Chapter 1

Painting the Broad Strokes of Stem Cell Science

In This Chapter

- Exploring the foundations for stem cell science
- Understanding what researchers know now
- Looking at what scientists still need to discover

Some stem cells researchers shake their heads in bemusement at the sudden public interest in their field. Thirty years ago, no one outside the scientific community had ever heard of stem cells. Today, stem cell scientists are sort of like the overnight singing sensations who have been performing at local nightclubs for years and suddenly has a No. 1 hit on the national charts. The general public has no idea how much work that singer put in before she was "discovered." Similarly, many people aren't aware of how much stem cell researchers have discovered about normal biological development and disease, or how those years of research have led them to the experiments and discoveries that are touted in the headlines today.

Finally, many people are unaware of how far stem cell research still has to go. Although scientists know a lot about human development, the workings of various genes, and the behavior of certain diseases, a lot of questions remain unanswered. And these aren't esoteric questions, either; they're questions like why some cells in the body's tissues never become specific cell types, what signals or mechanisms direct those cells to become active, and how cells malfunction in disease.

In this chapter, we provide a brief overview of what stem cell scientists have been doing all these years before their work generated such widespread interest. We explain why scientists do so much work with mice, fruit flies, and other animals, and how they translate their findings in animal studies into predictions (and subsequent testing to confirm those predictions) about what happens in humans. We also inventory the things that researchers think they know about various kinds of stem cells, as well as the things they're still trying to figure out.

Working with Animals and Other Organisms

Humans are a lot like yeast. No, this isn't the start of one of those joke e-mails your coworkers send you on a quiet Friday afternoon; it's a biological fact. Humans also are a lot like fruit flies, mice, and other animals and organisms that have *eukaryotic* (pronounce you-CARE-ee-ah-tic) cells — cells that have a distinct nucleus encased in a membrane. (*Prokaryotic* cells, such as those in bacteria, don't have a compartmentalized nucleus, but rather a less-defined *nucleoid region* that contains their DNA.) Amazing as it sounds, at the cellular level, many of the pathways and functions of eukaryotic cells are the same no matter what organism the cells are in.

Scientists have shown that some of the genes in yeast will function in human cells, and vice versa. This interchangeability of genes among different organisms is called *conservation;* that is, nature uses many of the same blueprints and mechanisms, at least at the cellular level, for a wide range of living creatures. In fact, different organisms are so similar at the cellular level that many of the genes that cause certain kinds of cancer were first discovered and studied in yeasts and fruit flies.

When it comes to fruit flies and worms, not only are the pathways inside cells very similar to those in humans, some of the pathways for communicating between cells or for instructing a cell to specialize are similar. For example, scientists know what genes are turned on in order to make a human neuron and wire it so that it communicates properly with other cells and tissues in part because they've studied these genetic mechanisms in fruit flies and worms.

Just because fruit flies don't look like humans — or just because fruit flies are insects and humans are mammals — doesn't mean they don't share some characteristics. From a scientific perspective, fruit flies, mice, and humans are like different motorized vehicles. Fruit flies are motorcycles; mice are compact cars; and humans are luxury sedans. The details of how you put each of these vehicles together differ greatly, but many of the basic mechanisms are the same, and a lot of the parts are the same (although they may not be the same size). And, in some cases, some of the parts are even interchangeable, as in the case of yeast and human genes.

Obviously, you can't take the throttle from a motorcycle and install it in a luxury sedan. But when the throttle on the motorcycle breaks, sometimes it can tell you a lot about how the Cadillac's acceleration mechanism might break. The same principle is what leads scientists to spend so much of their time working with yeasts, worms, fruit flies, and mice. These approaches are important because, in many cases, experimenting on human beings is unethical; the risks are too great.

Understanding the mouse's role in stem cell research

The mouse has arguably been the most important animal in stem cell research. In the early 1960s, Canadian researchers James Till and Ernest McCulloch were the first to prove that bone marrow contained stem cells. They exposed mice to high doses of radiation to kill the mouse's blood- and immune-forming system and then injected bone marrow cells into some of those mice. The mice that didn't receive new bone marrow cells died; the mice that received the transplants lived because the new bone marrow cells rebuilt their blood- and immune-forming systems.

Till and McCulloch also noted that the mice that received transplants developed small but visible nodules, or lumps, on their spleens, and that the sizes of the nodules were directly proportional to the number of bone marrow cells the mouse received in the transplant. The scientists theorized that these so-called *spleen colonies* originated with a single cell from the bone marrow transplant — perhaps a stem cell. They later proved that theory and, as their work continued, also proved that some cells in bone marrow are capable of reproducing themselves as well as generating specific cell types.

Till and McCulloch's work on mice is the basis for human bone marrow transplants, which are routinely used today to treat leukemia and some other kinds of blood disorders (see Chapter 13).

Embryonic stem cells also were first isolated from mice. In the early 1980s, researchers learned how to extract the inner cells from mouse *blastocysts* — a hollow ball of cells that forms a few days after an egg cell is fertilized — and grow them in Petri dishes or other containers. When these cells are grown properly *(*a process called *culturing),* they reproduce themselves — or *self-renew;* they don't adopt the characteristics of specialized cells until they're exposed to the appropriate biochemical signals. That work formed the foundation for isolating human embryonic stem cells in 1998, which in turn led to the "overnight sensation" phenomenon the field is experiencing today. (See Chapter 4 for more on embryonic stem cells.)

Using mice in today's labs

The mouse is still a critical component of many stem cell laboratories. Researchers manipulate mouse genes to see how specific genetic changes affect normal development or the progression of a disease. They create mice with defective immune systems so that they can inject them with human tumors to study different forms of cancer (see Chapter 8). And researchers focusing on leukemia and other diseases of the blood still study how abnormal blood cells and normal blood cells interact in mouse models of these diseases.

Researchers also test potential drugs and other therapies on mouse models. Till and McCulloch proved that you can save a mouse whose own blood- and immune-forming system has been destroyed by giving it an injection of new bone marrow stem cells. Patients with certain forms of leukemia and immune diseases undergo basically the same treatment today: Doctors kill their blood- and immune-forming systems with high doses of chemicals and radiation and then save the patient by injecting them with blood-forming stem cells that, ideally, settle into the patient's bone marrow and begin generating new blood cells — including the immune cells that normally circulate in the bloodstream. (Chapter 13 describes this process in detail.)

New discoveries from work on the mouse are announced all the time, and most of those discoveries have implications for how researchers can go about treating human ailments. The following sections describe two recent examples of this kind of work.

Figuring out how cells make skin

One of the key questions in stem cell research has been how stem cells know when it's time to stop reproducing themselves and start producing specialized cells for the tissue in which they reside. In some cases, stem cells seem to be able to divide into two structurally different cells — one that remains a stem cell and another, called a *progenitor cell,* that goes on to generate specialized cells. In other cases, though, a stem cell creates two progenitor cells — essentially giving up its ability to reproduce itself so it can create specialized cells instead. The details are still unclear, so this area of research is quite active, and it's important because work with skin cells reveals a lot of information about what may happen in other organ systems.

In most instances, researchers believe that certain proteins and other signaling or controlling molecules are responsible for directing cell specialization (although they still don't fully understand the signals that tell stem cells to produce progenitor cells). But, because the human body has some 200 different cell types, isolating the specific proteins (or other elements) that are responsible for creating each type of specialized cell is a monumental task.

In the past few years, researchers have identified some of the proteins that tell stem cells in the base layer of the skin to generate new skin cells. These discoveries relied on genetic engineering techniques in mouse embryos to turn off the genes that create specific types of proteins. When those mice were born, their skin was sometimes so badly deformed that it couldn't contain water, and the newborn mice quickly died of dehydration.

Although researchers are actively working to identify the specific molecules that control normal skin development, a lot of questions still remain unanswered, including how stem cells know when to make more skin cells.

Scientists know that skin stem cells make the decision to produce new skin cells daily because you shed dead skin cells every day and, if the stem cells in your skin didn't create replacement skin cells, you'd suffer the same fate as the genetically engineered mice. But the precise mechanism that tells stem cells to make more skin cells remains unknown.

Determining the role immune cells play in certain diseases

Researchers have recently discovered some of the genes that control creation of special immune cells in the blood known as Natural Killers, or NK cells. *NK cells,* a type of white blood cell, are formed by stem cells in the bone marrow, and they roam throughout the bloodstream, seeking out and attacking cells that are contaminated with cancer-initiating mutations, viruses, or harmful bacteria.

Researchers have long wondered whether NK cells play a pivotal role in autoimmune diseases like Type 1 diabetes (in which the immune system attacks and destroys the insulin-producing cells in the pancreas), multiple sclerosis, and other diseases. The theory is that maybe NK cells turn into rogues, attacking everything in sight instead of limiting their activities to truly infected or otherwise dangerous cells.

Some work suggests that it's possible to disable the genes that control NK cell production in mice, creating mice with no NK cells in their bodies (but all the other normal blood and immune cells). The mouse model gives scientists another tool for figuring out whether NK cells really are the bad guys in various autoimmune diseases and what role they play in inflammation, drug-resistant infections, and even transplant rejection. Knowing the genes that control NK production also opens the door to finding possible drug or gene therapies for autoimmune diseases.

Finally, researchers can use this mouse model to study potential new treatments for cancer. If certain chemicals can induce stem cells in the bone marrow to produce extra NK cells to attack tumors (but not healthy cells), such drugs may eventually reduce the need to use radiation and other chemicals that kill both cancerous and normal cells.

Any time you read of a new therapy or exciting development in stem cell research, you can almost guarantee that it came about through work on mouse or other animal models of the disease or developmental process. And researchers still use layers of specially treated mouse skin cells as a base on which to grow human embryonic stem cells. Scientists and bioengineers are investigating other methods to grow these cells, because using mouse cells raises concerns about contaminating the human cells with viruses or other unwanted elements, but, so far, mouse skin cells seem to be the most reliable for growing human embryonic stem cells.

Exploring What Scientists Know (And Don't Know) About Stem Cells

You could write a book on what researchers have already discovered about stem cells. (Oh, wait — we did, and you're reading it!) But even with all the work that's been done over the past 40 years or so, there are still quite a few holes in the body of stem cell knowledge. Some of those holes are pretty big, too.

Other chapters in this book detail what's known and unknown about stem cells in specific contexts. In the following sections, we provide an overview of what scientists have figured out about stem cells in general, and what they're still trying to discover about them.

Understanding stem cells' key properties

Stem cells have two key characteristics that distinguish them from other types of cells: They can reproduce themselves for long periods (self-renewal), and they can, under certain conditions in the body or in the lab, produce cells that eventually become specific types of cells — a process known as *differentiation* or *specialization*.

The following sections discuss what scientists know and don't know about embryonic and adult stem cells.

Looking at the unique abilities of embryonic stem cells

Embryonic stem cells don't technically exist in an embryo. In the normal course of development, the blastocyst fuses with the uterine wall, and the inner cell mass begins growing into all the different cell types of the fully developed body (see Chapter 4). In other words, although the cells in the inner cell mass grow and divide, they don't create more of themselves; instead, they create daughter cells that, in their turn, create cells with the structures and other elements they need to do their specific jobs. By the time a baby is born, his body doesn't have any cells that precisely mimic the cells in the inner cell mass — at least as far as researchers know.

In the lab, scientists can extract the inner cell mass from blastocysts that are created in the lab (not in a female's body) and prevent those cells from going through this specialization process. When these cells are grown properly, they renew themselves virtually indefinitely and never develop the unique characteristics of specialized cells (unless they're prompted to do so through changes in their growth environment). This process of developing stem cell lines from the inner cell mass is called *derivation*.

Scientists are investigating a number of methods to induce embryonic stem cells to differentiate into specific cell types. Some methods work pretty well; others aren't as reliable. But the ability to create the kinds of cells you want is enormously important in studying development and disease, and especially in testing potential treatments, because it allows you to study human cells and tissues instead of relying on animal sources. While animal models are useful and animals and humans share many biological characteristics, you don't really want to do all your hands-on training on a beater car if you plan to fix a luxury sedan.

Exploring adult stem cells

Although a fully developed body apparently doesn't normally have cells that can give rise to *any* type of cell, it does have some self-renewing cells that can generate specific types of differentiated cells. Researchers call these cells *adult stem cells,* to reflect the fact that they live in fully formed tissues. (The term adult stem cells has caused some confusion because they're in fetal tissues as well, so some researchers prefer to call them *tissue stem cells* or *somatic* stem cells; see Chapter 5 for more information.)

Researchers have found adult stem cells in a variety of tissues. The best-known are skin stem cells and blood-forming stem cells, but stem cells also have been identified in fatty tissue, the intestines, the liver, the lungs, and skeletal muscles, as well as in the brain, blood vessels, and even, it appears, in the heart muscle. Their job seems to be to replenish tissue cells as they wear out or die from age or normal wear and tear.

Researchers don't fully understand the signals that induce adult stem cells to form, or the signals that control their behavior. How do adult stem cells decide when it's time to renew themselves or make differentiated cells? With a few exceptions — notably skin and blood-forming stem cells — most adult stem cells seem to be inactive most of the time. On the face of it, you could assume that these cells don't do anything until they're activated by disease or injury. But if that were the case, why don't stem cells in the brain, for example, leap into a flurry of activity when someone suffers a stroke or sustains a head injury in a car accident? One possibility is that those stem cells actually do become active in response to an injury but don't have enough repair capacity to heal the injury. Another possibility: Stem cells in the brain don't have a repair function, but instead play a role in storing new information. Clearly, researchers need to do a lot more work to figure these things out.

Finally, some adult stem cells may be able to generate cells outside their own tissue type — a phenomenon called *transdifferentiation.* In the early 2000s, several research groups reported that certain kinds of blood-forming cells, which typically only create blood cells, can transdifferentiate into other kinds of tissue cells, including heart cells, brain cells, and liver cells. Further investigation, though, revealed that other processes may have been at work in those experiments.

Most stem cell scientists aren't convinced that stem cells actually can go outside their normal tissue types to produce other kinds of cells. Experiments haven't provided clear answers yet. If you inject blood-forming stem cells into a damaged heart muscle and the heart muscle gets better, does that mean the blood-forming stem cells began generating new heart muscle cells? Or did the blood-forming stem cells send signals to the heart's own stem cells and stimulate them to repair the tissue? Or did the blood-forming stem cells fuse with cells in the heart muscle and therefore adopt some of the structure and function of the heart muscle cells? No one knows for sure, and until researchers better understand what's really happening, these kinds of procedures are unlikely to become standard treatment.

Confirming that cells really are stem cells

Okay, you've extracted the inner cell mass of a blastocyst, put it in a dish with the appropriate chemicals and feeder cells, and let the cells grow and divide for a while. Or you've taken samples of the areas in skin where skin stem cells hang out and induced them to grow and divide for a while. How do you know that what you've grown really are stem cells?

Scientists use a number of strategies for demonstrating that suspected stem cells really are what the scientists think they are. For embryonic stem cells, researchers may do one or more of the following:

- Grow them for several months to ensure that they really are self-renewing.
- Examine the cells' surfaces, looking for markers that occur only in undifferentiated cells.
- Look for the presence of the OCT-4 protein and other signaling molecules typically produced by undifferentiated cells.
- Inject them into immune-compromised mice to see whether they form *teratomas,* a special kind of benign tumor that contains cells from all three main tissue layers (see Chapter 2).

To identify adult stem cells, scientists usually use one or more of the following techniques:

- Attaching special markers to cells in the tissue and seeing what kind of cells they generate
- Removing the cells from a living animal, such as a mouse, labeling them with special markers (see Chapter 4) and then injecting them into another animal to see whether they repopulate their specific tissue type
- Using genetic engineering methods to induce the cells to grow and divide in a dish and then inventorying the types of cells the original cells become

Not all stem cell researchers agree on which verification methods are the most useful or which ones should be standard for confirming that cells are indeed stem cells. This area of the science is still evolving, with different criteria needed for different types of cells; as researchers learn more about the cells themselves, it should become more clear which criteria are the most important for positively identifying cells as stem cells.

Figuring out how to use stem cells

In broad terms, researchers use stem cells to study development — both normal development of specific systems in the body and how diseases start and what happens as they progress. They've used stem cells to identify the so-called *master genes* that tell cells what to do and when to do it, as well as *transcription factors,* the proteins that turn those genetic instructions on and off. This kind of basic research helps scientists figure out how individual cells and collections of cells are supposed to work and what goes wrong in disease.

Armed with that knowledge, scientists can target their search for possible treatments to specific mechanisms. For example, researchers have discovered that cells that support motor neurons — the nerve cells that control movement — seem to play a critical role in Lou Gehrig's disease (see Chapter 9). Now they're working on ways to counter the possibly damaging activity of those supporting cells, such as replacing them with transplanted cells or finding drugs that rescue the motor neurons or reverse the toxicity of neighboring cells.

In some cases, stem cells may act as delivery agents instead of actually fixing a problem themselves. They could be used to deliver missing enzymes to the cells in the brain, for example, or growth factors that prod the body's own stem cells to begin making new specialized cells. Scientists also can use stem cells to reconstruct diseases in the lab and study those diseased cells to understand the molecular abnormalities that cause or lead to disease.

And, someday, stem cells may be used to grow replacement tissues — or even whole organs — in the lab. (Turn to Chapter 20 for ten reasonable possibilities in using stem cells for medical treatments.)

Looking at some unanswered questions

Scientists have been studying adult stem cells for more than 40 years and embryonic stem cells for more than 20 years. They've uncovered a lot about both kinds of stem cells, but there's a lot they still don't know.

Questions researchers are still seeking answers to include the following:

- How many kinds of adult stem cells are there?
- Where do adult stem cells live in specific tissues?
- What control mechanisms do stem cells use to maintain their self-renewal capabilities?
- What genetic mechanisms control stem cells' ability to make one or more kinds of differentiated cells?
- Why don't adult stem cells differentiate automatically when they're surrounded by differentiated cells?
- Why can embryonic stem cells grow and make more of themselves in the lab for a year or more, while most adult stem cells have far more limited self-renewing capabilities in a Petri dish?
- How do stem cells know when to make more of themselves and when to make cells for specific tissues?
- Why don't all stem cells "home in" to their proper location the way blood-forming stem cells do when they're transplanted into a living body (see Chapter 13)?
- If you introduce stem cells into specific tissues in a living body, do they stay where you put them, or do they wander aimlessly around the body's tissues?
- How long do transplanted stem cells stay in the body?
- If you reprogram adult cells to behave like embryonic stem cells (see Chapter 6), are the reprogrammed cells completely normal, or does the reprogramming process mess with the genetic instructions?
- In their normal environments (known as *niches),* can adult stem cells really make differentiated cells for tissues other than their tissue of origin?
- Is there a master adult stem cell — one that, like embryonic stem cells, can make any type of cell in the body?

Modern stem cell science is pretty young, so it's not surprising that researchers still don't know the answers to some relatively basic questions. As Lao Tzu, the father of Taoism, is credited with saying, "The wise man knows he doesn't know."