

## About this document

This primer on stem cells is intended for anyone who wishes to learn more about the biological properties of stem cells, the important questions about stem cells that are the focus of scientific research, and the potential use of stem cells in research and in treating disease. The primer includes information about stem cells derived from embryonic and non-embryonic tissues. Much of the information included here is about stem cells derived from human tissues, but some studies of animal-derived stem cells are also described.

The National Institutes of Health (NIH) developed this primer to help readers understand the answers to questions such as:

- ▶ What are stem cells?
- ▶ What are the different types of stem cells, and where do they come from?
- ▶ What is the potential for new medical treatments using stem cells?
- ▶ What research is needed to make such treatments a reality?

This document provides basic information about stem cells. More detailed discussion is available from the NIH stem cell reports at <http://stemcells.nih.gov/info/>. Quick answers to specific common queries can be found on the [Frequently Asked Questions](#) page.

Throughout “Stem Cell Basics,” many technical terms appear in **bold, underlined maroon** type. Click the term to see its definition in the Glossary at the end of the primer.

## I. Introduction: What are stem cells, and why are they important?

**Stem cells** have the remarkable potential to develop into many different cell types in the body during early life and growth. In addition, in many tissues they serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell.

Stem cells are distinguished from other cell types by two important characteristics. First, they are unspecialized cells capable of renewing themselves through **cell division**, sometimes after long periods of inactivity. Second, under certain physiologic or experimental conditions, they can be induced to become tissue- or organ-specific cells

with special functions. In some organs, such as the gut and bone marrow, stem cells regularly divide to repair and replace worn out or damaged tissues. In other organs, however, such as the pancreas and the heart, stem cells only divide under special conditions.

Until recently, scientists primarily worked with two kinds of stem cells from animals and humans: **embryonic stem cells** and non-embryonic **“somatic” or “adult” stem cells**. The functions and characteristics of these cells will be explained in this document. Scientists discovered ways to derive embryonic stem cells from early mouse embryos nearly 30 years ago, in 1981. The detailed study of the biology of mouse stem cells led to the discovery, in 1998, of a method to derive stem cells from human embryos and grow the cells in the laboratory. These cells are called **human embryonic stem cells**. The embryos used in these studies were created for reproductive purposes through ***in vitro* fertilization** procedures. When they were no longer needed for that purpose, they were donated for research with the informed consent of the donor. In 2006, researchers made another breakthrough by identifying conditions that would allow some specialized adult cells to be “reprogrammed” genetically to assume a stem cell-like state. This new type of stem cell, called **induced pluripotent stem cells (IPSCs)**, will be discussed in a later section of this document.

Stem cells are important for living organisms for many reasons. In the 3- to 5-day-old embryo, called a **blastocyst**, the inner cells give rise to the entire body of the organism, including all of the many specialized cell types and organs such as the heart, lung, skin, sperm, eggs and other tissues. In some adult tissues, such as bone marrow, muscle, and brain, discrete populations of adult stem cells generate replacements for cells that are lost through normal wear and tear, injury, or disease.

Given their unique regenerative abilities, stem cells offer new potentials for treating diseases such as diabetes and heart disease. However, much work remains to be done in the laboratory and the clinic to understand how to use these cells for **cell-based therapies** to treat disease, which is also referred to as **regenerative or reparative medicine**.

Laboratory studies of stem cells enable scientists to learn about the cells’ essential properties and what makes them different from specialized cell types. Scientists are already using stem cells in the laboratory to screen new drugs and to develop model systems to study normal growth and identify the causes of birth defects.

Research on **stem cells** continues to advance knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. Stem cell research is one of the most fascinating areas of contemporary biology, but, as with many expanding fields of scientific inquiry, research on stem cells raises scientific questions as rapidly as it generates new discoveries.

## II. What are the unique properties of all stem cells?

Stem cells differ from other types of cells in the body. All stem cells—regardless of their source—have three general properties: 1) they are capable of dividing and renewing themselves for long periods; 2) they are unspecialized; and 3) they can give rise to specialized cell types.

*Stem cells are capable of dividing and renewing themselves for long periods.* Unlike muscle cells, blood cells, or nerve cells—which do not normally replicate themselves—stem cells may replicate many times, or proliferate. A starting population of stem cells that proliferates for many months in the laboratory can yield millions of cells. If the resulting cells continue to be unspecialized, like the parent stem cells, the cells are said to be capable of long-term self-renewal.

Scientists are trying to understand two fundamental properties of stem cells that relate to their long-term self-renewal:

1. Why can embryonic stem cells proliferate for a year or more in the laboratory without differentiating, but most non-embryonic stem cells (adult stem cells) cannot; and
2. What factors in living organisms normally regulate stem cell proliferation and self-renewal?

Discovering the answers to these questions may make it possible to understand how cell proliferation is regulated during normal embryonic development or during the abnormal cell division that leads to cancer. Such information would also enable scientists to grow embryonic and non-embryonic stem cells more efficiently in the laboratory.

The specific factors and conditions that allow stem cells to remain unspecialized are of great interest to scientists. It has taken many years of trial and error to learn to derive and maintain stem cells in the laboratory without them spontaneously differentiating into specific cell types. For example, it took two decades to learn how to grow human embryonic stem cells in the laboratory following the development of conditions for growing mouse stem cells. Therefore, understanding the signals in a mature organism that cause a stem cell population to proliferate and remain unspecialized until the cells are needed. Such information is critical for scientists to be able to grow large numbers of unspecialized stem cells in the laboratory for further experimentation.

*Stem cells are unspecialized.* One of the fundamental properties of a stem cell is that it does not have any tissue-specific structures that allow it to perform specialized functions. For example, a stem cell cannot work with its neighbors to pump blood through the body

(like a heart muscle cell), and it cannot carry oxygen molecules through the bloodstream (like a red blood cell). However, unspecialized stem cells can give rise to specialized cells, including heart muscle cells, blood cells, or nerve cells.

*Stem cells can give rise to specialized cells.* When unspecialized stem cells give rise to specialized cells, the process is called **differentiation**. While differentiating, the cell usually goes through several stages, becoming more specialized at each step. Scientists are just beginning to understand the signals inside and outside cells that trigger each step of the differentiation process. The internal **signals** are controlled by a cell's **genes**, which are interspersed across long strands of DNA, and carry coded instructions for all cellular structures and functions. The external signals for cell differentiation include chemicals secreted by other cells, physical contact with neighboring cells, and certain molecules in the **microenvironment**. The interaction of signals during differentiation causes the cell's DNA to acquire **epigenetic** marks that restrict DNA expression in the cell and can be passed on through cell division.

Many questions about stem cell differentiation remain. For example, are the internal and external signals for cell differentiation similar for all kinds of stem cells? Can specific sets of signals be identified that promote differentiation into specific cell types? Addressing these questions may lead scientists to find new ways to control stem cell differentiation in the laboratory, thereby growing cells or tissues that can be used for specific purposes such as **cell-based therapies** or drug screening.

Adult stem cells typically generate the cell types of the tissue in which they reside. For example, a blood-forming adult stem cell in the bone marrow normally gives rise to the many types of blood cells. It is generally accepted that a blood-forming cell in the bone marrow—which is called a **hematopoietic stem cell**—cannot give rise to the cells of a very different tissue, such as nerve cells in the brain. Experiments over the last several years have purported to show that stem cells from one tissue may give rise to cell types of a completely different tissue. This remains an area of great debate within the research community. This controversy demonstrates the challenges of studying adult stem cells and suggests that additional research using adult stem cells is necessary to understand their full potential as future therapies.

### III. What are embryonic stem cells?

#### A. What stages of early embryonic development are important for generating embryonic stem cells?

**Embryonic stem cells**, as their name suggests, are derived from embryos. Most embryonic stem cells are derived from embryos that develop from eggs that have been fertilized *in vitro*—in an **in vitro fertilization** clinic—and then donated for research purposes with informed consent of the donors. They are *not* derived from eggs fertilized in a woman's body.

#### B. How are embryonic stem cells grown in the laboratory?

Growing cells in the laboratory is known as **cell culture**. Human embryonic stem cells (hESCs) are generated by transferring cells from a **preimplantation**-stage embryo into a plastic laboratory culture dish that contains a nutrient broth known as **culture medium**. The cells divide and spread over the surface of the dish. The inner surface of the culture dish is typically coated with mouse embryonic skin cells that have been treated so they will not divide. This coating layer of cells is called a **feeder layer**. The mouse cells in the bottom of the culture dish provide the cells a sticky surface to which they can attach. Also, the feeder cells release nutrients into the culture medium. Researchers have devised ways to grow embryonic stem cells without mouse feeder cells. This is a significant scientific advance because of the risk that viruses or other macromolecules in the mouse cells may be transmitted to the human cells.

The process of generating an embryonic stem cell line is somewhat inefficient, so lines are not produced each time cells from the preimplantation-stage embryo are placed into a culture dish. However, if the plated cells survive, divide, and multiply enough to crowd the dish, they are removed gently and plated into several fresh culture dishes. The process of re-plating or **subculturing** the cells is repeated many times and for many months. Each cycle of subculturing the cells is referred to as a **passage**. Once the cell line is established, the original cells yield millions of embryonic stem cells. Embryonic stem cells that have proliferated in cell culture for six or more months without differentiating, are **pluripotent**, and appear genetically normal are referred to as an **embryonic stem cell line**. At any stage in the process, batches of cells can be frozen and shipped to other laboratories for further culture and experimentation.

### ***C. What laboratory tests are used to identify embryonic stem cells?***

At various points during the process of generating embryonic stem cell lines, scientists test the cells to see whether they exhibit the fundamental properties that make them embryonic stem cells. This process is called characterization.

Scientists who study human embryonic stem cells have not yet agreed on a standard battery of tests that measure the cells' fundamental properties. However, laboratories that grow human embryonic stem cell lines use several kinds of tests, including:

- ▶ Growing and subculturing the stem cells for many months. This ensures that the cells are capable of long-term growth and self-renewal. Scientists inspect the cultures through a microscope to see that the cells look healthy and remain **undifferentiated**.
- ▶ Using specific techniques to determine the presence of transcription factors that are typically produced by undifferentiated cells. Two of the most important transcription factors are Nanog and Oct4. Transcription factors help turn **genes** on and off at the right time, which is an important part of the processes of cell **differentiation** and embryonic development. In this case, both Oct4 and Nanog are associated with maintaining the stem cells in an undifferentiated state, capable of self-renewal.
- ▶ Using specific techniques to determine the presence of particular cell surface markers that are typically produced by undifferentiated cells.
- ▶ Examining the chromosomes under a microscope. This is a method to assess whether the chromosomes are damaged or if the number of chromosomes has changed. It does not detect genetic mutations in the cells.
- ▶ Determining whether the cells can be re-grown, or subcultured, after freezing, thawing, and re-plating.
- ▶ Testing whether the human embryonic stem cells are pluripotent by 1) allowing the cells to differentiate spontaneously in cell culture; 2) manipulating the cells so they will differentiate to form cells characteristic of the three **germ layers**; or 3) injecting the cells into a mouse with a suppressed immune system to test for the formation of a benign tumor called a **teratoma**. Since the mouse's immune system is suppressed, the injected human stem cells are not rejected by the mouse immune system and scientists can observe growth and differentiation of the human stem cells. Teratomas typically contain a mixture of many differentiated or partly differentiated cell types—an indication that the embryonic stem cells are capable of differentiating into multiple cell types.

### D. How are embryonic stem cells stimulated to differentiate?

As long as the embryonic stem cells in culture are grown under appropriate conditions, they can remain undifferentiated (unspecialized). But if cells are allowed to clump together to form **embryoid bodies**, they begin to differentiate spontaneously. They can form muscle cells, nerve cells, and many other cell types. Although spontaneous differentiation is a good indicator that a culture of embryonic stem cells is healthy, the process is uncontrolled and therefore an inefficient strategy to produce cultures of specific cell types.

So, to generate cultures of specific types of differentiated cells—heart muscle cells, blood cells, or nerve cells, for example—scientists try to control the differentiation of embryonic stem cells. They change the chemical composition of the culture medium, alter the surface of the culture dish, or modify the cells by inserting specific genes. Through years of experimentation, scientists have established some basic protocols or “recipes” for the **directed differentiation** of embryonic stem cells into some specific cell types (Figure 1). (For additional examples of directed differentiation of embryonic stem cells, refer to the NIH stem cell reports available at <http://stemcells.nih.gov/info/2006report/> and <http://stemcells.nih.gov/info/2001report/2001report.htm>)

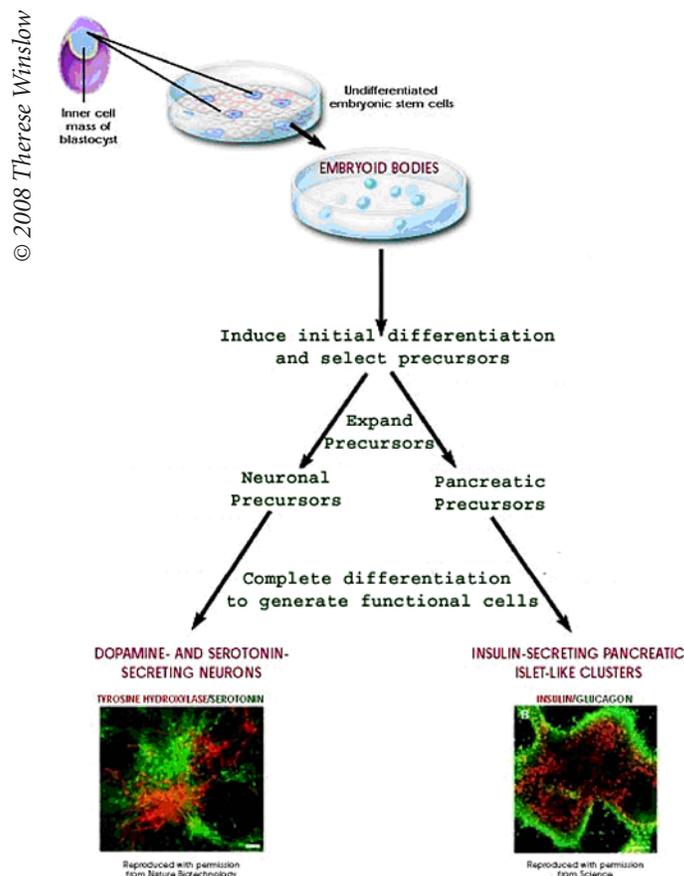


Figure 1. Directed differentiation of mouse embryonic stem cells.

If scientists can reliably direct the differentiation of embryonic stem cells into specific cell types, they may be able to use the resulting, differentiated cells to treat certain diseases in the future. Diseases that might be treated by transplanting cells generated from human embryonic stem cells include diabetes, traumatic spinal cord injury, [Duchenne's muscular dystrophy](#), heart disease, and vision and hearing loss.

#### IV. What are adult stem cells?

An [adult stem cell](#) is thought to be an [undifferentiated](#) cell, found among differentiated cells in a tissue or organ that can renew itself and can differentiate to yield some or all of the major specialized cell types of the tissue or organ. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found. Scientists also use the term [somatic stem cell](#) instead of adult stem cell, where somatic refers to cells of the body (not the germ cells, sperm or eggs). Unlike [embryonic stem cells](#), which are defined by their origin (cells from the preimplantation-stage embryo), the origin of adult stem cells in some mature tissues is still under investigation.

Research on adult stem cells has generated a great deal of excitement. Scientists have found adult stem cells in many more tissues than they once thought possible. This finding has led researchers and clinicians to ask whether adult stem cells could be used for transplants. In fact, adult hematopoietic, or blood-forming, stem cells from bone marrow have been used in transplants for 40 years. Scientists now have evidence that stem cells exist in the brain and the heart. If the differentiation of adult stem cells can be controlled in the laboratory, these cells may become the basis of transplantation-based therapies.

The history of research on adult stem cells began about 50 years ago. In the 1950s, researchers discovered that the bone marrow contains at least two kinds of stem cells. One population, called [hematopoietic stem cells](#), forms all the types of blood cells in the body. A second population, called [bone marrow stromal stem cells](#) (also called [mesenchymal stem cells](#), or skeletal stem cells by some) were discovered a few years later. These non-hematopoietic stem cells make up a small proportion of the [stromal cell](#) population in the bone marrow, and can generate bone, cartilage, fat, cells that support the formation of blood, and fibrous connective tissue.

In the 1960s, scientists who were studying rats discovered two regions of the brain that contained dividing cells that ultimately become nerve cells. Despite these reports, most scientists believed that the adult brain could not generate new nerve cells. It was not until the 1990s that scientists agreed that the adult brain does contain stem cells that are able to generate the brain's three major cell types—[astrocytes](#) and [oligodendrocytes](#), which are non-neuronal cells, and [neurons](#), or nerve cells.

### ***A. Where are adult stem cells found, and what do they normally do?***

Adult stem cells have been identified in many organs and tissues, including brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testis. They are thought to reside in a specific area of each tissue (called a “stem cell niche”). In many tissues, current evidence suggests that some types of stem cells are pericytes, cells that compose the outermost layer of small blood vessels. Stem cells may remain quiescent (non-dividing) for long periods of time until they are activated by a normal need for more cells to maintain tissues, or by disease or tissue injury.

Typically, there is a very small number of stem cells in each tissue, and once removed from the body, their capacity to divide is limited, making generation of large quantities of stem cells difficult. Scientists in many laboratories are trying to find better ways to grow large quantities of adult stem cells in cell culture and to manipulate them to generate specific cell types so they can be used to treat injury or disease. Some examples of potential treatments include regenerating bone using cells derived from bone marrow stroma, developing insulin-producing cells for type 1 diabetes, and repairing damaged heart muscle following a heart attack with cardiac muscle cells.

### ***B. What tests are used to identify adult stem cells?***

Scientists often use one or more of the following methods to identify adult stem cells: (1) label the cells in a living tissue with molecular markers and then determine the specialized cell types they generate; (2) remove the cells from a living animal, label them in cell culture, and transplant them back into another animal to determine whether the cells replace (or “repopulate”) their tissue of origin.

Importantly, it must be demonstrated that a single adult stem cell can generate a line of genetically identical cells that then gives rise to all the appropriate differentiated cell types of the tissue. To confirm experimentally that a putative adult stem cell is indeed a stem cell, scientists tend to show either that the cell can give rise to these genetically identical cells in culture, and/or that a purified population of these candidate stem cells can repopulate or reform the tissue after transplant into an animal.

### C. What is known about adult stem cell differentiation?

As indicated above, scientists have reported that adult stem cells occur in many tissues and that they enter normal **differentiation** pathways to form the specialized cell types of the tissue in which they reside.

*Normal differentiation pathways of adult stem cells.* In a living animal, adult stem cells are available to divide for a long period, when needed, and can give rise to mature cell types that have characteristic shapes and specialized structures and functions of a particular tissue. The following are examples of differentiation pathways of adult stem cells (Figure 2) that have been demonstrated *in vitro* or *in vivo*.

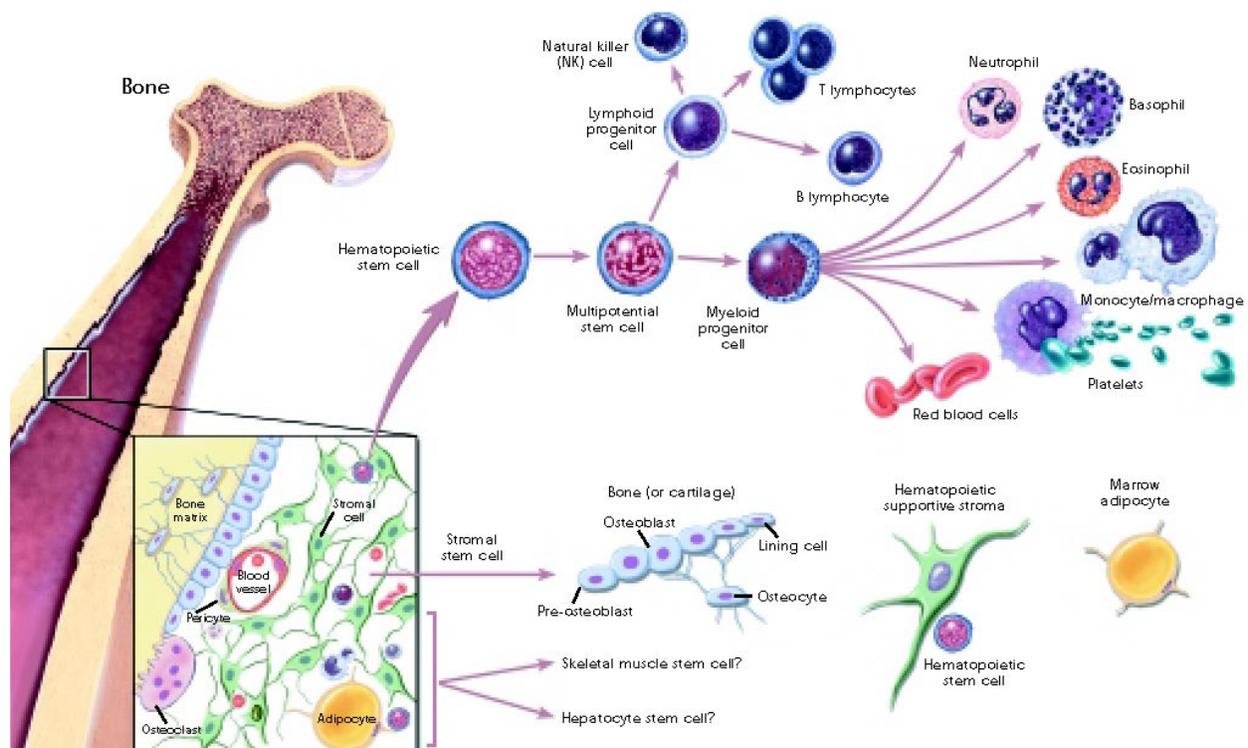


Figure 2. Hematopoietic and stromal stem cell differentiation.

- ▶ Hematopoietic stem cells give rise to all the types of blood cells: red blood cells, B lymphocytes, T lymphocytes, natural killer cells, neutrophils, basophils, eosinophils, monocytes, and macrophages.
- ▶ **Mesenchymal stem cells** have been reported to be present in many tissues. Those from bone marrow (bone marrow stromal stem cells, skeletal stem cells) give rise to a variety of cell types: bone cells (osteoblasts and osteocytes), cartilage cells (chondrocytes), fat cells (adipocytes), and stromal cells that support blood formation. However, it is not yet clear how similar or dissimilar mesenchymal cells derived

- from non-bone marrow sources are to those from bone marrow stroma.
- ▶ **Neural stem cells** in the brain give rise to its three major cell types: nerve cells (neurons) and two categories of non-neuronal cells—**astrocytes** and **oligodendrocytes**.
  - ▶ Epithelial stem cells in the lining of the digestive tract occur in deep crypts and give rise to several cell types: absorptive cells, goblet cells, Paneth cells, and enteroendocrine cells.
  - ▶ Skin stem cells occur in the basal layer of the epidermis and at the base of hair follicles. The epidermal stem cells give rise to keratinocytes, which migrate to the surface of the skin and form a protective layer. The follicular stem cells can give rise to both the hair follicle and to the epidermis.

*Transdifferentiation.* A number of experiments have reported that certain adult stem cell types can differentiate into cell types seen in organs or tissues other than those expected from the cells' predicted lineage (i.e., brain stem cells that differentiate into blood cells or blood-forming cells that differentiate into cardiac muscle cells, and so forth). This reported phenomenon is called **transdifferentiation**.

Although isolated instances of transdifferentiation have been observed in some vertebrate species, whether this phenomenon actually occurs in humans is under debate by the scientific community. Instead of transdifferentiation, the observed instances may involve fusion of a donor cell with a recipient cell. Another possibility is that transplanted stem cells are secreting factors that encourage the recipient's own stem cells to begin the repair process. Even when transdifferentiation has been detected, only a very small percentage of cells undergo the process.

In a variation of transdifferentiation experiments, scientists have recently demonstrated that certain adult cell types can be “reprogrammed” into other cell types *in vivo* using a well-controlled process of genetic modification (see [Section VI](#) for a discussion of the principles of reprogramming). This strategy may offer a way to reprogram available cells into other cell types that have been lost or damaged due to disease. For example, one recent experiment shows how pancreatic beta cells, the insulin-producing cells that are lost or damaged in diabetes, could possibly be created by reprogramming other pancreatic cells. By “re-starting” expression of three critical beta-cell genes in differentiated adult pancreatic exocrine cells, researchers were able to create beta cell–like cells that can secrete insulin. The reprogrammed cells were similar to beta cells in appearance, size, and shape; expressed genes characteristic of beta cells; and were able to partially restore blood sugar regulation in mice whose own beta cells had been chemically destroyed. While not transdifferentiation by definition, this method for reprogramming adult cells may be used as a model for directly reprogramming other adult cell types.

In addition to reprogramming cells to become a specific cell type, it is now possible to reprogram adult somatic cells to become like embryonic stem cells (induced pluripotent stem cells, iPSCs) through the introduction of embryonic genes. Thus, a source of cells can be generated that are specific to the donor, thereby avoiding issues of histocompatibility, if such cells were to be used for tissue regeneration. However, like embryonic stem cells, determination of the methods by which iPSCs can be completely and reproducibly committed to appropriate cell lineages is still under investigation.

#### ***D. What are the key questions about adult stem cells?***

Many important questions about adult stem cells remain to be answered. They include:

- ▶ How many kinds of adult stem cells exist, and in which tissues do they exist?
- ▶ How do adult stem cells evolve during development and how are they maintained in the adult? Are they “leftover” embryonic stem cells, or do they arise in some other way?
- ▶ Why do stem cells remain in an undifferentiated state when all the cells around them have differentiated? What are the characteristics of their “niche” that controls their behavior?
- ▶ Do adult stem cells have the capacity to transdifferentiate, and is it possible to control this process to improve its reliability and efficiency?
- ▶ If the beneficial effect of adult stem cell transplantation is a trophic effect, what are the mechanisms? Is donor cell-recipient cell contact required, secretion of factors by the donor cell, or both?
- ▶ What are the factors that control adult stem cell proliferation and differentiation?
- ▶ What are the factors that stimulate stem cells to relocate to sites of injury or damage, and how can this process be enhanced for better healing?

#### **V. What are the similarities and differences between embryonic and adult stem cells?**

Human embryonic and adult stem cells each have advantages and disadvantages regarding potential use for cell-based regenerative therapies. One major difference between adult and embryonic stem cells is their different abilities in the number and type of differentiated cell types they can become. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin.

Embryonic stem cells can be grown relatively easily in culture. Adult stem cells are rare in mature tissues, so isolating these cells from an adult tissue is challenging, and methods to expand their numbers in [cell culture](#) have not yet been worked out. This is an important distinction, as large numbers of cells are needed for stem cell replacement therapies.

Scientists believe that tissues derived from embryonic and adult stem cells may differ in the likelihood of being rejected after transplantation. We don't yet know whether tissues derived from embryonic stem cells would cause transplant rejection, since [the first phase 1 clinical trial](#) testing the safety of cells derived from hESCs has only recently been approved by the United States Food and Drug Administration (FDA).

Adult stem cells, and tissues derived from them, are currently believed less likely to initiate rejection after transplantation. This is because a patient's own cells could be expanded in culture, coaxed into assuming a specific cell type ([differentiation](#)), and then reintroduced into the patient. The use of adult stem cells and tissues derived from the patient's own adult stem cells would mean that the cells are less likely to be rejected by the immune system. This represents a significant advantage, as immune rejection can be circumvented only by continuous administration of immunosuppressive drugs, and the drugs themselves may cause deleterious side effects.

## VI. What are induced pluripotent stem cells?

[Induced pluripotent stem cells \(iPSCs\)](#) are adult cells that have been genetically reprogrammed to an embryonic stem cell-like state by being forced to express genes and factors important for maintaining the defining properties of embryonic stem cells. Although these cells meet the defining criteria for pluripotent stem cells, it is not known if iPSCs and embryonic stem cells differ in clinically significant ways. Mouse iPSCs were first reported in 2006, and human iPSCs were first reported in late 2007. Mouse iPSCs demonstrate important characteristics of pluripotent stem cells, including expressing stem cell markers, forming tumors containing cells from all three germ layers, and being able to contribute to many different tissues when injected into mouse embryos at a very early stage in development. Human iPSCs also express stem cell markers and are capable of generating cells characteristic of all three [germ layers](#).

Although additional research is needed, iPSCs are already useful tools for drug development and modeling of diseases, and scientists hope to use them in transplantation medicine. Viruses are currently used to introduce the reprogramming factors into adult cells, and this process must be carefully controlled and tested before the technique can lead to useful treatments for humans. In animal studies, the virus used to introduce the stem cell factors sometimes causes cancers. Researchers are currently investigating non-

viral delivery strategies. In any case, this breakthrough discovery has created a powerful new way to “de-differentiate” cells whose developmental fates had been previously assumed to be determined. In addition, tissues derived from iPSCs will be a nearly identical match to the cell donor and thus probably avoid rejection by the immune system. The iPSC strategy creates pluripotent stem cells that, together with studies of other types of pluripotent stem cells, will help researchers learn how to reprogram cells to repair damaged tissues in the human body.

## **VII. What are the potential uses of human stem cells and the obstacles that must be overcome before these potential uses will be realized?**

There are many ways in which human stem cells can be used in research and the clinic. Studies of human embryonic stem cells will yield information about the complex events that occur during human development. A primary goal of this work is to identify how undifferentiated stem cells become the differentiated cells that form the tissues and organs. Scientists know that turning genes on and off is central to this process. Some of the most serious medical conditions, such as cancer and birth defects, are due to abnormal cell division and differentiation. A more complete understanding of the genetic and molecular controls of these processes may yield information about how such diseases arise and suggest new strategies for therapy. Predictably controlling cell proliferation and differentiation requires additional basic research on the molecular and genetic signals that regulate cell division and specialization. While recent developments with iPS cells suggest some of the specific factors that may be involved, techniques must be devised to introduce these factors safely into the cells and control the processes that are induced by these factors.

Human stem cells could also be used to test new drugs. For example, new medications could be tested for safety on differentiated cells generated from human pluripotent cell lines. Other kinds of cell lines are already used in this way. Cancer cell lines, for example, are used to screen potential anti-tumor drugs. The availability of pluripotent stem cells would allow drug testing in a wider range of cell types. However, to screen drugs effectively, the conditions must be identical when comparing different drugs. Therefore, scientists will have to be able to precisely control the differentiation of stem cells into the specific cell type on which drugs will be tested. Current knowledge of the signals controlling differentiation falls short of being able to mimic these conditions precisely to generate pure populations of differentiated cells for each drug being tested.

Perhaps the most important potential application of human stem cells is the generation of cells and tissues that could be used for cell-based therapies. Today, donated organs and tissues are often used to replace ailing or destroyed tissue, but the need for transplantable tissues and organs far outweighs the available supply. Stem cells, directed to differentiate

into specific cell types, offer the possibility of a renewable source of replacement cells and tissues to treat diseases including Alzheimer’s disease, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis, and rheumatoid arthritis.

For example, it may become possible to generate healthy heart muscle cells in the laboratory and then transplant those cells into patients with chronic heart disease. Preliminary research in mice and other animals indicates that **bone marrow stromal cells**, transplanted into a damaged heart, can have beneficial effects. Whether these cells can generate heart muscle cells or stimulate the growth of new blood vessels that repopulate the heart tissue, or help via some other mechanism is actively under investigation. For example, injected cells may repair by secreting growth factors, rather than actually incorporating into the heart. Promising results from animal studies have served as the basis for a small number of exploratory studies in humans (see “[Can Stem Cells Mend a Broken Heart?](#)” on page 16). Other recent studies in **cell culture** systems indicate that it may be possible to direct the **differentiation** of embryonic stem cells or adult bone marrow cells into heart muscle cells (Figure 3).

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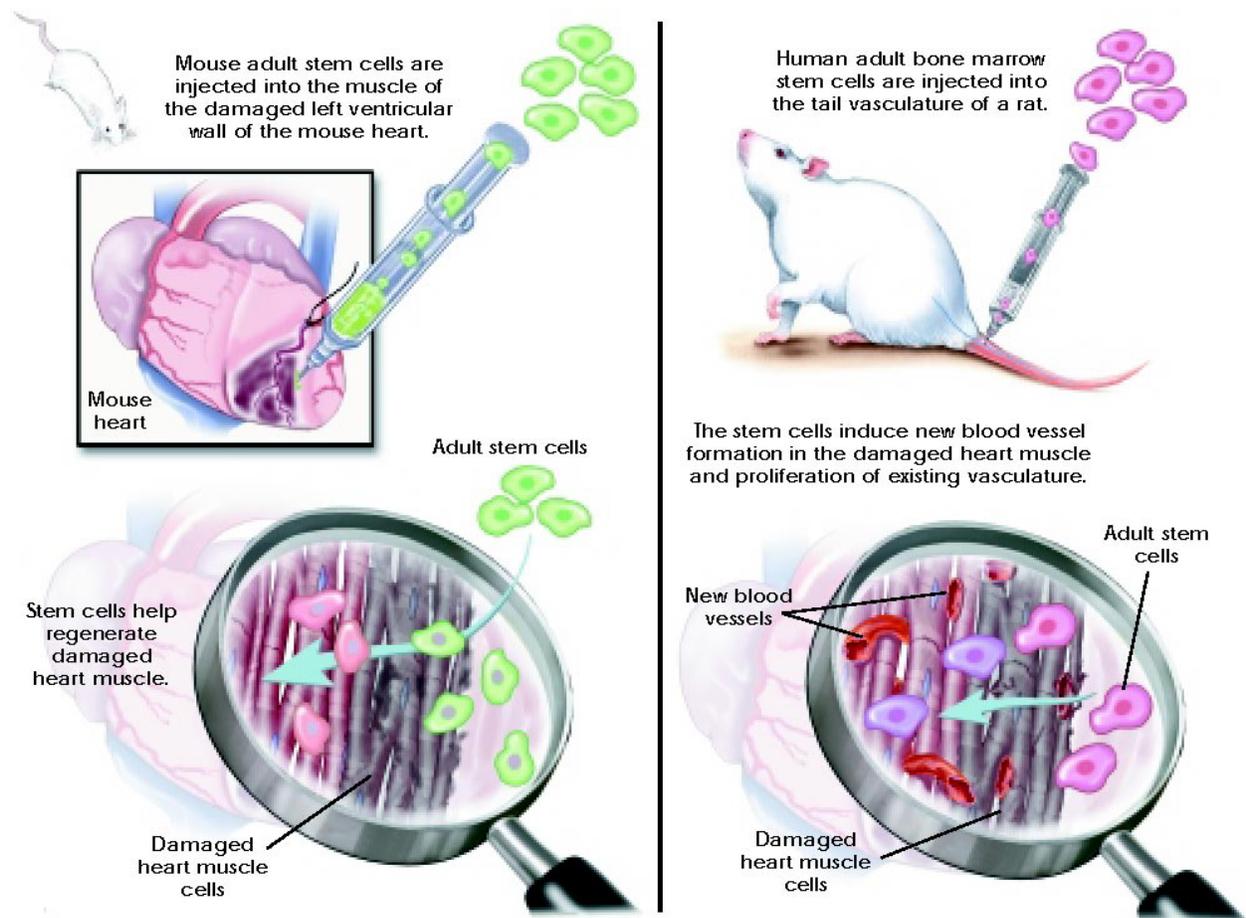


Figure 3. Strategies to repair heart muscle with **adult stem cells**.

## Can Stem Cells Mend a Broken Heart?: Stem Cells for the Future Treatment of Heart Disease

Cardiovascular disease (CVD), which includes hypertension, coronary heart disease, stroke, and congestive heart failure, has ranked as the number one cause of death in the United States every year since 1900 except 1918, when the nation struggled with an influenza epidemic. Nearly 2,600 Americans die of CVD each day, roughly one person every 34 seconds. Given the aging of the population and the relatively dramatic recent increases in the prevalence of cardiovascular risk factors such as obesity and type 2 diabetes, CVD will be a significant health concern well into the 21st century.

Cardiovascular disease can deprive heart tissue of oxygen, thereby killing cardiac muscle cells (cardiomyocytes). This loss triggers a cascade of detrimental events, including formation of scar tissue, an overload of blood flow and pressure capacity, the overstretching of viable cardiac cells attempting to sustain cardiac output, leading to heart failure, and eventual death. Restoring damaged heart muscle tissue, through repair or regeneration, is therefore a potentially new strategy to treat heart failure.

The use of embryonic and adult-derived stem cells for cardiac repair is an active area of research. A number of stem cell types, including embryonic stem (ES) cells, cardiac stem cells that naturally reside within the heart, myoblasts (muscle stem cells), adult bone marrow–derived cells including mesenchymal cells (bone marrow–derived cells that give rise to tissues such as muscle, bone, tendons, ligaments, and adipose tissue), endothelial progenitor cells (cells that give rise to the endothelium, the interior lining of blood vessels), and umbilical [cord blood cells](#), have been investigated as possible sources for regenerating damaged heart tissue. All have been explored in mouse or rat models, and some have been tested in larger animal models, such as pigs.

A few small studies have also been carried out in humans, usually in patients who are undergoing open-heart surgery. Several of these have demonstrated that stem cells that are injected into the circulation or directly into the injured heart tissue appear to improve cardiac function and/or induce the formation of new capillaries. The mechanism for this repair remains controversial, and the stem cells likely regenerate heart tissue through several pathways. However, the stem cell populations that have been tested in these experiments vary widely, as do the conditions of their purification and application. Although much more research is needed to assess the safety and improve the efficacy of this approach, these preliminary clinical experiments show how stem cells may one day be used to repair damaged heart tissue, thereby reducing the burden of cardiovascular disease.

In people who suffer from type 1 diabetes, the cells of the pancreas that normally produce insulin are destroyed by the patient's own immune system. New studies indicate that it may be possible to direct the differentiation of human embryonic stem cells in cell culture to form insulin-producing cells that eventually could be used in transplantation therapy for persons with diabetes.

To realize the promise of novel cell-based therapies for such pervasive and debilitating diseases, scientists must be able to manipulate stem cells so that they possess the necessary characteristics for successful differentiation, transplantation, and engraftment. The following is a list of steps in successful cell-based treatments that scientists will have to learn to control to bring such treatments to the clinic. To be useful for transplant purposes, stem cells must be reproducibly made to:

- ▶ Proliferate extensively and generate sufficient quantities of cells for making tissue.
- ▶ Differentiate into the desired cell type(s).
- ▶ Survive in the recipient after transplant.
- ▶ Integrate into the surrounding tissue after transplant.
- ▶ Function appropriately for the duration of the recipient's life.
- ▶ Avoid harming the recipient in any way.

Also, to avoid the problem of immune rejection, scientists are experimenting with different research strategies to generate tissues that will not be rejected.

To summarize, stem cells offer exciting promise for future therapies, but significant technical hurdles remain that will only be overcome through years of intensive research.

## VIII. Where can I get more information?

For a more detailed discussion of stem cells, see the [NIH's Stem Cell Reports](#). Check the [Frequently Asked Questions](#) page for quick answers to specific queries.

The following websites, which are not part of the NIH Stem Cell Information site, also contain information about stem cells. The NIH is not responsible for the content of these sites.

- ▶ <http://www.isscr.org/public> Stem cell information for the public from the International Society for Stem Cell Research (ISSCR).
- ▶ <http://www.nlm.nih.gov/medlineplus/stemcells.html> Medline Plus is a consumer health database that includes news, health resources, clinical trials, and more.
- ▶ <http://www.explorestemcells.co.uk> A United Kingdom–based resource for the general public that discusses the use of stem cells in medical treatments and therapies.
- ▶ <http://www.stemcellresearchnews.com> A commercial, online newsletter that features stories about stem cells of all types.

## Glossary

**Adult stem cell**—See *Somatic stem cell*.

**Astrocyte**—A type of supporting (glial) cell found in the nervous system.

**Blastocoel**—The fluid-filled cavity inside the blastocyst, an early, preimplantation stage of the developing embryo.

**Blastocyst**—A preimplantation embryo of about 150 cells produced by cell division following fertilization. The blastocyst is a sphere made up of an outer layer of cells (the trophoblast), a fluid-filled cavity (the blastocoel), and a cluster of cells on the interior (the inner cell mass).

**Bone marrow stromal cells**—A population of cells found in bone marrow that are different from blood cells.

**Bone marrow stromal stem cells (skeletal stem cells)**—A multipotent subset of bone marrow stromal cells able to form bone, cartilage, stromal cells that support blood formation, fat, and fibrous tissue.

**Cell-based therapies**—Treatment in which stem cells are induced to differentiate into the specific cell type required to repair damaged or destroyed cells or tissues.

**Cell culture**—Growth of cells *in vitro* in an artificial medium for experimental research.

**Cell division**—Method by which a single cell divides to create two cells. There are two main types of cell division depending on what happens to the chromosomes: mitosis and meiosis.

**Chromosome**—A structure consisting of DNA and regulatory proteins found in the nucleus of the cell. The DNA in the nucleus is usually divided up among several chromosomes. The number of chromosomes in the nucleus varies depending on the species of the organism. Humans have 46 chromosomes.

**Clone**—(v) To generate identical copies of a region of a DNA molecule or to generate genetically identical copies of a cell, or organism; (n) The identical molecule, cell, or organism that results from the cloning process.

1. In reference to DNA: To clone a gene, one finds the region where the gene resides on the DNA and copies that section of the DNA using laboratory techniques.
2. In reference to cells grown in a tissue culture dish: a clone is a line of cells that is genetically identical to the originating cell. This cloned line is produced by cell division (mitosis) of the original cell.
3. In reference to organisms: Many natural clones are produced by plants and (mostly invertebrate) animals. The term clone may also be used to refer to an animal produced by somatic cell nuclear transfer (SCNT) or parthenogenesis.

**Cloning**—See *Clone*.

**Cord blood stem cells**—See *Umbilical cord blood stem cells*.

**Culture medium**—The liquid that covers cells in a culture dish and contains nutrients to nourish and support the cells. Culture medium may also include growth factors added to produce desired changes in the cells.

**Differentiation**—The process whereby an unspecialized embryonic cell acquires the features of a specialized cell such as a heart, liver, or muscle cell. Differentiation is controlled by the interaction of a cell's genes with the physical and chemical conditions outside the cell, usually through signaling pathways involving proteins embedded in the cell surface.

**Directed differentiation**—The manipulation of stem cell culture conditions to induce differentiation into a particular cell type.

**DNA**—Deoxyribonucleic acid, a chemical found primarily in the nucleus of cells. DNA carries the instructions or blueprint for making all the structures and materials the body needs to function. DNA consists of both genes and non-gene DNA in between the genes.

**Ectoderm**—The outermost germ layer of cells derived from the inner cell mass of the blastocyst; gives rise to the nervous system, sensory organs, skin, and related structures.

**Embryo**—In humans, the developing organism from the time of fertilization until the end of the eighth week of gestation, when it is called a fetus.

**Embryoid bodies**—Rounded collections of cells that arise when embryonic stem cells are cultured in suspension. Embryoid bodies contain cell types derived from all 3 germ layers.

**Embryonic germ cells**—Pluripotent stem cells that are derived from early germ cells (those that would become sperm and eggs). Embryonic germ cells (EG cells) are thought to have properties similar to embryonic stem cells.

**Embryonic stem cells**—Primitive (undifferentiated) cells derived from a 5-day preimplantation embryo that are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers.

**Embryonic stem cell line**—Embryonic stem cells, which have been cultured under *in vitro* conditions that allow proliferation without differentiation for months to years.

**Endoderm**—The innermost layer of the cells derived from the inner cell mass of the blastocyst; it gives rise to lungs, other respiratory structures, and digestive organs, or generally “the gut”.

**Enucleated**—Having had its nucleus removed.

**Epigenetic**—Having to do with the process by which regulatory proteins can turn genes on or off in a way that can be passed on during cell division.

**Feeder layer**—Cells used in co-culture to maintain pluripotent stem cells. For human embryonic stem cell culture, typical feeder layers include mouse embryonic fibroblasts (MEFs) or human embryonic fibroblasts that have been treated to prevent them from dividing.

**Fertilization**—The joining of the male gamete (sperm) and the female gamete (egg).

**Fetus**—In humans, the developing human from approximately eight weeks after conception until the time of its birth.

**Gamete**—An egg (in the female) or sperm (in the male) cell. See also *Somatic cell*.

**Gastrulation**—The process in which cells proliferate and migrate within the embryo to transform the inner cell mass of the blastocyst stage into an embryo containing all three primary germ layers.

**Gene**—A functional unit of heredity that is a segment of DNA found on chromosomes in the nucleus of a cell. Genes direct the formation of an enzyme or other protein.

**Germ layers**—After the blastocyst stage of embryonic development, the inner cell mass of the blastocyst goes through

gastrulation, a period when the inner cell mass becomes organized into three distinct cell layers, called germ layers. The three layers are the ectoderm, the mesoderm, and the endoderm.

**Hematopoietic stem cell**—A stem cell that gives rise to all red and white blood cells and platelets.

**Human embryonic stem cell (hESC)**—A type of pluripotent stem cell derived from early-stage human embryos, up to and including the blastocyst stage. hESCs are capable of dividing without differentiating for a prolonged period in culture and are known to develop into cells and tissues of the three primary germ layers. See also *pluripotent*, *blastocyst*, and *germ layers*.

**Induced pluripotent stem cell (iPSC)**—A type of pluripotent stem cell, similar to an embryonic stem cell, formed by the introduction of certain embryonic genes into a somatic cell.

**In vitro**—Latin for “in glass”; in a laboratory dish or test tube; an artificial environment.

**In vitro fertilization**—A technique that unites the egg and sperm in a laboratory, instead of inside the female body.

**Inner cell mass (ICM)**—The cluster of cells inside the blastocyst. These cells give rise to the embryo and ultimately the fetus. The ICM cells may be used to generate embryonic stem cells.

**Long-term self-renewal**—The ability of stem cells to renew themselves by dividing into the same non-specialized cell type over long periods (many months to years) depending on the specific type of stem cell.

**Mesenchymal stem cells**—A term that is currently used to define non-blood adult stem cells from a variety of tissues, although it is not clear that mesenchymal stem cells from different tissues are the same.

**Meiosis**—The type of cell division a diploid germ cell undergoes to produce gametes (sperm or eggs) that will carry half the normal chromosome number. This is to ensure that when fertilization occurs, the fertilized egg will carry the normal number of chromosomes rather than causing aneuploidy (an abnormal number of chromosomes).

**Mesoderm**—Middle layer of a group of cells derived from the inner cell mass of the blastocyst; it gives rise to bone, muscle, connective tissue, kidneys, and related structures.

**Microenvironment**—The molecules and compounds such as nutrients and growth factors in the fluid surrounding a cell in an organism or in the laboratory, which play an important role in determining the characteristics of the cell.

**Mitosis**—The type of cell division that allows a population of cells to increase its numbers or to maintain its numbers.

The number of chromosomes remains the same in this type of cell division.

**Multipotent**—Having the ability to develop into more than one cell type of the body. See also *pluripotent* and *totipotent*.

**Neural stem cell**—A stem cell found in adult neural tissue that can give rise to neurons and glial (supporting) cells. Examples of glial cells include astrocytes and oligodendrocytes.

**Neurons**—Nerve cells, the principal functional units of the nervous system. A neuron consists of a cell body and its processes—an axon and one or more dendrites. Neurons transmit information to other neurons or cells by releasing neurotransmitters at synapses.

**Oligodendrocyte**—A supporting cell that provides insulation to nerve cells by forming a myelin sheath (a fatty layer) around axons.

**Parthenogenesis**—The artificial activation of an egg in the absence of a sperm; the egg begins to divide as if it has been fertilized.

**Passage**—In cell culture, the process in which cells are disassociated, washed, and seeded into new culture vessels after a round of cell growth and proliferation. The number of passages a line of cultured cells has gone through is an indication of its age and expected stability.

**Pluripotent**—Having the ability to give rise to all of the various cell types of the body. Pluripotent cells cannot make extra-embryonic tissues such as the amnion, chorion, and other components of the placenta. Scientists demonstrate pluripotency by providing evidence of stable developmental potential, even after prolonged culture, to form derivatives of all three embryonic germ layers from the progeny of a single cell and to generate a teratoma after injection into an immunosuppressed mouse.

**Polar Body**—A polar body is a structure produced when an early egg cell, or oogonium, undergoes meiosis. In the first meiosis, the oogonium divides its chromosomes evenly between the two cells but divides its cytoplasm unequally. One cell retains most of the cytoplasm, while the other gets almost none, leaving it very small. This smaller cell is called the first polar body. The first polar body usually degenerates. The ovum, or larger cell, then divides again, producing a second polar body with half the amount of chromosomes but almost no cytoplasm. The second polar body splits off and remains adjacent to the large cell, or oocyte, until it (the second polar body) degenerates. Only one large functional oocyte, or egg, is produced at the end of meiosis.

**Preimplantation**—With regard to an embryo, preimplantation means that the embryo has not yet implanted in the wall of the uterus. Human embryonic stem cells are derived from

preimplantation stage embryos fertilized outside a woman's body (*in vitro*).

**Proliferation**—Expansion of the number of cells by the continuous division of single cells into two identical daughter cells.

**Regenerative medicine**—A field of medicine devoted to treatments in which stem cells are induced to differentiate into the specific cell type required to repair damaged or destroyed cell populations or tissues. See also *cell-based therapies*.

**Reproductive cloning**—The process of using somatic cell nuclear transfer (SCNT) to produce a normal, full grown organism (e.g., animal) genetically identical to the organism (animal) that donated the somatic cell nucleus. In mammals, this would require implanting the resulting embryo in a uterus where it would undergo normal development to become a live independent being. The first animal to be created by reproductive cloning was Dolly the sheep, born at the Roslin Institute in Scotland in 1996. See also *Somatic cell nuclear transfer (SCNT)*.

**Signals**—Internal and external factors that control changes in cell structure and function. They can be chemical or physical in nature.

**Somatic cell**—Any body cell other than gametes (egg or sperm); sometimes referred to as “adult” cells. See also *Gamete*.

**Somatic cell nuclear transfer (SCNT)**—A technique that combines an enucleated egg and the nucleus of a somatic cell to make an embryo. SCNT can be used for therapeutic or reproductive purposes, but the initial stage that combines an enucleated egg and a somatic cell nucleus is the same. See also *Therapeutic cloning* and *Reproductive cloning*.

**Somatic (adult) stem cell**—A relatively rare undifferentiated cell found in many organs and differentiated tissues with a limited capacity for both self renewal (in the laboratory) and differentiation. Such cells vary in their differentiation capacity, but it is usually limited to cell types in the organ of origin. This is an active area of investigation.

**Stem cells**—Cells with the ability to divide for indefinite periods in culture and to give rise to specialized cells.

**Stromal cells**—Connective tissue cells found in virtually every organ. In bone marrow, stromal cells support blood formation.

**Subculturing**—Transferring cultured cells, with or without dilution, from one culture vessel to another.

**Surface markers**—Proteins on the outside surface of a cell that are unique to certain cell types and that can be visualized using antibodies or other detection methods.

**Teratoma**—A multi-layered benign tumor that grows from pluripotent cells injected into mice with a dysfunctional

immune system. Scientists test whether they have established a human embryonic stem cell (hESC) line by injecting putative stem cells into such mice and verifying that the resulting teratomas contain cells derived from all three embryonic germ layers.

**Therapeutic cloning**—The process of using somatic cell nuclear transfer (SCNT) to produce cells that exactly match a patient. By combining a patient's somatic cell nucleus and an enucleated egg, a scientist may harvest embryonic stem cells from the resulting embryo that can be used to generate tissues that match a patient's body. This means the tissues created are unlikely to be rejected by the patient's immune system. See also *Somatic cell nuclear transfer (SCNT)*.

**Totipotent**—Having the ability to give rise to all the cell types of the body plus all of the cell types that make up the extraembryonic tissues such as the placenta. See also *Pluripotent* and *Multipotent*.

**Transdifferentiation**—The process by which stem cells from one tissue differentiate into cells of another tissue.

**Trophectoderm**—The outer layer of the preimplantation embryo in mice. It contains trophoblast cells.

**Trophoblast**—The outer cell layer of the blastocyst. It is responsible for implantation and develops into the extraembryonic tissues, including the

placenta, and controls the exchange of oxygen and metabolites between mother and embryo.

**Umbilical cord blood stem cells**—Stem cells collected from the umbilical cord at birth that can produce all of the blood cells in the body (hematopoietic). Cord blood is currently used to treat patients who have undergone chemotherapy to destroy their bone marrow due to cancer or other blood-related disorders.

**Undifferentiated**—A cell that has not yet developed into a specialized cell type.



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