ENHANCEMENT CHAPTER

The Revolution in Cell Technology

Concept Outline

9e.1 Cloning animals, once thought impossible, isn't.

The Challenge of Cloning. As recently as 1997, scientists thought it was impossible to clone an adult animal. Then researchers announced the successful cloning of a lamb from a breast cell taken from an adult sheep.

Cloning Humans Is Not Going to Work Until a Key Problem Is Solved. Cloning of animals usually fails for lack of proper gene conditioning of the adult DNA used in the attempted cloning.

9e.2 Replacing defective genes in tissues remains an elusive goal.

Initial Attempts at Gene Therapy. It should be possible to cure hereditary disorders by replacing damaged genes in particular tissues. Early attempts, however, have failed.

More Promising Vectors. New viral vectors seem to avoid the problems experienced in earlier studies.

Ethical Issues Raised by Gene Interventions. Both somatic and germ-line gene therapy raise significant ethical issues.

9e.3 Replacing defective tissues is a promising but controversial possibility.

Embryonic Stem Cells. Some of the cells of a blastocyst, called embryonic stem cells, are capable of forming any tissue of the body. In mice, transplanted embryonic stem cells can replace injured or lost tissue.

Tissue-Specific Adult Stem Cells. Because the use of embryonic stem cells is controversial, researchers are also attempting to replace damaged tissues with later-stage stem cells isolated from adult tissues.

Therapeutic Cloning of Human Embryonic Stem Cells. To avoid immunological rejection of transplanted stem cells, researchers have proposed using a patient's own cells to obtain nuclei for a Dolly-style cloning procedure, creating a blastocyst that could be used as a source of embryonic stem cells that would not be embryonically rejected.

The Search for Pleuripotent Adult Stem Cells. There are teasing possibilities that some adult tissues may harbor small numbers of stem cells still able to become any body tissue.

Grappling with the Ethics of Stem Cell Research. The promise of stem cell research has rekindled debates about when human life begins. Few issues in modern science raise as many difficult ethical issues.

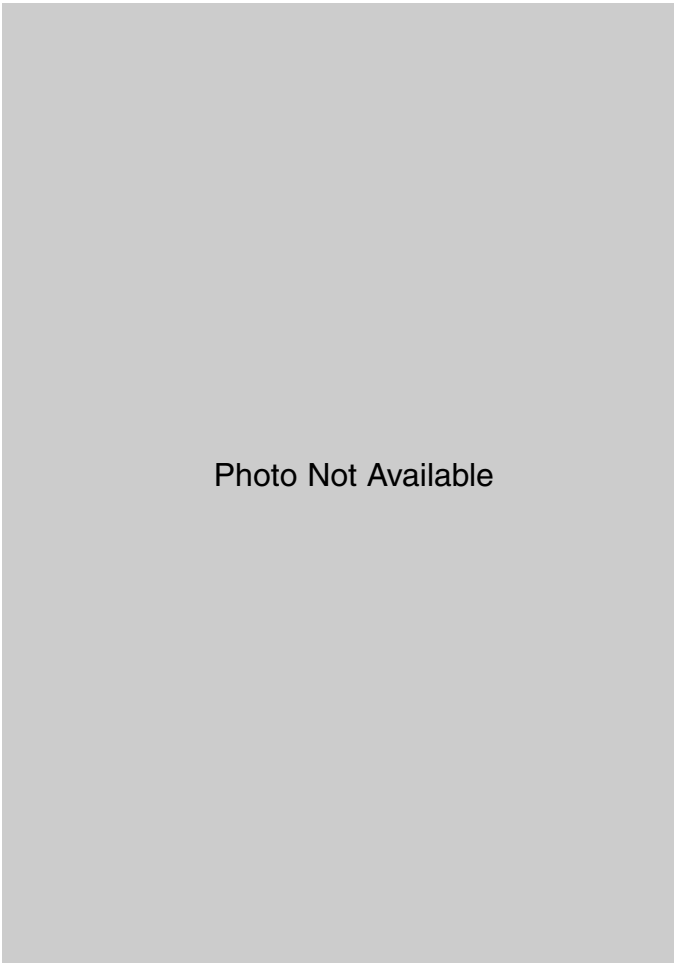


FIGURE 9e.1
Embryonic stem cells growing in tissue culture. Embryonic stem cells derived from early human embryos will grow indefinitely in tissue culture. When transplanted, they can sometimes be induced to form new cells of the adult tissue into which they have been placed. This suggests exciting therapeutic uses.

Molecular biology is such an integral part of our lives today that it is difficult to comprehend how very new is our knowledge of how genes work. In less than 50 years, we have come from Watson and Crick's discovery of the structure of DNA to a time when new developments in genetics are announced practically every week. Many of these developments, particularly those involving the biology of cells, impact human health very directly. In this chapter, you will encounter three areas where landmark progress is being made in cell technology: animal cloning, gene therapy, and stem cell research (figure 9e.1). While each of these might have been treated within different chapters of the text—chapters devoted to control of gene expression (Chapter 8), gene engineering (Chapter 9), or development (Chapter 28)—it seemed more desirable to pull them together, so the broad sweep of what is occurring would be more apparent. Advances in cell technology hold the promise of literally revolutionizing our lives.

9e.1 Cloning animals, once thought impossible, isn't.

The Challenge of Cloning

The promise of gene engineering has had little impact on farm animals. The difficulty in using genetic engineering to improve livestock is in getting enough animals. Breeding genetically improved individuals produces offspring only slowly, and recombination acts to undo the painstaking work of the genetic engineer. Ideally, one would like to "Xerox" many exact genetic copies of the desirable strain—but adult animals can't be cloned. At least it was commonly accepted that they couldn't be. Now, in a surprising development, the holy grail of agricultural genetic engineers seems within reach. In 1997, scientists announced the first successful cloning of differentiated vertebrate tissue.

Spemann's "Fantastical Experiment"

The idea of cloning animals was first suggested in 1938 by German embryologist Hans Spemann (called the "father of modern embryology") who proposed what he called a "fantastical experiment": remove the nucleus from an egg cell and put in its place a nucleus from another cell.

It was 14 years before technology advanced far enough for anyone to take up Spemann's challenge. In 1952, two American scientists, Robert Briggs and T. J. King, used very fine pipettes to suck the nucleus from a frog egg (frog eggs are unusually large, making the experiment feasible) and transfer a nucleus sucked from a body cell of an adult frog into its place. The experiment did not work when done this way, but partial success was achieved 18 years later by the British developmental biologist John Gurdon. In 1970 he inserted nuclei from advanced toad embryos and adult small intestine into toad eggs. The toad eggs developed into tadpoles, but almost all of them died before becoming adults.

The Path to Success

Although nuclear transplant experiments were attempted without success for decades, the technology continued to advance. Finally in 1984, Steen Willadsen, a Danish embryologist working in Texas, succeeded in cloning a sheep using a nucleus from a cell of an early embryo. This exciting result was soon replicated by others in a host of other organisms, including cattle, pigs, and monkeys.

Only early embryo cells seemed to work, however. Researchers became convinced that animal embryo cells become irreversibly "committed" after the first few cell divisions. After that, they concluded, nuclei from differentiated animal cells could not be used to clone entire organisms.

We now know their conclusion to have been unwarranted. The key advance for unraveling this puzzle was made in Scotland by geneticist Keith Campbell, a specialist in studying the cell cycle of agricultural animals. By the early 1990s, knowledge of how the cell cycle is controlled, advanced by cancer research, had led to an understanding that cells do not divide until conditions are appropriate. Just as a washing machine checks that the water has completely emptied before initiating the spin cycle, so the cell checks that everything needed is on hand before initiating cell division. Campbell reasoned, "Maybe the egg and the donated nucleus need to be at the same stage in the cell cycle."

This proved to be a key insight. In 1994 researcher Neil First, and in 1995 Campbell himself working with reproductive biologist Ian Wilmut, succeeded in cloning farm animals from advanced embryos by first starving the cells, so that they paused at the beginning of the cell cycle at the G_1 checkpoint. Two starved cells are thus synchronized at the same point in the cell cycle.

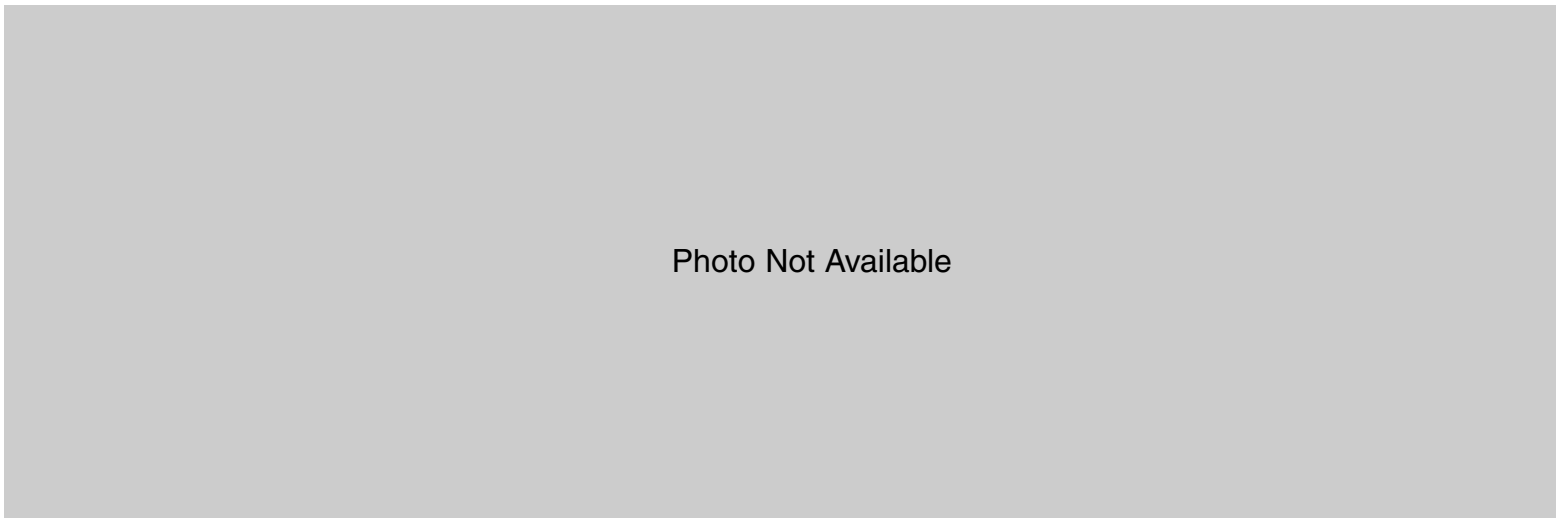


FIGURE 9e.2
Wilmut's animal cloning experiment. Wilmut combined a nucleus from a mammary cell and an egg cell (with its nucleus removed) to successfully clone a sheep.

Wilmut's Lamb

Wilmut then attempted the key breakthrough, the experiment that had eluded researchers since Spemann proposed it 59 years before: he set out to transfer the nucleus from an adult differentiated cell into an enucleated egg, and to allow the resulting embryo to grow and develop in a surrogate mother, hopefully producing a healthy animal.

Wilmut removed mammary cells from the udder of a six-year-old sheep (figure 9e.2). With tongue in cheek, the clone was named "Dolly" after the country singer Dolly Parton. The cells were grown in tissue culture; some were frozen so that in the future it would be possible with genetic fingerprinting to prove that a clone was indeed genetically identical to the six-year-old sheep.

In preparation for cloning, Wilmut's team reduced for five days the concentration of serum on which the sheep mammary cells were subsisting. In parallel preparation, eggs obtained from a ewe were enucleated, the nucleus of each egg carefully removed with a micropipette.

Mammary cells and egg cells were then surgically combined in January of 1996, the mammary cells being inserted inside the covering around the egg cell. Wilmut then applied a brief electrical shock. A neat trick, this causes the plasma membranes surrounding the two cells to become leaky, so that the contents of the mammary cell passes into the egg cell. The shock also jump-starts the cell cycle, initiating cell division.

After six days, in 30 of 277 tries, the dividing embryo reached the hollow-ball "blastula" stage, and 29 of these were transplanted into surrogate mother sheep. Approximately five months later, on July 5, 1997, one sheep gave birth to a lamb. This lamb, "Dolly," was the first successful clone generated from a differentiated animal cell.

The Future of Cloning

Wilmut's successful cloning of fully differentiated sheep cells is a milestone event in gene technology. Even though his procedure proved inefficient (only one of 277 trials succeeded), it established the point beyond all doubt that cloning of adult animal cells *can* be done. In the following four years, researchers succeeded in greatly improving the efficiency of cloning. Seizing upon the key idea in Wilmut's experiment—to clone a resting-stage cell—they returned to the nuclear transplant procedure pioneered by Briggs and King. It seemed to work well. Many different mammals have been cloned, including mice, pigs, and cattle. Unfortunately, as we will see, problems often develop in the cloned animals.

Transgenic cloning will likely have a major impact on medicine as well as agriculture. Animals with human genes can be used to produce rare hormones. For example, sheep that recently have been genetically engineered to secrete a protein called alpha-1 antitrypsin (helpful in relieving the symptoms of cystic fibrosis) into their milk may be cloned, greatly reducing the cost of producing this expensive drug.

It is impossible not to speculate on the possibility of cloning a human. There is no reason to believe such an experiment could not be made to work but many reasons to question whether it should be done. Because much of Western thought is based on the concept of human individuality, we can expect the possibility of human cloning to engender considerable controversy.

Recent experiments have demonstrated the possibility of cloning differentiated mammalian tissue, opening the door for the first time to practical transgenic cloning of farm animals.

Photo Not Available

Cloning Humans Is Not Going to Work until a Key Problem Is Solved

Since Dolly's birth in 1997, scientists have successfully cloned sheep, mice, cattle, goats, and pigs. Only a small percentage of the transplanted embryos survive to term, however, most dying late in pregnancy. Those that survive to be born usually die soon thereafter. Many become oversized, a condition known as *large offspring syndrome*.

The few cloned offspring that reach childhood face an uncertain future, as their development into adults tends to go unexpectedly haywire. For example, three heifers cloned by scientists at California State University at Chico were born healthy on March 9, 2001, but the calves died in April 2001 of abrupt immune system failure.

The Importance of Genomic Imprinting

What is going wrong? It turns out that to make a successful mammal, two kinds of information are required:

1. The genome says what *proteins are made.* The DNA sequences of the genes within an adult donor cell provide the basic information specifying a new human being—what proteins are to be made and how they are to be used to construct the adult body.

2. Reprogramming says when *proteins are made.* As human eggs and sperm mature, their DNA is conditioned by the parent female or male, a process called reprogramming. Chemical changes are made to the DNA that alter when particular genes are expressed without changing the genes' DNA sequences.

In the years since Dolly, scientists have learned a lot about reprogramming. It appears to occur by a process called *genomic imprinting*. While the details are complex, the basic mechanism of genomic imprinting is simple.

Like a book, a gene can have no impact unless it is read. Genomic imprinting works by blocking the cell's ability to read certain genes. A gene is locked in the "OFF" position by chemically altering some of the cytosine DNA units. Because this involves adding a $-CH_3$ group (a methyl group), the process is called *methylation*. After a gene has been methylated, the polymerase protein that is supposed to "read" the gene can no longer recognize it. The gene has been shut off (figure 9e.3).

Genomic imprinting can also lock genes in the "ON" position, permanently activating them. This process also uses methylation; in this case, however, it is not the gene that is blocked. Rather, a DNA sequence that normally would have prevented the gene from being read is blocked.

Genomic imprinting locks such a gene in the "ON" position by methylating the insulator sequence. Now the bulky protein cannot bind to it and shield the gene. No longer blocked, the helper proteins have the access they need to do their job, and the gene is expressed.

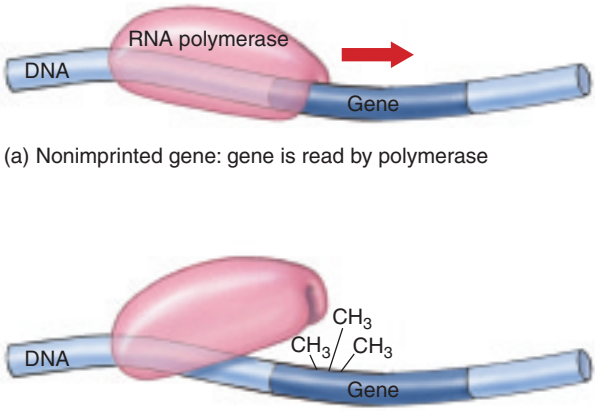


FIGURE 9e.3
Genomic imprinting. In mammalian reproductive cells (sperm and eggs), many genes with roles in early development are imprinted. For each of these genes, imprinting occurs either in the maternal side or the paternal side but never in both. Imprinting involves methylation—the addition of methyl groups. When methyl groups are added directly to the gene, the gene cannot be read and is silenced. Alternatively, gene expression can be activated by the methylation of genes encoding repressor proteins.

Why Cloning Fails

Normal human development depends on precise genomic imprinting. This chemical reprogramming of the DNA, which takes place in adult reproductive tissue, takes months for sperm and years for eggs.

During cloning, by contrast, the reprogramming of the donor DNA must occur within a few minutes. After the donor nucleus is added to an egg whose nucleus has been removed, the reconstituted egg begins to divide within minutes, starting the process of making a new individual.

Cloning fails because there is simply not enough time in these few minutes to get the reprogramming job done properly. Lorraine Young of the Roslin Institute in Scotland (Dolly's birthplace) reported in 2001, for example, that in Large Offspring Syndrome sheep, many genes have failed to become properly methylated.

Human cloning will not be practical until scientists figure out how to reprogram a donor nucleus, as occurs to DNA of sperm or eggs in our bodies. This reprogramming may be as simple as finding a way to postpone the onset of cell division after adding the donor nucleus to the enucleated egg, or may prove to be a much more complex process.

The point is, we don't have a clue today as to how to do it. In light of this undeniable ignorance, any attempt to clone a human is simply throwing stones in the dark, hoping to hit a target you cannot see.

Cloning of animals from adult tissue usually fails for lack of proper gene conditioning.

9e.2 Replacing defective genes in tissues remains an elusive goal.

Initial Attempts at Gene Therapy

The search for a way to introduce "healthy" genes into humans that lack them has gone on for 35 years. A trio of Nobel Prize winners (Ed Tatum, Joshua Lederberg, and Arthur Kornberg) suggested in 1964 that it should be possible to cure often-fatal genetic disorders like cystic fibrosis, muscular dystrophy, and multiple sclerosis by replacing the defective gene with a functional one.

Early Success

That such **gene transfer therapy** can work was first demonstrated in 1990. Two girls were cured of a rare blood disorder due to a defective gene for the enzyme adenosine deaminase. Scientists isolated working copies of this gene and introduced them into bone marrow cells taken from the girls. The gene-modified bone marrow cells were allowed to proliferate then were injected back into the girls. The girls recovered and stayed healthy. For the first time, a genetic disorder was cured by gene therapy.

The Rush to Cure Cystic Fibrosis

Like hounds to a hot scent, researchers set out to apply the new approach to one of the big killers, cystic fibrosis. The defective gene, labelled *cf*, had been isolated in 1989. Five years later, in 1994, researchers successfully transferred a healthy *cf* gene into a mouse with a defective one—they in effect had cured cystic fibrosis in a mouse. They achieved this remarkable result by adding the *cf* gene to a virus that infected the lungs of the mouse, carrying the gene with it "piggyback" into the lung cells. The virus chosen as the "vector" was adenovirus, a virus that causes colds and is very infective of lung cells. To avoid any complications, the lab mice used in the experiment had their immune systems disabled.

Very encouraged by these well-publicized preliminary trials with mice, several labs set out in 1995 to attempt to cure cystic fibrosis by transferring healthy copies of the *cf* gene into human patients. Confident of success, researchers added the human *cf* gene to adenovirus then squirted the gene-bearing virus into the lungs of cystic fibrosis patients. For eight weeks the gene therapy did seem successful, but then disaster struck. The gene-modified cells in the patients' lungs came under attack by the patients' own immune systems. The "healthy" *cf* genes were lost and with them any chance of a cure.

Problems with the Vector

Other attempts at gene therapy met with similar results, eight weeks of hope followed by failure. In retrospect, although it was not obvious then, the problem with these early attempts seems predictable. Adenovirus causes colds. Do you know anyone who has *never* had a cold? Due to previous colds, all of us have antibodies directed against adenovirus (figure 9e.4). We were introducing therapeutic genes in a vector our bodies are primed to destroy.

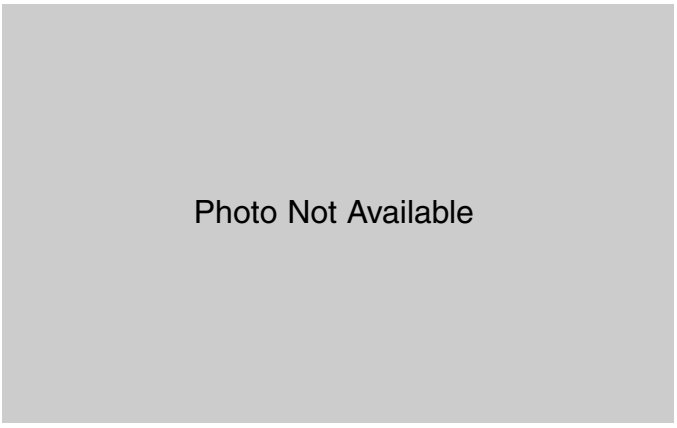


FIGURE 9e.4
Adenovirus (200,000×). This virus that causes the common cold has been used to carry healthy genes in clinical trials of gene therapy. Its use as a vector is problematic, however, as it is usually attacked and destroyed by the immune system of the host. In addition, it can cause severe immune reactions and can insert into the host's DNA at random places to cause mutations.

In 1995, the newly-appointed head of the National Institutes of Health (the NIH), Nobel winner Harold Varmus, held a comprehensive review of human gene therapy trials. Three problems became evident in the review: (1) The adenovirus vector being used in most trials elicits a strong immune response, leading to rejection of the added gene. (2) Adenovirus infection can, in rare instances, produce a very severe immune reaction, enough to kill. If many patients are treated, such instances can be expected to occur. (3) When the adenovirus infects a cell, it inserts its DNA into the human chromosome. Unfortunately, it does so at a random location. This means that the insertion events will cause mutations—by jumping into the middle of a gene, the virus inactivates that gene. Because the spot where adenovirus inserts is random, some of the mutations that result can be expected to cause cancer, certainly an unacceptable consequence.

Faced with these findings, Varmus called a halt to all further human clinical trials of gene therapy. "Go back to work in the laboratory," he told researchers, "until you get a vector that works."

In principle, it should be possible to cure hereditary disorders like cystic fibrosis by transferring a healthy gene into affected individuals. Early attempts using adenovirus vectors were not often successful.

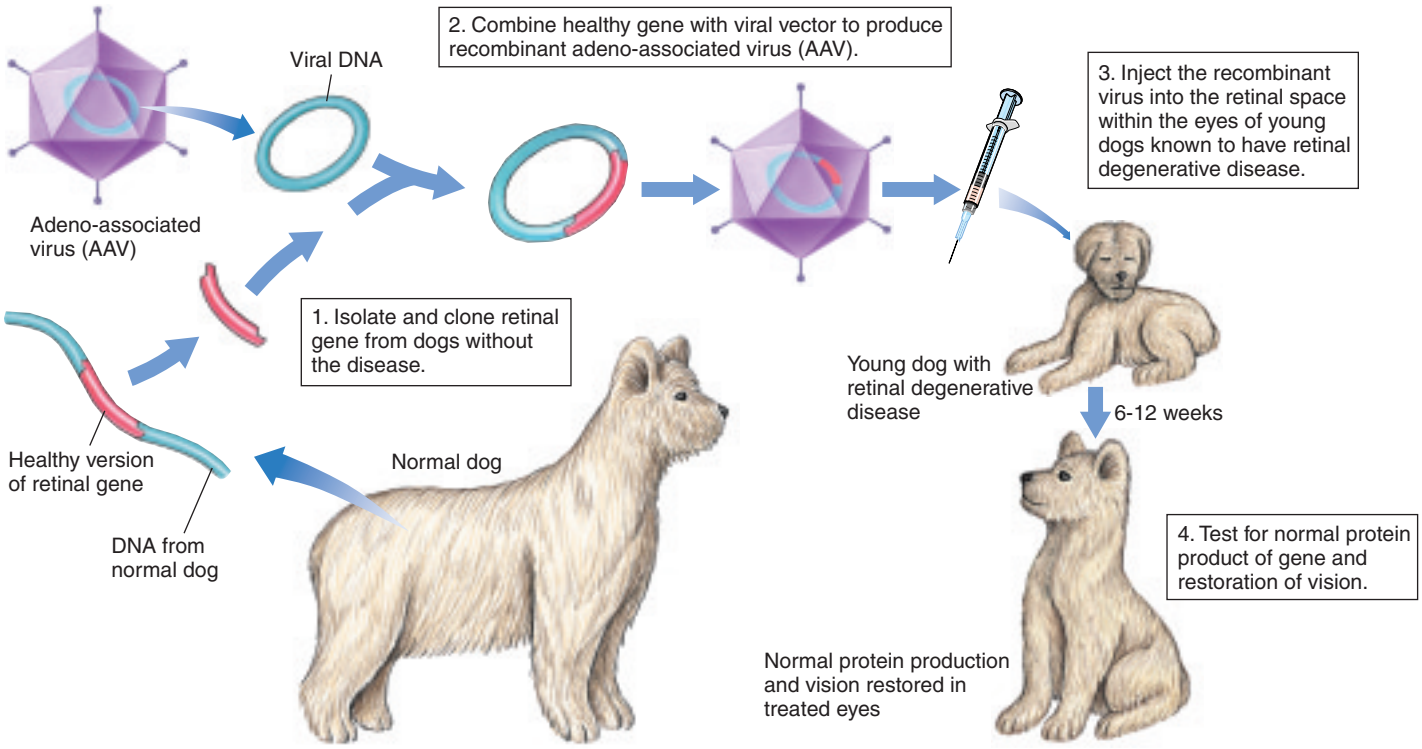


FIGURE 9E.5
Using gene therapy to cure a retinal degenerative disease in dogs. Recently, researchers were able to use genes from healthy dogs to restore vision in dogs blinded by an inherited retinal degenerative disease. This disease also occurs in human infants and is caused by a defective gene that leads to early vision loss, degeneration of the retinas, and blindness. In the gene therapy experiments, genes from dogs without the disease were inserted into 3-month-old dogs that were known to carry the defective gene and that had been blind since birth. Six weeks after the treatment, the dogs' eyes were producing the normal form of the gene's protein product, and by three months, tests showed that the dogs' vision was restored.

More Promising Vectors

Within a few years, researchers had a much more promising vector. This new gene carrier is a tiny parvovirus called *adeno-associated virus* (AAV). It has only two genes and needs adenovirus to replicate. To create a vector for gene transfer, researchers remove both of the AAV genes. The shell that remains is still quite infective and can carry human genes into patients. Importantly, AAV always enters the human DNA at the same place, a harmless location; thus, it does not produce cancer-causing mutations. In addition, AAV does not elicit a strong immune response—cells infected with AAV are not eliminated by a patient's immune system. Finally, AAV never elicits a dangerously strong immune response, so it is safe to administer AAV to patients.

Success with the AAV Vector

In 1999, AAV successfully cured anemia in rhesus monkeys. In monkeys, humans, and other mammals, red blood cell production is stimulated by a protein called erythropoietin (EPO). People with anemia (that is, low red blood cell counts), like dialysis patients, get regular injections of EPO. Using AAV to carry a souped-up EPO gene into the monkeys, scientists were able to greatly elevate their red blood cell counts, curing the monkeys of anemia—and they stayed cured.

A similar experiment using AAV cured dogs of a hereditary disorder leading to retinal degeneration and blindness. These dogs had a defective gene that produced a mutant form of a protein associated with the retina of the eye, and were blind. Injection of AAV bearing the needed gene into the fluid-filled compartment behind the retina restored their sight (figure 9e.5).

Human clinical trials are now underway again. In 2000, scientists performed the first gene therapy experiment for muscular dystrophy, injecting genes into a 35-year old South Dakota man. He is an early traveller on what is likely to become a well-travelled therapeutic highway. Trials are also underway for cystic fibrosis, rheumatoid arthritis, hemophilia, and a wide variety of cancers. The way seems open, the possibility of progress tantalizingly close.

New virus vectors like AAV avoid the problems of earlier vectors and offer promise of gene transfer therapy cures.

Ethical Issues Raised by Gene Interventions

The advent of highly publicized clinical trials of gene therapy has raised serious ethical issues that are being widely discussed within the medical and scientific communities. Medical ethicists tend to avoid the term "gene therapy," as these words seem to offer a cure to potential patients, when, in fact, today's procedures are still largely experimental. Ethicists prefer the term **gene intervention**. A gene intervention is any procedure that deliberately alters a person's genes, whether by modification of existing genes or by contributing additional genes.

When considering the ethics of gene interventions, it is important to draw a clear distinction between changes that will be hereditary and those that will not. There are two general possibilities that must be considered:

1. Somatic tissue. Gene intervention of somatic tissue is what we generally mean by "gene therapy." Such procedures attempt to correct problems with somatic tissues by adding "healthy" genes to their cells. Attempting to cure cystic fibrosis by adding healthy *cf* genes to lung and pancreas cells is a widely discussed example. Changes induced by gene interventions in somatic tissues are not inherited.

2. Germ-line tissue. Gene intervention of germ-line tissue has not yet been attempted in humans. Experiments have been carried out with mice for decades and in 2000 with a rhesus monkey (dubbed ANDi for "inserted DNA" read backwards). These experiments typically used an adenovirus vector and suffered from the problems associated with that vector: the genes inserted any old place in the genome, and often did not function correctly after insertion. New experiments using improved vectors like AAV can be expected to produce better results. Importantly, changes induced by gene interventions in germ-line tissue are inherited.

Ethical questions arise from both sorts of gene interventions, but their implications are far more profound when considering germ-line changes. Such changes may offer advantages over somatic therapies, as the changes are permanent. The children of a cystic fibrosis parent with modified germ-line tissue would be free of worry about contracting the disease, as would all their descendents. However, because the procedure is in effect altering the human genome, the possibility of unanticipated negative consequences must be evaluated carefully.

The Beneficence Principle

In the United States, every proposed gene intervention involving human subjects must be approved by the NIH. In trying to assess the ethical questions raised by gene interventions, NIH ethicists apply a broad test called the **beneficence principle**. Phrased broadly, the beneficence principle states that in reaching decisions about potential therapy, one should carefully weigh risks versus benefits. Thus, for example, it is important that results of earlier experiments carried out with laboratory animals be solid and promising before risking the life of a human patient. Gene transfer vectors like adenovirus greatly increase the risk of a procedure because of the possibility their insertion into a human chromosome might cause cancer in rare patients. Safer vectors like AAV reduce that risk.

Because the risks of germ-line gene intervention are difficult to assess ahead of time and must be born for many generations by babies who have had no say in the decision to undergo the therapy, it is particularly important that the patient suffer from a condition sufficiently "bad" to justify the risk. A potentially fatal hereditary disorder like cystic fibrosis or muscular dystrophy might be considered to satisfy the beneficence principle if animals trials work with a high probability of success.

But what about "enhancements" that seek to improve a person's genetic complement, such as adding a gene that increases life span (such a human gene has been recently reported)? Are "designer babies" OK? How about nonlethal disorders like hypercholesterolemia, a gene mutation leading to too-high-cholesterol that is the most common human genetic disorder? The risk would have to be very low before the beneficence principle would recommend such changes.

The Respect-for-Persons Principle

Ethicists apply a second principle, a test called the **respect-for-persons principle**, in trying to assess the desirability of gene interventions. This principle is particularly important in the case of germ-line interventions. The respect-for-persons principle states that the persons affected by the procedure have a right to make their own informed decisions. Prospective parents contemplating germ-line gene intervention have a right to a clear explanation of the risks, possible benefits, and particularly of the alternative ways available to have healthy children. Parents who know they carry a gene disorder might instead choose *in vitro* fertilization, for example, where the doctor can select a healthy embryo for implantation. In any ongoing study, parents, children, and future descendents must be allowed to discontinue their participation in the research study. Participation in any gene intervention must always be informed and free.

Gene therapy and other gene interventions raise a host of ethical issues, because the procedures involve human beings and affect real lives. These ethical issues are being actively evaluated by both researchers and clinicians.

9e.3 Replacing defective tissues is a promising but controversial possibility.

Embryonic Stem Cells

In 1981, mouse stem cells were first discovered to be pluripotent—to have the ability to form any body tissue, and even an adult animal—launching the era of stem cell research. After many years of failed attempts, human embryonic stem cells were isolated by James Thomson of the University of Wisconsin in 1998.

What is an embryonic stem cell? At the dawn of a human life, a sperm fertilizes an egg to create a single cell destined to become a child. As development commences, that cell begins to divide, producing after five or six days a small ball of a few hundred cells called a blastocyst. Described in Chapter 28, a blastocyst consists of a protective outer layer destined to form the placenta, enclosing an inner cell mass of **embryonic stem cells.** Each embryonic stem cell is capable by itself of developing into a healthy individual. In cattle breeding, for example, these cells are frequently separated by the breeder and used to produce multiple clones of valuable offspring.

Because they can develop into any tissue, these embryonic stem cells offer the exciting possibility of restoring damaged tissues, such as muscle or nerve tissue (figure 9e.6). Experiments have already been tried successfully in mice. Heart muscle cells grown from mouse embryonic stem cells have been successfully integrated with the heart tissue of a living mouse. This suggests that the damaged heart muscle of heart attack victims might be repairable with stem cells. In other experiments with mice, damaged spinal neurons have been partially repaired, suggesting a path to treating spinal injuries. DOPA-producing neurons of mouse brains whose progressive loss is responsible for Parkinsons disease have been successfully replaced with embryonic stem cells, as have the islet cells of the pancreas whose loss leads to juvenile diabetes.

These very promising experiments in mice suggest that embryonic stem cell therapy may hold great promise in treating a wide variety of human illnesses involving damaged or lost tissues. This research, however, is quite controversial, as embryonic stem cells are typically isolated from discarded embryos of reproductive clinics, where in vitro fertilization procedures typically produce many more human embryos than can be successfully implanted in the prospective mother's womb. This raises serious ethical issues among some citizens who regard life as starting at fertilization.

There is a second serious problem associated with using embryonic stem cells to replace defective or lost tissues. All of the successful experiments described above were carried out in mice whose immune systems had been disabled. Had these mice possessed fully functional immune systems, they almost certainly would have rejected the implanted stem cells as foreign. For such stem cell therapy to work in humans, this problem will need to be addressed and solved. The need to solve this problem is the primary impetus behind the push to develop therapeutic cloning, described later in this chapter.

Human embryonic stem cells offer the possibility of replacing damaged or lost human tissues, although the procedures are controversial.

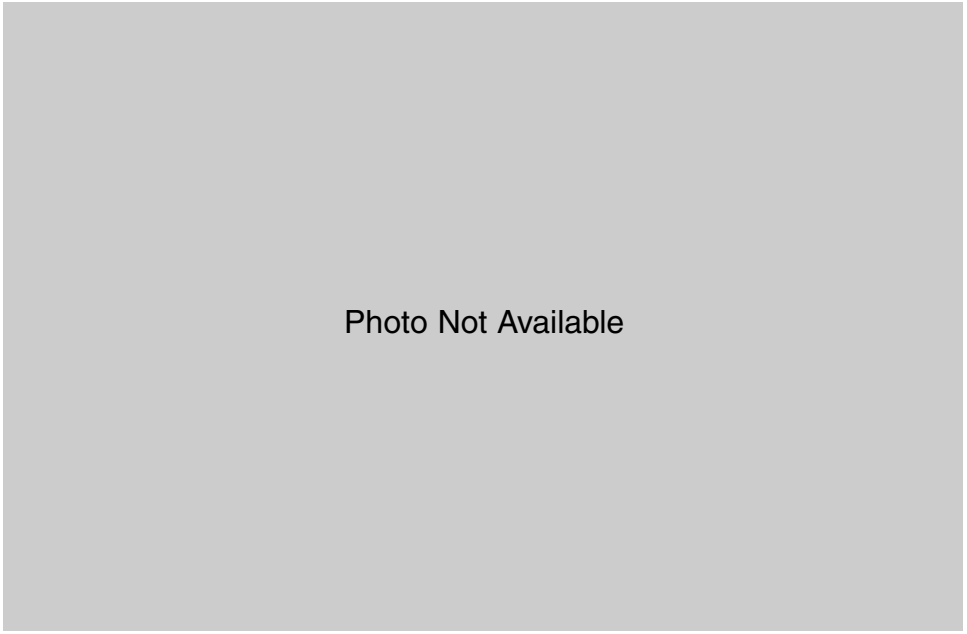


FIGURE 9e.6
Using embryonic stem cells to restore damaged tissue. Embryonic stem cells can develop into any body tissue. Methods for growing the tissue and using it to repair damaged tissue in adults, such as the brain cells of multiple sclerosis patients, heart muscle, and spinal nerves, are being developed.

Tissue-Specific Adult Stem Cells

New results promise an innovative way around the ethical maze presented by stem cells derived from embryos. Go back for a moment to what we were saying about how a human child develops. What happens next to the embryonic stem cells? They start to take different developmental paths. Some become destined to form nerve tissue and, after this decision is taken, cannot ever produce any other kind of cell. They are then called nerve stem cells. Others become specialized to produce blood, still others muscle. Each major tissue is represented by its own kind of **tissue-specific stem cell** (figure 9e.7). Now here's the key point: as development proceeds, these tissue-specific stem cells persist. Even in adults. So why not use these adult cells rather than embryonic stem cells?

Transplanted Tissue-Specific Stem Cells Cure Multiple Sclerosis in Mice

In pathfinding 1999 laboratory experiments by Dr. Evan Snyder of Harvard Medical School, tissue-specific stem cells were able to restore lost brain tissue. He and his coworkers injected neural stem cells (immediate descendants of embryonic stem cells able to become any kind of neural cell) into the brains of newborn mice with a disease resembling multiple sclerosis (MS). These mice lacked the cells that maintain the layers of myelin insulation around signal-conducting nerves. The injected stem cells migrated all over the brain and were able to convert themselves into the missing type of cell. The new cells then proceeded to repair the ravages of the disease by replacing the lost insulation of signal-conducting nerve cells. Many of the treated mice fully recovered. In mice at least, tissue-specific stem cells offer a treatment for MS.

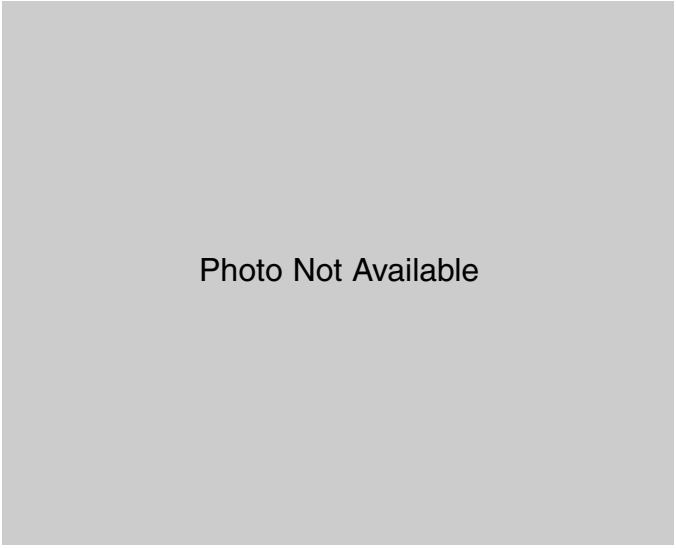
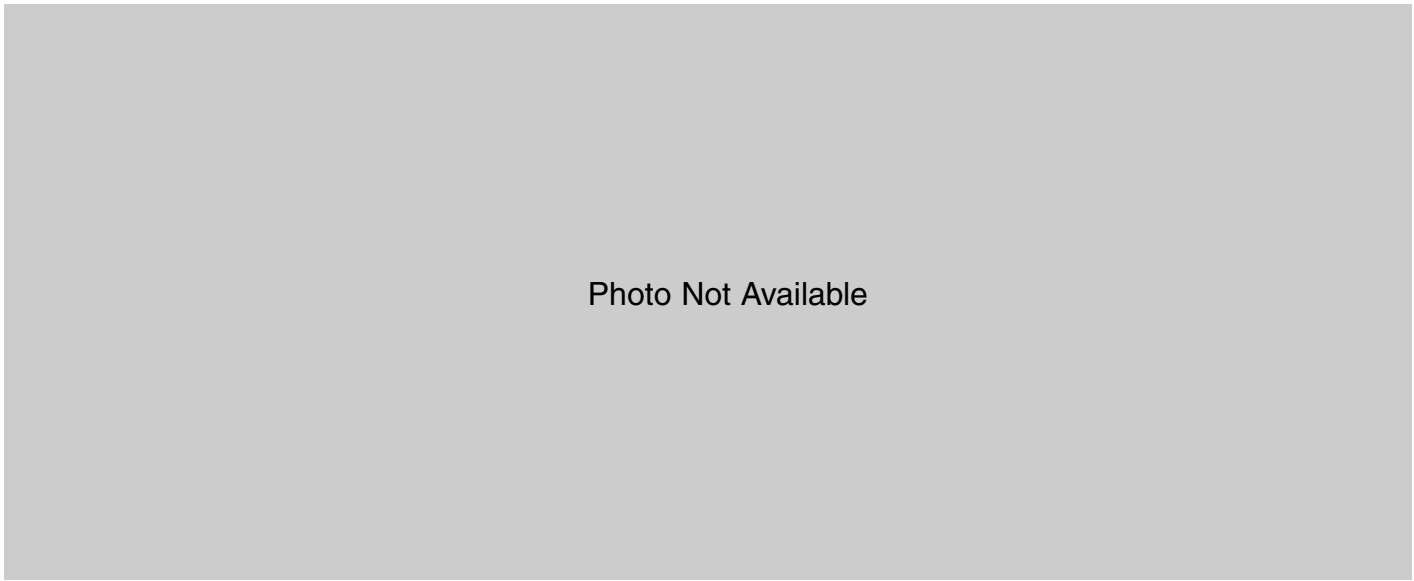


FIGURE 9e.7
Human embryonic stem cells. Stem cells removed from a six-day blastocyst can be established in culture and then maintained indefinitely.

The approach seems very straightforward and should apply to humans. Indeed, blood stem cells are already routinely used in humans to replenish the bone marrow of cancer patients after marrow-destroying therapy. The problem with extending the approach to other kinds of tissue-specific stem cells is that it has not always been easy to find the kind of tissue-specific stem cell needed.

Transplanted tissue-specific stem cells may allow us to replace damaged or lost tissue, offering cures for many disorders that cannot now be treated while avoiding the ethical problems posed by embryonic stem cells.



Therapeutic Cloning of Human Embryonic Stem Cells

By surgically transplanting embryonic stem cells, scientists have performed the remarkable feat of repairing disabled body tissues in mice. The basic strategy for repairing damaged tissues is to surgically transfer embryonic stem cells to the damaged area, where the stem cells can form healthy replacement cells. Stem cells transferred into mouse heart muscle develop into heart muscle cells, replacing cells dead from heart attack. Stem cells transferred into a mouse brain form neurons, offering hope that we eventually will learn to use embryonic stem cells to repair spinal injury. Embryonic stem cells of mice have been induced to become insulin-secreting pancreas cells. The new cells produce only about 2% as much insulin as normal cells do, so there is still plenty to learn, but the take-home message is clear: transplanted embryonic stem cells offer a path to cure type 1 diabetes.

While exciting, these advances in stem cell research were all experiments carried out in strains of mice without functioning immune systems. This prevents the mice from rejecting transplanted stem cells as "foreign." A human with a normal immune system might well refuse to accept transplanted stem cells simply because they are from another individual.

Early in 2001, a research team at the Rockefeller University reported a way around this potentially serious problem. Their solution? They isolate skin cells, then using the same procedure that created Dolly, they create an embryo from them. First they remove the nucleus from the skin cell, and then they insert it into an egg whose nucleus has already been removed. The egg with its skin cell nucleus is allowed to form a 120-cell embryo. The embryo is then destroyed, its cells used as embryonic stem cells for transfer to injured tissue.

Using this procedure, which they called **therapeutic cloning**, the researchers succeeded in making cells from the tail of a mouse convert into the dopamine-producing cells of the brain that are lost in Parkinsons disease.

Therapeutic cloning successfully addresses the key problem that must be solved before stem cells can be used to repair human tissues damaged by heart attack, nerve injury, diabetes (figure 9e.8), or Parkinsons, which is immune acceptance. Since stem cells are cloned from the body's own tissues in therapeutic cloning, they pass the immune system's "self" identity check, and the body readily accepts them.

The first cloning of a human embryo from adult skin cells was reported in November 2001. Designed to allow therapeutic cloning, the procedure is controversial, for fear the embryo could be brought to term by inserting it into a human uterus. Actually, without first solving the gene imprinting problem, this would be impossible, and the researchers argue that they had no such intention—rather, they had wished to obtain stem cells for therapeutic cloning. In fact, their human clones did not survive long enough to make stem cells. There is little doubt that the controversy over human therapeutic cloning will continue.

Therapeutic cloning involves initiating blastocyst development from a patient's tissue using nuclear transplant procedures, then using these embryonic stem cells to replace the patient's damaged or lost tissue.

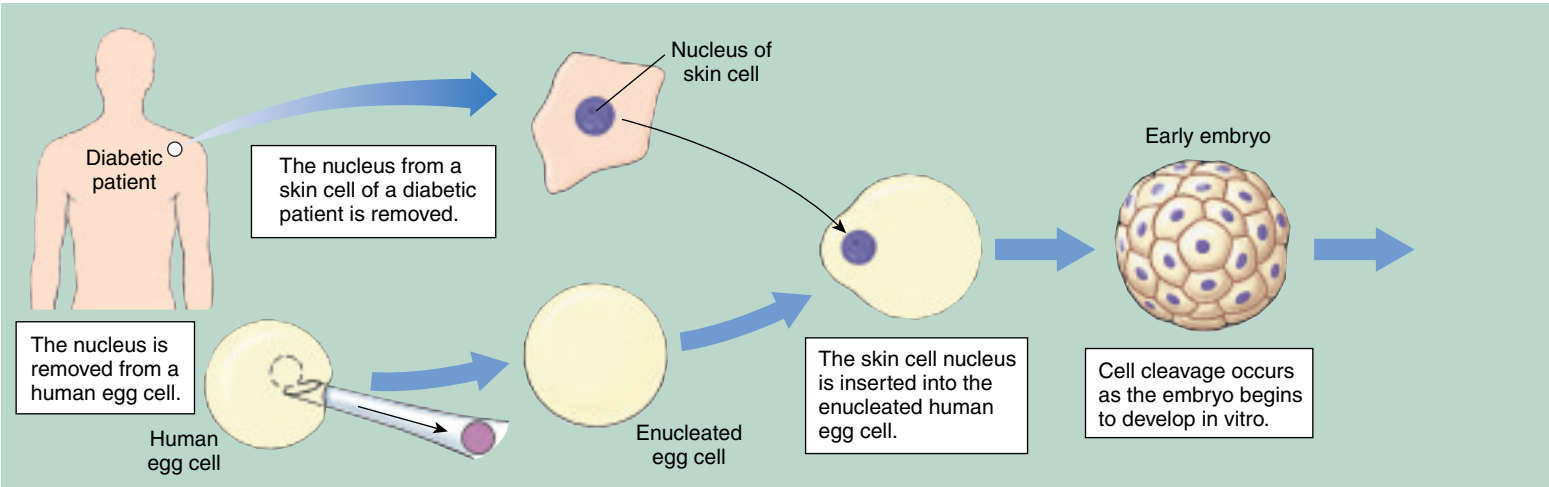


FIGURE 9e.8
How human embryos might be used for therapeutic cloning. Therapeutic cloning differs from reproductive DNA cloning in that after the initial similar stages, the embryo is destroyed and its embryonic stem cells are extracted, grown in culture, and added to a tissue of the individual who provided the DNA. In reproductive cloning, by contrast, the embryo is preserved to be implanted and grown to term in a surrogate mother. It is this latter procedure that was done in cloning Dolly the sheep. Human cells were first cloned in November 2001 in a failed attempt to obtain stem cells for therapeutic cloning procedures such as outlined in this figure.

The Search for Pleuripotent Adult Stem Cells

The difficult ethical issues raised by human therapeutic cloning could be largely avoided if the stem cells did not have to be harvested from an embryo. Imagine, for example, that it were possible to find stem cells able to become any other kind of cell—technically known as pleuripotent stem cells—somewhere in the body of an *adult* human.

In 2001, researchers reported that they had found just such cells in the bone marrow of mice. They transplanted single stem cells from mouse bone marrow into the marrow of individuals whose marrow had been destroyed. After 11 months, the one stem cell had given rise to descendant cells that had migrated throughout the body, forming new bone, blood, lung, esophagus, stomach, intestine, liver, and skin cells (figure 9e.9). The bone marrow stem cells appear to have the properties of the long-sought pleuripotent adult stem cells. Many labs are trying to repeat this exciting preliminary result.

Many of the ethical issues concerning the use of human embryonic stem cells for research can be avoided if cells with similar developmental potential can be found in adult tissues. Some results suggest this may be possible.

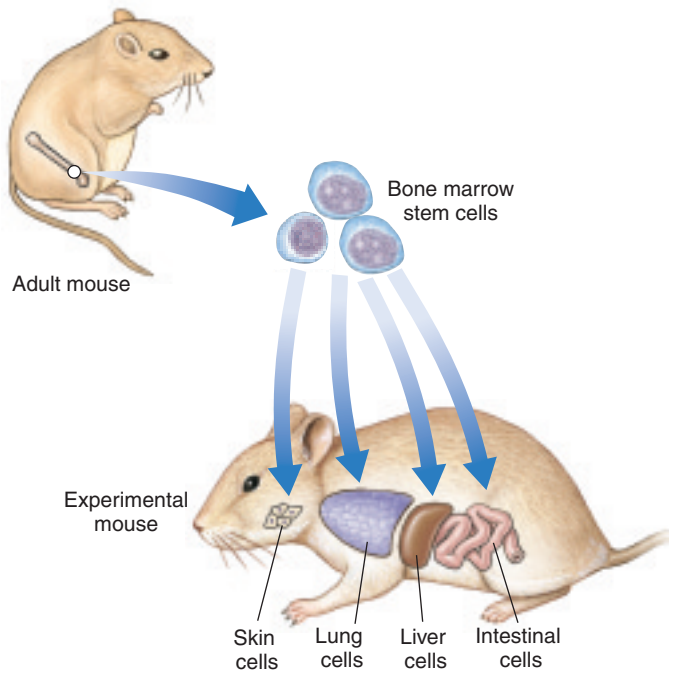
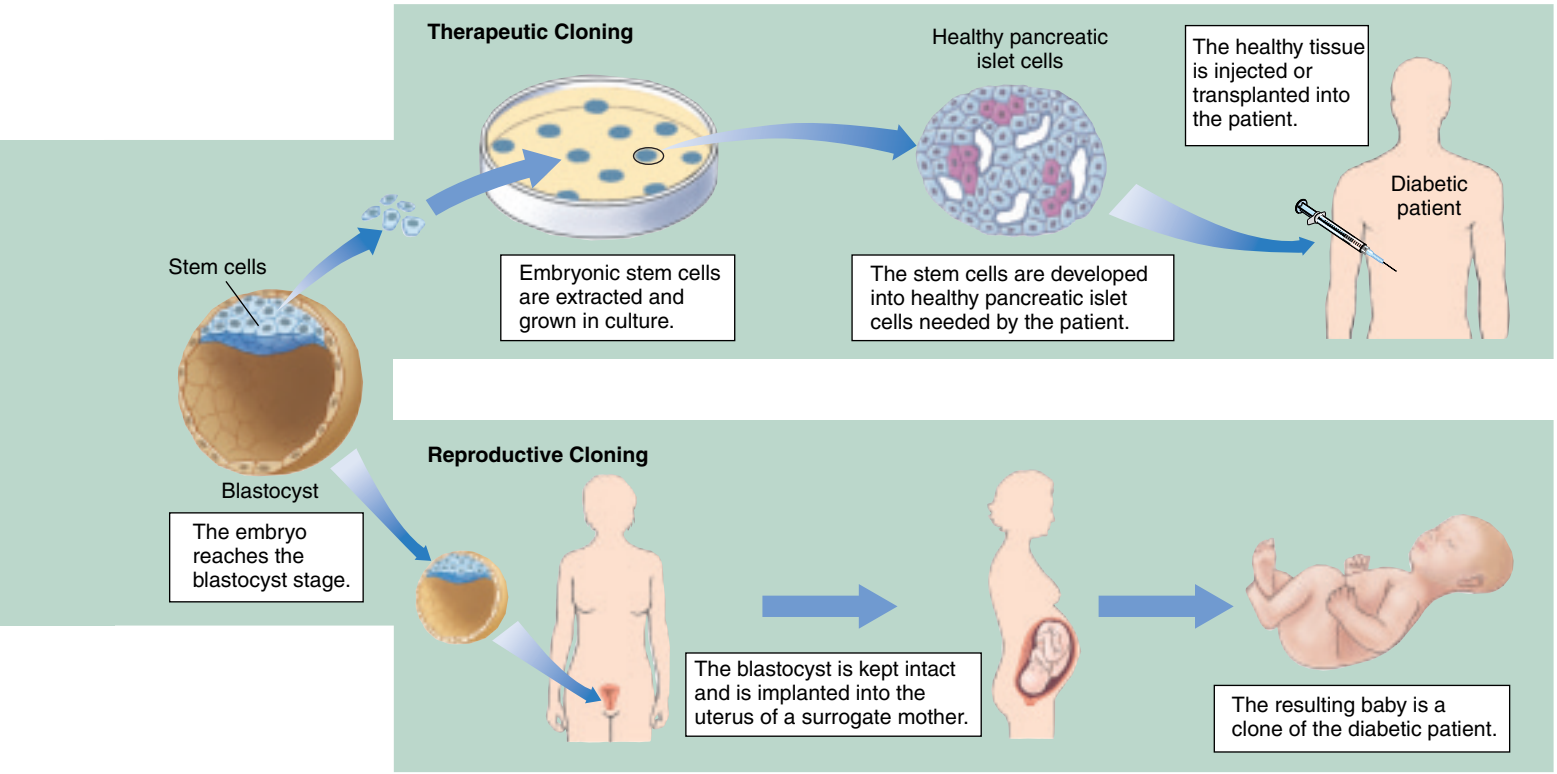


FIGURE 9e.9
Pleuripotent stem cells. In May, 2001, a single cell from the bone marrow of a mouse was demonstrated to have added functional cells to the lungs, liver, intestine, and skin of an experimental mouse.



Grappling with the Ethics of Stem Cell Research

Few advances in science have proven as controversial as embryonic stem cell research and the possibility of using therapeutic cloning to generate them. The relevant facts are straightforward. Human embryonic stem cells retain the potential to become any tissue in the body and thus have enormous promise for treating a wide range of diseases. Human embryonic stem cells are very difficult to isolate and establish in culture, but a few dozen lines have been successfully obtained from the inner cell mass of six-day blastocysts. It is important to isolate the cells at this early stage, before development begins the process of restricting what sorts of tissues the stem cells can become. The blastocysts are obtained from reproductive clinics, which routinely produce excess embryos in the process of helping infertile couples have children by *in vitro* fertilization.

However, obtaining embryonic stem cells destroys the early embryo in the process; for this reason, stem cell research raises profound ethical issues. The timeless question of when human life begins cannot be avoided when human embryos are being deliberately destroyed. What is the moral standing of a six-day human embryo? In resolving the tension between scientific knowledge and moral sensibilities, religious, philosophical, and cultural issues all come into play. Table 9e.1 illustrates the range of issues being discussed.

It will come as no surprise that government, which funds much of modern biomedical research, has become embroiled in the controversy. In Britain, reproductive cloning is banned, but stem cell research and therapeutic cloning to obtain clinically useful stem cells are both permitted. Because the research is funded by the government, there is careful ethical supervision of all research by a variety of governmental oversight committees. Britain's Human Fertilization and Embryology Authority (HFEA), for example, is a panel of scientists and ethicists accountable to parliament, which oversees government-funded stem cell research. Similar arrangements are being established in Japan and France. Germany, by contrast, discourages all stem cell research.

In the United States, the situation is ambivalent. American stem cell research is chiefly carried out in private research labs using no government funds and thus are subject to no ethical oversight. This leaves American scientists pretty much free to do what they want, so long as they use private money. Federal funds were made available in the summer of 2001 for research on the small number of existing human embryonic stem cell lines. In what has become a very political contest between those favoring increased stem cell research and those opposing all such research on ethical grounds, it seems certain that federal government policies with regard to stem cell research will fluctuate for some time to come.

Table 9e.1 The Ethics of Stem Cell Research

1. DESTRUCTION OF HUMAN EMBRYOS

opponents A human life begins at the moment egg and sperm are united, so destroying an embryo to harvest embryonic stem cells is simply murder, and morally wrong. Benefits to others, however great, cannot justify the destruction of a human life.

proponents While human embryos should be treated with respect, the potential for saving lives that embryonic stem cell research offers is also a strong moral imperative. The blastocysts being used to obtain stem cells were created to help infertile couples conceive and would have been destroyed in any event. Besides, it is not clear that an individual life begins at fertilization. An early embryo can split, leading to the birth of identical twins, so it can be argued that individuality begins some days after fertilization.

2. POSSIBILITY OF FUTURE ABUSE

opponents Permitting embryonic stem cell research may open the door to further ethically-objectionable research. Certainly the development of embryonic stem cell therapies will lead to a cry for therapeutic cloning, so that the therapies can actually be employed in clinical situations. This creation of embryos specifically for the production of embryonic stem cells is morally wrong. In addition, therapeutic cloning to obtain clinical embryonic stem cells is unnatural, as it involves producing a viable human embryo without fertilization. If therapeutic use of the results of stem cell research is unacceptable, then there is little use in carrying out the research in the first place. It only delays and excalibrates the morally difficult choice posed by therapeutic cloning. Even more disturbing, it opens the door to reproductive cloning—the production of human babies from cloned embryos. This moral nightmare will always be a threat if an absolute line is not drawn preventing all cloning.

proponents In practice, only a few hundred cell lines are likely to be required to carry out embryonic stem cell research. The derivation of human cell lines, which is difficult and expensive to do, would be limited to these few lines. Continuous destruction of embryos would be neither desirable nor likely. Therapeutic cloning presents a separate and more complex ethical issue. Because fertilization is not involved, the blastocyst might better be thought of as an "activated egg" rather than as an embryo. Such a distinction has biological merit and avoids the ethical issues posed by human reproductive cloning, which should be banned.

3. ALTERNATIVE SOURCES OF STEM CELLS

opponents Why not use stem cells derived from adult tissues? These stem cells raise no difficult ethical issues and can lead to the same medical benefits.

proponents Adult stem cells simply cannot do the job. By the time embryonic stem cells have developed into adult stem cells, they have lost much of their developmental versatility and so lack the range of medical capabilities necessary for regenerative medicine. Also, adult stem cells are not very prolific and have proven to be difficult to use in therapeutic procedures on experimental laboratory animals.

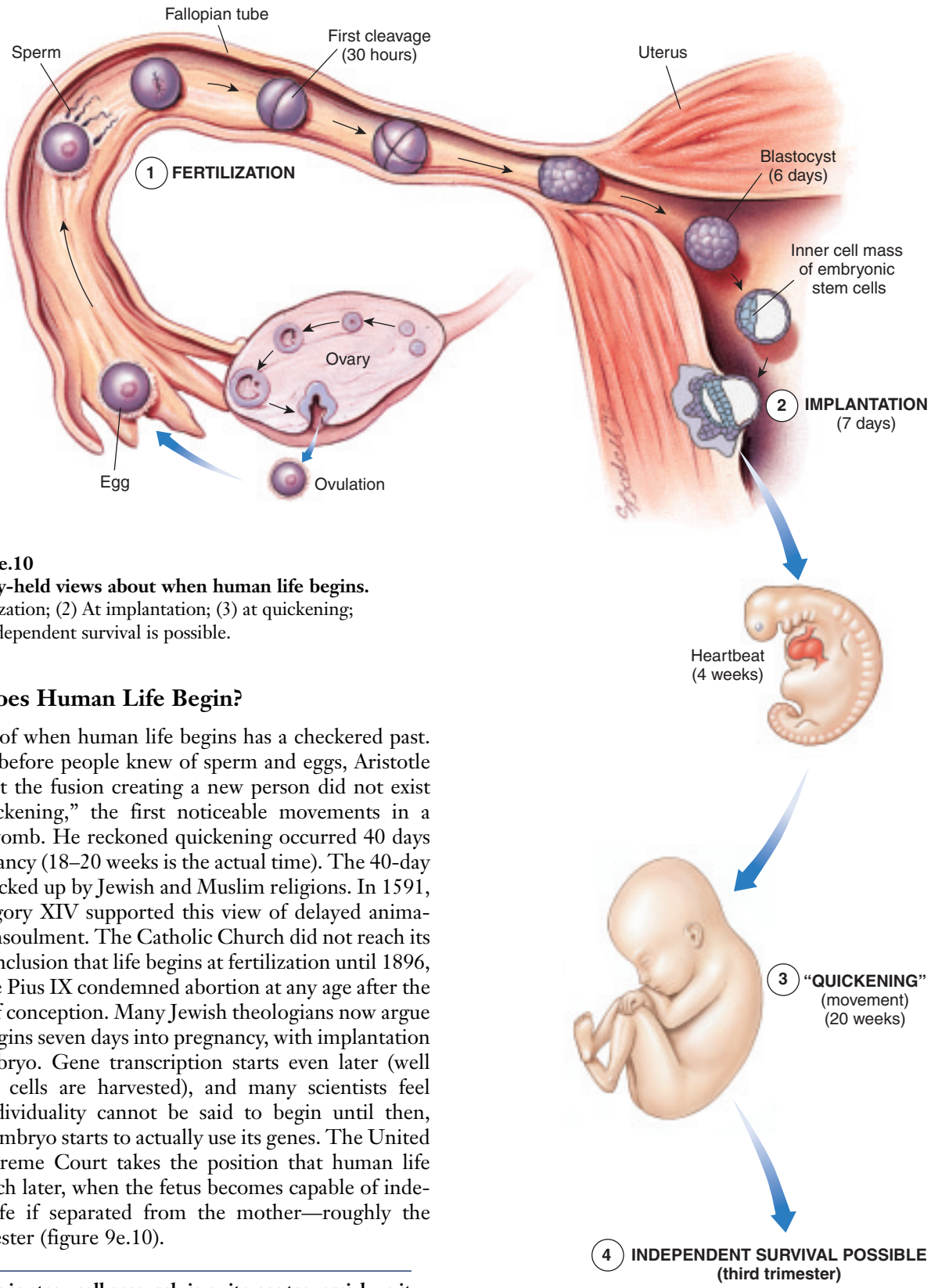


FIGURE 9e.10
Four widely-held views about when human life begins.
(1) At fertilization; (2) At implantation; (3) at quickening; (4) when independent survival is possible.

When Does Human Life Begin?

The story of when human life begins has a checkered past. Centuries before people knew of sperm and eggs, Aristotle argued that the fusion creating a new person did not exist until "quickening," the first noticeable movements in a woman's womb. He reckoned quickening occurred 40 days into pregnancy (18–20 weeks is the actual time). The 40-day rule was picked up by Jewish and Muslim religions. In 1591, Pope Gregory XIV supported this view of delayed animation and ensoulment. The Catholic Church did not reach its current conclusion that life begins at fertilization until 1896, when Pope Pius IX condemned abortion at any age after the moment of conception. Many Jewish theologians now argue that life begins seven days into pregnancy, with implantation of the embryo. Gene transcription starts even later (well after stem cells are harvested), and many scientists feel human individuality cannot be said to begin until then, when the embryo starts to actually use its genes. The United States Supreme Court takes the position that human life begins much later, when the fetus becomes capable of independent life if separated from the mother—roughly the third trimester (figure 9e.10).

Embryonic stem cell research is quite controversial, as it involves many ethical issues, not the least of which is when human life begins.