch of between 5 and 35 CAG's in a row. In afflicted individuals,

Keynote 18.1



X-Inactivation

Figure 18.2



Two calico cats

blastomeres, by the time they become adults they will have differences in the sequence of their DNA.

A second factor that is at play is that many processes in cells do not occur predictably, even when they are under the control of DNA. Female calico cats (Figure 18.2) exemplify this principle nicely. These beautiful animals have a complex coat color pattern consisting of patches of different color. The patches reflect the fact that some coat color genes are on the X chromosome.

Let me elaborate. Female mammals have two X chromosomes, but in most cells

only one is in play. That is, the genes on one X chromosome are transcribed normally. The other appears to be nearly completely inactive. The shutdown of one or the other X chromosome occurs early in development and is random (Figure 18.3). If a coat color variant of a gene is on the chromosome that isn't operational, it won't be expressed. The ultimate result of this process is the complex pattern of colors observed. Even if two calico cats begin life with the same DNA, the random nature of the X chromosome inactivation would produce animals that looked very different.

There are other operations going in cells that are stochastic. DNA can't dictate the fact that every step in every chemical pathway will be identical.

Finally, cloning won't produce exact duplicates of the donor because of the environment. How children are reared, the circumstances of their lives, and improbable and random events probably contributes greatly to the development of their personalities, possibly more than their genetic endowment. We don't know exactly how much the environment is responsible for differences between twins, but it is far from negligible. If a megalomaniac wants to make an exact copy of him/herself, he/she is bound to be disappointed. As Silver puts it:

What the egomaniac will end up with is a baby … that will grow up into a boy that won't listen to him. So he's not going to get what he expected. He's not going to achieve immortality. He's just going to have a son. He's not going to be able to control the life of that son. When people understand the little that cloning does, most of these kinds of people will lose interest in the technology. It's not going to accomplish what they think it's going to accomplish.

In other words, **cloning isn't resurrection**. Everyone is going to die, cloning isn't the path to eternal life.

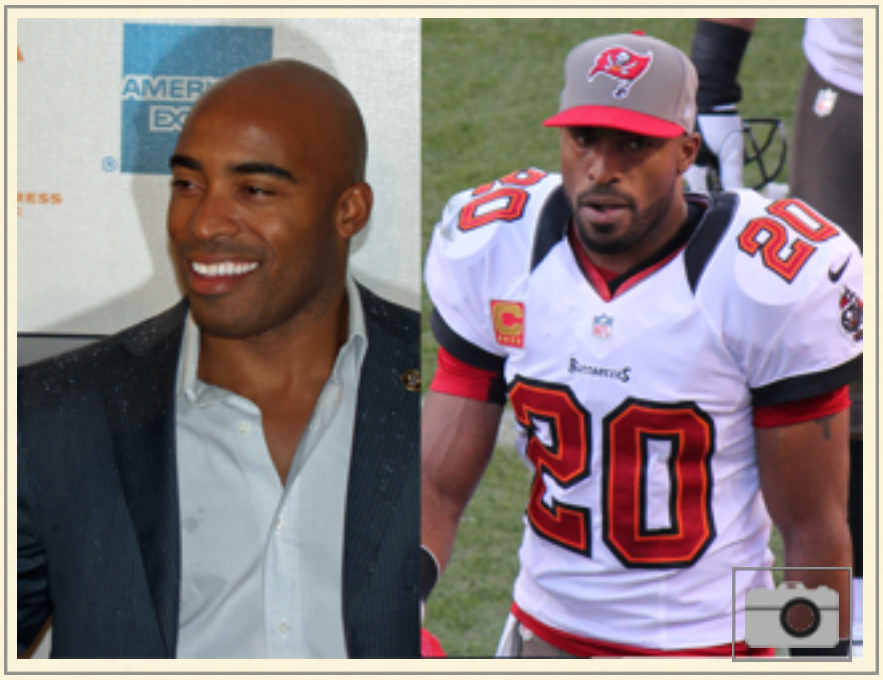
Silver goes on:

So you may ask me, "Then why would anybody use it if you're not going to be able to guarantee the child is going to turn out in a particular way?" My answer is that the only people who will end up really using this are people who can't have biological children another way and are going to be using this to have biologically children, because what most normal people want is unpredictable biological children. They want this genetic link to their children. And if that's why they're doing it, not expecting anything except to have a child that may not listen to them, that I don't have a problem with that use of the technology.

Four of the sheep cloned by Wilmut and colleagues were clear demonstrations that genes aren't all empowering. The rams Cedric, Cecil, Cyril and Tuppence were cloned from embryonic cells grown in culture (not adult cells, from which Dolly was derived) that came from the same individual. While they look more or less alike, they differ somewhat in size and considerably in temperament. If genetically identical sheep can be so disparate, it's very likely that cloned humans will exhibit at least as many differences.

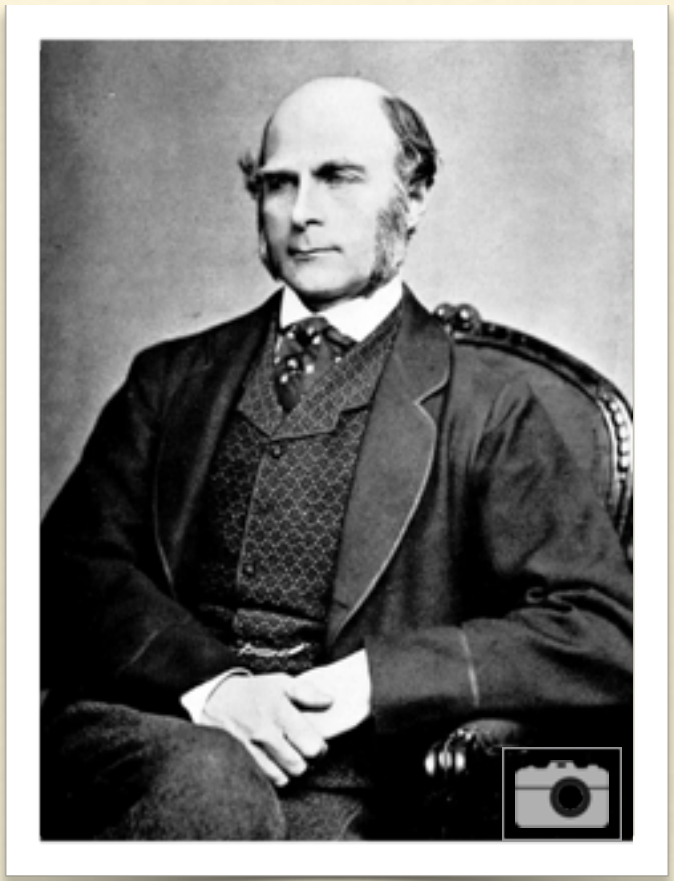
What's the evidence that genetic determination isn't destiny in humans? How do we know that human clones will be different from one another in many ways? The best data comes from analysis of monozygotic twins. As I remarked at the beginning of the chapter, these are naturally occurring clones, siblings that begin life with the same genetic complement. How different can two monozygotic twins be?

Figure 18.4



Tiki (left) and Ronde Barber

Figure 18.5



Francis Galton
(1822- 1911)

For one thing, their fingerprints aren't the same. Some scientists attribute that to such things as the different position of the twin in the womb. Other monozygotic twins are different enough so that computer recognition software can distinguish between them. Microsoft's Windows 10 "Hello" program is claimed to be able to accomplish this feat. And then there's the case of Tiki and Ronde Barber (Figure 18.4). They're former NFL football players.

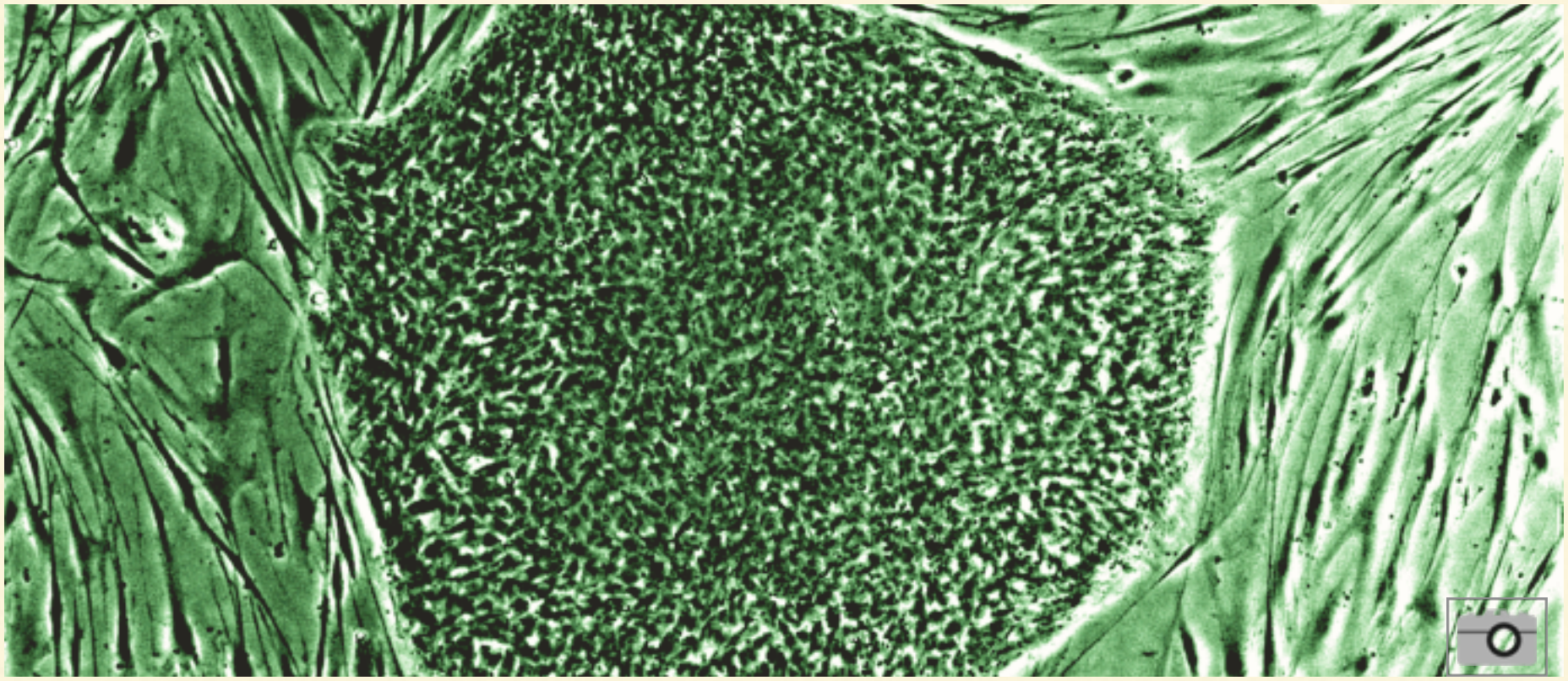
Tiki was a halfback for the NY Giants and Ronde a cornerback for the Tampa Bay Buccaneers. One's a defensive player the other offensive. How much more different can two monozygotic twins be? :)

Twins have been the object of intense scientific analysis for over 140 years. Francis Galton (Figure 18.5), who among other accomplishments coined the phrase "nature verses nurture", published a highly influential paper in 1875 that tried to tease apart the role of environment and heredity in intelligence using twins as subject material. Unfortunately his studies were problematic because he wasn't aware of the difference between identical and fraternal twins. Later generations of scientists have recognized the power of studying twins for the analysis of the genetic contribution to some characteristic. In fact, it is still an active area of research.

SECTION 3

Stem Cells

The different kinds of stem cells are described in this section.



19

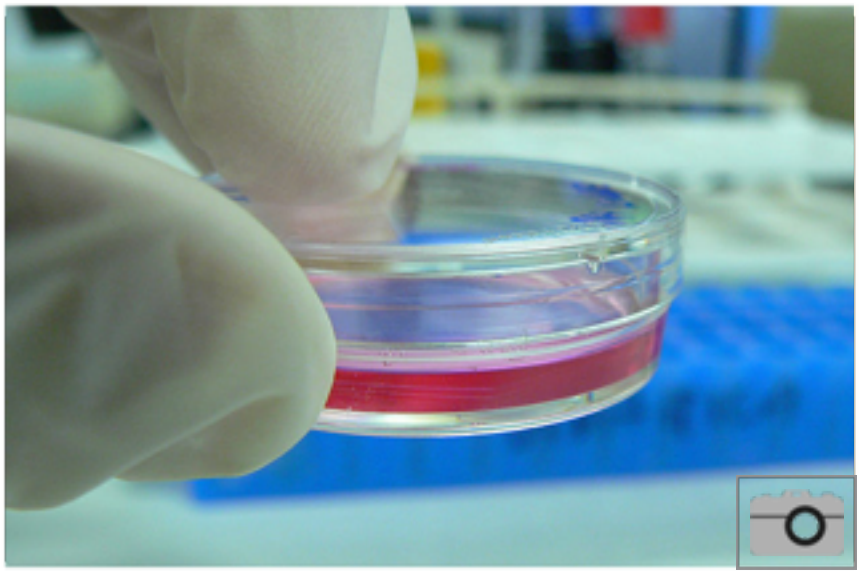
Stem Cells

As development proceeds in mammals most cells become specialized and, at the same time, less capable of reproducing themselves. However, there are some cells, rare ones, that retain both the ability for self renewal and the property of being able to generate more than one kind of differentiated offspring. Cells like these are called stem cells.

Not everyone in the cell biology community is comfortable with this definition. For example, there are adult cells called “progenitors”, that many scientists do not regard as true stem cells. They seem to be the descendants of stem cells. They do give rise to differentiated offspring, but seem to be transients, with a reduced capacity for dividing and forming a large range of different cell types. The distinction between stem and progenitor cells seems a subject for discussion by specialists, but it is probably best to restrict the definition of stem cells to those that last the lifetime of the organism (see the discussion by Melton in “Essentials of Stem Cell Biology”, second edition).

The fertilized egg (zygote) and its immediate descendants are not true stem cells by this last definition. They're "totipotent", capable of giving rise to the entire spectrum of cell types in an organism, but they don't endure through post-embryonic development. At later developmental stages, when a preponderance of cells have taken on specific functions and lost the ability to divide, stem cells form and persist through old age. In all, there appear to be four different types of stem cells: adult, embryonal carcinoma, embryonic, and induced pluripotent stem cells. Cancer cells are yet another kind of cell that share some of the characteristics of stem cells, but I'll only discuss these tangentially.

Figure 19.1



Cells in culture

CellCulture

A short digression. All of the cell types that I'm going to discuss are amplified by growing them in culture (Figure 19,1). It wasn't until the 20th century that biologists were able to get animal cells to grow outside the body for prolonged periods of time. Mammalian cells are particularly difficult to grow, requiring an incubator that can maintain them at exactly 37°C and, often, in a high concentration of CO_2. They also need a complex medium to thrive, typically a

Sidebar 19.1

Ross Harrison

(1870 - 1959)

It is widely acknowledge that Ross Harrison was the first scientist to culture cells. Harrison developed the technique in order to resolve a dispute about the nature

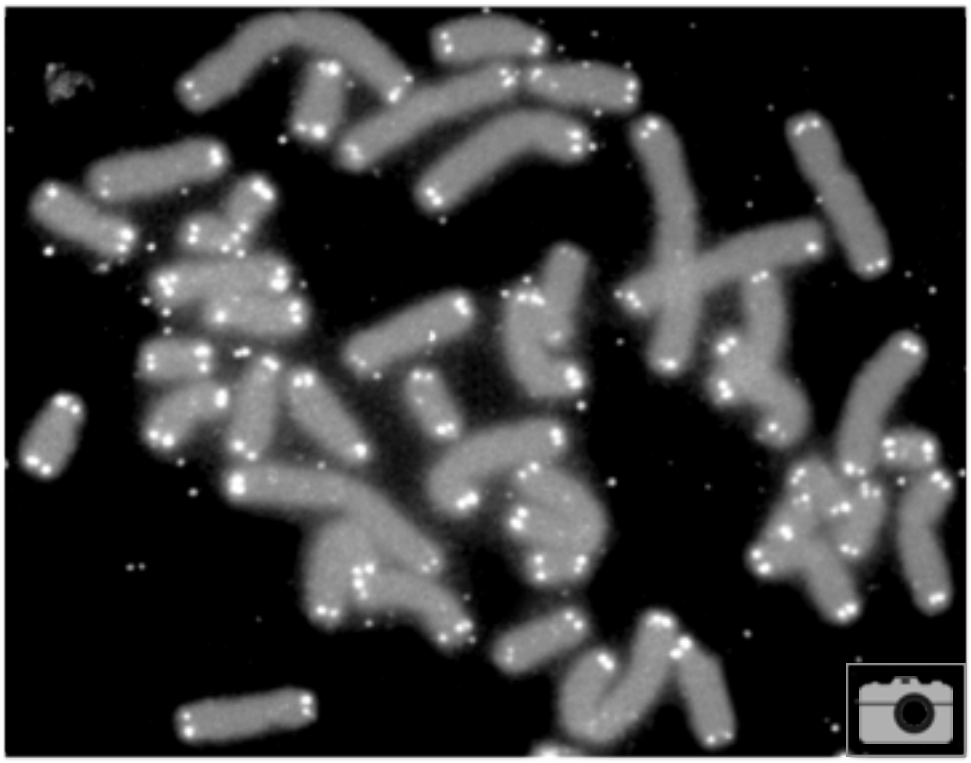
mix of nutrients, salts, and growth factors. And they must be passaged often, meaning that when they outgrow their surroundings, they must be removed and placed elsewhere with more room.

Cellular Aging

Despite the complex equipment and sophisticated growth media available to biologists, cells taken from the body of vertebrates will only grow for a limited number of generations before undergoing senescence and death, a phenomenon discovered by Leonard Hayflick at the Wistar Institute in Philadelphia in the early 1960's.

Hayflick was widely ridiculed for asserting that cells have a limited lifespan. Many of the his most prominent contemporaries felt that if his technique was better, or that if he improved his culture conditions, the cells would flourish for an infinite number of generations. Hayflick devised a clever experiment to prove them wrong. He mixed male cells that had been growing in culture for 40 generations with female cells that were only 10 generations old. He cultured the mixture until cells began to stop growing and die. It turned out that only the male cells were senescent (he could distinguish male from female cells, by the presence of a Y chromosome). Since the female cells continued to grow, it was unlikely that the culture medium was at fault. The mostly likely explanation was that it was the age of the cells. Within a few years, this experiment was repeated at many other laboratories, and it is now generally accepted that cells taken from an adult have a limited life span, typically 50 or so generations, in culture.

Figure 19.2



Chromosomes stained at their telomeres

The mechanism of cellular senescence is partially understood. It has to do with

Sidebar 19.2

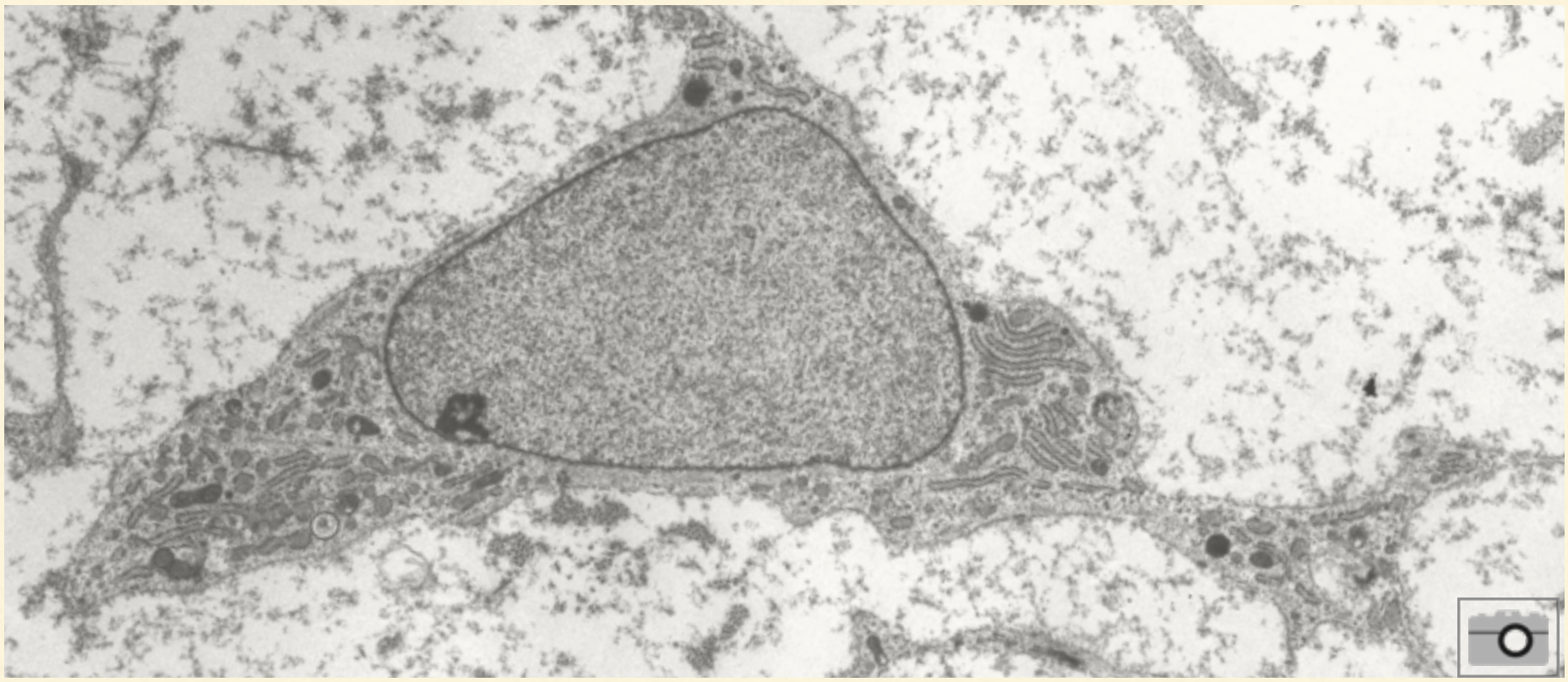
Telomeres

Most familiar animals and plant chromosomes consist of a single molecule of double-stranded linear DNA and associated proteins. "Linear" in this case refers to the fact that the molecule has two free ends; it is not circular as are many bacterial and viral chromosomes.

The ends of most linear chromosomes are special. They carry short repetitive DNA sequences. Human chromosomes ends, for example, are composed of a six nucleotide repeat, TTAGGG, repeated thousands of times. These repetitive

DNA replication. When DNA duplicates it loses bases from one end of each strand. To compensate for this loss, chromosomes bear special repetitive structures on their ends called "telomeres" (Figure 19.2). At every division, some of these telomeric sequences are lost, but there is little consequence since the telomeres carry no genetic information. However, if the cell continues to divide the telomeres may ultimately be lost. At that point the cell is in trouble. As the DNA gets shorter and shorter, vital genes may be affected. The cell will cease growing, sicken, and eventually die.

However, there are some cells that will grow indefinitely in culture. They are said to be "immortal". These cells make use of an enzyme, called "telomerase", that renews telomeres. They are maintained no matter how many times the DNA replicates. Embryonic stem cells, induced pluripotent stem cells, and cancer cells fall into this category, but not adult stem cells.



20

Adult Stem Cells

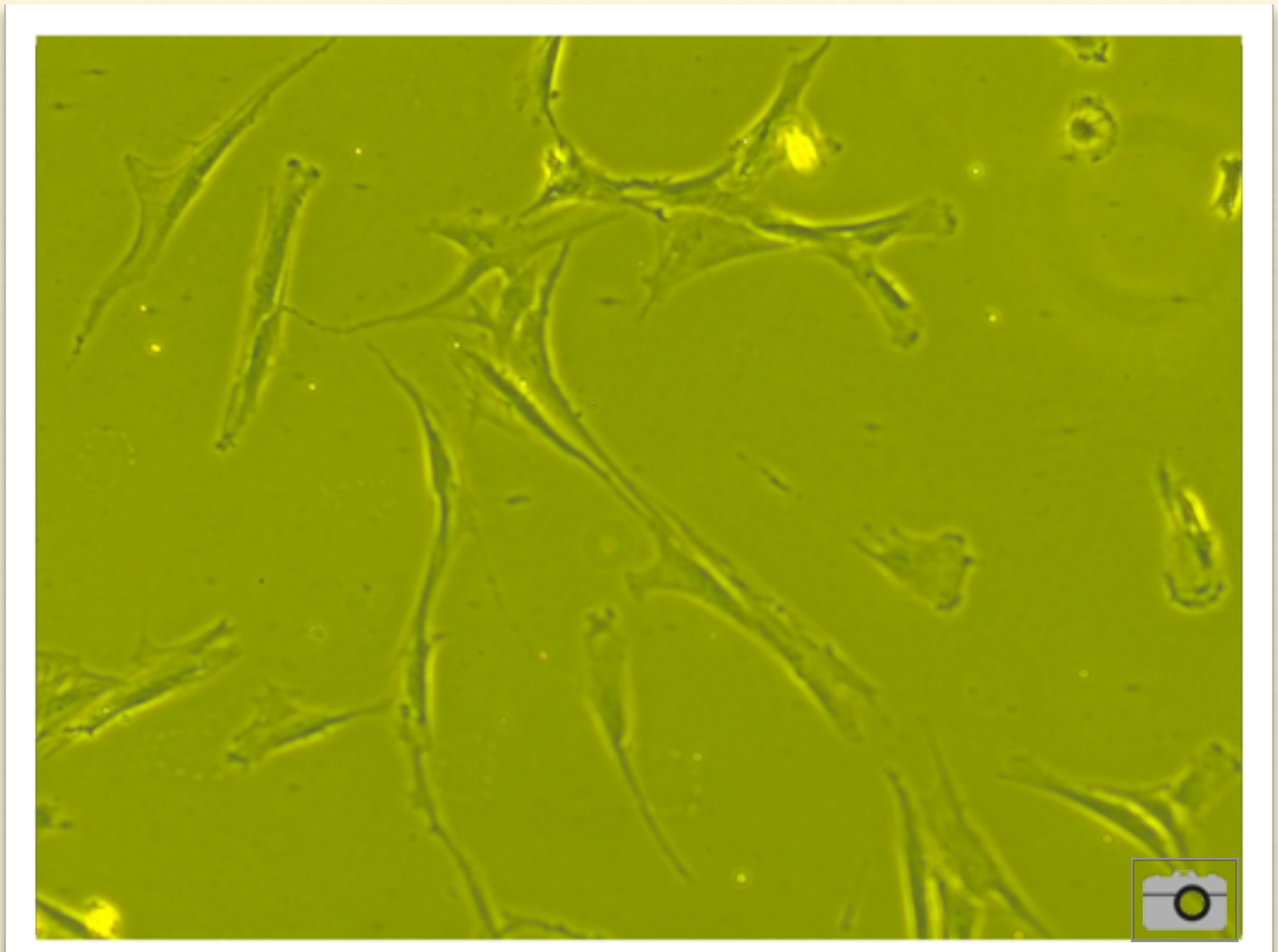
When your skin is injured, new cells appear that participate in its repair. The cells that do this job are adult skin stem cells. Red blood cells are constantly wearing out and being destroyed. New ones appear to take their place. They derive from hematopoietic cells in the bone marrow. The billions of sperm that a human male produces each day come from stem cells in the testes. The fact is that it appears that almost all organs carry a store of stem cells that regularly repair and replace injured and worn out constituents. These adult stem cells are relatively few in number. Most of the trillions of cells in a mature human are differentiated and many appear incapable of further division. Stem cells are so rare, that for some time, except for the blood forming cells in bone marrow, most members of the scientific community didn't know they existed. And to this day, their origin remains a mystery.

While there is a 100 or more year history of experiments and theories suggested the existence of stem cells in adults, the first rigorous demonstration of their reality came from

studies with mice initiated by two Canadian scientists: Ernest. A. McCulloch and James Till. They reported their results in a paper in the journal “Nature” a little more than 50 years ago. They demonstrated that the bone marrow contained cells that could give rise to both red and white blood cells. These adult hematopoietic cells are termed “pluripotent”. That is, they are capable of self renewal and differentiation into one or a few kinds of cells, but not all types.

Somewhat later, another type of pluripotent stem cell was discovered that also appeared to have potential use for therapy. Called, mesenchymal stem cells (MSC’s), they were discovered in the 1960’s and grown in the laboratory of Arnold Caplan (who coined their name) and associates in the 1980’s (Figure 20.1). They can be isolated from a variety of sources including skin, tooth pulp, umbilical cord tissue, amniotic fluid, and fat (finally, a use for fat!). In fact, it’s been suggested that all tissues carry a small

Figure 20.1



Mesenchymal stem cell

complement of MSC's. They have the ability to differentiate into bone, fat, and connective tissue, but their clinical usefulness may not wholly be dependent on their "steminess" (more on this later). Upwards of 350 clinical trials involving MSC's are currently underway.

Disadvantages of Adult Stem Cells for Therapy

It was first thought that adult stem cells could be extraordinarily useful for therapy; to renew damaged or worn out tissues in critical organs. But to date, other than bone marrow transplants, the promise of adult stem cells for therapy hasn't been achieved. First, these cells are present in relatively small numbers. And trying to proliferate them in culture to the extent where they may be useful has proven fraught with difficulties. Second, they have a limited repertoire of developmental potential. That is, they appear to be restricted in the kind of cells that they can form. Third, in practice they have had difficulty in integrating into the tissue under repair, a process called "engraftment". Fourth, after injection, they die off rapidly. And, because they don't proliferate well, they are not readily replaced. Finally, adult stem cells, like all cells that are grown in culture have the problem of mutations, changes in DNA sequence. Whenever a cell divides, there is the high probability that a mutation will occur. Some mutations may lead to the risk of these cells becoming cancerous.

Advantages of Adult Stem Cells for Therapy

Despite their limitations, adult stem cells have great potential for therapy as well as some advantages over the other stem cell types. First, because they have a limited capacity to produce different cell types, there is little danger they'll run off and differentiate into something that is undesirable for their intended use. This is the other side of the coin with respect to their limited proliferative capacity. In the years that adult stem cells have been used therapeutically, there have been remarkably few instances where they did harm. A second advantage is that can be procured directly from the patient undergoing therapy. Such so-called "autologous" stem cells should not be rejected upon transplantation as might happen with foreign (heterologous) cells. Thirdly, even when heterologous stem cells have been tried for therapy, they have proved to be well tolerated by the immune system. There are some great advantages to doing such so called "allogeneic" stem cell transplants. Cells can be prepared well ahead of time and can be optimized for the task at hand.

A great many clinical trials are currently underway with the intent of using adult stem cells as therapeutic agents. But if people are interested in undergoing such treatment, they needn't wait for FDA approval. It is possible to get injections of adult stem cells to remedy a host of ailments in unlicensed clinics that have sprung up around the world. A web search of "stem cell clinics" will yield hundreds of results. How well do these therapies work? Without the use of double blind, placebo based studies, it is hard to tell. I discuss the use of adult stem cells with regard to specific diseases in a later chapter.

Umbilical Cord Stem Cells

There's another kind of cell that is being widely used. It is usually classified as an adult stem cell, although it originates from newborns: umbilical cord stem cells. Blood that is obtained from the umbilical cord shortly after the birth of a baby is relatively rich in stem cells.

Drs. Eliane Gluckman in Paris, France and Hal Broxmeyer in Indiana were the first to use cord blood to treat a disease. The patient was a young boy suffering from Fanconi's anemia, a genetic disease that had resulted in the failure of his bone marrow to produce blood cells. The donor was his new born sister. The transplant was a success and the patient is still alive and well, more than a quarter century later.

Since that event, umbilical cord cells have been used in more than 30,000 instances as an alternative to bone marrow transplants as a source for hemopoietic cells, mostly in children. They have several advantages for this purpose. They're relatively easy to procure. They can be frozen, banked, and stored for long periods. They seem to somewhat less prone to rejection than adult stem cells. And they also may be more hardy and proliferative.

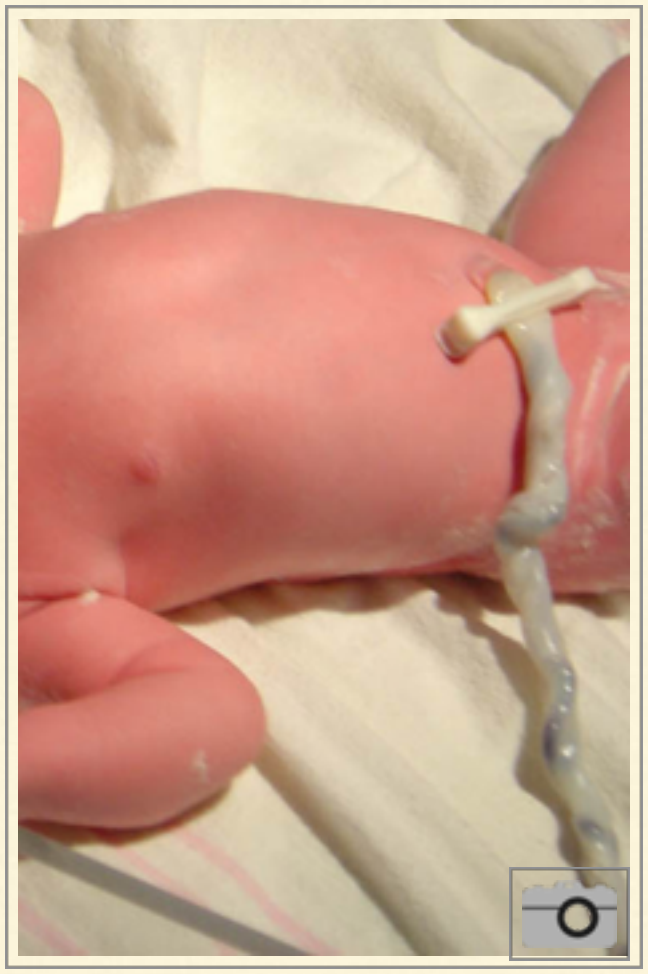
Because umbilical stem cells seem so advantageous, many parents have been encouraged to bank their children's umbilical blood in the event of the child contracting an illness for which stem cell therapy would be appropriate. There are about 200 private cell banking world wide. All told, they have in storage about four million units of cord blood. However, despite their popularity, banking of stems cells in private facilities for autologous purposes has generated some controversy.

One concern seems to be financial. The process is expensive. Private firms charge upwards of $1,200 for collecting cells and one to two hundred dollars a year for storage. Sometimes an additional fee is charged for testing and treatment. The

companies that are engaged in this enterprise say that the price is cheap. They assert that parents are buying insurance in the event that their child contracts an illness that can be treated with their own stem cells.

As with all insurance, one has to calculate costs verses benefits. The key question seems to be: What are the chances that a child will develop an illness that might be remedied by the stored cells? The answer is difficult to ascertain, partly because it isn't clear what diseases can be effectively treated with umbilical stem cells. In particular, some diseases are not suitable for the autologous transfers. If a child bears a genetic mutation, transfer of his/her own cells may only contribute more cells with the same defect. For example, Fanconi's anemia can't be cured with autologous umbilical stem cells because the condition is genetic. In the original Gluckman/Broxmeyer transplant, the donor was a near relative who was tested and found to be disease free.

Figure 20.2



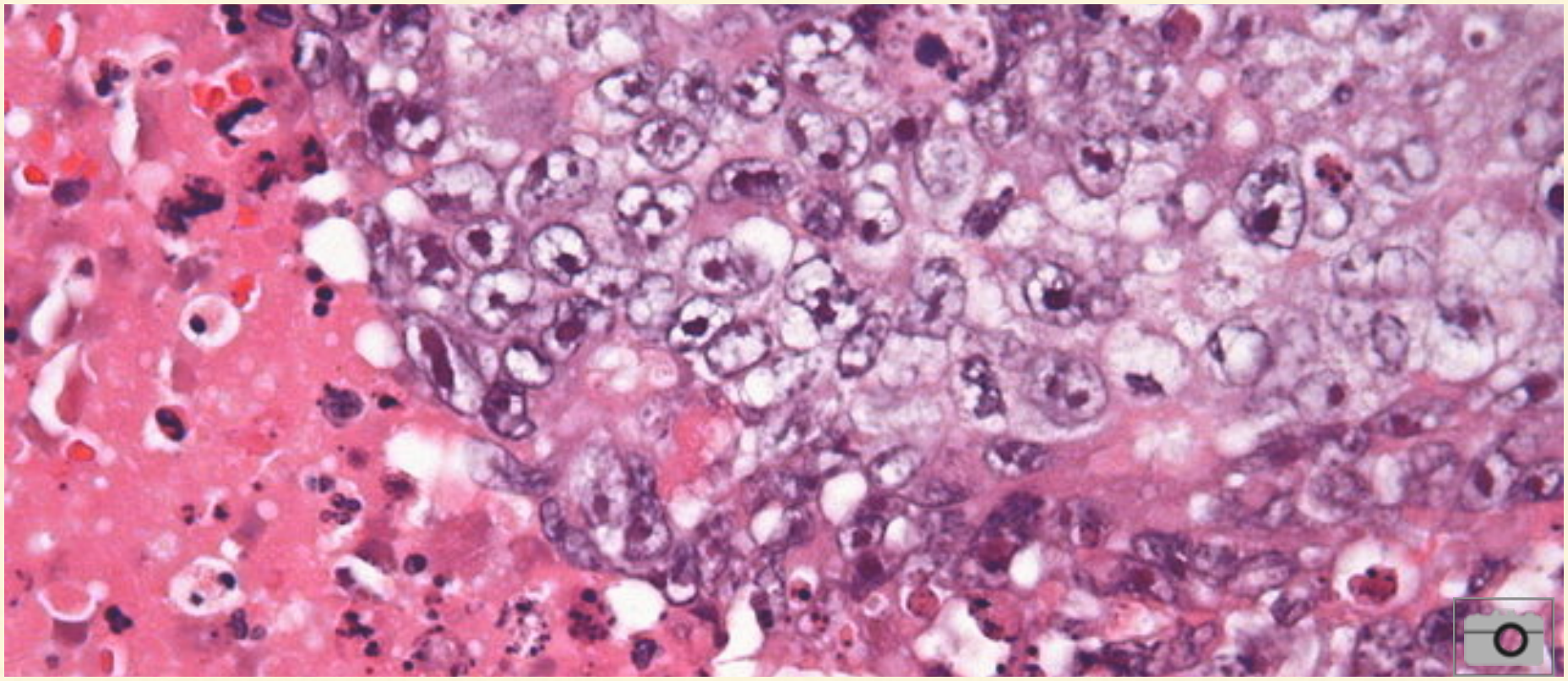
Human umbilical cord

On the other hand, it seems much more reasonable to collect and bank umbilical stem cells in a private facility if a member of one's family is known to have a condition for which stem cell therapy is appropriate.

An alternative to private banking is to contribute umbilical cord blood to a **public** facility. Both the American Academy of Pediatrics and the American Association of Obstetrics and Gynecology recommend this option. There is no direct charge to the parents. Of course, the cells are not available solely for use by the donor. But if enough people make such donations, the chances that cells with a good match will be available will increase. In addition, in some facilities donated umbilical blood samples can be used for transplantation to a family member if a relative develops a medical condition that warrants its use.

In addition to hemopoietic stem cells, umbilical cord blood also carries high concentrations of mesenchymal stem cells.

It has been suggested that such cells from umbilical cords might be more effective for therapy than ordinary adult stem cells. In fact, I was surprised to learn the results of a survey done at the behest of the Parent's Guide to Cord Blood Foundation that examined the use to which autologous umbilical cord cells were put. Some 82% were used to treat brain disorders including brain injuries and cerebral palsy. Whether these treatments were effective isn't clear. But a large number of clinical trials are underway in an effort to find out whether cord stem cells, whether autologous or heterologous, are of clinical utility. We're still awaiting definitive word.

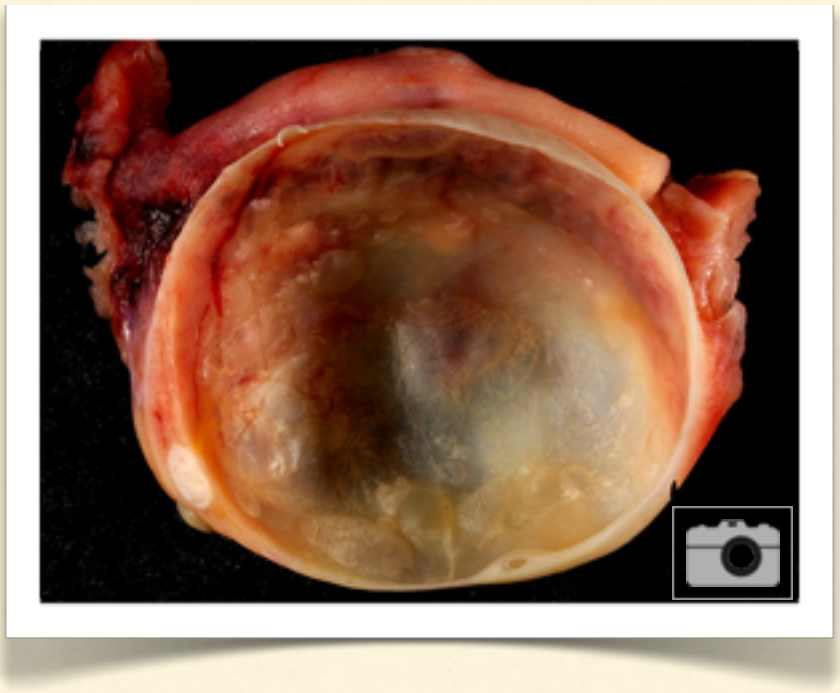


21

Embryonal Carcinoma Cells

Many women are familiar with ovarian cysts, generally benign tumors – teratomas – that form mostly in the ovary. There is a male counterpart that develops in the testis. Male tumors are often malignant and are termed

Figure 21.1

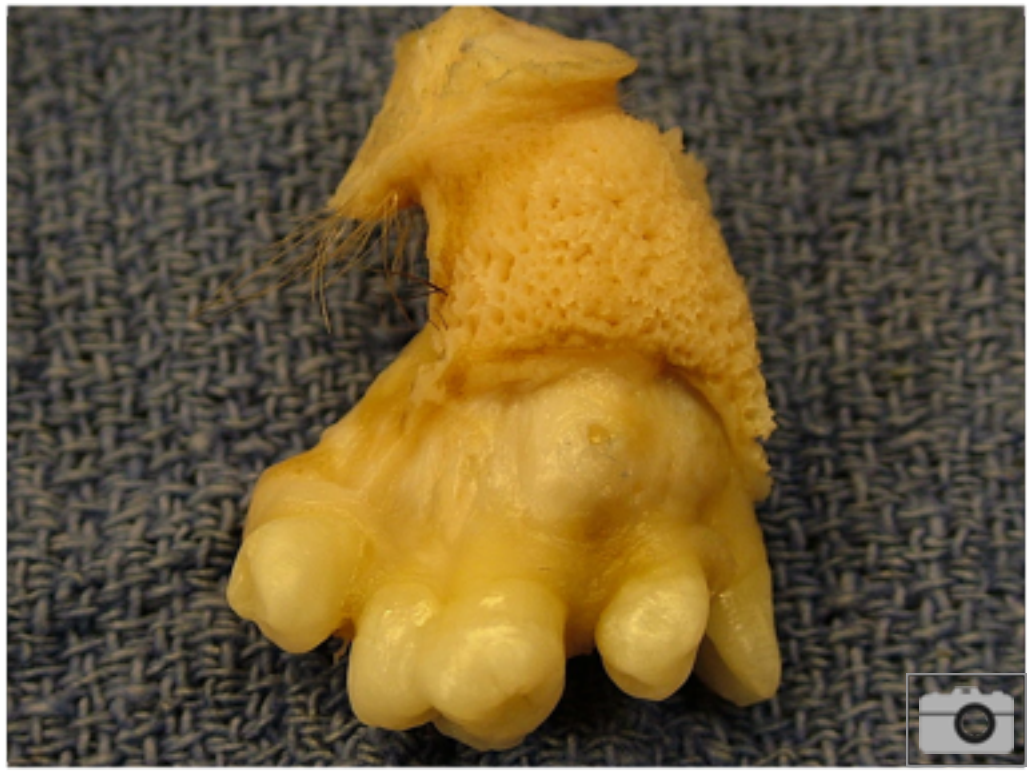


Benign ovarian cyst

teratocarcinomas. The distinction between the terms “teratoma” and “teratocarcinoma” is somewhat controversial. However, the consensus seems to be that teratomas are benign tumors while teratocarcinomas are cancerous. For convenience, I’m going to refer to these both these tumors as “teratomas”, whether or not they are malignant.

Teratoma is a term that derives from the Greek word “teratos”, which means monster, a fitting description of the bizarre mix of tissues and organs that these tumors carry. A typical teratoma might contain hair, muscle, nerves, bones, and even teeth. More than a dozen tissue types may be present. They have fascinated biologists for hundreds of years, but they occurred too infrequently for detailed analysis.

Figure 21.2



Ovarian teratoma showing teeth, skin, and hairs

Leroy Stevens

That all changed in the early 1950’s. Leroy (“Roy”) Stevens was a postdoctoral fellow in the Jackson Laboratory in Bar Harbor, Maine, an institution renowned for its focus on mouse genetics. The laboratory had obtained a grant from a tobacco company, awarded with the object of determining whether it was the paper in cigarettes or the tobacco that was the cancer causing agent. Stevens dutifully treated many mice with various components derived from cigarettes. In the course of these investigations, he was surprised to find that the scrotums of a few males of a particular strain (#129) were enlarged due to internal growths. Upon careful microscopic examination, the growths were found to be composed of tissues from many adult organs, including muscle,

bone, and intestine. In short, they were teratomas.

Stevens mated mice that seemed to produce tumors to each other for many generations. Eventually, 30% of the mice that were so selected developed testicular cancer. Sometime later, he found that he could obtain an unlimited supply of the teratomas by dissecting the genital ridge (the area in an early embryo that gives rise to the testes) from one of these cancer prone strains and transplanting it to the testes of an immunologically compatible mouse. He was now armed with material that would allow him to ask a crucial question: What kind of cell was responsible for forming the teratomas?

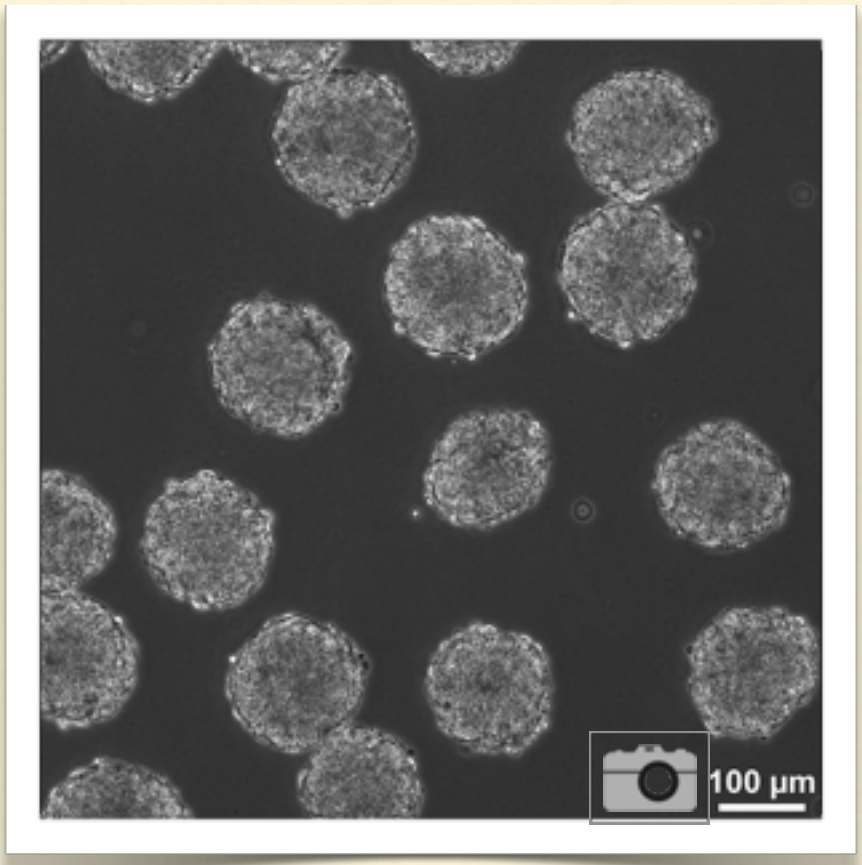
He knew, of course, that the cells were coming from the testes. But the testes contained many cell types, including the germ cells that would give rise to the sperm. Which ones produced the tumors?

To answer this question he did an ingenious experiment. He introduced a mutant gene into strain #129 mice that severely curtailed the production of germ cells but had no apparent effect on the other cells of the developing gonad. When genital ridges from this strain were transplanted into the testes they formed very few teratomas. This was strong evidence that the germ cells themselves, the future sperm, were responsible for teratoma formation.

Mouse teratoma cells had another fascinating property: they could be passaged by injection into other mice. When introduced into the abdominal cavity, they formed so-called "embryoid bodies", groups of cells that looked to all the world like early mouse embryos.

Some years later, scientists succeeded in growing the cells from these tumors in culture ("*in vitro*", literally, on glass, even though most modern petri plates are made of plastic). They could be grown indefinitely and

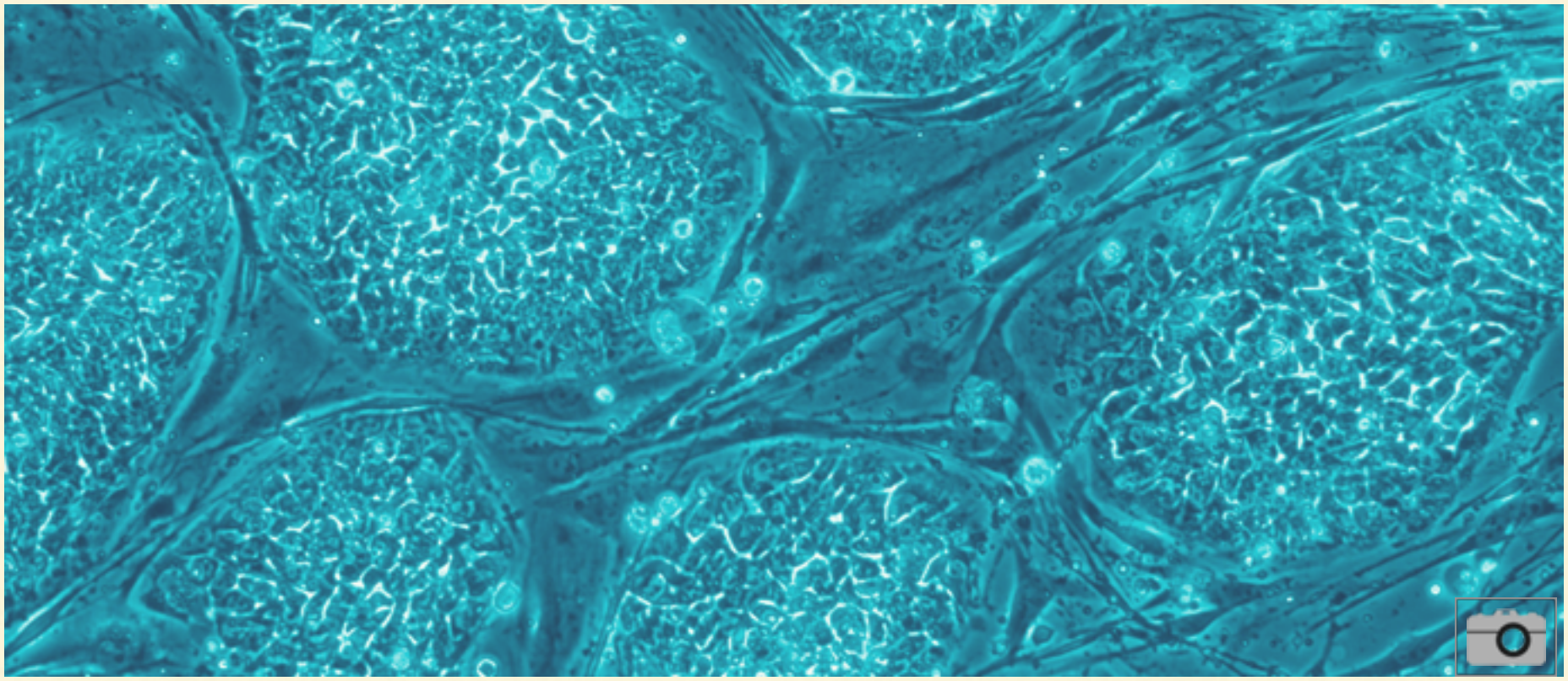
Figure 21.3



Embryoid bodies.

hence readily studied. Cells so cultured looked and acted like embryonic cells and, like embryonic cells could, under certain conditions, differentiate. For example, when injected into a normal mouse blastocyst, and incorporated into the inner cell mass, they were able to differentiate into many different cell types. Even in culture, when exposed to the proper conditions, they could be induced into becoming nerves and muscles depending on the chemicals added to them. Astonishingly, they even could form embryoid bodies under some circumstances.

Now termed embryonic carcinoma cells (EC cells), these mouse teratoma cells often bore multiple mutations and chromosome aberrations. But despite this handicap, they were studied extensively as models for early development and differentiation. As these studies advanced, it appeared that EC cells were very similar to those of the inner cell mass of normal embryos. The question arose: Could inner cell mass cells be cultured like EC cells? If they could, scientists would no longer have to depend on teratoma-derived cells as models for early development. They could study the real thing.



22

Embryonal Stem Cells

In 1981, two laboratories, Gail Martin's at the University of California in San Francisco and Martin Evans' at Cambridge in England, more or less simultaneously reported that they could grow cells from the inner cell mass of a mouse in culture. Martin coined the term "embryonal stem cells" (ES) for these cells. Their success seemed to depend on the use of a "feeder layer", mouse cells that were rendered incapable of division upon which the ES cells could grow. Apparently, the mouse feeder cells were producing some unknown "growth factors" that enabled the ES cells to divide and thrive.

ES cells could reproduce and be maintained in petri dishes indefinitely. However, they showed a tendency to differentiate spontaneously. Subsequently, it was found that a small protein called "leukemia inhibitory factor" was necessary to prevent differentiation.

When injected into blastocysts of mouse embryos, the ES cells proved "pluripotent", able to form the hundreds of different kinds of cells typically found in a mouse. The only

Figure 22.1

Figure 22.2

cell types that they were unable to form were the cells of the trophoblast, which contribute to a large portion of the presumptive placenta.

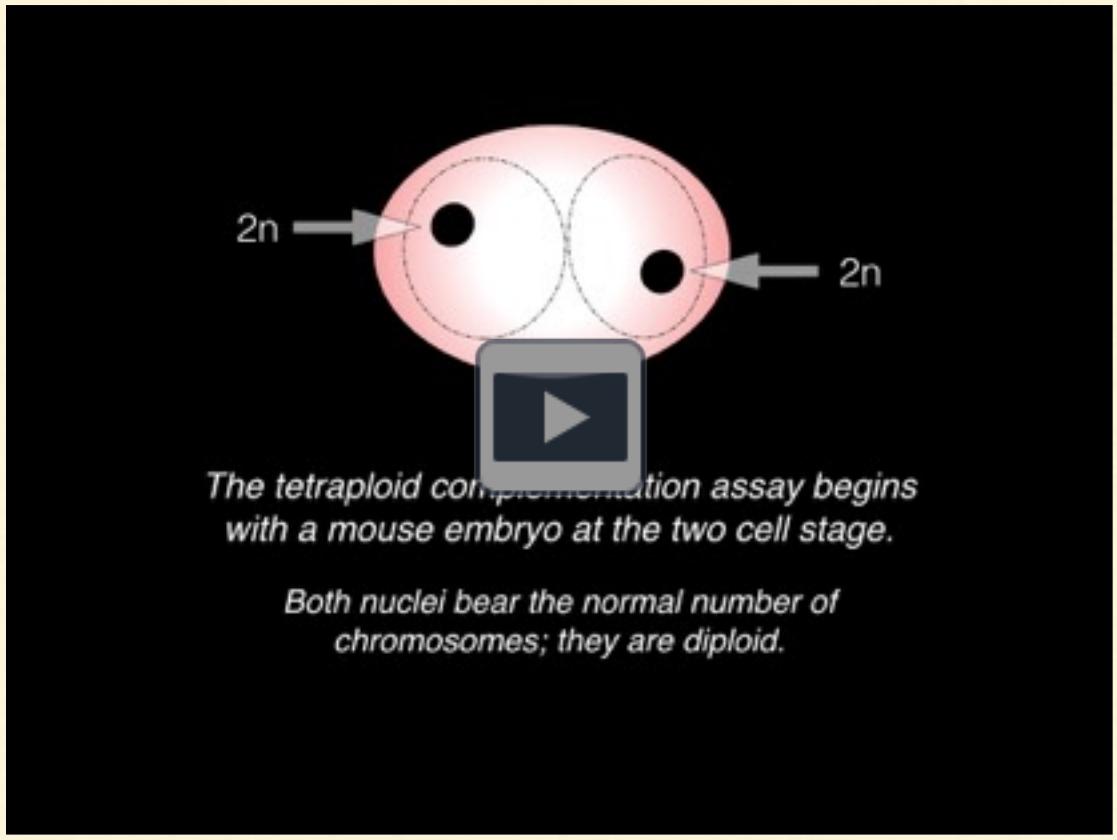
Injection into blastocysts is just one of several methods for determining if an ES cell has retained totipotency after extensive growth in culture. Such assays are needed because developmental potential may change with time. Another procedure for determining whether cells are totipotent is to see if injected cells can form teratomas when injected into adult mice. A third method, somewhat more stringent, is to see if injected ES cells are capable of contributing to the germ line after injection into blastocysts.

Tetraploid Complementation

A still more rigorous test of totipotency in mice is the tetraploid complementation assay, developed in 1990 (Slideshow 22.1) . In this clever technique, two cell stage mouse embryonic cells are fused by subjecting them to an electric current. The resultant single cell is tetraploid (4n), meaning that it has twice the normal number of chromosomes. It will divide and form a blastocyst which, when transferred into mice, can implant into the uterine wall but cannot develop further, apparently because of its abnormal chromosomal constitution. If, however, a diploid embryonic cell from another mouse is mixed with the tetraploid ones, the normal cell can form a mouse embryo, one that entirely derives from the normal cell. In fact, an entire mouse may develop

to term and beyond using this technique. If a mouse does develop it must mean that the contributed cell, the non-tetraploid one, must have had the capacity to form all tissues, and is therefore totipotent.

Slideshow 22.1 Tetraploid Complementation Assay



Click on the images to change slides.

It took more than 15 years after the discovery of ES cells in mice to find the equivalent cells in humans. Some of the reasons for this delay were undoubtedly non-scientific. Biologists didn't want to deal with the legal and political issues that the studies were bound to encounter. And funding for this kind of research was not available from the federal government in the United States for many years. Recall that the prospective ES cells had to come from a living human blastocyst.

Nevertheless, in 1998 James Thompson's laboratory at the University of Wisconsin succeeded in establishing five ES cell lines from human blastocysts. The embryos were obtained from an *in vitro* fertilization center.

The human ES cells had most of the characteristics of their mouse relatives. For obvious reasons, one couldn't test them for totipotency using the tetraploid complementation assay, or for that matter, by many of the other techniques commonly used. But they could be tested for the ability to differentiate by injection into immuno-compromised mice embryos, where they grew into teratomas that had many differentiated cell types. They also differentiated when removed from the mouse feeder layer that they were grown on or when leukemia inhibitory factor was removed.

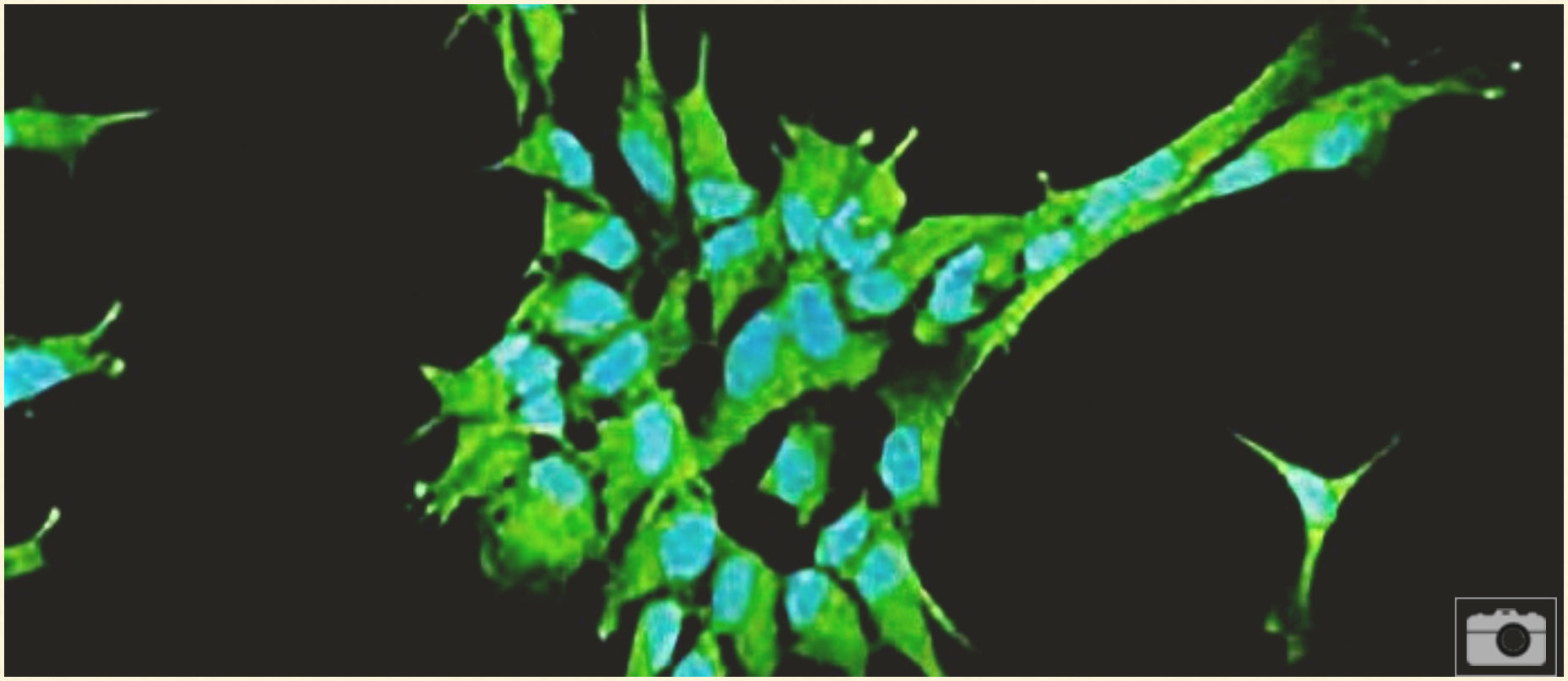
ES Cell Advantages and Drawbacks

ES cells seemed to offer great potential for therapy. But they have several drawbacks –

political, ethical, and practical – as well. Their key advantage is that they have virtually unlimited potential for forming any cell type that might be required for therapy. On the other hand, they must be derived from human embryos. These embryos are "left over" from *in vitro* fertilization attempts and would otherwise be frozen and eventually discarded. They are never taken from aborted embryos. However, in the course of gathering the starting material for embryonal stem cells, there is no question that embryos must be destroyed. While some people believe that life begins at conception, and that this destruction therefore means that "babies" are being killed, this is not a universally held conviction. People of different cultures and religions have different views on the subject. Nevertheless, the use of human early embryos for obtaining embryonal stem cells have been a matter of great political and religious controversy that has resulted in the field moving slower than it otherwise might. During the administration of President George Bush stem cell research that utilized embryonal stem cells was greatly curtailed. More recently, a federal court in 2012 upheld the legality of using embryonal stem cells.

Embryonal stem cells have other disadvantages for their wide spread adoption for clinical use. Since they are obtained from embryos that are not related to those who would benefit from their use, they are by definition allogeneic, meaning that there may be immunological issues with their use. Another disadvantage derives from their pluripotency. They have the potential to form all kinds of cells, including teratomas. If a therapy was successful but resulted in the formation of a dangerous tumor, that would be devastating to both the patient and the physician.

To overcome the most serious non-scientific objection to the use of embryonal stem cells, that they had to be derived from living embryos, a breakthrough was required, a way of forming ES cells that didn't require the use of embryos. A new technique that seemed to offer such a breakthrough came from a laboratory in Japan less than a decade ago.



23

Induced Pluripotent Stem Cells

As we've seen, John Gurdon and Ian Wilmut showed convincingly that all cells of multicellular organisms, regardless of how different they appear and what function they serve, carry a full complement of genetic information in their nuclei. But if they don't have different genes, what causes them to become different? The answer turned out to be conceptually simple, but complicated in practice. To appreciate some of these complexities, let me briefly add to our discussion of the basic principles of molecular biology presented previously.

Some More Molecular Biology

We've already seen that genes are sections of DNA – thousands or tens of thousands of nucleotides long – consisting of a specific sequence of bases. These sequences are copied into RNA, a process called "transcription". Each gene copy bears the instructions for the construction of a particular protein. And proteins are the keys to how cells operate. Different cell types have different constellations of proteins that enable them to perform distinct tasks. All this

should be old hat. If not, you might want to review Chapter 4.

A crucial question arises: How does the cell choose which genes to copy (transcribe)? How does it know to make cell type specific proteins appropriate to the occasion?

The scheme that organisms use to accomplish this task was worked out in the 1960's in bacteria. Of course bacteria are single cell organisms and don't have tissues or different cell types in a single individual. But they do need to synthesize specific proteins in response to changing conditions. How do they do that?

3D Molecule 1

Two transcription factors bound to DNA
Oct2 is shown in green; Sox2 in yellow. The two strands of DNA are drawn in red and blue.

Transcription Factors

Here's how. There's a group of special proteins whose job it is to recognize specific genes and attach to them. Once bound, they either block or activate the machinery that does the copying of DNA into RNA. In animals and plants, these proteins are called "transcription factors". This process is commonly called "turning genes on and off", a phrase that indicates that genes are either being prevented from being read or stimulated to do so.

In early development, the egg carries a set of transcriptional factors that allow certain specific sets of genes to be transcribed. Some of the proteins specified by these genes promote cell division and discourage differentiation. Other genes specify additional transcription factors that, in turn, control the copying of more genes. A cascade of events ensues, where even more transcription factors get synthesized or released, promoting differentiation of various cell types.

Shinya Yamanaka

Induced pluripotent stem cells (iPS cells) are cells that were once differentiated but have been induced to return to an undifferentiated state. How are cells persuaded to change their behavior in this way? From the description above, you might guess how to accomplish this task. One has only to remove the transcription factors that made the cell differentiated and substitute those from an earlier developmental stage in which the cell was pluripotent. In fact, that was what was accomplished in 2006. A Nobel Prize was awarded to Shinya Yamanaka of Kyoto University based on that simple line of reasoning.

Figure 23.1

Shinya Yamanaka

Yamanaka's Background

Shinya Yamanaka was born in Osaka in 1962, His family ran a factory that manufactured parts for sewing machines. Yamanaka at first gravitated toward engineering. He considered entering the family business, but discouraged by his father, he decided to become a physician. More specifically, an orthopedic surgeon. He was partly motivated by the fact that a good deal of his childhood was spent recovering from sports injuries due to his interest in judo and running. He earned an MD degree in 1987 but found that he hadn't the skills to become a successful surgeon. Moreover, medicine frustrated him. He found that he and his fellow physicians stood by helplessly when confronting many ailments.

So, he went back to school again. This time he pursued a Ph.D degree in Pharmacology at Osaka University's Graduate School of Medicine, hoping to become a basic scientist, reasoning that by

working on the fundamental foundations of disease he could do more for the health of his fellows than by helping individuals. At the age of 31, he completed his degree. The next step up the academic ladder for a budding research scientist was to take a postdoctoral appointment. He wanted to study molecular genetics. Specifically, the molecular genetics of mice. However, his options were limited. He was a failed surgeon with little expertise in molecular biology and a degree from a not very well known university. He applied for many positions, and ended up in San Francisco at the Gladstone Institutes.

It turned out to be a serendipitous event. The Gladstone Institutes use stem cells to tackle cardiovascular, neurological, and immunological diseases. They're associated with the University of California at San Francisco. He learned how to introduce genes into mice and how to "knockout" genes already present. And he became acquainted with mouse embryonic stem cells.

After a successful stint as a postdoctoral fellow in the US, he returned to Japan, first to Osaka University, and later, in 1999, at the Nara Institute of Science and Technology (NAIST). At NAIST he headed his own laboratory and began to work seriously with embryonic stem cells. It was there also that he set his long term goal of creating ES-like cells from somatic cells without using embryos. It took him only seven years to succeed.

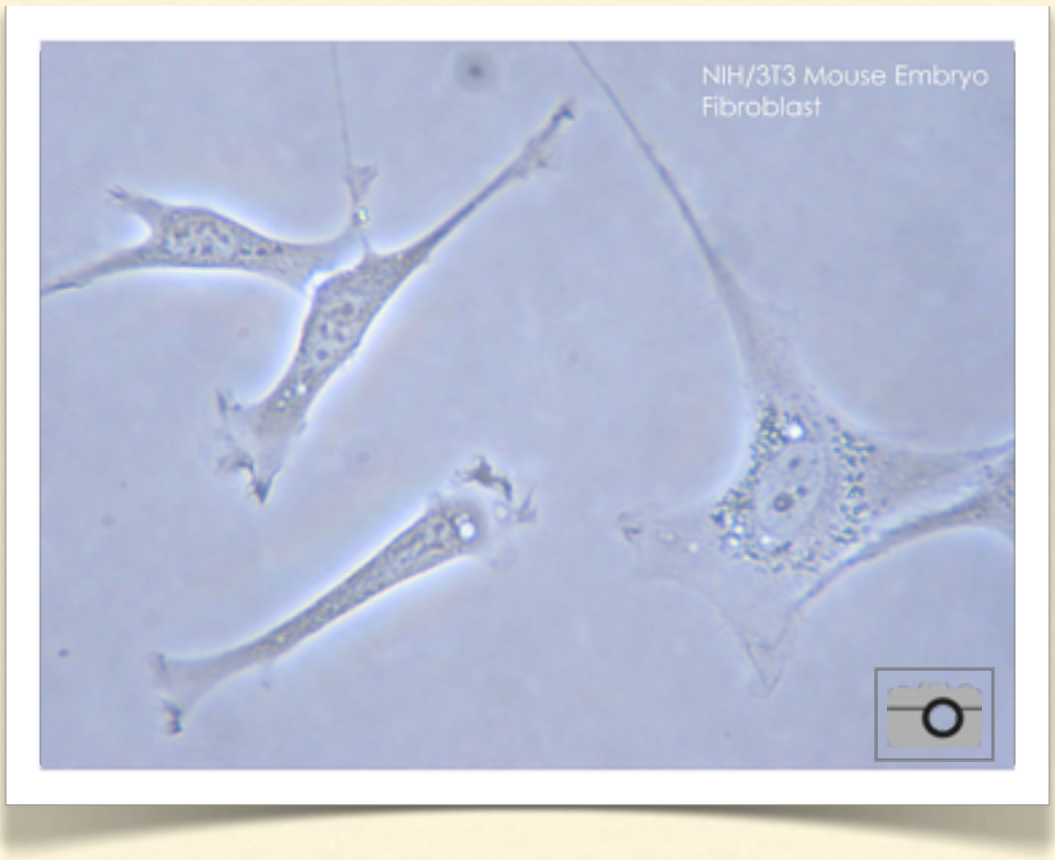
Yamanaka's discoveries were based on three previous developments. The first, as we have seen, was the demonstration that cells do not irreversibly lose genetic information as they differentiate. As Gurdon and Wilmut showed, somatic cells can became pluripotent when their nuclei were transferred into eggs. This meant that oocytes carried some substances, probably proteins, that were responsible for reprogramming somatic cells from their original fate to one where they became dedifferentiated. These findings inspired Yamanaka to search for such substances in embryonic stem cells, which also seem to carry reprogramming factors.

Second, he was aware of earlier work that indicated that embryos carried "master regulatory genes", DNA sequences that encode transcription factors that regulate the expression of other transcription factors. In turn, these other transcription factors control gene expression of other genes, including genes for other transcription factors. In this way, complicated genetic regulatory circuits are established and differentiation can be directed along specific pathways. He knew

that some of these master regulatory genes appeared to be critical in determining pluripotency and he determined to find the ones that played that role.

Third, he was familiar with the work of Gail Martin and Martin Evans on the establishment and maintenance of embryonic stem cells. If he were to succeed in getting differentiated cells to become pluripotent, he would have to use their methods to sustain them.

Figure 23.2



Mouse fibroblasts

Yamanaka took a brute force approach. He looked through the literature and selected 24 transcription factor genes that were active in cells that were pluripotent. He then introduced all 24 one by one into cultured mouse fibroblasts (a common type of differentiated cell that is found in connective tissue). In order to determine whether any of the cells had been directed toward an embryonic state, he monitored the expression of FBX 15, a gene of unknown function that is expressed in early mouse development but which can entirely be dispensed with. He reasoned that if FBX 15 is "turned on", it would indicate that the fibroblasts had become dedifferentiated. None of the introduced transcription factor genes seemed to work when used singly. By contrast, when all 24 were introduced at once, a small fraction of the cells responded by expressing the FBX 15 gene. They grew well in culture and, encouragingly, resembled embryonic stem cells.

Their next step was to withdraw individual genes from the 24 to see which ones could be dispensed with. A total of 14 could be omitted without much effect. Of the remaining 10, four seemed critical. To quote Yamanaka, "These data demonstrate that iPS cells can be induced ... by the introduction of four transcription factors,

...". For this outstanding contribution, Yamanaka was awarded the Nobel Prize for Medicine in 2012 (along with one of the scientists who inspired his work, John Gurdon).

While the iPS cells looked strikingly like embryonic stem cells, how similar were they? A number of sensitive biochemical tests were carried out and the results indicated that the two cell types were similar, but not identical. Encouragingly, iPS cells were found to be pluripotent by several biological tests.

Just a year later, Yamanaka's and two other laboratories were able to make iPS cells from human cells in culture. Other improvements to iPS generation soon followed. It became apparent that it a relatively easy task for a laboratory to produce iPS cells by a variety of techniques similar to that used by Yamanaka. However, the question remains as to how similar iPS cells are to embryonic stem cells and what role the iPS cells will play in future therapies.

Another Route to iPS Cells?

As I've noted, it took Yamanaka and his coworkers considerable effort to discover the recipe for generating iPS cells. Some years later, another group announced a simpler way of producing these cells. In two paper that were published in late January 2014 in Nature, Haruko Obokata and a string of co-authors at the RIKEN Center for Developmental Biology in Kobe, Japan (CDB) in collaboration with scientists at the Brigham and Women's Hospital in Massachusetts, announced that they could make iPS cells by simply exposing cells to stressful conditions. They called their method, "STAP", for "stimulus-triggered acquisition of pluripotency". No longer would laboratory workers have to introduce a complicated mix of transcription factors into cells in order to turn them into stem cells. Simply adding a little acid to cells in culture would do the trick.

Their papers met with immediate excitement and some skepticism. Researchers desperately wanted to believe that STAP worked, but it seemed much too good to be true. An informal poll conducted shortly after publication of the paper on Paul Knoepfler's web site revealed that the scientific community was more or less evenly split on whether they believed in STAP or not

Within a week of its publication several red flags appeared. One of the figures in the paper looked suspect. It seemed to be photographically altered. Another figure appeared to be a copy from an earlier publication. In a related development,

there seemed to be evidence of plagiarism in Obokata's doctoral thesis.

Of course, the gold standard in science is reproducibility. To that end, two weeks after the publication of the Obokata papers, the editors of Nature took the highly unusual step of asking ten prominent stem cell research laboratories to try to replicate the STAP results. None could. At about the same time, the CDB launched an internal investigation. They found that misconduct had occurred. Obokata appealed the CDB's verdict, but to no avail. In July, Nature retracted both papers. Subsequently, scientists at the CDB attempted to repeat Obokata's experiments. But even with her help they failed. It now appears that stressing cells doesn't cause pluripotency. STAP isn't real. Obokata had faked her results.

Figure 23.3

Yoshiki Sasai
(1962 - 2014)

Repercussions

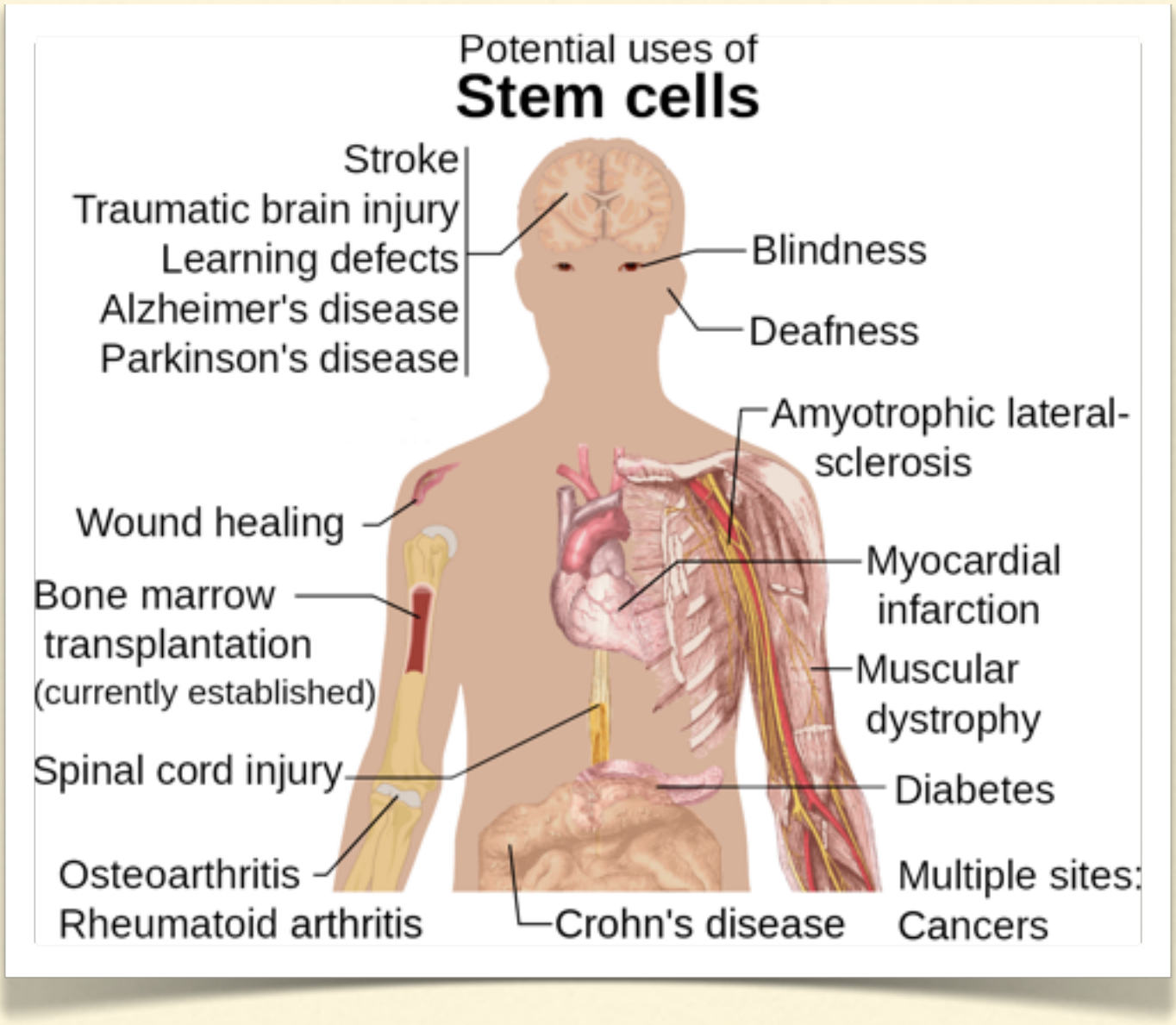
The STAP scandal spread and affected more of the community. One of the senior coauthors on both of the STAP papers was Charles Vacanti from Brigham and Women's Hospital with whom Obokata had studied for two years before earning her doctorate in Japan. As it happens, Vacanti had proposed a concept similar to STAP back in 2001. It had not been particularly well received. When Obokata's experiments came under fire, he supported her results, even publishing a protocol for generating STAP cells on his website. However, his recipe didn't work any better than Obokata's. In September 2014, he took a one years leave of absence from Brigham and Women's Hospital and stepped down as the Chair of the Anesthesiology Department there. Some believe that he bears more of the blame for the STAP fiasco than others who have been more harshly treated.

Undoubtedly the person who was most affected by the scandal was Yoshiki Sasai, a world renowned and highly respected stem cell scientist and the Deputy Director of the CDB. Sasai, a coauthor on the STAP papers, was found innocent of wrong doing

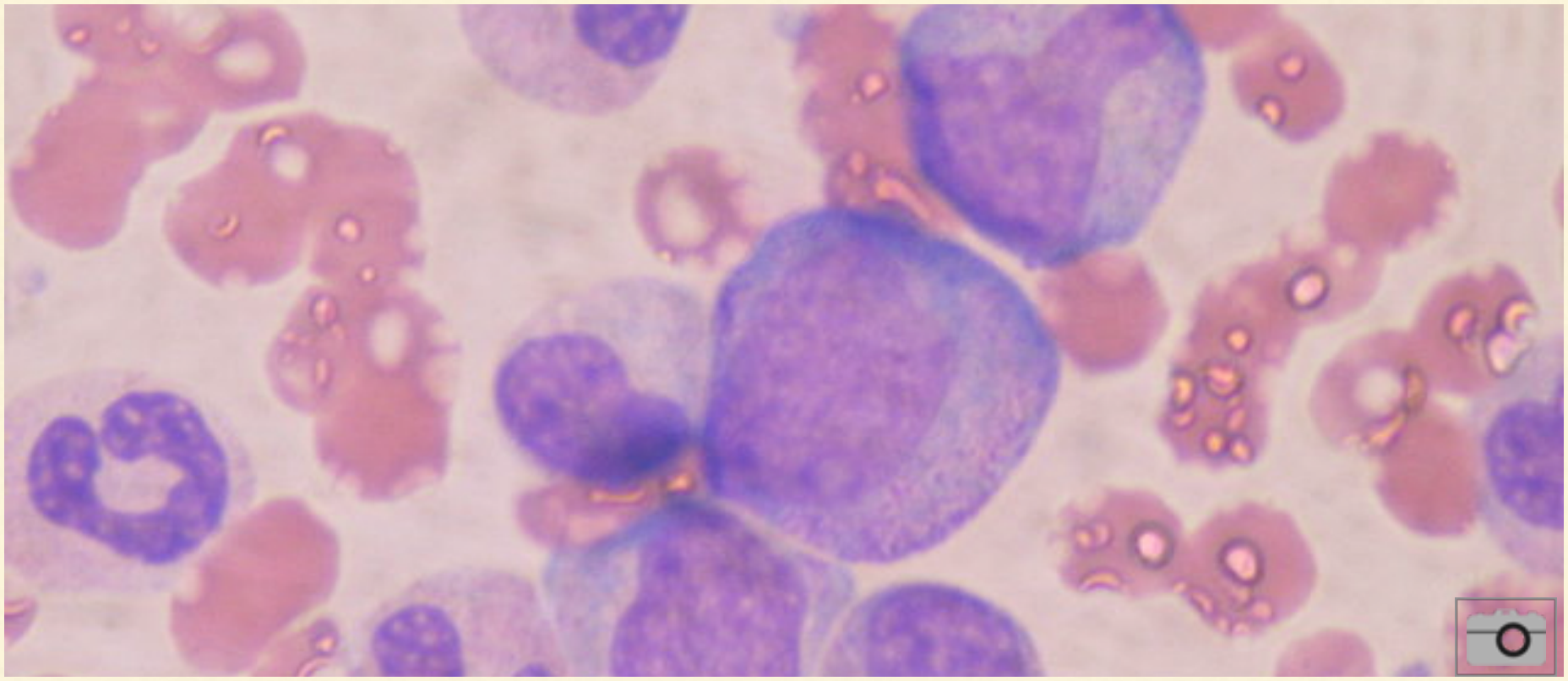
but the internal investigating committee did note that he failed to oversee Obokata's research adequately. The Japanese press was particularly quick to assess blame for the whole affair, and the tabloid press attacked Sasai viciously. On August 5, 2014 Sasai committed suicide by hanging. He left several notes. A lawyer representing Sasai's family, did not release the contents of the note to the press, but at a conference said that that Sasai was "worn out by the unjust bashing in the mass media and the responsibility he felt towards RIKEN and his laboratory".

SECTION 4

Stem Cell Therapies



How stem cells can be used to study and remedy disease.



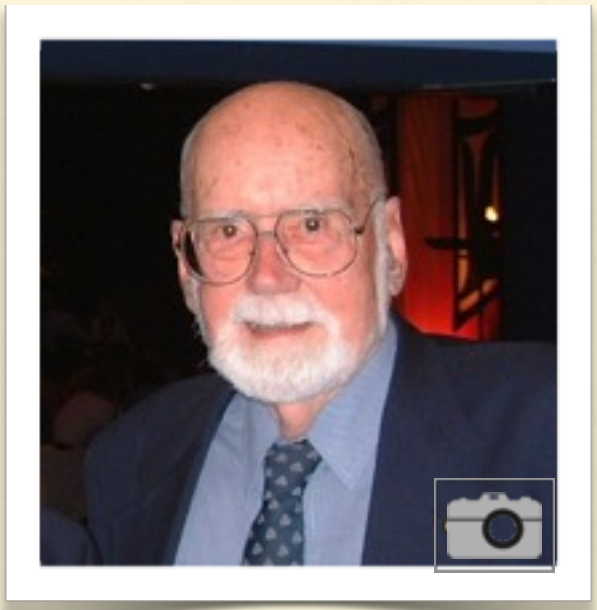
24 Blood Diseases

The marrow is the soft tissue that lies within bones. It contains stem cells that are the main source of not only the oxygen conveying red blood cells, but also infection fighting white cells and the platelets that aid in blood clotting. Blood marrow is essential for life. That's because red cells, white cells, and platelets must continually be renewed. Their lifetimes are measured in weeks and months. If they're not replenished, we soon die.

Lymphomas (including multiple myeloma, Hodgkins and non-Hodgkins type) and leukemia (many type) are cancers of the lymphatic and hematopoietic systems. They are fairly common diseases, especially among children, resulting in about 500,000 deaths worldwide each year. The five year survival rate for most of these cancers depends on the specific type involved and the agents used to treat the disease, but, in general, is fairly high. However, when ordinary therapeutic methods fail, stem cell therapy can be used.

E. Donnall Thomas, the "father" of bone marrow transplantation, knew from experiments in mice carried out prior to 1950 that high doses of radiation destroyed the stem cells in bones. Other studies demonstrated that animals so treated could be saved by the infusion of live marrow cells. Thomas reasoned that if a patient with leukemia or lymphoma were irradiated with a lethal dose of X-rays, both the normal and cancerous cells would be killed. If normal stem cells could be reintroduced after the cancer was destroyed, blood cell production would be restored, and the patient would be cured.

Figure 24.1



E. Donnall Thomas (1920-2012) Thomas shared the Nobel Prize in Physiology or Medicine with Joseph E. Murray in 1990 for the development of cell and organ transplantation (bone marrow transplantation) as a treatment for leukemia.

The principle behind this idea seemed sound. The problem was that in practice, the scheme didn't work – at least not at first. Any newly introduced cells were recognized as foreign by the immune system and rejected. Without a source for renewal of their blood, patients would die of what was euphemistically called "secondary disease". The only long term successes came when an identical twin was the marrow donor. That demonstrated that the general strategy was correct, but that a very close immunological match was required.

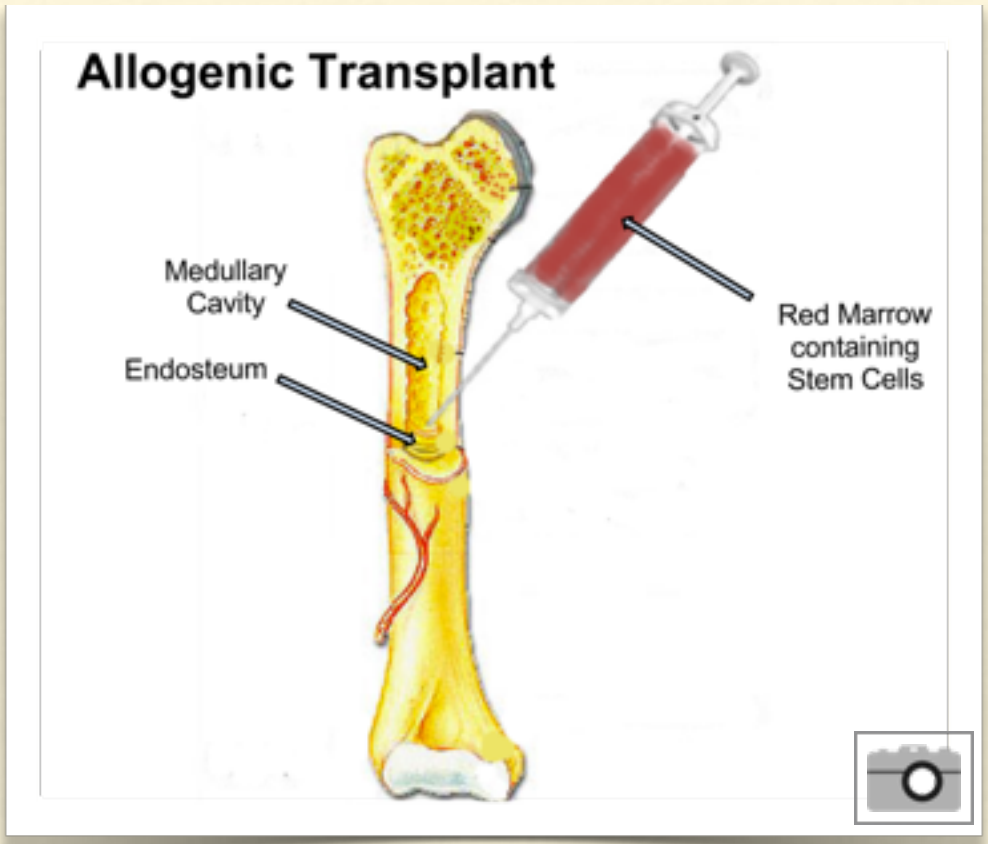
With increased understanding of the immune system and better antibiotics to fight infection, a few successful transplants were recorded in the early 1970's. These patients were very ill at the time of treatment. By the end of the decade, there was evidence that the procedure would be more successful when applied to patients who weren't as sick, whose disease was in partial remission. About half the patients were then being cured.

There have been many other improvements over the years. Immunosuppressive drugs have proved extremely helpful. Refinements have occurred in matching donors with recipients. National and international networks for donor registry have been developed. More than four million people have been listed. They have resulted in a great increase in unrelated

donor transplants and many more successes.

Donor stem cells may be obtained from bone marrow, the major site of blood production in the body. Alternatively, stem cells may be gathered from peripheral blood. In the latter case, the number of stem cells is normally small, and they may have to be augmented by giving donors a growth factor to stimulate stem cell production.

Figure 24.2 Lorem Ipsum dolor amet, consectetur



Bone marrow transplant

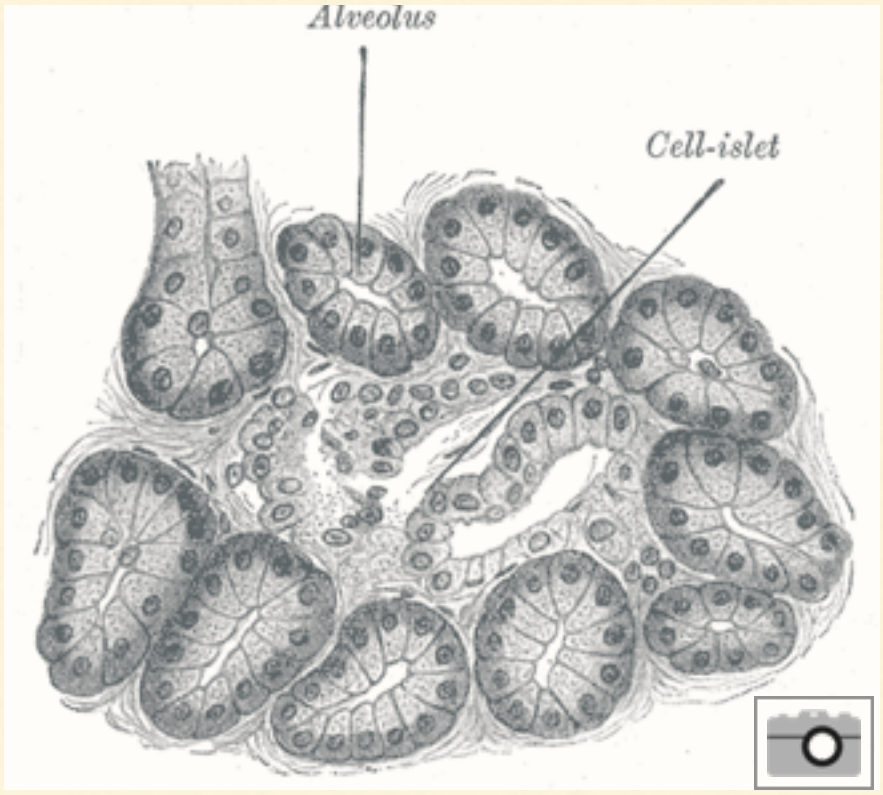
Recently, a blood donor contacted a friend of mine. She vividly and movingly described her experience. I reproduce her FaceBook post here.

Last week I was able to donate peripheral blood stem cells (like marrow) to a patient in need through Be The Match. The patient's immune system will essentially be replaced with mine. It was a quick process: from the time I was notified I was a match to the actual donation was maybe a month and a half. The discomfort to me was relative - the 4 days of injections leading up to donation day weren't painless and they made me feel like I was coming down with a nasty flu, but it couldn't have been nearly as bad as my recipient's. The actual donation wasn't horrible either - it was a day long process of being hooked up to a machine that filtered the stem cells out of my bloodstream and I walked out of the hospital feeling much better than when I walked in. Hopefully by now the patient has had the infusion of my stem cells and begins to recover soon. I should get an update on their well being in the next 30-45 days and in a year we can meet if we so choose.

This was an amazing experience. It is surreal to think that I may have helped save someone's life and I am grateful that I had the opportunity. I am also very thankful for the people that I met along the way at Be The Match and the Mayo Clinic. I also couldn't have done this without the support and love from my family and all my friends.

If you are interested in registering to become a bone marrow or PBSC donor, please go to www.bethematch.org.

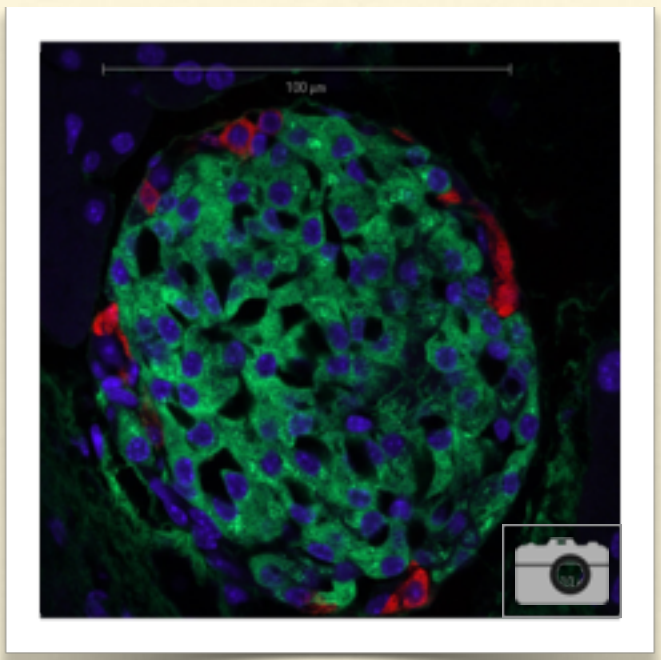
Stem cell therapy may also be used for other blood diseases, including sickle cell anemia and thalassemia. In fact, stem cell transplants from peripheral blood and bone marrow are the only widely practiced stem cell therapeutic procedure carried out today. The number of transplants has been staggering. In 2013, an organization called "The Worldwide Network for Blood and Marrow Transplantation" reported that the one millionth transplant had occurred in December, 2012. The procedure has been remarkably effective in remedying a large number of disorders.



25 Diabetes

Diabetes affects almost 400 million people worldwide. It is a disease that is characterized by a high level of blood sugar over prolonged periods. Other symptoms include frequent urination, thirst, and hunger. There are two common varieties: types 1 and 2. Type 1 results from a failure of pancreatic beta cells (Figure 25.1) to produce the hormone insulin. It used to be called "childhood diabetes" and seems to be an autoimmune disease. The body, for unknown reasons, attacks and destroys its own insulin-producing cells in the pancreas. The type 1 diabetes variety accounts for less than 10% of all people with

Figure 25.1



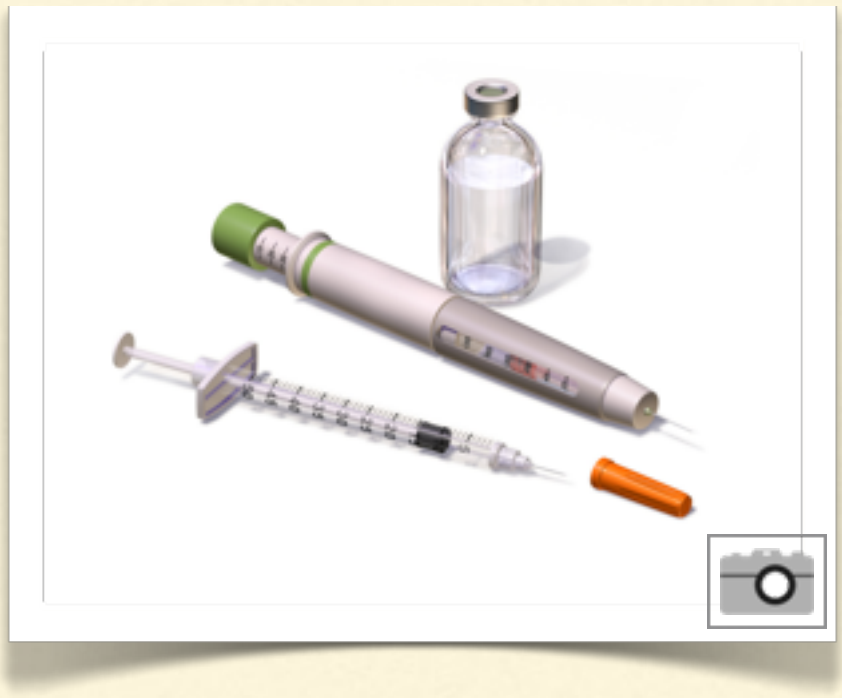
Pancreatic islet
The beta cells are stained green.

the disease.

The more common form of the disease, type 2, formally called "adult-onset diabetes", comes about when the body's cells exhibit decreased sensitivity to insulin. In an effort to compensate, the pancreatic beta cells churn out more insulin, sometimes harming themselves in the process. The result is that some afflicted individuals, while not wholly deficient, don't synthesize sufficient quantities of the hormone.

Type 2 diabetes is often associated with obesity, inactivity, and poor diet, and is increasing in incidence. Individuals with both disorders must monitor their blood sugar levels, regulate their diet, and take regular injections of insulin in order to maintain normal blood sugar amounts (Figure 25.2). Despite these measures, and because it is difficult to adjust the dosage of insulin precisely, serious health conditions arise in patients with the disease.

Figure 25.2



Insulin syringe and pen

A small number of type 1 patients, about 300, have tried to manage their disease by an alternative path. They have had pancreatic islet cells from human cadavers transplanted into their livers. This procedure, the so-called "Edmonton Protocol" has the advantage that when it works, the new cells can sense the level of sugar in the blood and regulate it properly. Almost 2/3 of the transplant recipients respond positively, achieving insulin independence after about a year.

However, a number of factors make this treatment less beneficial then it would appear. For one thing, the scarcity of donors combined with the fact that islet cells must be harvested rather quickly after death limits the amount of useful tissue. This is exacerbated by the fact that the isolation and preparation of the cells is inefficient. Further, immunosuppressive drugs must be used in most cases because of the limited pool of matching donors. These drugs, in turn, may lead to complications.

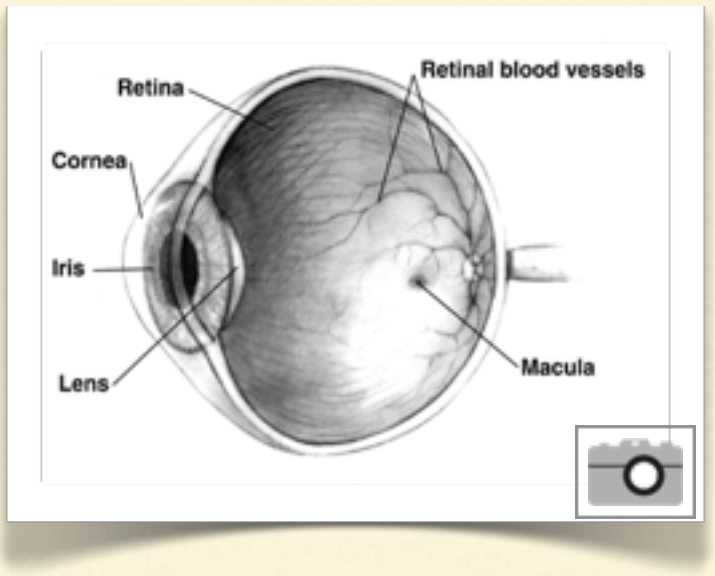
These issues made it obvious that a different source of beta cells would be of huge benefit. Hence the large research effort that has been invested in developing a stem cell therapy for diabetes. Recently, two research teams have announced significant progress along these lines. ViaCyte is a SanDiego, California company that has been working to develop a device that uses embryonic stem cells to deliver insulin to diabetics with type 1 disease. The company takes human embryonic stem cells and causes them to differentiate into insulin secreting cells. They then encapsulate the cells in a device (Figure 25.1) that allows blood plasma to enter, but restricts the movement of cells in and out. Essentially they have made a miniature pancreas that is protected from attack by cells of the immune system. The device can be implanted into the body of a diabetic patient. Phase I trials began in the fall of 2014. No results have been published to date.

The second breakthrough has great clinical implications, but is much less further along in development. Douglas Melton has been searching for a cure for diabetes for 20 years. Both his son and daughter have the disease. After all this time, he and the members of his laboratory at Harvard University have developed a complicated brew of materials that turn either embryonic stem cells or induced pluripotent cells into pancreatic beta cells. By adding 11 different factors and using five different growth media, he succeeded in reproducibly turning pluripotent stem cells in the beta cells. In order to use the cells for treating patients with type 1 disease, he must figure out how to protect them from the same autoimmune reaction that killed them in the first place. ViaCyte's device might prove very useful in this regard. Meanwhile, being able to grow large quantities of the cells in the lab will allow Melton and others to study the molecular basis for the disease.

26 Retinal Diseases

Stargardt's disease, age-related macular degeneration (Figure 26.2), and retinitis pigmentosa are three diseases that affect the retina. They're very common. It's estimated that about one third of all elderly people over 75 show symptoms of one of these conditions to some degree. And although they can be treated, no cures are presently available. It should come as no surprise that stem cells are

Figure 26.1



Cross section of the human eye

excellent candidates to help restore vision for people with these maladies.

The eye has a great advantage in this regard (Figure 26.1). It is surgically accessible. It's relatively straight forward to monitor the movement of introduced cells. It is a small organ, requiring relatively few cells for effective therapy. And, perhaps most importantly, the eye is considered to be immune advantaged, meaning that when foreign cells are introduced into it, they are less likely to be attacked by the body's immune system.

Figure 26.2

Click on the photo to see the same view with macular degeneration (National Eye Institute)

ES Cells

The first attempts to use non-fetal stem cells to help remediate retinal disease in humans were initiated by a company called Advanced Cell Technology (now Ocata Therapeutics Inc). They reported in October, 2014 the results of a phase 1/2 study of nine patients with Stargardt's disease and nine with age-related macular degeneration. The patients were administered 50,000, 100,000, or 150,000 retinal pigment cells in suspension that were derived from embryonic stem cells. After 22 months, the company found no evidence of abnormal tissue growth or teratoma formation. The cells did not cause inflammation nor were they rejected. Encouragingly, patches of pigmented cells were found in the majority of treated eyes compared to untreated ones. And more than half the patients reported an increase in visual acuity. But the results were not dramatic, and no "cure" resulted.

A similar study enrolled four patients, two with Stargardt's disease and two with macular degeneration. Again, no adverse effects were noted. There was slight improvement in visual acuity in three of the four patients, but the sample size was too small to be statistically significant.

iPS Cells

Another group at the RIKEN Institute in Japan led by Masayo Takahashi is using induced pluripotent stem cells to treat wet type age related macular degeneration. On September 7, 2014, one patient, a 77 year

old woman. received retinal pigment cells derived from her own skin cells. The transplant did not appear to harm the patient. RIKEN had originally planned to inject iPS cells into six additional patients, but the project was halted at just one. Apparently, it did not make economic sense to use autologous cells for transplantation. Apparently, it took too long to bring the cells to the point where they could be utilized safely, and it was too expensive. Takahashi is currently working on a proposal to develop a library of retinal pigment cells from a multitude of donors, with the aim of matching cells to patients so that rejection, if it occurs, would be minimized.

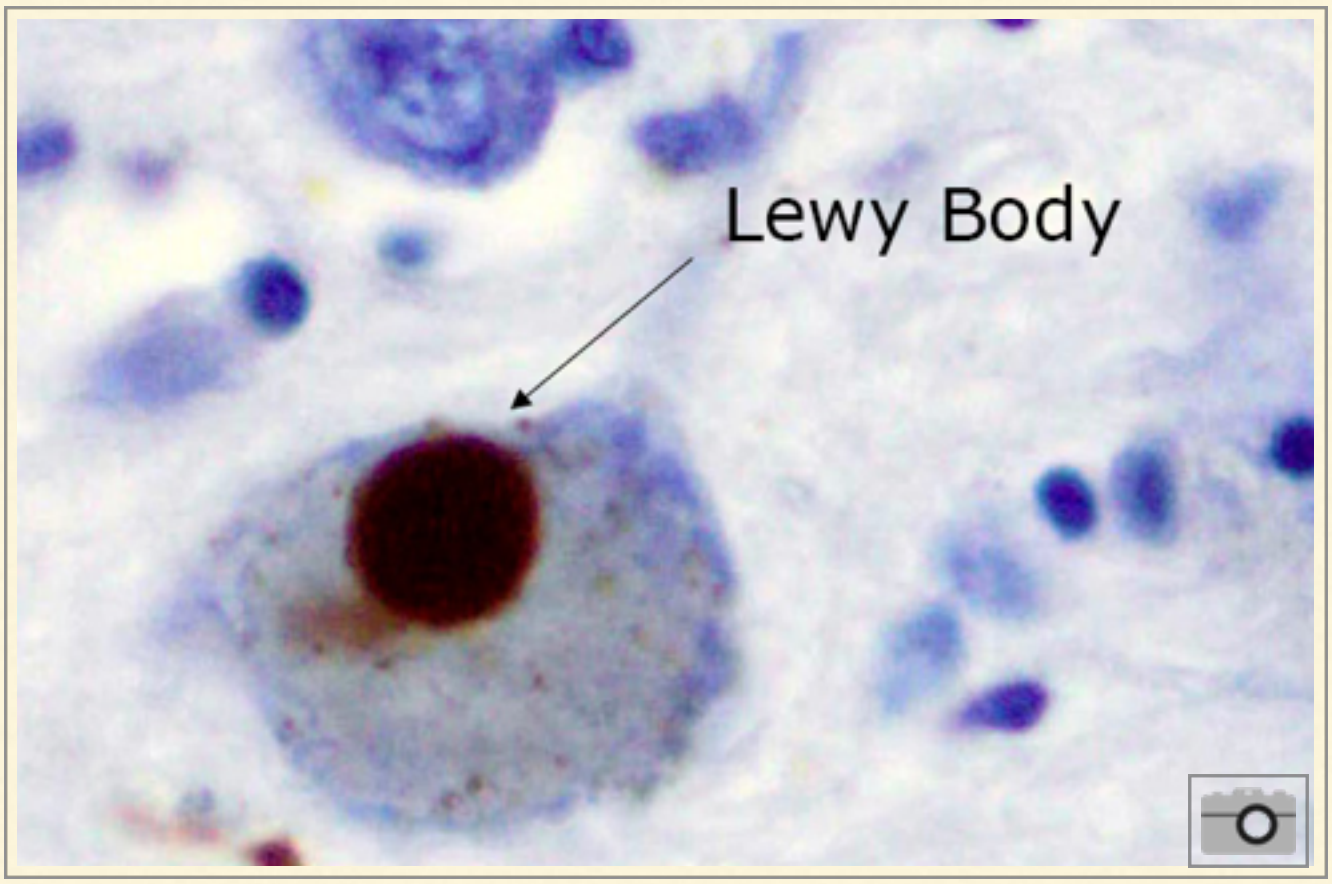
Adult Stem Cells

Adult human central system stem cells have been used in an attempt to remedy macular degeneration. An ongoing phase I/II study conducted by StemCells Inc. has demonstrated that such treatments appeared to be safe and showed a modest degree of efficacy.

Other Studies

Macular degeneration results when pigmented epithelial cells age and don't operate as they normally should. In turn, when these cells fail, the rod and cone photoreceptor cells of the eye become damaged and, eventually, blindness occurs.

For patients with advanced forms of the disorder, where photoreceptor cells have already been lost, transplanting retinal pigment epithelial cells doesn't help. A group at University College in London has succeeded in transplanting photoreceptor precursor cells into mice that lack rods (necessary for vision in dim light), and restoring some night vision, but no clinical trials of similar procedures are ongoing in people.

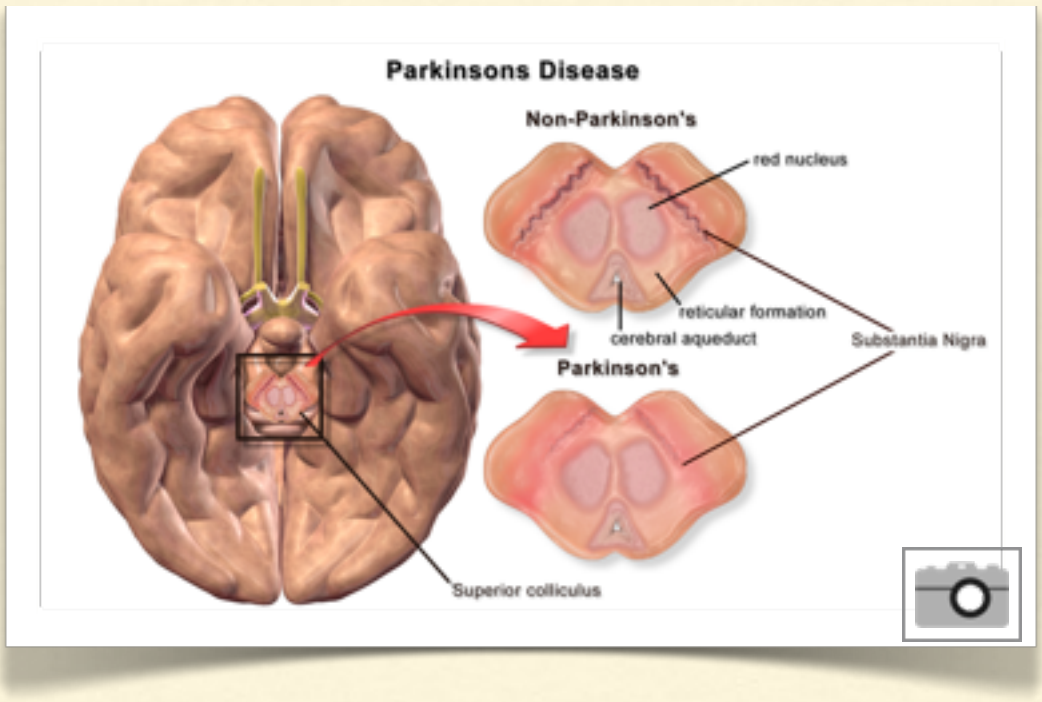


27

Parkinson's Alzheimer's

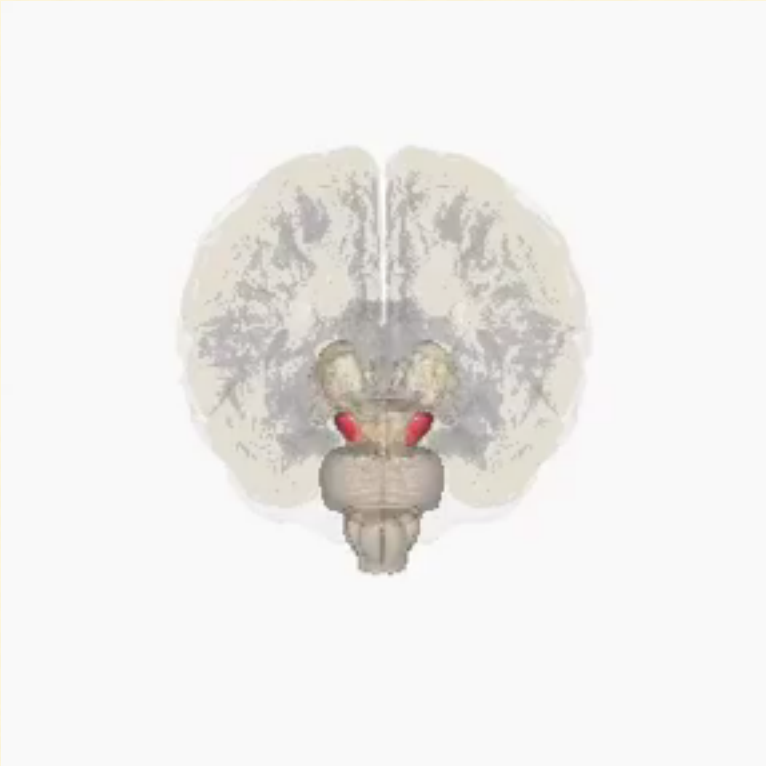
Named after an English physician who first described the disease in 1817, Parkinson's disease affects four to six million people worldwide, about a million in the United States. Some 10-15% of cases appear to have a genetic basis with variations in at least 13 genes traced to various symptoms.

Figure 27.1



Substantia nigra from a normal individual and from a patient with Parkinson's disease

Movie 27.1



Substantia nigra

The disorder is associated with a loss of function of certain cells in an area of the brain called the substantia nigra, (Figure 27.1, Movie 27.1) which produce the neurotransmitter, dopamine. A distinctive feature of the disease is the presence of characteristic inclusions – called Lewy bodies – containing the protein, alpha synuclein, in affected neurons of the brain (see chapter front piece).

Figure 27.2

Front and side views of a man portrayed to be suffering from Parkinsons disease. These are woodcut reproductions published in 1879.

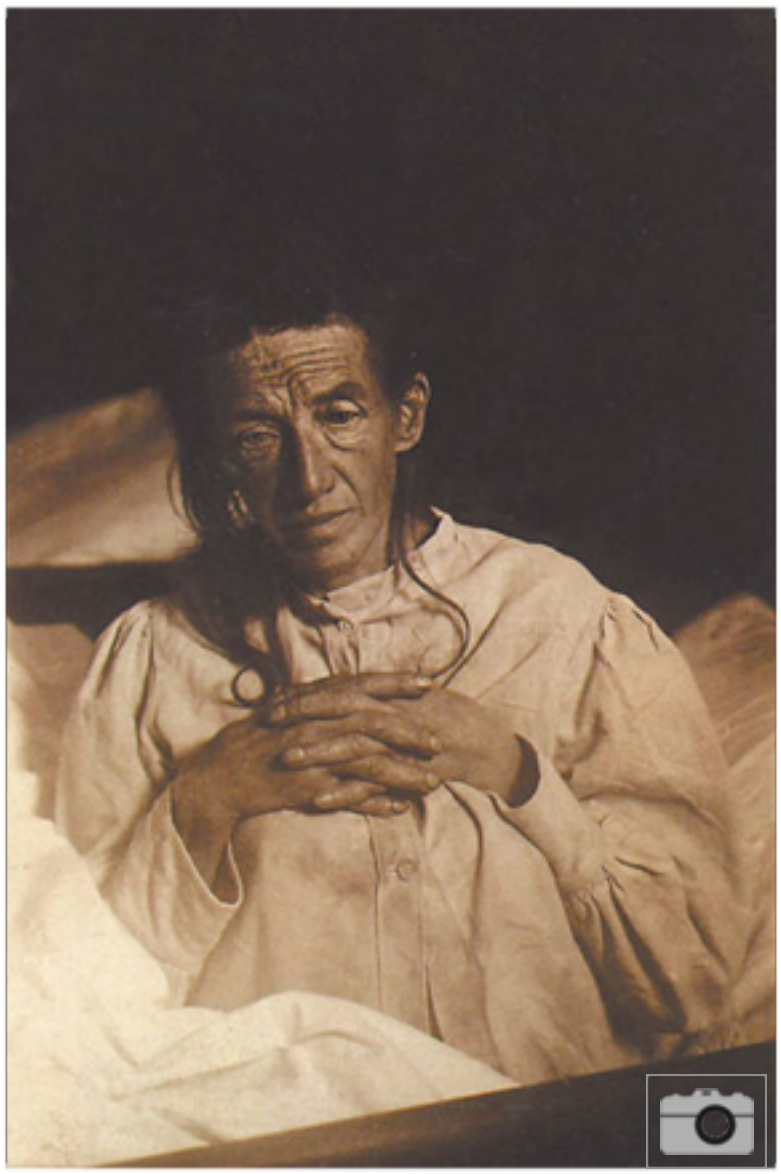
Onset usually occurs after age 50 and is progressive. The symptoms vary, but often include shaking, slowness of movement, and difficulty with walking. In more advanced stages of the disease, dementia occurs, often associated with hallucinations. There is no cure but there are some ameliorative treatments available.

Progenitor cells

derived from fetuses had been used as therapy for Parkinson's disease more than a decade ago. Some encouraging results were obtained. Two patients were followed for 15 and 18 years after transplantation. Both slowly regained motor function and neither has had to take any medication. These results gave proof of principle that stem cell therapy would work. However, the need for tissues from aborted fetuses, some troubling side effects, and the lack of statistically significant improvements (most patients did not get better) made further use of fetal cells untenable. Embryonic and induced pluripotent stem cells have moved into the breech and there have been many recent developments. I'll describe just one.

The results of a study appeared late in 2014 that dealt with the use of human stem cells in rats. It got considerable publicity. The work was done at the University of Lund in Sweden. In these experiments, immunological impaired rats were injected with a substance that destroyed their substantia nigra, thereby making them animal "models" of Parkinson's. They were then injected with human embryonic stem cells that had been coaxed into becoming nervous tissue. According to Malin Parmar the lead investigator in the study:

Figure 27.3

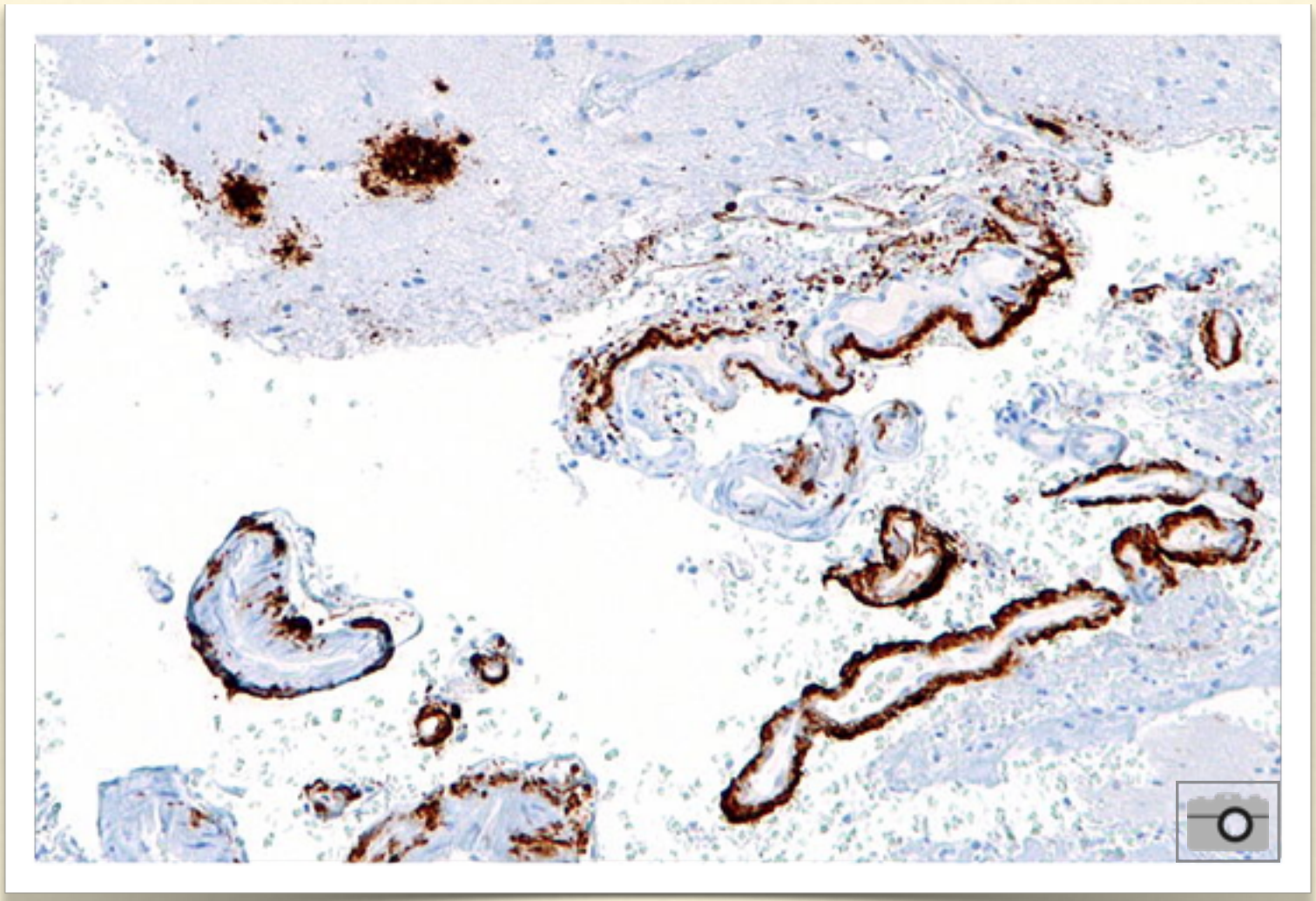


Augusta Deter, the first patient described with Alzheimer's disease. Alois Alzheimer began studying her symptoms in 1901.

"These cells have the same ability as the brain's normal dopamine cells to not only reach, but also to connect to their target area over longer distances. This has been our goal for some time, and the next step is to produce the same cells under the necessary regulations for human use."

Induced pluripotent stem cells would appear to be good candidates for Parkinson's therapy. And indeed, they are being intensively investigated by a group in

Figure 27.4



Amyloid plaques (brown staining regions) from the brain of an individual with Alzheimer's disease

Japan led by Dr. Jun Takahashi. He's the husband of Masayo Takahashi whom we met in the previous chapter. Quite a power couple! Jun planned on using autologous iPS cells as a basis for treatment, but, like his wife, changed course, and it developing a library of allogenic cells. This shift in strategy has delayed the project.

A number of other laboratories are preparing to use induced embryonic stem cells to treat Parkinson's. However, as of December, 2014 no clinical trials had begun.

Alzheimer's Disease

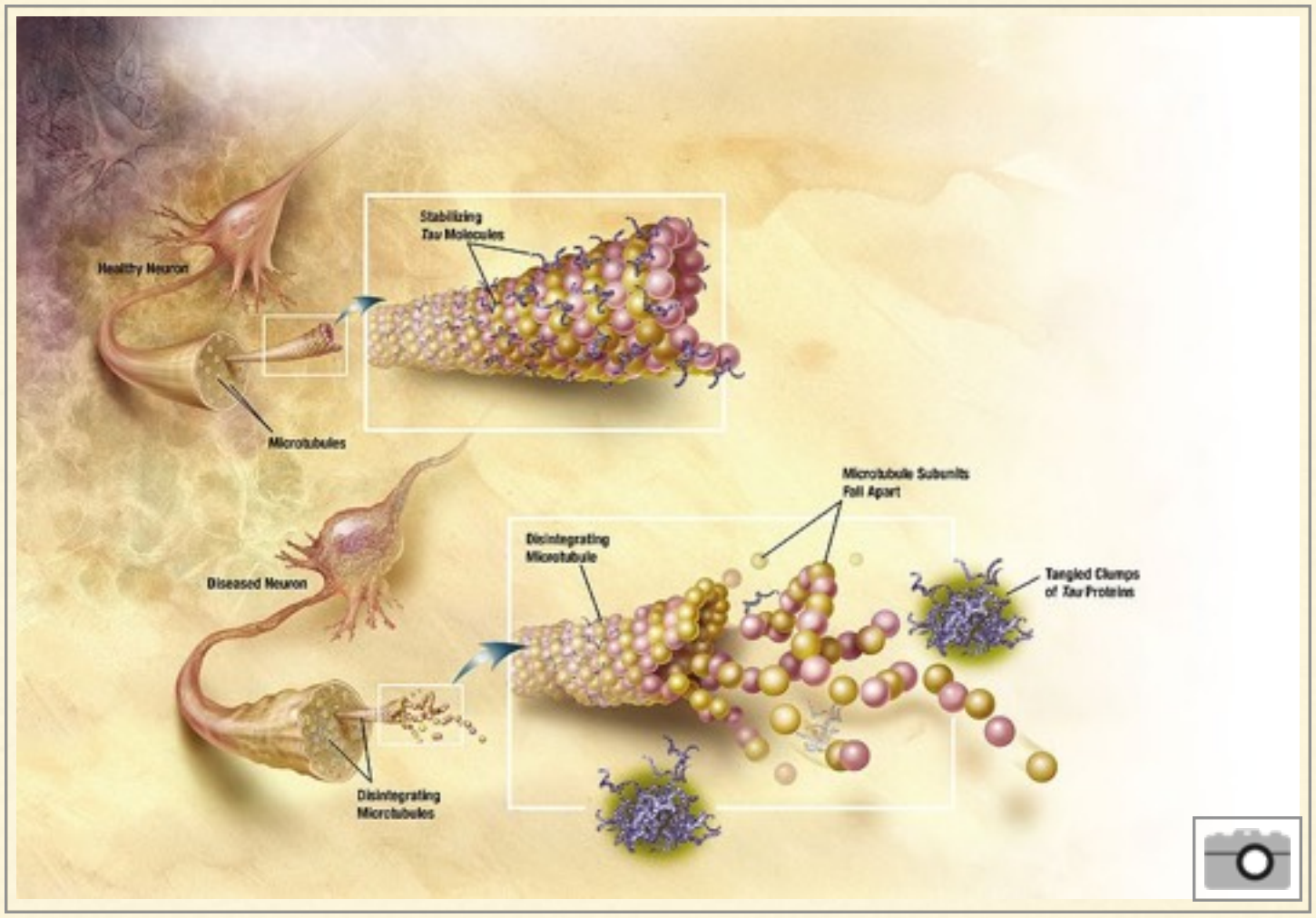
This devastating neurological disorder is the most common form of dementia, affecting an estimated 100 million people or more throughout the world. It is named after a German psychiatrist, Alois Alzheimer, who described a 50 year old woman with the disease in 1901 (Figure 27.3). Typically, Alzheimer's symptoms start to appear after the age of 65, but an uncommon form of the disease, called "early onset", may begin 15 or 20 years sooner. Late onset or sporadic Alzheimer's disease increases in incidence with age.

Some estimates are that nearly half of all people over 85 will show symptoms of the disorder. Since the average lifespan in developed countries is approaching this number, the economic and social consequences are terrifying.

protein), a protein whose function whose function is currently unknown but is being actively investigated. The fragments, called amyloid beta, or Aβ, originate from enzymatic cleavage of APP.

The tangles, also first found by Alzheimer,

Figure 27.5



Tau protein - Normal function and formation of neurofibrillary tangles

At the cellular level, Alzheimer's is usually characterized by the presence of two abnormal structures: amyloid plaques and neurofibrillary tangles. The plaques are found in the spaces between diseased and dying neurons (Figure 27.4). First associated with the disease state by Alzheimer, they are aggregates of fragments of APP (amyloid precursor

are, unlike plaques, found *inside* nerve cells. They are composed of aggregates of a protein called "tau". Tau is a normal constituent of microtubules (Chapter 3). However, in Alzheimer's disease an abnormal number of phosphate groups get attached to tau and it aggregates to form the tangles characteristic of the disorder (Figure 27.5).

While there is not unanimity in the scientific community, many investigators feel that amyloid beta is the major culprit in the development of Alzheimer's disease. One piece of evidence is that that the gene responsible for APP synthesis is located on chromosome 21 in humans. People with Down Syndrome (caused by the presence of a supernumerary 21st chromosome) and who therefore produce an excess of APP often fall victim to Alzheimer's disease before the age of 50. The observation that many people with early onset Alzheimer's exhibit mutations in the APP gene is additional evidence for APP's role in the disorder.

However, while they no doubt play some role, it is not clear that the plaques themselves are the proximate cause of the disease. One hypothesis that is gaining traction in the medical community is that Aβ fragment dimers, trimers, and other oligomers are the toxic principle that kill nerve cells. The plaques, which represent much larger aggregates of the Aβ fragment, may not be as dangerous. They may be consequences of the disease rather then a causative factor.

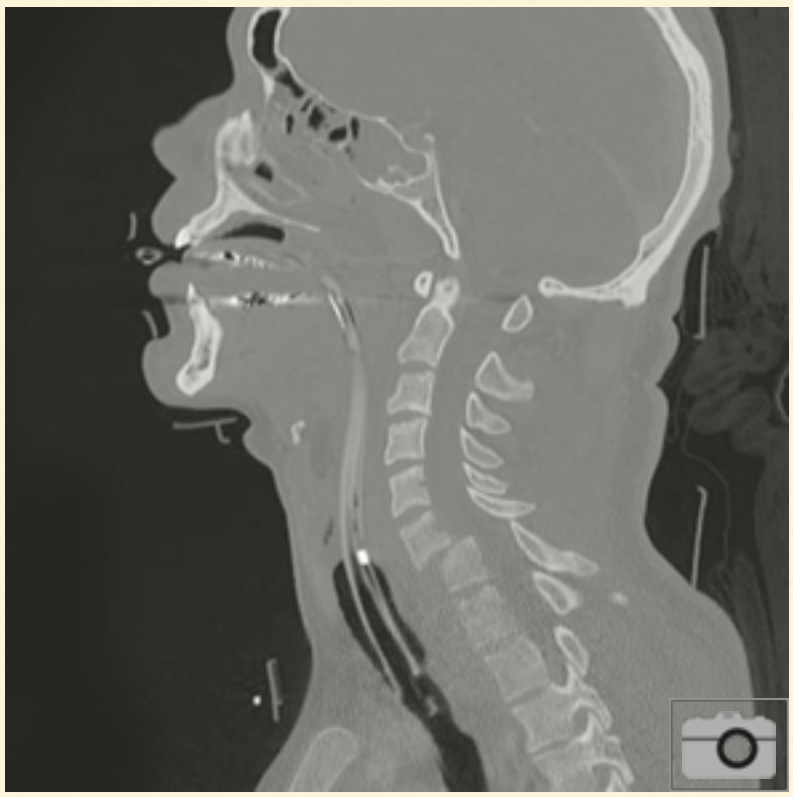
As for tau, one hypothesis is that the increased amount of Aβ oligomers leads, in some manner, to an increase in phosphorylation and the consequent aggregation of tau into the tangles seen in the interior of diseased cells. Exactly how this occurs isn't known.

It should be clear that we have along way to go in understanding the basis for Alzheimer's disease. That hasn't stopped people from trying a variety of treatments, including using stem cells. But stem cell therapy poses a number of challenges. For example, if healthy neurons are introduced into a diseased brain, it isn't clear that they would integrate properly. There is the gruesome possibility that if they do, they may disrupt the connections already established, thereby wiping out previous memories.

A possible alternative would be to make use of the homing property of stem cells to deliver drugs to the brain of afflicted individuals. The idea would be to load stem cells with therapeutic agents that could be precisely delivered to selected portions of the nervous system (See chapter 29).

The future of stem cell therapy is uncertain, but it is clear that stem cells are already being effectively used as a model system to study the molecular basis of Alzheimer's. Scientists are removing cells from adults with either the early onset or sporadic form of the disorder, turning them into stem cells via Yamanaka's

protocols, and then changing these iPS cells into neurons that can be grown and studied in culture.



28

Spinal Cord Repair

Injury to the spinal column commonly comes from motor vehicle accidents (42%), falls (27%), violence (15%), and sports activities (8%). Most of the injured are men, with an average age in their early thirties. About 12,000 such

Figure 28.1

Christopher Reeve, at a conference at MIT, in which he discussed the potential benefits of stem cell therapy.

incidents occur in the United States yearly, and hundreds of thousands of Americans live with spinal cord damage of

some kind. World wide, the numbers are even more staggering.

When severe, spinal cord trauma may be debilitating, producing loss of mobility and lack of sensation. If the injury occurs in the neck, the entire body may be paralyzed. Such an injury was suffered while horseback riding by Christopher Reeve, the actor who is known primarily for his role in the movies as Superman (Figure 28.1). Spinal injuries that occur below the neck may not result in total paralysis, sparing the arms, but paralysis and lack of sensation may occur in the legs, and impaired bowel and sexual functions may occur.

Prior to 1945, people with severe spinal cord injuries were restricted to wheel chairs or bedridden, and the resultant inactivity caused problems in breathing and blood clotting, as well as sores and infection. A shortened life expectancy was the inevitable outcome. More recently, many of these difficulties have been alleviated, and mortality rates have decreased dramatically. But for most injuries to the spinal cord, full restoration of activity cannot be achieved. Stem cell treatments offer the possibility of restoring mobility and feeling.

Mesenchymal Stem Cells

Most of the clinical trials aimed at remediation of injury to the spinal cord have utilized adult mesenchymal stem cells derived from bone marrow. These cells are relatively easy to harvest from the injured patient. Because they are autologous, there is little chance of immunological complications and no need for the use of immune suppression. A smaller number of studies used fetal stem cells or mesenchymal stem cells that are derived from tissues other than bone marrow.

Some 14 phase I trials have been held between 2006 and 2013. A total of nearly 900 people have been treated. Overall, the procedure has proved relatively safe, with few adverse effect. But the small numbers in each trial limited the ability of the investigators to assess the statistical significance of the treatment. However, what little evidence we have suggest that there were some improvements, mostly among those patients that didn't have complete paralysis of lack of sensation. This is encouraging news.

One more point that I've made before but one that bears repeating. The introduced cells seemed to work not because they ingrafted into the injury, but rather because they appeared to move to the site of injury (or were placed there) and

secreted one or more substances that aided in recovery (See the next chapter).

Embryonic Stem Cells

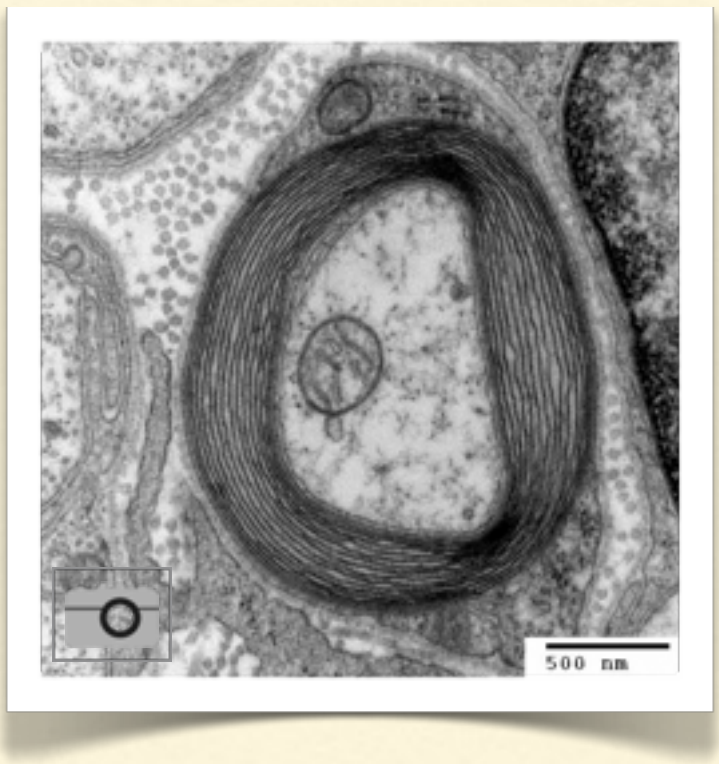
The very first clinical trial of an embryonic stem cell therapy ever carried in the United States was begun in 2009. The therapy being evaluated was directed at spinal cord injury. The Geron Corporation in Menlo Park, California, was using a therapy (developed by Hans Keirstead of the University of California at Irvine) consisting of embryonic stem cells that had been turned into oligodendrocyte precursors, a type of cell that builds the myelin sheath around nerves (Figure 28.3).

Tests in rats injected with cells shortly after injury were very encouraging. On the basis of these animal trials, Geron proposed a phase 1 study in humans. The preliminary experiments leading up to the trial, the company said, cost $45 million. Their application to the FDA ran to 22,000 pages. When finally approved, the first patient was a young man from Alabama named T. J. Atchison who had been injured in an automobile accident.

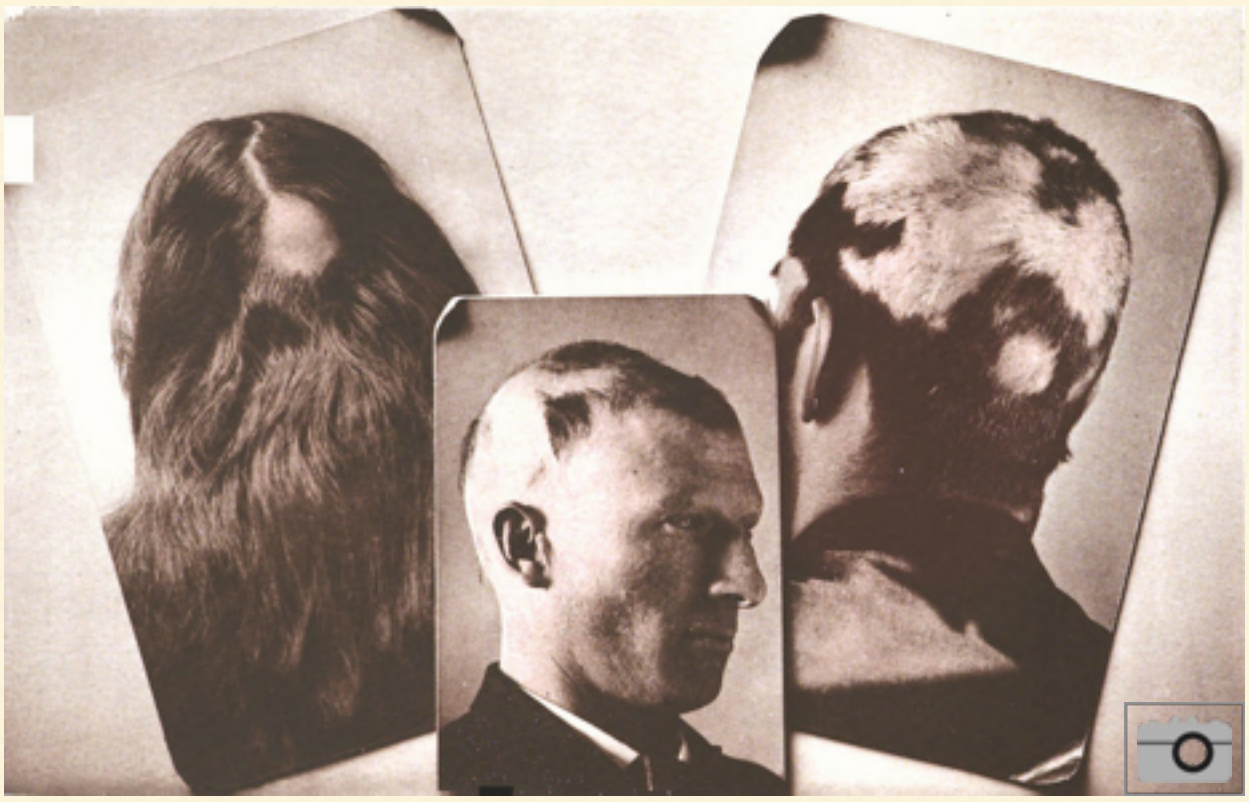
This tale does not have a storybook ending. The good news is that the procedure didn't do T.J. any harm. It was feared that the stem cells might form tumors. They did not. The bad news is that Atchison has not recovered.

Nevertheless, the outlook for remediation of spinal cord injury is bright. Rodents have shown improvements after human embryonic stem cell transplants. And, as noted, the procedure seems safe in humans. But more research is needed to understand how the body responds to injury; how (and if) the body's own stem cells function in repair and whether this can be augmented; what the best timing is for administering the cells; and what kind of cells are most effective for transplantation.

Figure 28.2



Transmission electron micrograph of a myelinated axon. The myelin layer (concentric) surrounds the axon of a neuron.



29

Immuno-modulation Drug Delivery

In addition to their proven use in bone marrow transplants, there have been numerous reports of other successful outcomes after stem cell therapy. However, in many cases when clinicians looked to see if the introduced cells had directly contributed to the organs or tissues being treated, they were surprised to find that they had not. In particular, the effectiveness of mesenchymal stem cells, at least in some cases, seems to derive from their ability to home to sites of injury and to secrete potent molecules that modulate inflammation and promote growth. Chronic obstructive pulmonary disease (COPD), asthma, emphysema, pulmonary fibrosis, and lung injury cased by radiation are conditions in which mesenchymal stem cells were introduced, found to not significantly engraft, yet relieve symptoms. Clinical trials are underway that make use of this property of stem cells to treat a variety of disorders.

Because stem cells, particularly mesenchymal stem cells, preferentially migrate to damaged tissues, Some scientist

have proposed using them to precisely deliver pharmaceuticals to injured sites. More than 50 proposed, ongoing, and completed clinical trials are listed on the government's web site (clinical trials.gov) that take this approach.

Even more exciting is the observation that mesenchymal stem cells traffic to sites of tumor growth. Recently, a collaboration between groups at Johns Hopkins and the Harvard Medical School devised a means of loading mesenchymal stem cells with the drug proaerolysin, a protein obtained from bacteria (3D Molecule 1). Proaerolysin is harmless, but when cleaved by a proteolytic enzyme it becomes extremely poisonous and will kill cells. The scientists involved with this project modified the toxin through genetic engineering such that cleavage can only be carried out by prostate specific antigen (PSA) - a proteolytic enzyme found, as its name indicates, in the prostate gland. The idea is that loaded mesenchymal stem cells will be attracted to sites where prostate cells have proliferated and deliver proaerolysin into the tumor. Once inside the cell, the inactive toxin will be activated by cleavage by PSA. Even if the prostate tumor has metastasized, the proaerolysin will still work as long as the cells carry PSA. In addition, the scientists point out that PSA in circulation in the blood is inactive due to the fact that it is complexed with specific blood borne inhibitors. Because of that, damage to non-prostate tissue should be minimized.

3D Molecule 1

Proaerolysin
Image from the Protein Date Base (1Pre)

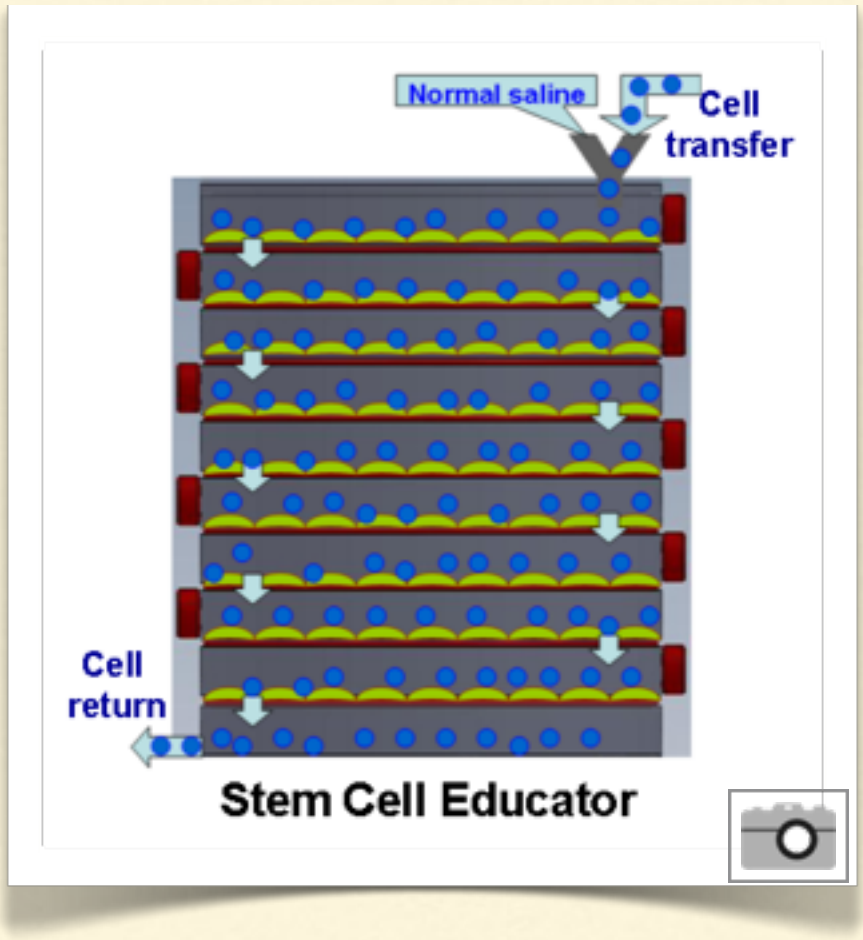
This is an ingenious, but untested strategy. No clinical trials have yet been set up. While the therapy is still quite experimental, it demonstrates the great

potential of using stem cells for drug delivery.

Still another approach that uses the immunomodulatory abilities of stem cells, this time umbilical cord cells, is also under investigation. It's called "Stem Cell Educator Therapy" and was developed by Dr. Yong Zhao from the University of Illinois at Chicago (Figure 29.1). Intended to help people afflicted with autoimmune diseases, it makes use of a device called a "Stem Cell Educator". In Zhao's own words:

"We developed a procedure for Stem Cell Educator therapy in which a patient's blood is circulated through a closed-loop system that separates lymphocytes from the whole blood and briefly co-cultures them with adherent CB-SCs [cord blood stem cells] before returning them to the patient's circulation."

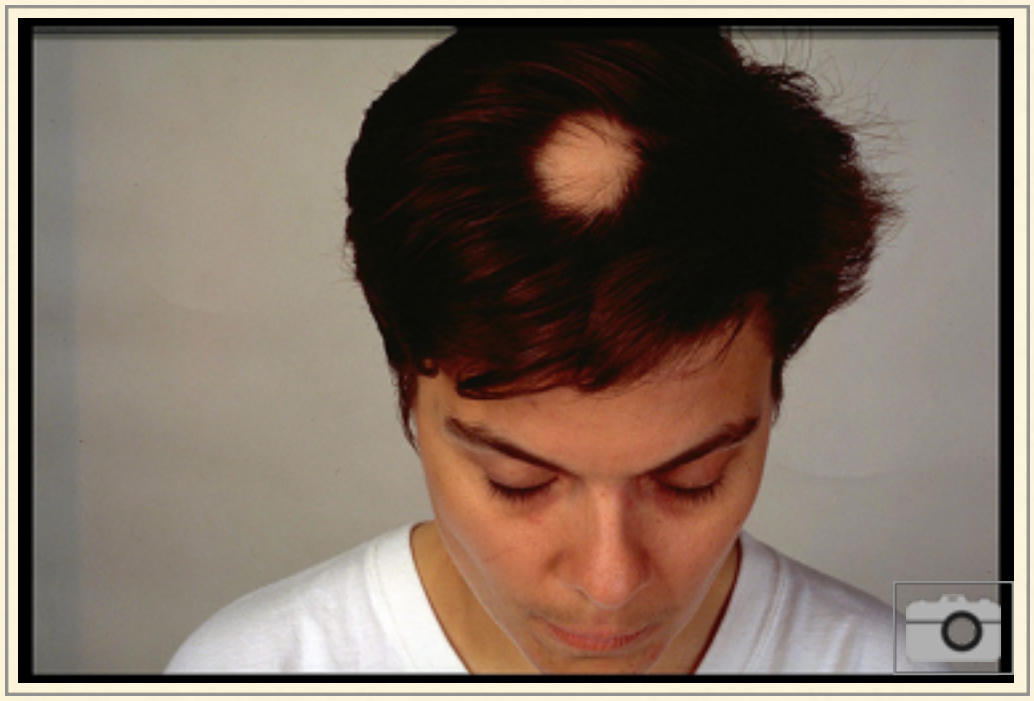
Figure 29.1



Stem Cell Educator Apparatus

In other words, lymphocytes from the patient are fed into the top of the educator apparatus, exposed to cord blood stem cells, and collected at the bottom. The cord cells are retained. The idea is that this treatment changes the behavior of the immune system of patients with autoimmune diseases like type I diabetes so that they no longer react with the patients own insulin producing cells. Phase I/II clinical trials were reported to be quite successful in remediating both type I and type II

Figure 29.2



Patient with alopecia areata

diabetes.

Another autoimmune disease that has been successfully treated with educated stem cells is alopecia areata, a condition that manifests itself as bald patches on the head of afflicted individuals (Figure 29.2). A clinical trial that enrolled a total of nine individuals resulted in improvement in eight patients. The hair of two completely bald patients was fully replaced and retained after two years.

30

Disease Modeling

Modeling human disorders in animals like mice, rats, and non-human primates has been a classic strategy for understanding the underlying mechanism of specific diseases as well as for developing and testing new therapies. But of course, the response of these animals to various pharmaceutical agents may be differ from that of humans. That's one of the reasons that animal models are not always successful in predicting how drugs will work in the clinic. Human stem cells taken from diseased individuals and placed in culture provide alternative routes toward understanding the basis of disease and for testing of new drugs. While this tactic too suffers from some disadvantages, it offers new possibilities, particularly for investigating the pathology of complex diseases that affect the nervous system. Embryonic stem cells have been used to model two devastating neural disorders: Huntington's disease and fragile X syndrome. Both disorders originate from mutations that result in the expansion of a sequence of three bases in DNA.

Huntington's Disease

Huntington's disease or Huntington's chorea is a neurodegenerative disorder effecting about 30,000 people in North America. It is progressive, first resulting in loss of muscle coordination, then the ability to talk, walk, and eat. Eventually it causes dementia and death. The initial time of onset varies, but it usually manifests itself between 35 and 45 years of age, although rare individuals may exhibit first signs of the disease in their 20's and some as late as their middle 50's. Life expectancy after the onset of symptoms is about 25 years.

Huntington's disease is a genetic disorder. Only one defective copy of a specific gene, called "huntingtin", is necessary for the disease to manifest itself. The huntingtin gene is located on the fourth chromosome in humans. It encodes a protein that is over 3,000 amino acids long. The gene has an interesting feature: it carries a repetitive sequence of the bases GAC near the beginning of its coding sequence. GAC codes for the amino acid glutamine (designated by the single letter "Q"), and

Figure 30.1

```
MATLEKLMKA FESLKSFQQQ QQQQQQQQQQ QQQQQQQQPP PPPPPPPPPQ
        60          70          80          90         100
LPQPPPQAQP LLPQPQPPPP PPPPPPGPAV AEEPLHRPKK ELSATKKDRV
       110         120         130         140         150
NHCLTICENI VAQSVRNSPE FQKLLGIAME LFLLCSDDAE SDVRMVADEC
       160         170         180         190         200
LNKVIKALMD SNLPRLQLEL YKEIKKNGAP RSLRAALWRF AELAHLVRPQ
       210         220         230         240         250
KCRPYLVNLL PCLTRTSKRP EESVQETLAA AVPKIMASFG NFANDNEIKV
       260         270         280         290         300
LLKAFIANLK SSSPTIRRTA AGSAVSICQH SRRTQYFYSW LLNVLLGLLV
```

Partial Sequence of the Huntington Protein
The letters indicate the various amino acids in the beginning of the protein. The sequence of 21 red "Q"s at the top are expanded in effected individuals.

the protein has a run of glutamines near one end as indicated by the red "Q"'s in Figure 30.1. Normal individuals have about 25 to 35 consecutive GAC's in the huntingtin gene while those who suffer from Huntington's disease may carry 40 to 100. There is a correlation between the number of repeats and the age of onset of symptoms.

It's not known why the increase in the number of glutamine amino acids within the huntingtin protein results in the death of nerve cells. To investigate that issue, two embryonic stem cells derived from blastocysts with moderate expansion of CAG repeats were obtained after *in vitro* fertilization. Interestingly, almost all parameters that they examined were normal. This is important information and their study illustrates the potential of using these kinds of cells for understanding the molecular basis of the disease.

Figure 30.2

Boy with Fragile X Syndrome

Fragile X Syndrome

Fragile X syndrome is an inherited disease that is the most widespread cause of autism and intellectual disability among boys. Large protruding ears, elongated faces, and low muscle tone are common characteristics of the disorder. Individuals exhibiting the fragile X syndrome usually have more than 200 repeats of the sequence CGG in the FMR1 gene, while normal individuals carry only about 30. Unlike Huntington's disease, the repeats in the FMR1 gene do not affect the amino acid sequence of its encoded protein. Instead the increased number of repeats seem to attract an enzyme that modifies DNA. The modification is believed to constrict the DNA (the "fragile" part of the name derives from this phenomenon) and shut down transcription of the gene. When an embryonic cell line was established from a

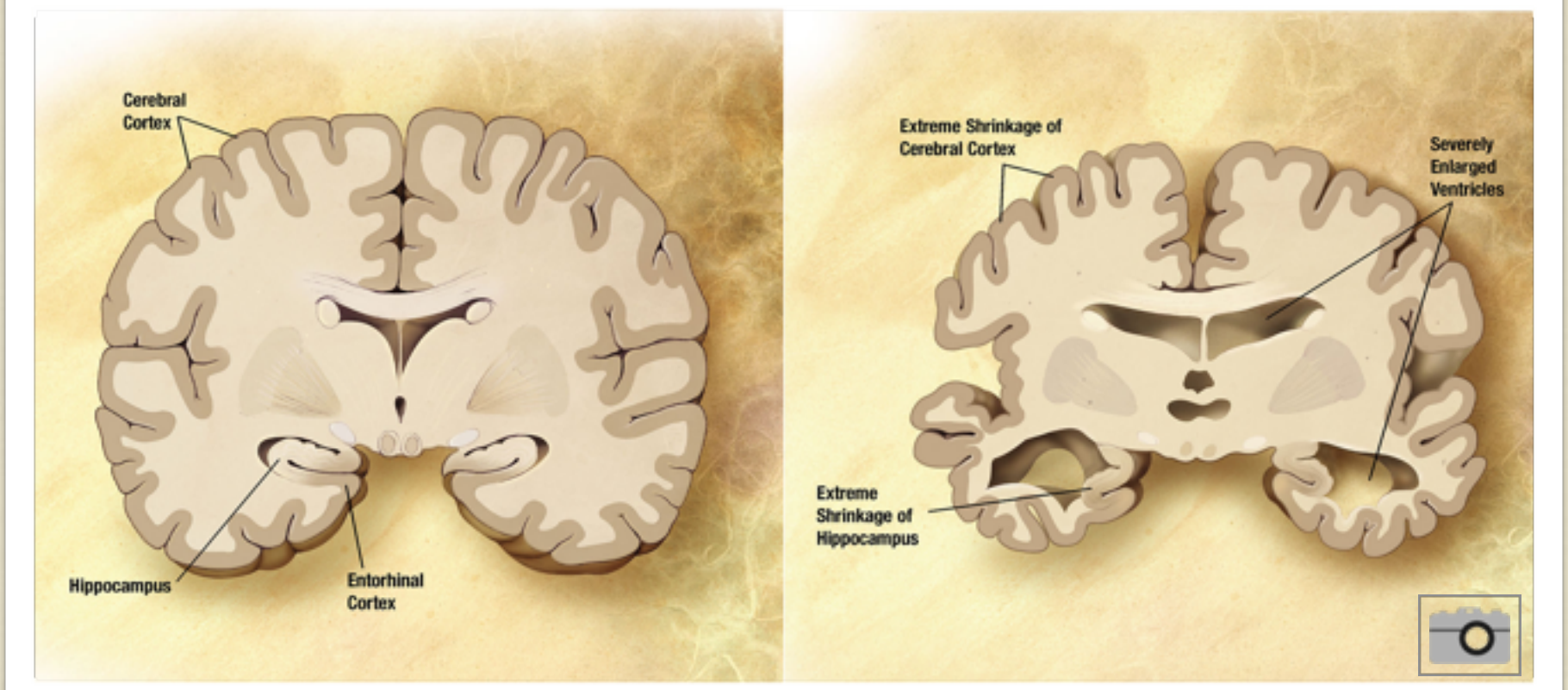
fragile X blastocyst, it was found that the FRM1 gene's transcription was unaffected. Only when the cells differentiated into neurons did shutdown occur. Again, this observation would have been much more difficult to make without the existence of a stem cell line in which to study the disease at its earliest stage.

pluripotent stem cells offer the possibility of taking tissue directly from affected individuals of any age, even when the underlying genetic basis is unknown.

Alzheimer's Disease

Alzheimer's disease is a good example. I've already covered some of the characteristics

Figure 30.3



Comparison of the brain of Alzheimer's patient (right) with that of an unaffected individual

While embryonic stem cells have proved helpful for modeling genetic diseases caused by a mutation in a single gene, they are less useful for studying sporadic disorders or for those with a complex genetic basis. That's because embryonic cells are not available for culture from diseased children or adults. Induced

of this devastating condition in Chapter 27. As mentioned, a cure for the disorder hasn't been developed, using stem cells or other treatments. However, stem cells do offer the possibility of investigating the biochemical basis for the disease. Most cases of Alzheimer's disease are sporadic and of unknown origin, but a few have a

well established genetic foundation. A group from Japan (Kondo et al., 2013) studied a genetical determined early onset form of the disease. The particular disease variant that they investigated is caused by a rare mutation in the APP gene itself (most genetic forms of Alzheimer's disease are caused by mutations in the enzymes that cleave APP). Unexpectedly, demented individuals carrying the mutation don't exhibit plaques in the cells of their brains. They do, however cleave APP. Pieces of the cleaved mutant APP protein were found to bind together, although in very small aggregates (oligomers); so small as to not be visible as clumps.

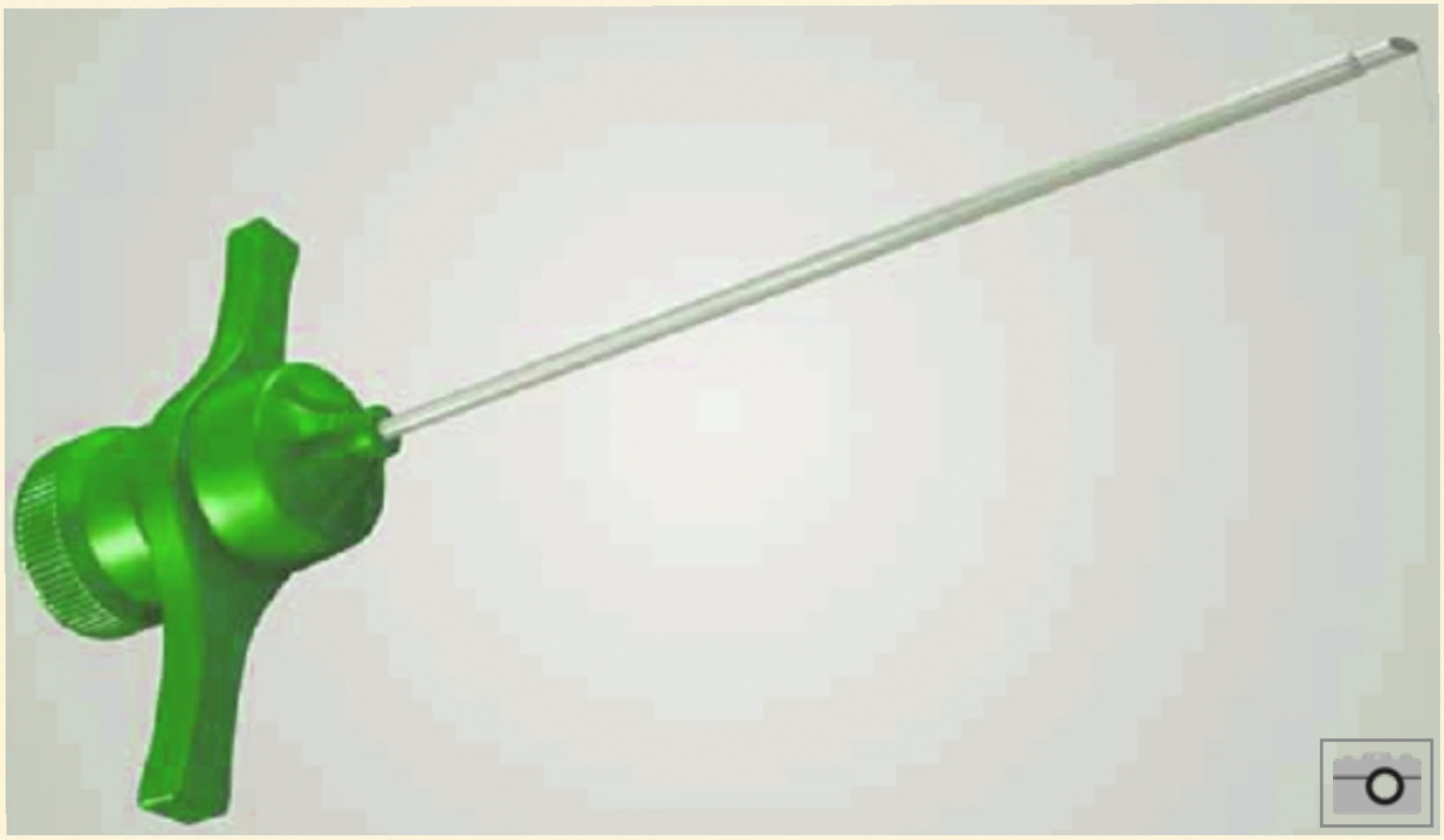
Cleavage of amyloid precursor protein (APP) into Aβ. The APP protein is embedded in the cell membrane. Two enzymes, β- and γ-secretase, cleave the protein into the Aβ fragment.

To model the disease, iPS cells from a single individual carrying the mutation were turned into neurons. When these were examined it was found that they had high levels of oligomers of APP. And when these neurons were tested, they were found to be in distress as evidenced by an increase in the level of enzymes that are elevated upon stress. These results indicated, in agreement with previous studies, that it is not the amyloid plaques themselves that cause Alzheimer's disease, but smaller aggregates of the APP protein.

Another promising line of inquiry comes from work done by Drs. Rudolph Tanzi and Doo Yeong Kim, researchers from the Massachusetts General Hospital, Harvard Medical School, in Charlestown, Massachusetts. They were examining human embryonic stem cells that they had turned into pre-Alzheimer's nerve cells in culture by the addition of two familial Alzheimer's genes. Try as they might, they could not get the cells to make the tangles and plaques characteristic of the disorder. In desperation, they decided that the fault was the liquid medium in which the cells were growing. Perhaps, they reasoned, if the cells were grown in a semi-solid gel, more like the environment of the brain, they would behave as expected. It worked! Within six weeks they could detect the presence of plaques. Two weeks later, tangles appeared. Moreover, if the formation of Aβ oligomers were blocked using antibodies, the tangles failed to appear.

Tanzi and Kim's work lend additional support to the view that Aβ production is the driving force behind Alzheimer's disease, producing not only plaques but tangles as well. Because it is much easier to assay the effect of potential drugs on cells

in culture rather then in the brain itself,
their work may allow for faster
development of treatments.



31 Veterinary Uses

It's proved relatively easy to employ stem cells for therapeutic purposes in non-human animals because the regulatory environment isn't as stringent. In the United States the Food and Drug Administration (FDA) oversees the use of stem cells in humans. While they have responsibility for doing the same for those practicing veterinary medicine, the regulations on the use of cell based products in animals have been unclear. However, in late July, 2014, the FDA issued a draft document entitled, "Guidance for Industry Cell-Based Products for Animal Use". A summary of the major points covered by the document appeared in the draft guidance:

• Clarifies FDA's position that it has jurisdiction over cell-based products meeting the definition of a new animal drug;

• Clarifies FDA's current thinking on how existing regulations apply to cell-based products;

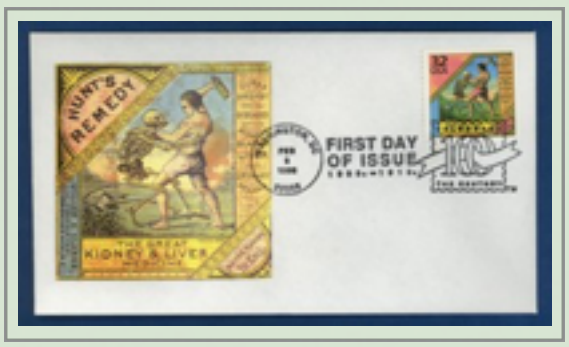
• Establishes a common vocabulary, including definitions, for cell-based products;

• Establishes a risk-based category structure for cell-based products; and

- **Encourages industry to communicate and interact with FDA early in product development."**

FDA

It's not surprising that guidelines for the use of stem cell therapy in animals have taken so long time to develop. The FDA whose origins trace to the 19th century, first began regulating drugs in 1906.



They originally monitored the efficacy and safety of relatively simple chemical

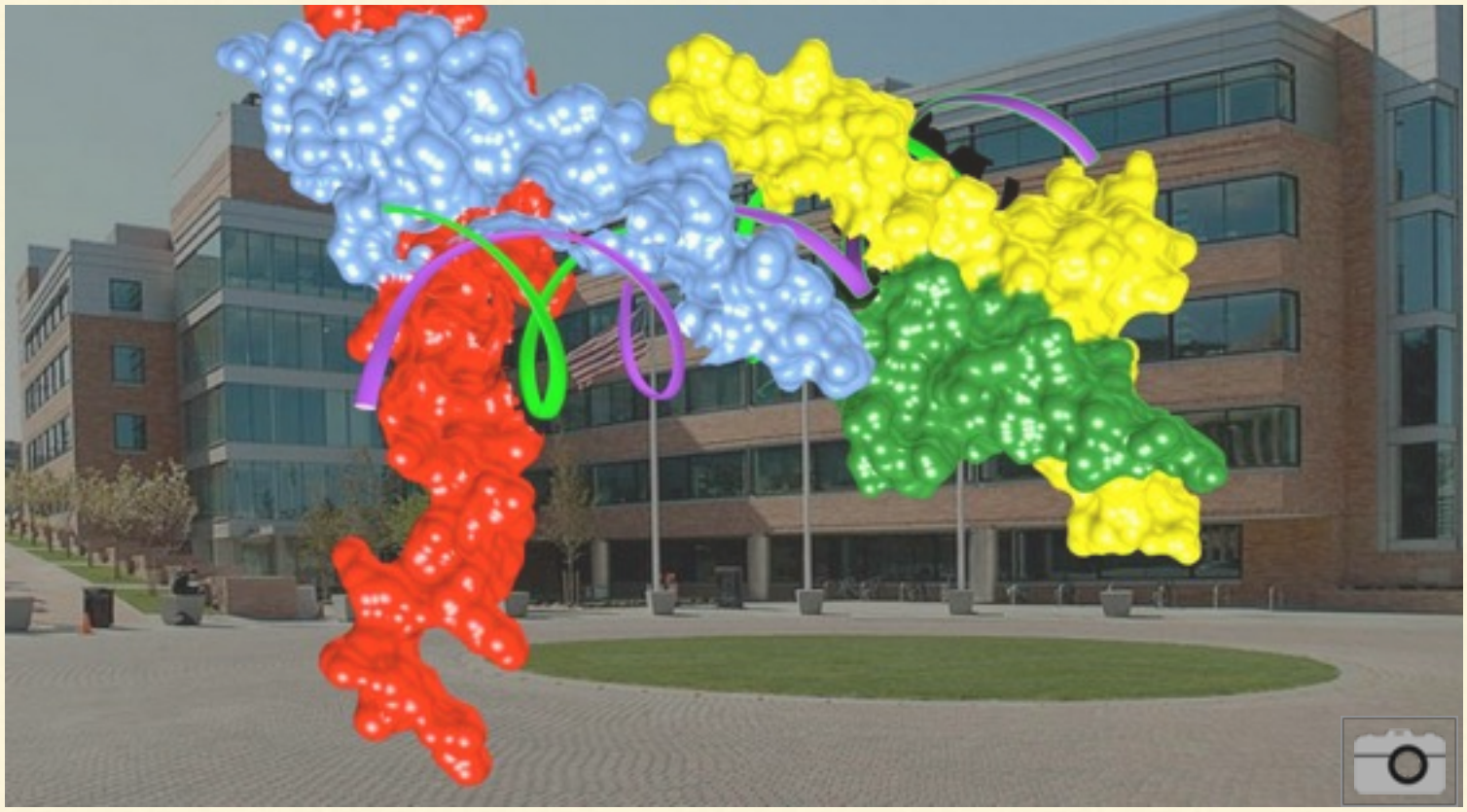
The FDA requested that responses to its draft guidance be sent to the agency by September, 2014. As of May, 2015, no official document had been issued.

The previous lack of regulation meant that in many cases carefully controlled studies were often neglected. That's unfortunate because companion animals (dogs, cats, and horses) are capable of serving as models for a variety of human diseases. For one thing, they have a longer lifespan than mice or rats. And their physiology more closely resembles humans than it does that of rodents. Without data from properly conducted studies, reliance is often placed on anecdotal evidence meaning that effective treatments might be missed and ineffective ones might go undetected.

Because of this lack of regulatory oversight, stem cell therapies in companion animals have mushroomed. Numerous commercial enterprises have sprung up to offer stem cell services to people responsible for animal care. For example, Vet-Stem, a California company that claims to practice regenerative medicine, has treated 8,000 horses, dogs and cats since opening in 2004. Another firm, MediVet America, provides kits for stem cell injections in horses. These therapies have not been restricted to farm animals and pets. Bottle nose dolphins, pigs, and even tigers have also been treated with stem cells.

Most treatments use mesenchymal stem cells obtained from a variety of sources. As discussed previously, it isn't clear that the transplanted cells actually engraft. There is a reasonable probability that most of the reported successful results have been due to secondary effects. Nevertheless, as in humans, it appears that the treatment isn't harmful and may have some efficacy. In one of the most cited studies, some 141 racehorses from the United States and United Kingdom were treated with

autologous mesenchymal stem cells for flexor tendon injury. They were followed for three years after they were treated. There was no control group, so accurate comparisons are difficult, but the treated horses appeared to have done better after a return to racing then previous generations of horses treated in more conventional ways.



32

Trans-differentation

Transdifferentiation

While all types of stem cells seem destined to be useful for therapy, induced pluripotent stem cells have particularly bright prospects. A few mature cells can be removed from an adult who suffers from a disorder, turned into embryonic-like cells with the Yamanaka protocol, and subsequently coaxed into differentiating into cells that may be used to repair that malady. Unfortunately, at present this procedure is both complicated and lengthy. Scientists have wondered whether it could be simplified and short circuited. Why not take ordinary cells, from skin or connective tissue for example, and directly guide them to a specific fate without first forcing them through an embryonic-like state?

Turning one kind of differentiated cell into another without going through an embryonic stage has been variously called “transdifferentiation” , “direct reprogramming”, or “lineage reprogramming”. It has several potential advantages over the use of induced pluripotent stem cells for therapy. It can

be faster. It can be simpler, requiring one step instead of two. And because the cells never appear to become embryo-like, there would seem to be less danger of tumor formation.

The first such conversion was reported way back in 1987 in a publication that emanated from the Fred Hutchison Cancer Center in Seattle. Harold Weintraub and associates introduced a gene called MyoD into mouse fibroblasts and succeeded in turning them, in one step, into cells that resembled muscle. Weintraub later found that the MyoD gene encoded a protein that was a transcription factor (an illustration of the MyoD protein against a backdrop of the Fred Hutchison Cancer Institute is shown in the figure at the head of this chapter). In fact, MyoD is a master regulatory protein that proved capable of directing several kinds of differentiated cells to become muscle.

Over the years, a variety of cell types have been made to switch identities. Muscle cells (or at least muscle progenitor cells) have been persuaded to form fat cells. Liver cells have become pancreatic cells and vice versa. And many more.

While these conversions worked, they were generally inefficient. Moreover, it wasn't always clear which transcription factors would be most effective in directing a particular cell in a specific direction. To get at these issues, cell biologists have turned to a variant of Yamanaka's strategy; that is, introducing many transcription factors *en masse* into cells and then dropping out the ones that weren't contributing to the desired changes.

One approach is exemplified by studies of a team of researchers headed by Marius Wernig at Stanford University. They introduced 19 transcription factors into fibroblasts and succeeded in turning them into neurons. Like Yamanaka, they subsequently dropped out individual proteins in an effort to identify the ones that would work most efficiently. either individually or in concert. A group of three turned out to be optimal.

A similar experiment was carried out with cardiac fibroblasts by a group led by Deepak Srivastava, at the Gladstone Institute of Cardiovascular Disease in San Francisco, California.

Cardiac fibroblasts are plentiful, making up some 70% of the volume of the human heart. Fully differentiated cells, they play a role in maintaining the heart's three dimensional structure and passing signals among cardiac cells. They also form scar tissue after cardiac damage.

Srivastava and colleagues used Yamanaka's strategy to uncover a combination of transcription factors that could convert mouse heart fibroblasts into functioning cardiac muscle cells. They started with 14 and whittled this set down to three. Amazingly, some of the cells subjected to these three transcription factors actually began to spontaneously beat after 6-8 weeks. Careful analysis of the process indicated that the fibroblasts did not first regress to a more embryonic-like state before becoming muscle cells.

This last point is somewhat controversial. It isn't clear whether in all cases transdifferentiation takes a cell from differentiated state A to state B in one step, or whether the cell has first to move back to a less differentiated, more embryonic, state before undergoing the change. The best evidence seems to indicate that some cells can transdifferentiate directly, especially those with a similar embryonic origin, while others must take a step back. In either case, the cells do not have to revert to an embryonic state before undergoing transdifferentiation.

The potential of turning fibroblasts into functional heart muscle cells in culture opens up exciting possibilities for treatment of heart disease. Even more intriguing is the possibility of doing the same in place, turning cardiac fibroblasts into cardiac muscle cells within the functioning heart. Or more generally, changing the fate of any cells in the body at the direction of the physician.

One problem with this approach is that direct reprogramming has generally required the introduction of a new set of genes into cells. Usually, a virus is employed to convey genes coding transcription factors into the targets. Therapeutic applications are limited with this approach. Recently, a group from Shanghai University in China reported that a cocktail of seven small molecules when added to human foreskin fibroblasts in culture could, within eight days, turn them into nerve cells. The reprogrammed cells look and act like neurons and don't appear to have gone through an embryonic stage before making the transition from fibroblasts.

However, despite the enormous potential that direct reprogramming offers, these are early times. It still isn't known whether transdifferentiation can be used to generate the wide variety of cell types that will be useful in the clinic, or whether reprogrammed cells will prove as beneficial for therapy as those generated by other techniques. And, while the procedure

works well for cells in culture, it isn't known how feasible transdifferentiation will be when attempted directly in the body.

33

Engineering Organs

The first successful transplant of an organ took place at the Peter Bent Brigham Hospital (later called the Brigham and Women's Hospital) in Boston. A kidney was removed from Ronald Herrick and placed in his identical twin brother, Robert. Robert, who had been dying from chronic kidney disease survived for eight more years. He married his nurse and fathered two children. Joseph Murray, who performed the operation, shared the Nobel Prize for Medicine in 1990 with E. Donnall Thomas, who was instrumental in developing bone marrow stem cell transplants.

In later years, lungs, livers, hearts and other organs have been transplanted. In the United States alone, about 28,000 patients undergo organ transplant surgery of some kind each year. About half the organs transplants are kidneys; a quarter are livers. Despite the large number of transplant surgeries, upwards of 100,000 people are waiting for a donated organ, and the gap between available donors and patients awaiting treatment continues to widen.

Transplant surgery was greatly accelerated with the development of immune suppressor drugs, particularly cyclosporin. But rejection is still a major hurdle as is the deficit in donors. Organ engineering, the combination of stem cells and artificial matrices to build tissues and organs, holds the prospects of bypassing both these difficulties.

Both embryonic stem cells and induced pluripotent stem cells have great promise for use in engineering organs. Both are pluripotent and can be manipulated to form specific cell types. Both are immortal, capable of growing for many generations to large numbers in culture. iPS cells have the further advantage that the donor and the recipient of the engineered organ is the same, thus avoiding rejection. As noted previously, both cell types have the disadvantage that they can grow out of control, posing the possibility of forming tumors. And too, embryonic stem cells must come from embryos that must be destroyed, raising ethical problems for some. These ethical issues may be bypassed with induced pluripotent stem cell that can be reprogrammed from somatic cells but this has been a recent development with unknown risks. In short, while prospects for using stem cell for building organs are excellent, there are many difficulties to be overcome and questions to be answered before they can be routinely used in the clinic.

Organoids

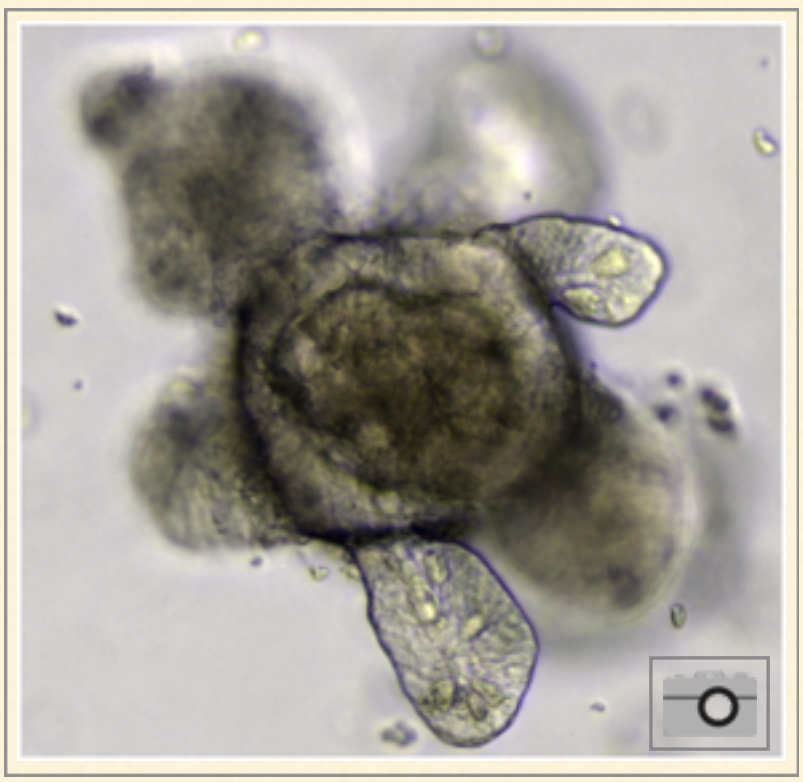
Back in 2012, the journal "Nature" ran a feature article entitled "Tissue engineering: The brainmaker". The piece described, in glowing terms, the work of Yoshiki Sasai of the RIKEN Center for Developmental Biology in Kobe, Japan. If that name doesn't sound familiar, you might want to take another look at chapter 23.

Sasai was a prolific scientist who was the first to take advantage of an apparent deficiency of embryonic stem cells. When left alone in culture they had a tendency to differentiate into nerve cells. Sasai came to recognize that cocktail used to grow embryonic stem cells tended to inhibit this process. He found that if he devised his own recipe, leaving out blood serum and other growth promoting factors, and in addition, allowing the cells to float, the outcome was that the cells aggregated into tiny spheres. These carried recognizable tissues of the developing brain, including cerebral cortex and hypothalamus. By adding a few pinches of specific growth factors, he was able to get these embryonic nerves to form retinas and other early eye

tissues that are characteristic outcroppings of the primitive brain.

Sasai's successes demonstrated that embryonic cells were somehow preprogrammed to interact and form complex structures. If they were just left alone, they could follow specific developmental pathways. His work had a great impact. It encouraged others to try to cajole embryonic cells to differentiate in other directions, to form other kinds of tissues. He was celebrated and widely acclaimed. It's a tragedy that he saw fit to take his own life.

Figure 33.1



Intestinal organoid

After Sasai's initial reports, others jumped on the bandwagon. Embryonic kidneys, livers, pancreases, stomaches. prostate glands, hearts, and lungs have been created in various laboratories. Keep in mind however, that these are tiny structures, similar to organs from embryos, and devoid of nerves and blood vessels (Figure 33.1). The absence of a blood supply is the reason that the size of the organoids are small. And while they are useful for probing development and to test drugs, they can't substitute for real organs. For that, something more sophisticated must be fashioned.

Complex Organs

To date, experience has shown that to build an artificial organ of any complexity and size a three dimensional skeleton must be assembled for cells to bind to, grow upon, and organize around. Most cell types will not even survive if they are not anchored to a matrix of some kind. The materials that have been used to fabricate such a framework fall into three categories. First, there are natural polymers, often proteins. Collagen is a good example, It is a protein that makes up a good part of tendons, cartilage, ligaments and skin. Second, synthetic polymers such as polyglycolic acid or polylactic acid have been used. A third material, one with perhaps the most potential is decellularized tissue. This framework is produced by removing the cells from a tissue, leaving behind a kind of

skeleton of collagen and other proteins into which added stem cells can infiltrate and grow. The advantage of this approach is that the exact architecture of the tissue is preserved. It's not necessary to try to duplicate it. Bladders, blood vessels, trachea, and heart valves have been decellularized and utilized in this way to produce the corresponding structures in rats and pigs.

There is great need to fabricate even more complex structures such as kidneys that require intricate connections to the blood supply and nervous system. In 2013, close to 5,000 individuals died while awaiting a kidney transplant. At the same time, about 2,500 of the kidneys harvested for transplants from deceased people have proven unsuitable for use. Some were unusable because of structural problems caused by disease or aging; others because of inadequate care in maintaining the organs after harvest.

In 2013, a group at Harvard headed by Harald Ott, prepared scaffolds from rat, pig and human kidneys.

The technique that they used seems relatively straightforward. They pumped a solution of detergents into the kidneys through the renal artery to clear out cells. Examination of the kidney after this treatment showed that 90% of the DNA had been removed, but that most of the collagen remained (Figure 33.2). No cells were detected after perfusion and kidney architecture seemed to be preserved. In particular, the blood vessels, which would be necessary for subsequent oxygenation of added cells, remained. Testing showed that the scaffolds were nonfunctional, as they should have been since they contained no live cells.

They used two kinds of cells to repopulate the rat matrices: human endothelial cells derived from umbilical cord blood and rat epithelial cells from neonatal kidneys. After perfusing the cells for several days, they were able to show that the kidneys exhibited partial function when cultured in a chamber. More encouraging was that when transplanted into rats, the "artificial" kidneys were well accepted and produced some urine.

More recently, Ott's laboratory has begun to fabricate artificial limbs. They knew that approximately 1.5 million people in the United States have lost a limb and that artificial prostheses don't restore full function. Having access to a biological replacement, especially one that was derived from the cells of the afflicted individual, would be a great advance.

Using their work with kidneys as a guide, they removed the cells from a rat (Figure

33.3) limb from which the skin was removed by perfusing it with detergents. After decellurization they infused a mixture of mouse muscle progenitor cells, mouse fibroblasts, and human umbilical

Again, electrical stimulation seemed to produce movement here too. Finally, they did the same experiment on a baboon limb, with similar success.

These, and similar breakthroughs, have a

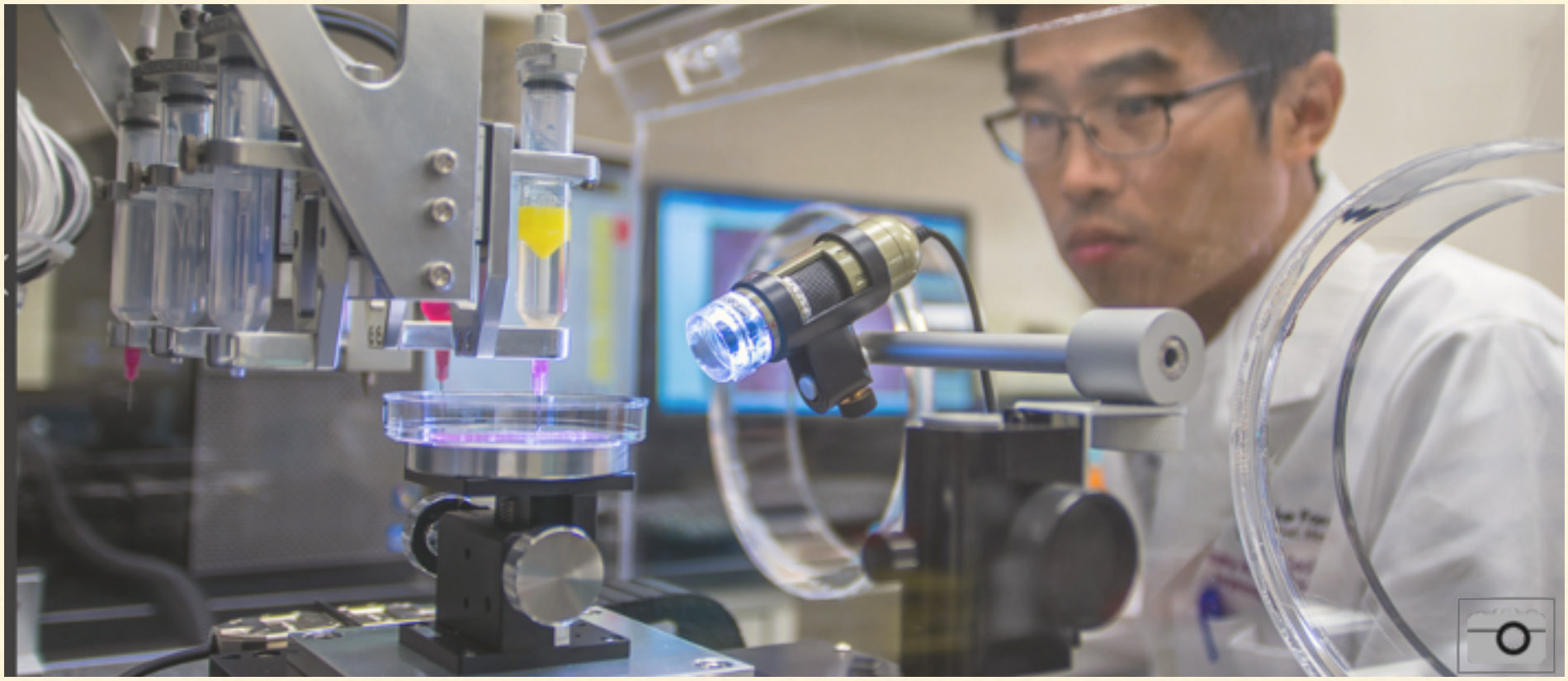
Figure 33.2

Decellularized Rat Limb

cells into the matrix. After five days, they began to electrically stimulate the limb. Some time later, they applied rat skin grafts to it.

This procedure resulted in the formation of muscle and vascular tissues in the appropriate locations. Upon stimulation, the muscles moved and exerted force. They also transplanted the limb to a rat whose own forearm had been removed.

long way to go before they can be applied to humans, but they are encouraging. The possibility of using induced pluripotent stem cells to repopulate acellular matrices is exciting. The hurdles that must be overcome include finding ways of generating the appropriate cells to repopulate the scaffold, getting nerve cells to grow from the host to the graft, and making sure that the added stem cells do not produce tumors.



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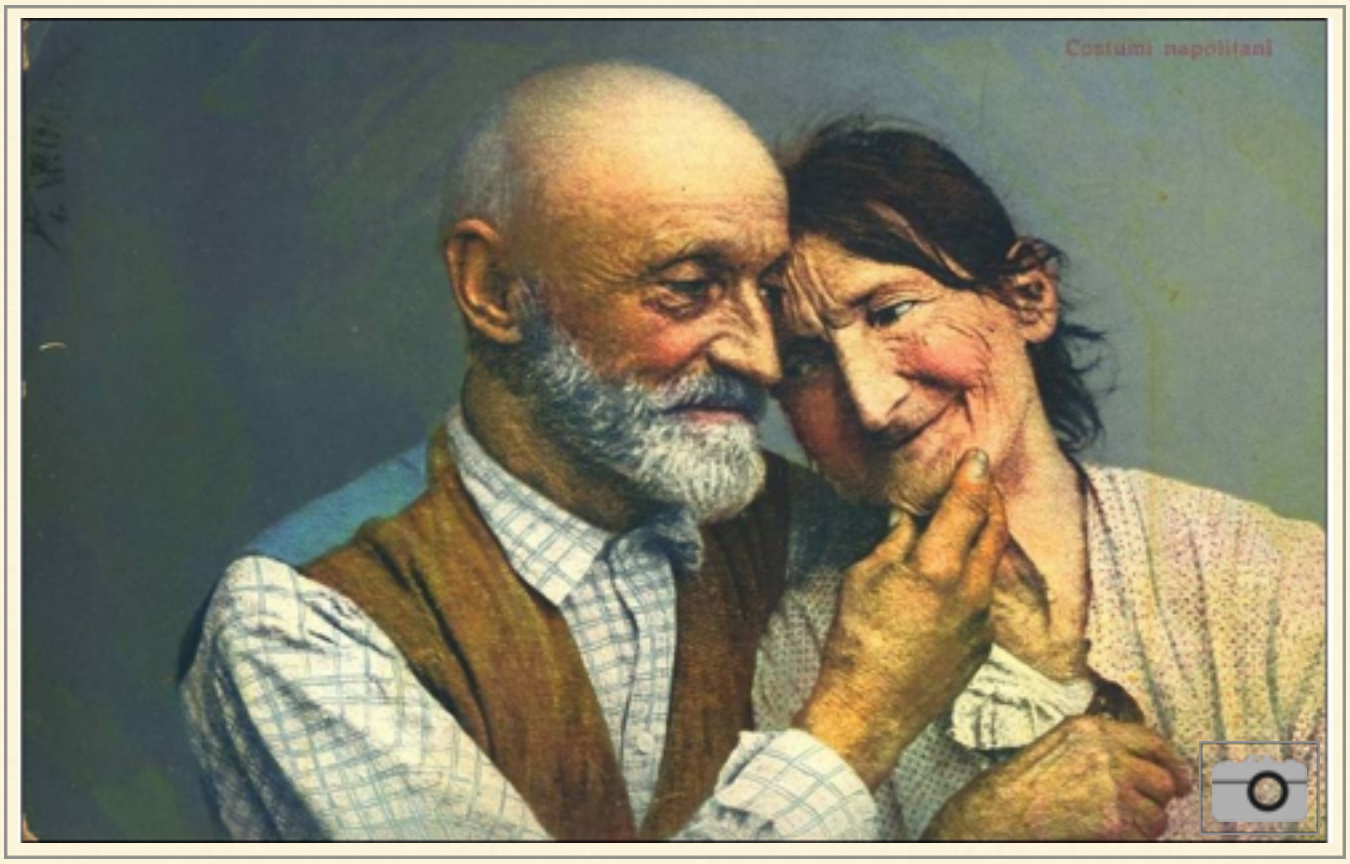
3D Printing

Three dimensional printing was invented by Charles Hull in 1986. He called the process “stereolithography”. In its original form it involved the building up of a three dimensional object by successive deposition of layers of some substance under the direction of a computer. Hull’s first machine used an ultraviolet beam to repeatedly polymerize thin layers of liquid in specific areas, one layer serving as the foundation for the next. His technique has been largely superseded by one called “fused filament fabrication” where a plastic is heated to its melting point and deposited as a liquid that immediately hardens upon extrusion. This technique and related ones have been used to manufacture industrial parts, archeological objects, molecular models, personal products, and jewelry, among other objects. Three dimensional printers have become faster, cheaper, and more readily available in the last few years, and are predicted to have a great impact in the future in many areas.

Biologists first began investigating the practicality of using three dimensional printing for the manufacture of biological materials using modified commercially available inkjet printers. These devices form characters by precisely depositing minuscule droplets of pigment on paper. In order to work with biologics the printers had to be altered so that they could deposit cells to form tissues and organs.

Adapting three dimensional printers to create living materials has been a challenge. Inkjet printers work by forcibly ejecting liquids out of a tiny orifice using sonic, electrical, or heat energy. In general, cells don't take kindly to be mistreated in this way. However, some investigators have been able to overcome some of these concerns by modifying commercial machines. For example, a group of scientists at Wake Forest and Cornell have constructed a device that deposits cells encapsulated in a "Jello"-like matrix on injured skin. One study carried out in mice used human amniotic stem cells in this context. These fetal cells have characteristics that lie somewhere between embryonic stem cells and mesenchymal stem cells. As such, they have the capacity to form a variety of cell types but can't form teratomas. Moreover, they can be easily grown in large numbers in culture. They also are not recognized as foreign by the mouse's immune system. Confirming some of the studies cited earlier, the cells don't engraft very well. However, they do promote healing, at least better than controls. The authors speculate that they are effective because they secrete proteins that stimulate existing cells to thrive.



35

Aging

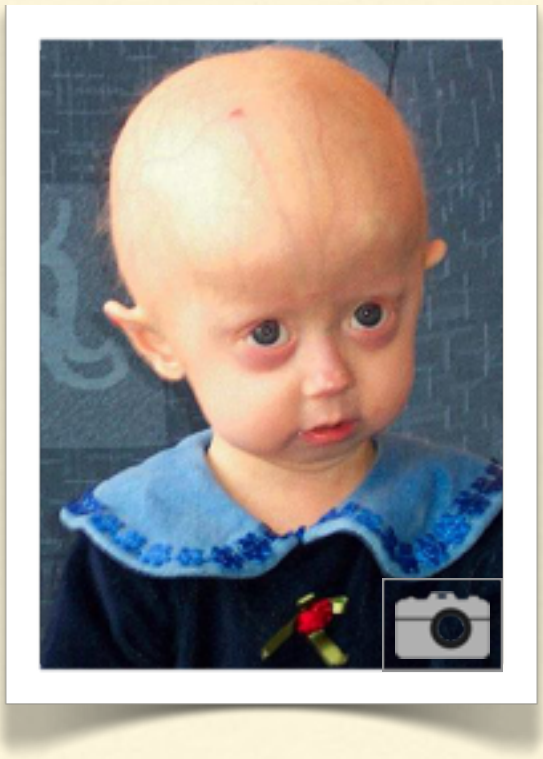
After we reach our mid and late thirties, our faculties begin to diminish and our ability to ward off disease lessens. This phenomenon appears to be part of an inevitable progression, but no one knows why it happens or how to prevent it. One theory of aging, of course not the only one, is that our stem cells become less capable of renewal as time passes. There is, in fact, good evidence that the stem cells in several tissues, including blood, brain, skin, and muscle, exhibit decreased functionality with age. These tissues respond to injury more slowly, exhibit lessened proliferative ability, and show diminished function. Because of this, the theory goes, as tissues and organs wear out they aren't as readily replaced.

If indeed stem cell senescence is responsible for aging, there is the possibility of slowing or even reversing the process. New stem cells might be transplanted or old ones rejuvenated. However, keep in mind that the scientific community is deeply divided on the question of whether

the signs of aging displayed by stem cells is a good model for the aging of individuals.

What's the mechanism that causes stem cells to lose function as we age? It's almost certain that more than one factor is at work. An idea that's been around since the 1970's is that stem cells (and others) are damaged by so-called "reactive oxygen species". These are chemical forms of oxygen (for example, peroxides) that are generated in mitochondria as a byproduct of normal respiration. They are capable of causing widespread damage when they react with DNA and proteins. According to the theory, a positive feedback loop, is set up when such damage occurs. Malfunctioning mitochondria produce even more reactive oxygen species wrecking more havoc that in turn injures mitochondria even further. The result ultimately is a vicious cycle that results in cell failure and death.

Figure 35.1



Young Boy with Hutchison-Gilford Progeria Syndrome

Several studies have demonstrated that indeed there are higher levels of reactive oxygen species in aged mesenchymal stem cells. In addition, when scientists interfered with the enzymes that are meant to deal with these reactive oxygen species increased damage to stem cells were observed.

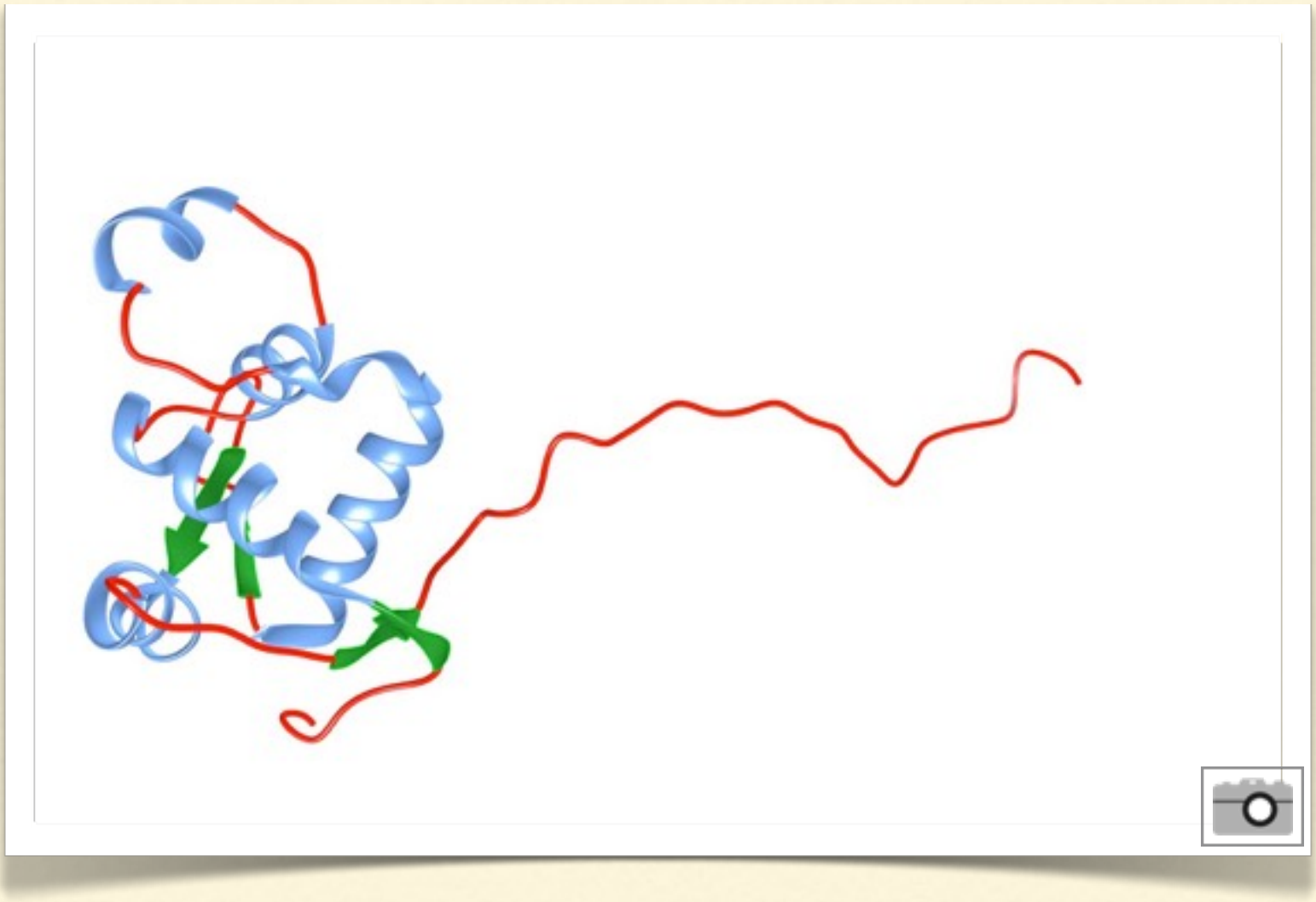
It is also possible to add substances to stem cells that help them combat the ravages of reactive oxygen species. These treatments seem to work, at least in cells in culture, but it isn't clear that will be effective in combating aging.

There are other factors that might influence stem cell proliferation in addition to reactive oxygen species. The telomere shortening that occurs when DNA replicates (See Chapter 19) may result in damage to those stem cells that divide frequently. Mutations in DNA may accumulate in stem cells during replication. The process of translation is not error free, and the mistakes that occur when proteins are synthesized may cause vicious cycles of injury as well. These

difficulties may also occur in cells other than stem cells, thereby contributing to the idea that aging isn't due to a single factor.

One way of investigating the mechanism of aging is to examine individuals who exhibit what appears to be premature aging due to some hereditary disorder. It would probably be better to examine humans who have genetic conditions that lead to eternal youth, or who age much more slowly than normal. Unfortunately, people like these seem to appear only in works of fiction. Hereditary diseases that mimic some characteristics of aging are called progerias ("pro" - premature; "geria" - old age). One such disease is called Hutchison-Gilford progeria syndrome. The molecular basis of the disease is known. A gene called "lamin A" is defective in afflicted individuals. Normally, the protein specified by this gene forms a part of the structure of the nucleus. A defect in the gene causes changes in the shape of nuclei and problems with a variety of nuclear processes. People who develop this disorder exhibit symptoms at a young age that include premature arteriosclerosis and atrophy of the musculature in their blood

Figure 35.2



WRN Protein

vessels. They have a sharply reduced lifespan, usually less than 20 years (Figure 36.1)

Another progeria that is associated with a variety of premature aging signs is Werner syndrome, sometime called "adult progeria" in contrast to Hutchison-Gilford progeria that is often called "childhood progeria". The onset of problems associated with Werner syndrome begins at puberty when individuals fail to show the growth spurt characteristic of adolescence. As the disorder advances, afflicted individuals may grey prematurely and lose their hair. In their twenties and thirties they are more prone to diseases that occur later in life including diabetes, cataracts, cancer, and osteoporosis. Most die from cancer or heart disease in their forties or fifties. Werner syndrome is a rare disease, affecting one person in a million. It is associated with mutations in the WRN gene located on chromosome eight in humans.

The study of progerias is a good illustration of the role that stem cells can play in probing the mechanism of disease in general, and aging in particular. In one study, cell biologists took fibroblasts from a patient with Hutchison-Gilford progeria and, using Yamanaka's protocols, succeeded in reprogramming them to become induced pluripotent stem cells. Surprisingly, when compared to induced pluripotent stem cells from a normal individual, they appeared identical, even after prolonged periods in cell culture, not showing the ravages of age that are symptomatic of the disease.

Perhaps that shouldn't have been so much of a surprise. It had previously been shown that embryonic cells don't express the lamin A protein. If the protein isn't expressed, it shouldn't matter whether it's abnormal or not.

However, when the induced pluripotent cells from Hutchison-Gilford progeria patients were induced to differentiate into muscle cells of blood vessels, they **did** show abnormal senescence. A related study demonstrated that when these same cells were made to form mesenchymal stem cells, these too exhibited the premature senescence of Hutchison-Gilford progeria.

With regard to Werner syndrome, similar results were obtained with embryonic stem cells in which the WRN gene had been removed by genetic engineering. They remained normal when maintained as embryonic cells, but showed premature aging when induced to form mesenchymal stem cells.

These results seem to strongly suggest that embryonic stem cells and their induced pluripotent stem cell counterparts aren't affected by progeria, but that mesenchymal stem cells and other more developmentally restricted cell types are.

While the results from these studies seem straightforward, some have questioned whether they tell us anything about normal aging. After all, critics maintain, the progerias are extremely rare and it's probable that few of us harbor mutations in either the lamin A or WRN gene. One possible responses to these criticisms is that perhaps the WRN gene isn't expressed to the same extent in older individuals. And perhaps the aberrant lamin A protein characteristic of Hutchison-Gilford syndrome forms more readily as we get older. In fact, there is some evidence to suggest that both events occur. In that case, it might be that these two progerias are good models of what is happening to us during senescence and that it may be possible to alleviate some aging issues by supplying people with stem cells that could make up for these problems.

Speaking personally, as a rapidly aging individual, I'm hoping that the studies being carried out with progeria are leading in the right direction. And I'm rooting for stem cell advances that come from these studies to come to the clinic; the sooner the better.

36

Heart Repair

Heart disease can be devastating, both in its individual effects, and for its impact on society. Something on the order of 17 million people over the globe die of heart disease each year. It's the leading cause of death in the United States, and has been in almost very year since 1900 (the one exception was 1918, the year of the great influenza epidemic). Researchers and clinicians, realizing the extent of the problem, have worked mightily to try to devise an effective treatment.

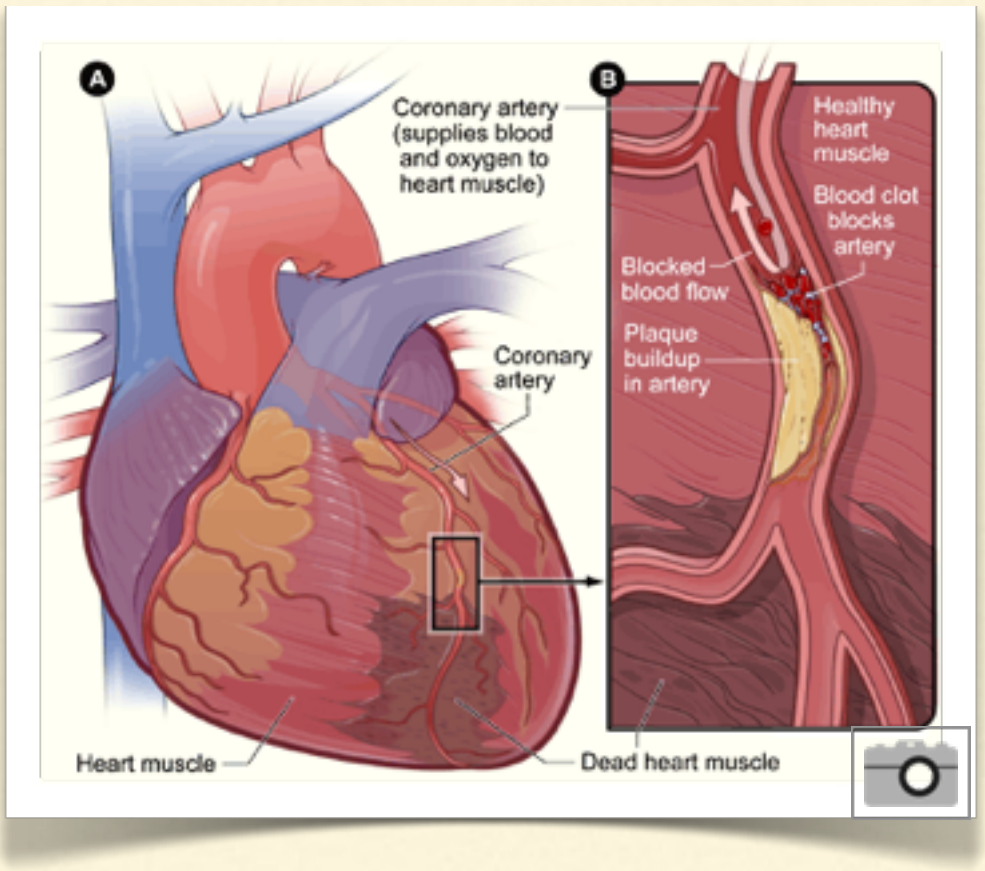
Cardiovascular disease often emanates from arteriosclerosis, the accumulation of plaque on the arteries that feed the heart. When sufficiently advanced, these vessels may be blocked, causing a cessation of blood flow, and a heart attack (Figure 36.1). The trouble is that the heart is particularly sensitive to a lack of oxygen. When circulation is cut off to it, portions die. If the affected individual survives, the dead cells are replaced with scar tissue that may impede normal contraction. The result can be inadequate circulation or arrhythmias. Moreover, scar

tissue may interfere with possible repair processes that otherwise might have come into play.

In amphibians, zebra fish, and neonatal mice, the heart can, to a considerable extent, repair itself, but for many years, it was thought that the human heart lacked that capacity. The consensus had been that mature adult heart cells are unable to divide. While there had been clear evidence that under some circumstances the heart can grow in size, it was thought that enlargement was due to heart cells growing bigger, not to the birth of new cells. However, in the 1990's, Piero Anversa (Figure 36.2) and colleagues reported that dead rodent heart cells were being replaced to the extent of perhaps 1 to 2% a year.

Several other studies have confirmed this phenomenon in adult humans. More recent research has shown that the rate of replenishment is much lower than that originally reported by Anversa in mice. The pace of replacement also seems to diminish with age.

Figure 36.1



Cartoon of a heart attack

There has been some controversy concerning exactly which cells contribute to heart regeneration. It is not known whether a population of non-cardiac stem or progenitor cells are the causative agents or, alternatively, whether mature heart cells dedifferentiate to become the source of new cells.

In either case, it seems firmly established that **limited** cardiac cell division does occur in the adult human heart. That's encouraged many laboratories to try to spur further heart regeneration by a variety of means, but principally by the addition of stem cells.

An apparent breakthrough occurred in 2001. In an article published in the journal "Nature", Anversa and colleagues, then at New York Medical College, contended that certain bone marrow cells when introduced into female mouse hearts resulted in regeneration in 40% of the cases. Given the technical challenge of injecting cells into a tiny heart that is beating some 600 times a minute, the fact that not every mouse responded was not surprising.

Two years later, the Anversa laboratory showed that there were cells in the heart itself that were capable of initiating repair. Moreover, these cells bore the same distinctive protein on their surfaces as the cells that he had previously used from the bone marrow.

These findings were seized upon by the clinical community. It appeared that hearts could be repaired by the injection of adult stem cells. Given that the alternative treatments for heart failure were either extremely expensive (heart transplants) or largely ineffective (drugs), the technique began to be widely employed. The first results were very encouraging. A group in Düsseldorf, Germany led by Bodo-Eckehard Strauer reported that the technique was safe and effective as early as 2002.

Over the years a large number of clinical trials have attempted to replicate these initial results by injection of autologous bone marrow cells into the heart. Almost universally, they procedure has proved safe and, in many cases, claimed to be effective. Most of the studies were small, and were not tested against a placebo, but, nevertheless, with nearly 1,000 patients involved, they strongly suggested that bone marrow stem cells seemed to work.

More recently, the treatment of heart disease using stem cells has become increasingly controversial, chaotic, and troubled. Beside the usual scientific disagreements, there have been charges of scientific misconduct. Lawsuits and institutional investigations have contaminated the scientific arena. They have placed the entire field under a cloud.

For example, Strauer's initial clinical studies carried out more than a decade ago were found to be riddled with problems. The Heinrich-Heine-University in Düsseldorf found evidence of scientific misconduct by Strauer (who retired in 2009). And an analysis of the clinical trials conducted under Strauer's supervision found numerous errors. None of Strauer's papers have been retracted, but they are widely disbelieved.

Meanwhile, Anversa's results were also undergoing increased scrutiny. Efforts to replicate some of his studies in some other laboratories were unsuccessful. In 2014, the Brigham and Women's Hospital in Boston, an affiliate of Harvard Medical School where he had moved in 2008, began a misconduct investigation into his laboratory's work. Anversa and a coworker responded with a suit alleging that the investigation was "procedurally and legally flawed". While acknowledging that some data had been fabricated in two previous papers, they claimed that the responsible party was a coworker and that they had no knowledge of the misdeed. The suit was dismissed. Anversa has since left Harvard, and has supposedly taken a position in Switzerland.

There have been some recent scientific developments that have cast further doubt on some of Anversa's cardiac stem cell studies as well. In 2014 two papers appeared that were pertinent to the dispute. The first provided excellent evidence that the cells that Anversa claimed to act to regenerate heart tissue did so but to a very limited extent. The second found that bone marrow derived stem cells did not significantly contribute to the injured heart. And a large scale "meta study" of 49 different clinical trials of bone marrow effectiveness (Strauer's were omitted) found numerous "discrepancies". Those trials with the least discrepancies showed that the treatments had no positive outcomes, while those with the most numerous problems seemed to be show the most effectiveness. But even this latter group, only showed improvements of less than 8%.

These 49 trials reflect more than a decade of studies that have utilized adult stem cells from bone marrow for cardiac therapy. These investigations continued despite the data that indicated that bone marrow derived stem cells didn't actually contribute to cardiac tissue in injured hearts. But scientists argued that the few reported successes that the technique produced were encouraging enough to proceed. They maintained that it didn't matter how the transplants worked. As long as they stimulated repair they were worth doing. But did they work? In order to resolve the question, the community called for a large carefully conducted clinical trial to determine once and for all whether bone marrow stem cell infusion into the heart was an effective treatment for cardiac disease.

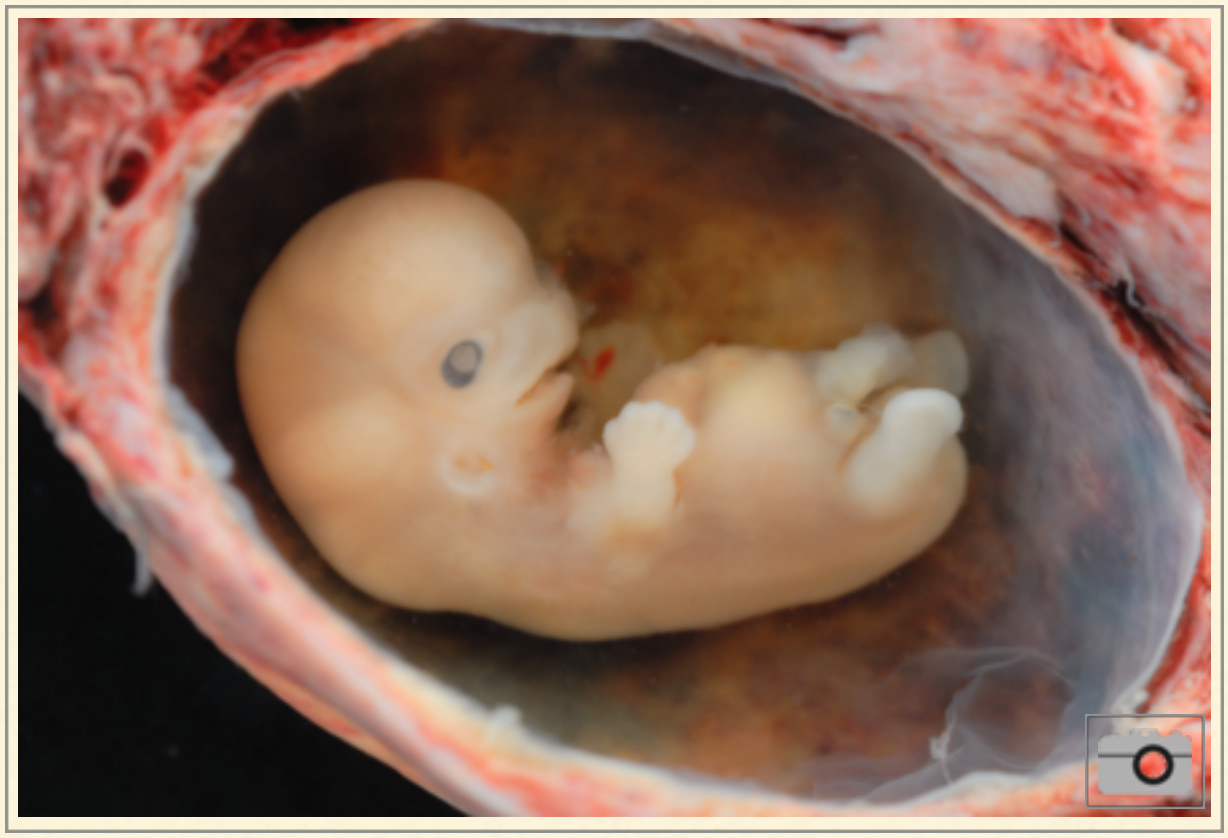
Three are ongoing. The largest, called "BAMI" (Bone Acute Myocardial Infarction), involves over 3,000 patients from 10 European countries. In most

cases, autologous transplants are being used. To date, no results have been published.

As noted, the BAMI study was begun on the basis of the initial results with bone marrow cells from Anversa's laboratory. More recently, some physicians have turned to another source of stem cells that derive directly from the heart in the hope that they may produce better results. Cardiospheres are clusters of cells that spontaneously grow out from pieces of heart tissue placed in culture. They're reminiscent of the neural organoids of chapter 33. First discovered in 2004, these tiny spherical bodies apparently consist of heart stem cells in their center, and differentiating heart muscle cells at their periphery. Preliminary experiments with rodents have been promising and a phase I clinical trial involving patients with diminished heart function called (CADUCEUS - **Ca**rdiosphere-**d**erived a**u**tologous stem **ce**lls) has provided evidence that these cells are safe for therapy. While less than two dozen patients were involved, the study concluded that the procedure resulted in a decrease in the size of heart scars and improved cardiac function. The results were deemed sufficiently encouraging to warrant further investigation.

Another approach that I've already discussed is to use transdifferentiation to turn the scar tissue formed by fibroblasts after heart injury into heart cells using cocktails similar to those developed by Yamanaka. Still another tack is use induced pluripotent stem cells. Experiments using both strategies are just getting underway.

In summary, the status of cardiac repair via stem cell therapy is in flux. Some very basic scientific questions remain unanswered. There is much controversy among the scientists working in the field. There have been some ugly incidents. Misconduct and fraud have been alleged. All this is not surprising given the stakes. Here's hoping that these issues become resolved and that further progress is made because a cure for heart disease would be a discovery of enormous import.



37 Ethical Issues and Practical Matters

Abortion

When does life begin? The major ethical issues concerning the use of embryonic stem cells swirl around this question. Human embryonic stem cells have to be procured from human blastocysts. Are they alive? When cells are taken from them are "babies" being killed? Opponents of the use of stem cells argue that life begins at conception and that the use of embryonic stem cells involves the death of a human being.

When does an individual become alive? It's not a question that can be answered scientifically. Scientists can't know. Does a human become alive when their brain first forms? When their brain begins to function? When their heart starts beating? When the fetus can survive on its own? At birth?

What is known is that, shortly after conception, a *potential* new individual forms. Because of recombination and random chromosome assortment during germ cell formation, this prospective person has a unique

combination of genes. It is almost certain that this particular combination will have never appeared on earth previously and will probably never occur again. For that reason alone, even if one believes that life begins sometime post conception, destruction of an embryo isn't something that should be dismissed lightly. It is, to my mind, a reasonable justification for the opposition to premature termination of a pregnancy.

But, again in my opinion, that doesn't mean that the destruction of embryos is **never** justified. That's especially true with regard to the issue of stem cells. The embryos that are used to derive embryonic stem cells come exclusively from laboratories engaged in *in vitro* fertilization (IVF). When a couple decides to use IVF, many oocytes are removed from the woman and fertilized in the laboratory. All the embryos that form cannot be transplanted into the prospective mother. The "extra" ones are frozen away in liquid nitrogen. Most are never used. They are discarded along with other "biological waste". Supporters of embryonic stem cells point out that it makes sense to use them to obtain material that can treat people with terrible illnesses or to derive cell lines that can be studied in order to understand the basis of devastating diseases. The alternative is to throw them in the trash. Or to let them freeze to death. Or to ban IVF.

The ethical issues concerning the use of embryonic stem cells has not been settled, but the question of the legality of the procedure has. In 2012, a federal court ruled that funding for embryonic stem cells is legal and the Supreme Court declined to hear an appeal of the case.

Mutations

DNA replication is extraordinarily accurate. The best estimates are that only one incorrect base is inserted for every 1 - 10 billion correct ones. This number is very small, but one mistake per billion adds up quickly given that there are about six billion bases in the human genome and many opportunities for DNA to replicate. In fact, an error rate of this magnitude means that there are about a half dozen mistakes made every time a human cell divides. Such errors are called mutations. And almost all mutations are harmful, or at best, innocuous.

Stem cells of all kinds are initially present in very small amounts in the tissues from which they're obtained. In order to secure sufficient cells for treatment, they must be expanded in tissue culture. In turn, that means that they must undergo multiple additional rounds of DNA replication, inevitably generating mutations at every

generation. The more cell divisions, the more errors. These mutations are random. They may be single base changes or, more infrequently, wholesale chromosomal aberrations. In either case, they may have large effects on cells. For example, they may kill or severely damage a cell. Or, more frighteningly, they may turn a stem cell into a cancer cell. In particular, mutations may occur in adult stem cells that change them from cells that have a limited life span to ones which are "immortal", a change that can start them on a path toward forming tumors (see Chapter 19).

Immunological Issues

One great disadvantage of using embryonic stem cells for therapy is that they are allogeneic, meaning that they must be derived from an individual other than the one being treated (autologous transplants use tissues from the recipient). This raises the issue of rejection by the immune system which sees the transplanted cells as foreign and tries to destroy them. There are several ways of coping with this problem. Immune suppressive drugs may be used, although they pose other complications. An alternate strategy that has been proposed is to prepare banks of stem cells whether embryonic, induced pluripotent, or adult, derived from many individuals, and try to get a good immunological match between the recipient and the cells. It should be pointed that adult stem cells seem to be somewhat immunoprivileged, and don't get readily rejected by the host.

Cost and Efficacy

If you go on line, it is easy to find dozens of centers, both in the United States and abroad, that are injecting stem cells into people. They claim to cure all manner of diseases or to improve your looks. Apart from bone marrow transplants, which have been used for 50 years and are of proven effectiveness, there is very little hard evidence that they work. They are not FDA approved. The leading hospitals in the country don't offer the treatments. Insurance companies will not pay for the therapy. The cost is high, ranging upwards of thousands of dollars per injection.

On the other hand, testimonials for miracle cures abound. Famous and highly paid athletes indorse the treatments. Even politicians have gotten into the act. A crucial question is: If you have a disease for which there is no cure, and you or your loved one is suffering, why not take a chance on a stem cell injection? The International Society for Stem Cell Research, an independent, nonprofit, non-governmental organization formed to foster exchange of information concerning

stem cells, has published a set of ten suggestions for prospective patients contemplating undergoing stem cell therapy. Here is a quick summary of some of their advice, not necessarily in the order that they were presented.

First, as I mentioned above, there are few widely accepted diseases for which stem cell therapy is approved. Second, be wary of testimonials. People will claim benefits from all manner of treatments. The placebo effect is very powerful. There really is no substitute for double blind controlled studies. Third, be wary if a clinic claims that the stem cells will work on a broad range of unrelated conditions, Fourth, just because stem cells are derived from the patients body doesn't mean that they are safe. And finally, there may actually be something to lose by undergoing an unproven treatment. Besides the possibility of serious complications, undergoing a treatment may prevent someone from participating in a future clinical study. And the financial costs may impose hardships on patients and their families.

Unfortunately, people with limited choices grasp for any possibility of help, even when they know that the chances of success are slim. To be told that clinical trials are underway and that proof of effectiveness is around the corner is not really helpful. Under those circumstances, rational arguments tend to have little sway. And there are always unscrupulous people waiting in the wings to take advantage of the situation.

Summary

38

Summary

Cloning and stem cells are the foci of this book. How are these two fields related? When scientists clone an animal by transferring a nucleus from one of its differentiated cells into an oocyte, they're asking the nucleus to undergo a massive reprogramming; to transit from a cell where it had overseen a stable differentiated state to one where it has to revert to a condition where it directs the rest of development. It has to go from a cell that was committed to supporting the production of a limited set of proteins to another where it is ultimately capable of dictating formation of all the proteins that the organism requires. Some of the questions that I've raised in this regard are:

- When in the course of development do cells become committed to a given differentiated state?
- How do they manage this feat?
- Can the commitment to differentiation be reversed?
- What's the history of discovery in this area and who were the scientists involved?

- What are the prospects of cloning humans by reversing differentiation of a committed cell?

Reprogramming is essential to the understanding of stem cells too. The developmental potential of stem cells must be taken advantage of if they are to be useful for therapy and diagnosis of disease. Some of the questions addressed in regard to stem cells are:

- What kind of stem cells are there and how do they differ from one another?
- What are the advantages and drawbacks of each kind of stem cell for therapy?
- What are the practical and ethical issues involved in stem cell use?
- What is the potential utility of stem cells in the near future and beyond?

Mesenchymal stem cells have a relatively narrow range of possible fates. That can be an advantage in some circumstances. They've been used to treat a variety of disorders with little evidence of harmful effects, but with limited hard evidence of success.

Embryonal stem cells have a much broader developmental potential. They have been restricted in their use, to some extent, because of ethical issues. In addition, they have the potential to form cancers.

Induced pluripotential stem cells are cell whose developmental potential has been changed so that they are capable of forming many other cell types. They have little ethical baggage, but are a relatively recent development and are still being actively studied. Because they can be derived from the patient who requires the stem cell treatment, they offer the possibility of treatment without the possibility of immune rejection. However, this last argument is losing some of its power because of the length of time it takes to grow these cells and the expense involved.

One of the points that I stressed in the book is that cloning shouldn't be particularly frightening. Many of the fruits and vegetables that we routinely consume are clones. And of course, identical twins are clones. It seems highly unlikely that some crazed dictator would use cloning to produce multiple copies of himself. But even if he did, it's not certain that the clones would have the political views of his "parent".

Another point that I tried to make is that stem cell science is in its infancy. While there have been some revolutionary advances, the community is still feeling its way. If appears that many problems will have to overcome before stem cells will be

routinely used in the doctor's office. But with time, we can look forward to possible cures for cancer, dementia, heart disease, diabetes and a whole host of other illnesses.

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Chromatin

The entirety of chromosomes in a cell, composed of DNA and protei
the term refers to the chromosomes during “interphase”, when they a
and so thin that they are impossible to identify individually under the
microscope.

Related Glossary Terms

Chromosome

Index Find Term

Chapter 3 - Cell Anatomy

Chromosome

A term meaning “colored body”. Chromosomes are composed of one
double-stranded DNA and a variety of proteins. Chromosomes are vis
cells are preparing to divide because they coil, shorten, and increase in
thickness. Actually, their length remains the same no matter the state

Related Glossary Terms

Chromatin