4. THE ADULT STEM CELL

For many years, researchers have been seeking to understand the body's ability to repair and replace the cells and tissues of some organs, but not others. After years of work pursuing the how and why of seemingly indiscriminant cell repair mechanisms, scientists have now focused their attention on adult stem cells. It has long been known that stem cells are capable of renewing themselves and that they can generate multiple cell types. Today, there is new evidence that stem cells are present in far more tissues and organs than once thought and that these cells are capable of developing into more kinds of cells than previously imagined. Efforts are now underway to harness stem cells and to take advantage of this new found capability, with the goal of devising new and more effective treatments for a host of diseases and disabilities. What lies ahead for the use of adult stem cells is unknown, but it is certain that there are many research questions to be answered and that these answers hold great promise for the future.

WHAT IS AN ADULT STEM CELL?

Adult stem cells, like all stem cells, share at least two characteristics. First, they can make identical copies of themselves for long periods of time; this ability to proliferate is referred to as long-term self-renewal. Second, they can give rise to mature cell types that have characteristic morphologies (shapes) and specialized functions. Typically, stem cells generate an intermediate cell type or types before they achieve their fully differentiated state. The intermediate cell is called a precursor or progenitor cell. Progenitor or precursor cells in fetal or adult tissues are partly differentiated cells that divide and give rise to differentiated cells. Such cells are usually regarded as "committed" to differentiating along a particular cellular development pathway, although this characteristic may not be as definitive as once thought [82] (see Figure 4.1. Distinguishing Features of Progenitor/Precursor Cells and Stem Cells).

Adult stem cells are rare. Their primary functions are to maintain the steady state functioning of a cell—called homeostasis—and, with limitations, to replace cells that die because of injury or disease [44, 58]. For example, only an estimated 1 in 10,000 to 15,000 cells in the bone marrow is a hematopoietic (blood-forming) stem cell (HSC) [105]. Furthermore, adult stem cells are dispersed in tissues throughout the mature animal and behave very differently, depending on their local environment. For example, HSCs are constantly being generated in the bone marrow where they differentiate into mature types of blood cells. Indeed, the primary role of HSCs is to replace blood cells [26] (see Chapter 5. Hematopoietic Stem Cells). In contrast, stem cells in the small intestine are stationary, and are physically separated from the mature cell types they generate. Gut epithelial stem cells (or precursors) occur at the bases of crypts—deep invaginations between the mature, differentiated epithelial cells that line the lumen of the intestine. These epithelial crypt cells divide fairly often, but remain part of the stationary group of cells they generate [93].

Unlike embryonic stem cells, which are defined by their origin (the inner cell mass of the blastocyst), adult stem cells share no such definitive means of characterization. In fact, no one knows the origin of adult stem cells in any mature tissue. Some have proposed that stem cells are somehow set aside during fetal development and restrained from differentiating. Definitions of adult stem cells vary in the scientific literature range from a simple description of the cells to a rigorous set of experimental criteria that must be met before characterizing a

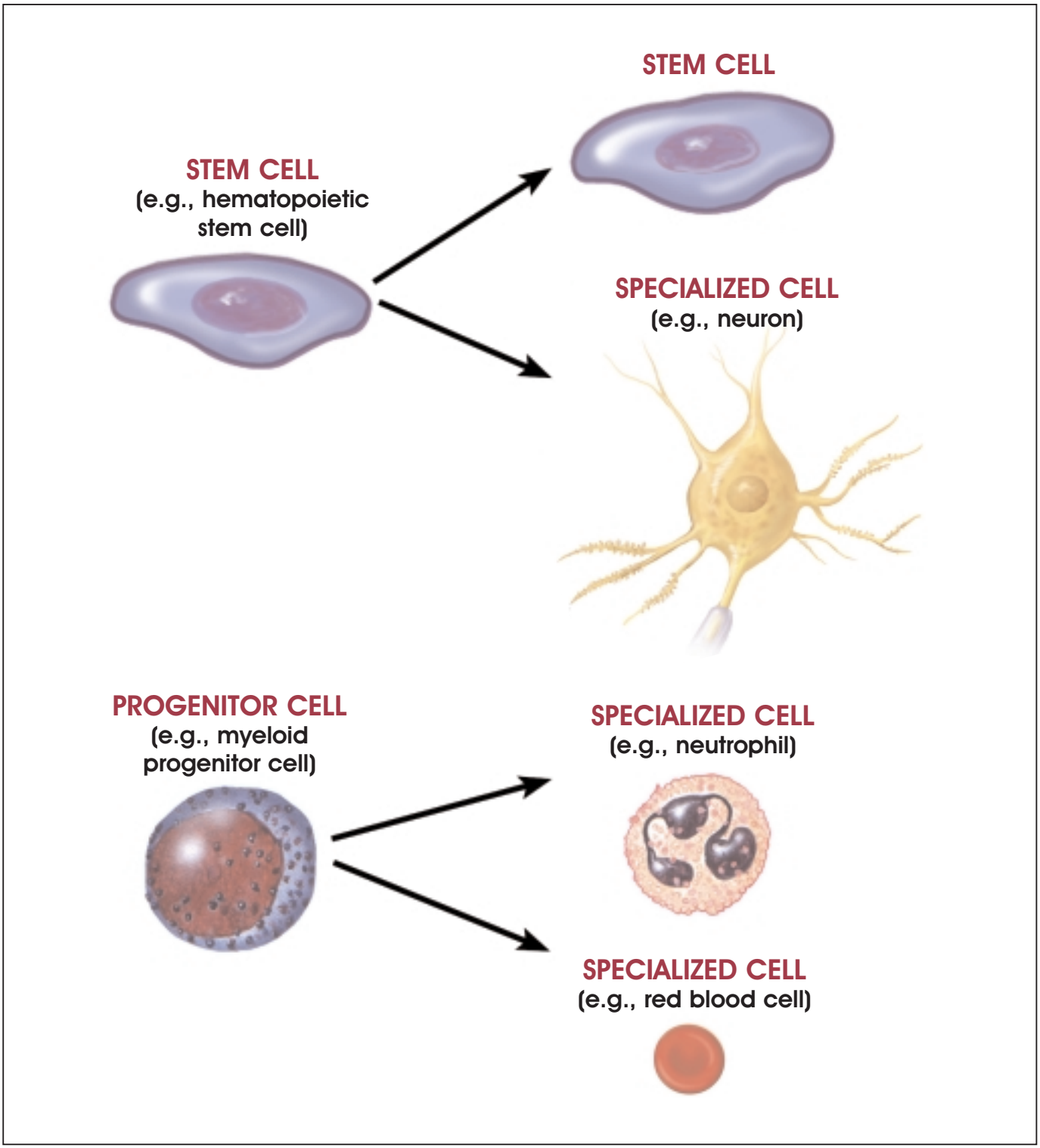

Figure 4.1. Distinguishing Features of Progenitor/Precursor Cells and Stem Cells. A stem cell is an unspecialized cell that is capable of replicating or self renewing itself and developing into specialized cells of a variety of cell types. The product of a stem cell undergoing division is at least one additional stem cell that has the same capabilities of the originating cell. Shown here is an example of a hematopoietic stem cell producing a second generation stem cell and a neuron. A progenitor cell (also known as a precursor cell) is unspecialized or has partial characteristics of a specialized cell that is capable of undergoing cell division and yielding two specialized cells. Shown here is an example of a myeloid progenitor/precursor undergoing cell division to yield two specialized cells (a neutrophil and a red blood cell).

particular cell as an adult stem cell. Most of the information about adult stem cells comes from studies of mice. The list of adult tissues reported to contain stem cells is growing and includes bone marrow, peripheral blood, brain, spinal cord, dental pulp, blood vessels, skeletal muscle, epithelia of the skin and digestive system, cornea, retina, liver, and pancreas.

In order to be classified as an adult stem cell, the cell should be capable of self-renewal for the lifetime of the organism. This criterion, although fundamental to the nature of a stem cell, is difficult to prove *in vivo*. It is nearly impossible, in an organism as complex as a human, to design an experiment that will allow the fate of candidate adult stem cells to be identified *in vivo* and tracked over an individual's entire lifetime.

Ideally, adult stem cells should also be clonogenic. In other words, a single adult stem cell should be able to generate a line of genetically identical cells, which then gives rise to all the appropriate, differentiated cell types of the tissue in which it resides. Again, this property is difficult to demonstrate *in vivo*; in practice, scientists show either that a stem cell is clonogenic *in vitro*, or that a purified population of candidate stem cells can repopulate the tissue.

An adult stem cell should also be able to give rise to fully differentiated cells that have mature phenotypes, are fully integrated into the tissue, and are capable of specialized functions that are appropriate for the tissue. The term phenotype refers to all the observable characteristics of a cell (or organism); its shape (morphology); interactions with other cells and the non-cellular environment (also called the extracellular matrix); proteins that appear on the cell surface (surface markers); and the cell's behavior (e.g., secretion, contraction, synaptic transmission).

The majority of researchers who lay claim to having identified adult stem cells rely on two of these characteristics—appropriate cell morphology, and the demonstration that the resulting, differentiated cell types display surface markers that identify them as belonging to the tissue. Some studies demonstrate that the differentiated cells that are derived from adult stem cells are truly functional, and a few studies show that cells are integrated into the differentiated tissue *in vivo* and that they interact appropriately with neighboring cells. At present, there is, however, a paucity of research, with a few notable exceptions, in which researchers were able to conduct studies of genetically identical (clonal) stem cells. In order to fully characterize the regenerating and self-renewal capabilities of the adult stem cell, and therefore to truly harness its potential, it will be important to demonstrate that a single adult stem cell can, indeed, generate a line of genetically identical cells, which then gives rise to all the appropriate, differentiated cell types of the tissue in which it resides.

EVIDENCE FOR THE PRESENCE OF ADULT STEM CELLS

Adult stem cells have been identified in many animal and human tissues. In general, three methods are used to determine whether candidate adult stem cells give rise to specialized cells. Adult stem cells can be labeled *in vivo* and then they can be tracked. Candidate adult stem cells can also be isolated and labeled and then transplanted back into the organism to determine what becomes of them. Finally, candidate adult stem cells can be isolated, grown *in vitro* and manipulated, by adding growth factors or introducing genes that help determine what differentiated cells types they will yield. For example, currently, scientists believe that stem cells in the fetal and adult brain divide and give rise to more stem cells or to several types of precursor cells, which give rise to nerve cells (neurons), of which there are many types.

It is often difficult—if not impossible—to distinguish adult, tissue-specific stem cells from progenitor cells, which are found in fetal or adult tissues and are partly differentiated cells that divide and give rise to differentiated cells. These are cells found in many organs that are generally thought to be present to replace cells and maintain the integrity of the tissue. Progenitor cells give rise to certain types of cells—such as the blood cells known as T lymphocytes, B lymphocytes, and natural killer cells—but are not thought to be capable of developing into all the cell types of a tissue and as such are not truly stem cells. The current wave of excitement over the existence of stem cells in many adult tissues is perhaps fueling claims that progenitor or precursor cells in those tissues are instead stem cells. Thus, there are reports of endothelial progenitor cells, skeletal muscle stem cells, epithelial precursors in the skin and digestive system, as well as some reports of progenitors or stem cells in the pancreas and liver. A detailed summary of some of the evidence for the existence of stem

cells in various tissues and organs is presented later in the chapter.

ADULT STEM CELL PLASTICITY

It was not until recently that anyone seriously considered the possibility that stem cells in adult tissues could generate the specialized cell types of another type of tissue from which they normally reside—either a tissue derived from the same embryonic germ layer or from a different germ layer (see Table 1.1. Embryonic Germ Layers From Which Differentiated Tissues Develop). For example, studies have shown that blood stem cells (derived from mesoderm) may be able to generate both skeletal muscle (also derived from mesoderm) and neurons (derived from ectoderm). That realization has been triggered by a flurry of papers reporting that stem cells derived from one adult tissue can change their appearance and assume characteristics that resemble those of differentiated cells from other tissues.

The term plasticity, as used in this report, means that a stem cell from one adult tissue can generate the differentiated cell types of another tissue. At this time, there is no formally accepted name for this phenomenon in the scientific literature. It is variously referred to as "plasticity" [15, 52], "unorthodox differentiation" [10] or "transdifferentiation" [7, 54].

Approaches for Demonstrating Adult Stem Cell Plasticity

To be able to claim that adult stem cells demonstrate plasticity, it is first important to show that a cell population exists in the starting tissue that has the identifying features of stem cells. Then, it is necessary to show that the adult stem cells give rise to cell types that normally occur in a different tissue. Neither of these criteria is easily met. Simply proving the existence of an adult stem cell population in a differentiated tissue is a laborious process. It requires that the candidate stem cells are shown to be self-renewing, and that they can give rise to the differentiated cell types that are characteristic of that tissue.

To show that the adult stem cells can generate other cell types requires them to be tracked in their new environment, whether it is *in vitro* or *in vivo*. In general, this has been accomplished by obtaining the stem cells from a mouse that has been genetically engineered to express a molecular tag in all its cells. It is then necessary to show that the labeled adult stem cells have adopted key structural and biochemical characteristics of the new tissue they are claimed to have generated. Ultimately—and most importantly—it is necessary to demonstrate that the cells can integrate into their new tissue environment, survive in the tissue, and function like the mature cells of the tissue.

In the experiments reported to date, adult stem cells may assume the characteristics of cells that have developed from the same primary germ layer or a different germ layer (see Figure 4.2. Preliminary Evidence of Plasticity Among Nonhuman Adult Stem Cells). For example, many plasticity experiments involve stem cells derived from bone marrow, which is a mesodermal derivative. The bone marrow stem cells may then differentiate into another mesodermally derived tissue such as skeletal muscle [28, 43], cardiac muscle [51, 71] or liver [4, 54, 97].

Alternatively, adult stem cells may differentiate into a tissue that—during normal embryonic development—would arise from a different germ layer. For example, bone marrow-derived cells may differentiate into neural tissue, which is derived from embryonic ectoderm [15, 65]. And—reciprocally—neural stem cell lines cultured from adult brain tissue may differentiate to form hematopoietic cells [13], or even give rise to many different cell types in a chimeric embryo [17]. In both cases cited above, the cells would be deemed to show plasticity, but in the case of bone marrow stem cells generating brain cells, the finding is less predictable.

In order to study plasticity within and across germ layer lines, the researcher must be sure that he/she is using only one kind of adult stem cell. The vast majority of experiments on plasticity have been conducted with adult stem cells derived either from the bone marrow or the brain. The bone marrow-derived cells are sometimes sorted—using a panel of surface markers—into populations of hematopoietic stem cells or bone marrow stromal cells [46, 54, 71]. The HSCs may be highly purified or partially purified, depending on the conditions used. Another way to separate population of bone marrow cells is by fractionation to yield cells that adhere to a growth substrate (stromal cells) or do not adhere (hematopoietic cells) [28].

To study plasticity of stem cells derived from the brain, the researcher must overcome several problems. Stem cells from the central nervous system (CNS),

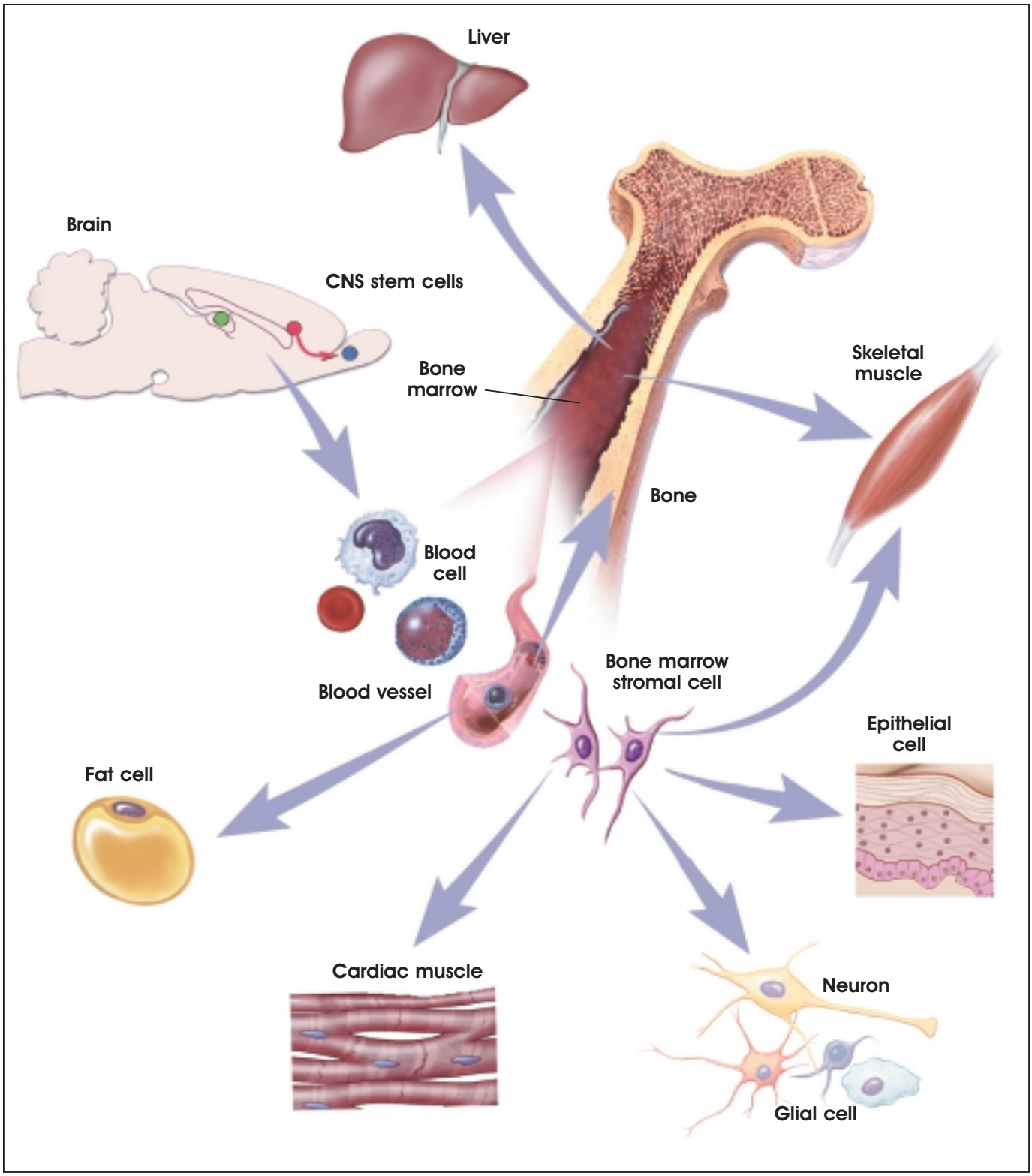

Figure 4.2. Preliminary Evidence of Plasticity Among Nonhuman Adult Stem Cells.

unlike bone marrow cells, do not occur in a single, accessible location. Instead, they are scattered in three places, at least in rodent brain—the tissue around the lateral ventricles in the forebrain, a migratory pathway for the cells that leads from the ventricles to the olfactory bulbs, and the hippocampus. Many of the experiments with CNS stem cells involve the formation of neurospheres, round aggregates of cells that are sometimes clonally derived. But it is not possible to observe cells in the center of a neurosphere, so to study plasticity *in vitro*, the cells are usually dissociated and plated in monolayers. To study plasticity *in vivo*, the cells may be dissociated before injection into the circulatory system of the recipient animal [13], or injected as neurospheres [17].

What is the Evidence for Plasticity?

The differentiated cell types that result from plasticity are usually reported to have the morphological characteristics of the differentiated cells and to display their characteristic surface markers. In reports that transplanted adult stem cells show plasticity *in vivo*, the stem cells typically are shown to have integrated into a mature host tissue and assumed at least some of its characteristics [15, 28, 51, 65, 71]. Many plasticity experiments involve injury to a particular tissue, which is intended to model a particular human disease or injury [13, 54, 71]. However, there is limited evidence to date that such adult stem cells can generate mature, fully functional cells or that the cells have restored lost function *in vivo* [54]. Most of the studies that show the plasticity of adult stem cells involve cells that are derived from the bone marrow [15, 28, 54, 65, 77] or brain [13, 17]. To date, adult stem cells are best characterized in these two tissues, which may account for the greater number of plasticity studies based on bone marrow and brain. Collectively, studies on plasticity suggest that stem cell populations in adult mammals are not fixed entities, and that after exposure to a new environment, they may be able to populate other tissues and possibly differentiate into other cell types.

It is not yet possible to say whether plasticity occurs normally *in vivo*. Some scientists think it may [14, 64], but as yet there is no evidence to prove it. Also, it is not yet clear to what extent plasticity can occur in experimental settings, and how—or whether—the phenomenon can be harnessed to generate tissues that may be useful for therapeutic transplantation. If the phenomenon of plasticity is to be used as a basis for generating tissue for transplantation, the techniques for doing it will need to be reproducible and reliable (see Chapter 10. Assessing Human Stem Cell Safety). In some cases, debate continues about observations that adult stem cells yield cells of tissue types different than those from which they were obtained [7, 68].

EXPERIMENTAL EVIDENCE OF ADULT STEM CELLS AND PLASTICITY

Adult Stem Cells of the Nervous System

More than 30 years ago, Altman and Das showed that two regions of the postnatal rat brain, the hippocampus and the olfactory bulb, contain dividing cells that become neurons [5, 6]. Despite these reports, the prevailing view at the time was that nerve cells in the adult brain do not divide. In fact, the notion that stem cells in the adult brain can generate its three major cell types—astrocytes and oligodendrocytes, as well as neurons—was not accepted until far more recently. Within the past five years, a series of studies has shown that stem cells occur in the adult mammalian brain and that these cells can generate its three major cell lineages [35, 48, 63, 66, 90, 96, 104] (see Chapter 8. Rebuilding the Nervous System with Stem Cells).

Today, scientists believe that stem cells in the fetal and adult brain divide and give rise to more stem cells or to several types of precursor cells. Neuronal precursors (also called neuroblasts) divide and give rise to nerve cells (neurons), of which there are many types. Glial precursors give rise to astrocytes or oligodendrocytes. Astrocytes are a kind of glial cell, which lend both mechanical and metabolic support for neurons; they make up 70 to 80 percent of the cells of the adult brain. Oligodendrocytes make myelin, the fatty material that ensheathes nerve cell axons and speeds nerve transmission. Under normal, *in vivo* conditions, neuronal precursors do not give rise to glial cells, and glial precursors do not give rise to neurons. In contrast, a fetal or adult CNS (central nervous system—the brain and spinal cord) stem cell may give rise to neurons, astrocytes, or oligodendrocytes, depending on the signals it receives and its three-dimensional environment within the brain tissue.

There is now widespread consensus that the adult mammalian brain does contain stem cells. However, there is no consensus about how many populations of CNS stem cells exist, how they may be related, and how they function *in vivo*. Because there are no markers currently available to identify the cells *in vivo*, the only method for testing whether a given population of CNS cells contains stem cells is to isolate the cells and manipulate them *in vitro*, a process that may change their intrinsic properties [67].

Despite these barriers, three groups of CNS stem cells have been reported to date. All occur in the adult rodent brain and preliminary evidence indicates they also occur in the adult human brain. One group occupies the brain tissue next to the ventricles, regions known as the ventricular zone and the subventricular zone (see discussion below). The ventricles are spaces in the brain filled with cerebrospinal fluid. During fetal development, the tissue adjacent to the ventricles is a prominent region of actively dividing cells. By adulthood, however, this tissue is much smaller, although it still appears to contain stem cells [70].

A second group of adult CNS stem cells, described in mice but not in humans, occurs in a streak of tissue that connects the lateral ventricle and the olfactory bulb, which receives odor signals from the nose. In rodents, olfactory bulb neurons are constantly being replenished via this pathway [59, 61]. A third possible location for stem cells in adult mouse and human brain occurs in the hippocampus, a part of the brain thought to play a role in the formation of certain kinds of memory [27, 34].

Central Nervous System Stem Cells in the Subventricular Zone. CNS stem cells found in the forebrain that surrounds the lateral ventricles are heterogeneous and can be distinguished morphologically. Ependymal cells, which are ciliated, line the ventricles. Adjacent to the ependymal cell layer, in a region sometimes designated as the subependymal or subventricular zone, is a mixed cell population that consists of neuroblasts (immature neurons) that migrate to the olfactory bulb, precursor cells, and astrocytes. Some of the cells divide rapidly, while others divide slowly. The astrocyte-like cells can be identified because they contain glial fibrillary acidic protein (GFAP), whereas the ependymal cells stain positive for nestin, which is regarded as a marker of neural stem cells. Which of these cells best qualifies as a CNS stem cell is a matter of debate [76].

A recent report indicates that the astrocytes that occur in the subventricular zone of the rodent brain act as neural stem cells. The cells with astrocyte markers appear to generate neurons *in vivo*, as identified by their expression of specific neuronal markers. The *in vitro* assay to demonstrate that these astrocytes are, in fact, stem cells involves their ability to form neurospheres—groupings of undifferentiated cells that can be dissociated and coaxed to differentiate into neurons or glial cells [25]. Traditionally, these astrocytes have been regarded as differentiated cells, not as stem cells and so their designation as stem cells is not universally accepted.

A series of similar *in vitro* studies based on the formation of neurospheres was used to identify the subependymal zone as a source of adult rodent CNS stem cells. In these experiments, single, candidate stem cells derived from the subependymal zone are induced to give rise to neurospheres in the presence of mitogens—either epidermal growth factor (EGF) or fibroblast growth factor-2 (FGF-2). The neurospheres are dissociated and passaged. As long as a mitogen is present in the culture medium, the cells continue forming neurospheres without differentiating. Some populations of CNS cells are more responsive to EGF, others to FGF [100]. To induce differentiation into neurons or glia, cells are dissociated from the neurospheres and grown on an adherent surface in serum-free medium that contains specific growth factors. Collectively, the studies demonstrate that a population of cells derived from the adult rodent brain can self-renew and differentiate to yield the three major cell types of the CNS cells [41, 69, 74, 102].

Central Nervous System Stem Cells in the Ventricular Zone. Another group of potential CNS stem cells in the adult rodent brain may consist of the ependymal cells themselves [47]. Ependymal cells, which are ciliated, line the lateral ventricles. They have been described as non-dividing cells [24] that function as part of the blood-brain barrier [22]. The suggestion that ependymal cells from the ventricular zone of the adult rodent CNS may be stem cells is therefore unexpected. However, in a recent study, in which two molecular tags—the fluorescent marker DiI, and an adenovirus vector carrying *lacZ* tags—were used to label the ependymal cells that line the entire CNS ventricular system of adult rats, it was shown that these cells could, indeed, act as stem cells. A few

days after labeling, fluorescent or *lacZ*$^+$ cells were observed in the rostral migratory stream (which leads from the lateral ventricle to the olfactory bulb), and then in the olfactory bulb itself. The labeled cells in the olfactory bulb also stained for the neuronal markers βIII tubulin and Map2, which indicated that ependymal cells from the ventricular zone of the adult rat brain had migrated along the rostral migratory stream to generate olfactory bulb neurons *in vivo* [47].

To show that DiI$^+$ cells were neural stem cells and could generate astrocytes and oligodendrocytes as well as neurons, a neurosphere assay was performed *in vitro*. DiI-labeled cells were dissociated from the ventricular system and cultured in the presence of mitogen to generate neurospheres. Most of the neurospheres were DiI$^+$; they could self-renew and generate neurons, astrocytes, and oligodendrocytes when induced to differentiate. Single, DiI$^+$ ependymal cells isolated from the ventricular zone could also generate self-renewing neurospheres and differentiate into neurons and glia.

To show that ependymal cells can also divide *in vivo*, bromodeoxyuridine (BrdU) was administered in the drinking water to rats for a 2- to 6-week period. Bromodeoxyuridine (BrdU) is a DNA precursor that is only incorporated into dividing cells. Through a series of experiments, it was shown that ependymal cells divide slowly *in vivo* and give rise to a population of progenitor cells in the subventricular zone [47]. A different pattern of scattered BrdU-labeled cells was observed in the spinal cord, which suggested that ependymal cells along the central canal of the cord occasionally divide and give rise to nearby ependymal cells, but do not migrate away from the canal.

Collectively, the data suggest that CNS ependymal cells in adult rodents can function as stem cells. The cells can self-renew, and most proliferate via asymmetrical division. Many of the CNS ependymal cells are not actively dividing (quiescent), but they can be stimulated to do so *in vitro* (with mitogens) or *in vivo* (in response to injury). After injury, the ependymal cells in the spinal cord only give rise to astrocytes, not to neurons. How and whether ependymal cells from the ventricular zone are related to other candidate populations of CNS stem cells, such as those identified in the hippocampus [34], is not known.

Are ventricular and subventricular zone CNS stem cells the same population? These studies and other leave open the question of whether cells that directly line the ventricles—those in the ventricular zone—or cells that are at least a layer removed from this zone—in the subventricular zone are the same population of CNS stem cells. A new study, based on the finding that they express different genes, confirms earlier reports that the ventricular and subventricular zone cell populations are distinct. The new research utilizes a technique called representational difference analysis, together with cDNA microarray analysis, to monitor the patterns of gene expression in the complex tissue of the developing and postnatal mouse brain. The study revealed the expression of a panel of genes known to be important in CNS development, such as *L3-PSP* (which encodes a phosphoserine phosphatase important in cell signaling), *cyclin D2* (a cell cycle gene), and *ERCC-1* (which is important in DNA excision repair). All of these genes in the recent study were expressed in cultured neurospheres, as well as the ventricular zone, the subventricular zone, and a brain area outside those germinal zones. This analysis also revealed the expression of novel genes such as A16F10, which is similar to a gene in an embryonic cancer cell line. A16F10 was expressed in neurospheres and at high levels in the subventricular zone, but not significantly in the ventricular zone. Interestingly, several of the genes identified in cultured neurospheres were also expressed in hematopoietic cells, suggesting that neural stem cells and blood-forming cells may share aspects of their genetic programs or signaling systems [38]. This finding may help explain recent reports that CNS stem cells derived from mouse brain can give rise to hematopoietic cells after injection into irradiated mice [13].

Central Nervous System Stem Cells in the Hippocampus. The hippocampus is one of the oldest parts of the cerebral cortex, in evolutionary terms, and is thought to play an important role in certain forms of memory. The region of the hippocampus in which stem cells apparently exist in mouse and human brains is the subgranular zone of the dentate gyrus. In mice, when BrdU is used to label dividing cells in this region, about 50% of the labeled cells differentiate into cells that appear to be dentate

gyrus granule neurons, and 15% become glial cells. The rest of the BrdU-labeled cells do not have a recognizable phenotype [90]. Interestingly, many, if not all the BrdU-labeled cells in the adult rodent hippocampus occur next to blood vessels [33].

In the human dentate gyrus, some BrdU-labeled cells express NeuN, neuron-specific enolase, or calbindin, all of which are neuronal markers. The labeled neuron-like cells resemble dentate gyrus granule cells, in terms of their morphology (as they did in mice). Other BrdU-labeled cells express glial fibrillary acidic protein (GFAP) an astrocyte marker. The study involved autopsy material, obtained with family consent, from five cancer patients who had been injected with BrdU dissolved in saline prior to their death for diagnostic purposes. The patients ranged in age from 57 to 72 years. The greatest number of BrdU-labeled cells were identified in the oldest patient, suggesting that new neuron formation in the hippocampus can continue late in life [27].

Fetal Central Nervous System Stem Cells. Not surprisingly, fetal stem cells are numerous in fetal tissues, where they are assumed to play an important role in the expansion and differentiation of all tissues of the developing organism. Depending on the developmental stage of an animal, fetal stem cells and precursor cells—which arise from stem cells—may make up the bulk of a tissue. This is certainly true in the brain [48], although it has not been demonstrated experimentally in many tissues.

It may seem obvious that the fetal brain contains stem cells that can generate all the types of neurons in the brain as well as astrocytes and oligodendrocytes, but it was not until fairly recently that the concept was proven experimentally. There has been a long-standing question as to whether or not the same cell type gives rise to both neurons and glia. In studies of the developing rodent brain, it has now been shown that all the major cell types in the fetal brain arise from a common population of progenitor cells [20, 34, 48, 80, 108].

Neural stem cells in the mammalian fetal brain are concentrated in seven major areas: olfactory bulb, ependymal (ventricular) zone of the lateral ventricles (which lie in the forebrain), subventricular zone (next to the ependymal zone), hippocampus, spinal cord, cerebellum (part of the hindbrain), and the cerebral cortex. Their number and pattern of development vary in different species. These cells appear to represent different stem cell populations, rather than a single population of stem cells that is dispersed in multiple sites. The normal development of the brain depends not only on the proliferation and differentiation of these fetal stem cells, but also on a genetically programmed process of selective cell death called apoptosis [76].

Little is known about stem cells in the human fetal brain. In one study, however, investigators derived clonal cell lines from CNS stem cells isolated from the diencephalon and cortex of human fetuses, 10.5 weeks post-conception [103]. The study is unusual, not only because it involves human CNS stem cells obtained from fetal tissue, but also because the cells were used to generate clonal cell lines of CNS stem cells that generated neurons, astrocytes, and oligodendrocytes, as determined on the basis of expressed markers. In a few experiments described as "preliminary," the human CNS stem cells were injected into the brains of immunosuppressed rats where they apparently differentiated into neuron-like cells or glial cells.

In a 1999 study, a serum-free growth medium that included EGF and FGF2 was devised to grow the human fetal CNS stem cells. Although most of the cells died, occasionally, single CNS stem cells survived, divided, and ultimately formed neurospheres after one to two weeks in culture. The neurospheres could be dissociated and individual cells replated. The cells resumed proliferation and formed new neurospheres, thus establishing an *in vitro* system that (like the system established for mouse CNS neurospheres) could be maintained up to 2 years. Depending on the culture conditions, the cells in the neurospheres could be maintained in an undifferentiated dividing state (in the presence of mitogen), or dissociated and induced to differentiate (after the removal of mitogen and the addition of specific growth factors to the culture medium). The differentiated cells consisted mostly of astrocytes (75%), some neurons (13%) and rare oligodendrocytes (1.2%). The neurons generated under these conditions expressed markers indicating they were GABAergic, [the major type of inhibitory neuron in the mammalian CNS responsive to the amino acid neurotransmitter, gamma-aminobutyric acid (GABA)]. However, catecholamine-like cells that express tyrosine hydroxylase (TH, a critical enzyme in the dopamine-synthesis pathway)

could be generated, if the culture conditions were altered to include different medium conditioned by a rat glioma line (BB49). Thus, the report indicates that human CNS stem cells obtained from early fetuses can be maintained *in vitro* for a long time without differentiating, induced to differentiate into the three major lineages of the CNS (and possibly two kinds of neurons, GABAergic and TH-positive), and engraft (in rats) *in vivo* [103].

Central Nervous System Neural Crest Stem Cells. Neural crest cells differ markedly from fetal or adult neural stem cells. During fetal development, neural crest cells migrate from the sides of the neural tube as it closes. The cells differentiate into a range of tissues, not all of which are part of the nervous system [56, 57, 91]. Neural crest cells form the sympathetic and parasympathetic components of the peripheral nervous system (PNS), including the network of nerves that innervate the heart and the gut, all the sensory ganglia (groups of neurons that occur in pairs along the dorsal surface of the spinal cord), and Schwann cells, which (like oligodendrocytes in the CNS) make myelin in the PNS. The non-neural tissues that arise from the neural crest are diverse. They populate certain hormone-secreting glands—including the adrenal medulla and Type I cells in the carotid body—pigment cells of the skin (melanocytes), cartilage and bone in the face and skull, and connective tissue in many parts of the body [76].

Thus, neural crest cells migrate far more extensively than other fetal neural stem cells during development, form mesenchymal tissues, most of which develop from embryonic mesoderm as well as the components of the CNS and PNS which arises from embryonic ectoderm. This close link, in neural crest development, between ectodermally derived tissues and mesodermally derived tissues accounts in part for the interest in neural crest cells as a kind of stem cell. In fact, neural crest cells meet several criteria of stem cells. They can self-renew (at least in the fetus) and can differentiate into multiple cells types, which include cells derived from two of the three embryonic germ layers [76].

Recent studies indicate that neural crest cells persist late into gestation and can be isolated from E14.5 rat sciatic nerve, a peripheral nerve in the hindlimb. The cells incorporate BrdU, indicating that they are dividing *in vivo*. When transplanted into chick embryos, the rat neural crest cells develop into neurons and glia, an indication of their stem cell-like properties [67]. However, the ability of rat E14.5 neural crest cells taken from sciatic nerve to generate nerve and glial cells in chick is more limited than neural crest cells derived from younger, E10.5 rat embryos. At the earlier stage of development, the neural tube has formed, but neural crest cells have not yet migrated to their final destinations. Neural crest cells from early developmental stages are more sensitive to bone morphogenetic protein 2 (BMP2) signaling, which may help explain their greater differentiation potential [106].

Stem Cells in the Bone Marrow and Blood

The notion that the bone marrow contains stem cells is not new. One population of bone marrow cells, the hematopoietic stem cells (HSCs), is responsible for forming all of the types of blood cells in the body. HSCs were recognized as a stem cells more than 40 years ago [9, 99]. Bone marrow stromal cells—a mixed cell population that generates bone, cartilage, fat, fibrous connective tissue, and the reticular network that supports blood cell formation—were described shortly after the discovery of HSCs [30, 32, 73]. The mesenchymal stem cells of the bone marrow also give rise to these tissues, and may constitute the same population of cells as the bone marrow stromal cells [78]. Recently, a population of progenitor cells that differentiates into endothelial cells, a type of cell that lines the blood vessels, was isolated from circulating blood [8] and identified as originating in bone marrow [89]. Whether these endothelial progenitor cells, which resemble the angioblasts that give rise to blood vessels during embryonic development, represent a bona fide population of adult bone marrow stem cells remains uncertain. Thus, the bone marrow appears to contain three stem cell populations—hematopoietic stem cells, stromal cells, and (possibly) endothelial progenitor cells (see Figure 4.3. Hematopoietic and Stromal Stem Cell Differentiation).

Two more apparent stem cell types have been reported in circulating blood, but have not been shown to originate from the bone marrow. One population, called pericytes, may be closely related to bone marrow stromal cells, although their origin remains elusive [12]. The second population of blood-born stem cells, which occur in four species of

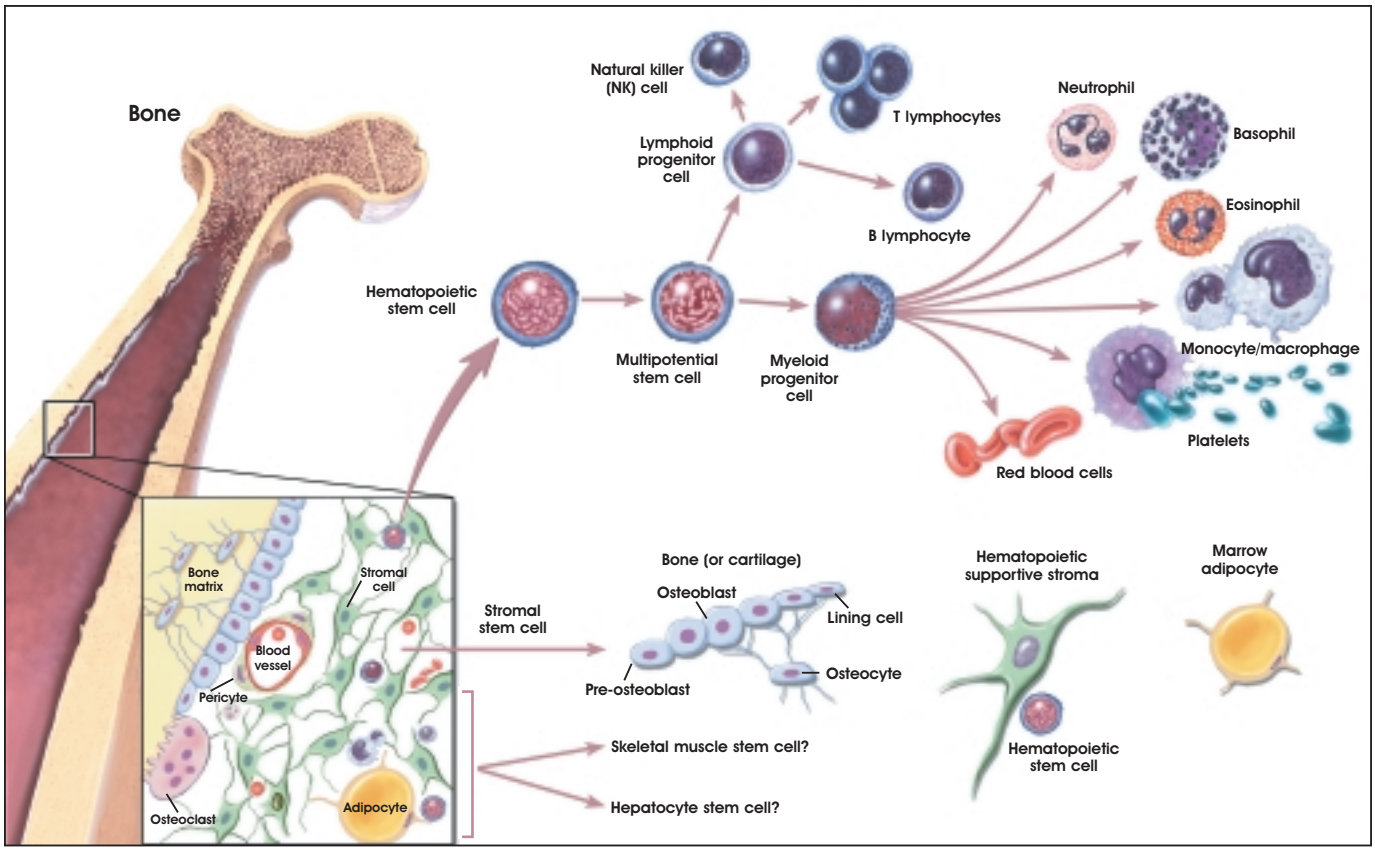

Figure 4.3. Hematopoietic and Stromal Stem Cell Differentiation.

animals tested—guinea pigs, mice, rabbits, and humans—resemble stromal cells in that they can generate bone and fat [53].

Hematopoietic Stem Cells. Of all the cell types in the body, those that survive for the shortest period of time are blood cells and certain kinds of epithelial cells. For example, red blood cells (erythrocytes), which lack a nucleus, live for approximately 120 days in the bloodstream. The life of an animal literally depends on the ability of these and other blood cells to be replenished continuously. This replenishment process occurs largely in the bone marrow, where HSCs reside, divide, and differentiate into all the blood cell types. Both HSCs and differentiated blood cells cycle from the bone marrow to the blood and back again, under the influence of a barrage of secreted factors that regulate cell proliferation, differentiation, and migration (see Chapter 5. Hematopoietic Stem Cells).

HSCs can reconstitute the hematopoietic system of mice that have been subjected to lethal doses of radiation to destroy their own hematopoietic systems. This test, the rescue of lethally irradiated mice, has become a standard by which other candidate stem cells are measured because it shows, without question, that HSCs can regenerate an entire tissue system—in this case, the blood [9, 99]. HSCs were first proven to be blood-forming stem cells in a series of experiments in mice; similar blood-forming stem cells occur in humans. HSCs are defined by their ability to self-renew and to give rise to all the kinds of blood cells in the body. This means that a single HSC is capable of regenerating the entire hematopoietic system, although this has been demonstrated only a few times in mice [72].

Over the years, many combinations of surface markers have been used to identify, isolate, and purify HSCs derived from bone marrow and blood. Undifferentiated HSCs and hematopoietic progenitor cells express c-kit, CD34, and H-2K. These cells usually lack the lineage marker Lin, or express it at very low levels ($Lin^{-/low}$). And for transplant purposes, cells that are $CD34^{+}$ $Thy1^{+}$ Lin^{-} are most likely to contain stem cells and result in engraftment.

Two kinds of HSCs have been defined. Long-term HSCs proliferate for the lifetime of an animal. In young adult mice, an estimated 8 to 10 % of long-term HSCs enter the cell cycle and divide each day. Short-term HSCs proliferate for a limited time, possibly a few months. Long-term HSCs have high levels of telomerase activity. Telomerase is an enzyme that helps maintain the length of the ends of chromosomes, called telomeres, by adding on nucleotides. Active telomerase is a characteristic of undifferentiated, dividing cells and cancer cells. Differentiated, human somatic cells do not show telomerase activity. In adult humans, HSCs occur in the bone marrow, blood, liver, and spleen, but are extremely rare in any of these tissues. In mice, only 1 in 10,000 to 15,000 bone marrow cells is a long-term HSC [105].

Short-term HSCs differentiate into lymphoid and myeloid precursors, the two classes of precursors for the two major lineages of blood cells. Lymphoid precursors differentiate into T cells, B cells, and natural killer cells. The mechanisms and pathways that lead to their differentiation are still being investigated [1, 2]. Myeloid precursors differentiate into monocytes and macrophages, neutrophils, eosinophils, basophils, megakaryocytes, and erythrocytes [3]. *In vivo*, bone marrow HSCs differentiate into mature, specialized blood cells that cycle constantly from the bone marrow to the blood, and back to the bone marrow [26]. A recent study showed that short-term HSCs are a heterogeneous population that differ significantly in terms of their ability to self-renew and repopulate the hematopoietic system [42].

Attempts to induce HSC to proliferate *in vitro*—on many substrates, including those intended to mimic conditions in the stroma—have frustrated scientists for many years. Although HSCs proliferate readily *in vivo*, they usually differentiate or die *in vitro* [26]. Thus, much of the research on HSCs has been focused on understanding the factors, cell-cell interactions, and cell-matrix interactions that control their proliferation and differentiation *in vivo*, with the hope that similar conditions could be replicated *in vitro*. Many of the soluble factors that regulate HSC differentiation *in vivo* are cytokines, which are made by different cell types and are then concentrated in the bone marrow by the extracellular matrix of stromal cells—the sites of blood formation [45, 107]. Two of the most-studied cytokines are granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3) [40, 81].

Also important to HSC proliferation and differentiation are interactions of the cells with adhesion molecules in the extracellular matrix of the bone marrow stroma [83, 101, 110].

Bone Marrow Stromal Cells. Bone marrow (BM) stromal cells have long been recognized for playing an important role in the differentiation of mature blood cells from HSCs (see Figure 4.3. Hematopoietic and Stromal Stem Cell Differentiation). But stromal cells also have other important functions [30, 31]. In addition to providing the physical environment in which HSCs differentiate, BM stromal cells generate cartilage, bone, and fat. Whether stromal cells are best classified as stem cells or progenitor cells for these tissues is still in question. There is also a question as to whether BM stromal cells and so-called mesenchymal stem cells are the same population [78].

BM stromal cells have many features that distinguish them from HSCs. The two cell types are easy to separate *in vitro*. When bone marrow is dissociated, and the mixture of cells it contains is plated at low density, the stromal cells adhere to the surface of the culture dish, and the HSCs do not. Given specific *in vitro* conditions, BM stromal cells form colonies from a single cell called the colony forming unit-F (CFU-F). These colonies may then differentiate as adipocytes or myelosupportive stroma, a clonal assay that indicates the stem cell-like nature of stromal cells. Unlike HSCs, which do not divide *in vitro* (or proliferate only to a limited extent), BM stromal cells can proliferate for up to 35 population doublings *in vitro* [16]. They grow rapidly under the influence of such mitogens as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and insulin-like growth factor-1 (IGF-1) [12].

To date, it has not been possible to isolate a population of pure stromal cells from bone marrow. Panels of markers used to identify the cells include receptors for certain cytokines (interleukin-1, 3, 4, 6, and 7) receptors for proteins in the extracellular matrix, (ICAM-1 and 2, VCAM-1, the alpha-1, 2, and 3 integrins, and the beta-1, 2, 3 and 4 integrins), etc. [64]. Despite the use of these markers and another stromal cell marker called Stro-1, the origin and specific identity of stromal cells have remained elusive. Like HSCs, BM stromal cells arise from embryonic mesoderm during development, although no specific precursor or stem cell for stromal cells has been isolated and identified.

One theory about their origin is that a common kind of progenitor cell—perhaps a primordial endothelial cell that lines embryonic blood vessels—gives rise to both HSCs and to mesodermal precursors. The latter may then differentiate into myogenic precursors (the satellite cells that are thought to function as stem cells in skeletal muscle), and the BM stromal cells [10].

In vivo, the differentiation of stromal cells into fat and bone is not straightforward. Bone marrow adipocytes and myelosupportive stromal cells—both of which are derived from BM stromal cells—may be regarded as interchangeable phenotypes [10, 11]. Adipocytes do not develop until postnatal life, as the bones enlarge and the marrow space increases to accommodate enhanced hematopoiesis. When the skeleton stops growing, and the mass of HSCs decreases in a normal, age-dependent fashion, BM stromal cells differentiate into adipocytes, which fill the extra space. New bone formation is obviously greater during skeletal growth, although bone "turns over" throughout life. Bone forming cells are osteoblasts, but their relationship to BM stromal cells is not clear. New trabecular bone, which is the inner region of bone next to the marrow, could logically develop from the action of BM stromal cells. But the outside surface of bone also turns over, as does bone next to the Haversian system (small canals that form concentric rings within bone). And neither of these surfaces is in contact with BM stromal cells [10, 11].

Adult Stem Cells in Other Tissues

It is often difficult—if not impossible—to distinguish adult, tissue-specific stem cells from progenitor cells. With that caveat in mind, the following summary identifies reports of stem cells in various adult tissues.

Endothelial Progenitor Cells. Endothelial cells line the inner surfaces of blood vessels throughout the body, and it has been difficult to identify specific endothelial stem cells in either the embryonic or the adult mammal. During embryonic development, just after gastrulation, a kind of cell called the hemangioblast, which is derived from mesoderm, is presumed to be the precursor of both the hematopoietic and endothelial cell lineages. The embryonic vasculature formed at this stage is transient and consists of blood islands in the yolk sac. But hemangioblasts, per se, have not been isolated from the embryo and their existence remains in question. The process of forming new blood vessels in the embryo is called vasculogenesis. In the adult, the process of forming blood vessels from pre-existing blood vessels is called angiogenesis [50].

Evidence that hemangioblasts do exist comes from studies of mouse embryonic stem cells that are directed to differentiate *in vitro*. These studies have shown that a precursor cell derived from mouse ES cells that express Flk-1 [the receptor for vascular endothelial growth factor (VEGF) in mice] can give rise to both blood cells and blood vessel cells [88, 109]. Both VEGF and fibroblast growth factor-2 (FGF-2) play critical roles in endothelial cell differentiation *in vivo* [79].

Several recent reports indicate that the bone marrow contains cells that can give rise to new blood vessels in tissues that are ischemic (damaged due to the deprivation of blood and oxygen) [8, 29, 49, 94]. But it is unclear from these studies what cell type(s) in the bone marrow induced angiogenesis. In a study which sought to address that question, researchers found that adult human bone marrow contains cells that resemble embryonic hemangioblasts, and may therefore be called endothelial stem cells.

In more recent experiments, human bone marrow-derived cells were injected into the tail veins of rats with induced cardiac ischemia. The human cells migrated to the rat heart where they generated new blood vessels in the infarcted muscle (a process akin to vasculogenesis), and also induced angiogenesis. The candidate endothelial stem cells are $CD34^+$ (a marker for HSCs), and they express the transcription factor GATA-2 [51]. A similar study using transgenic mice that express the gene for enhanced green fluorescent protein (which allows the cells to be tracked), showed that bone-marrow-derived cells could repopulate an area of infarcted heart muscle in mice, and generate not only blood vessels, but also cardiomyocytes that integrated into the host tissue [71] (see Chapter 9. Can Stem Cells Repair a Damaged Heart?).

And, in a series of experiments in adult mammals, progenitor endothelial cells were isolated from peripheral blood (of mice and humans) by using antibodies against CD34 and Flk-1, the receptor for VEGF. The cells were mononuclear blood cells (meaning they have a nucleus) and are referred to as MB^{CD34+} cells and MB^{Flk1+} cells. When plated in tissue-culture

dishes, the cells attached to the substrate, became spindle-shaped, and formed tube-like structures that resemble blood vessels. When transplanted into mice of the same species (autologous transplants) with induced ischemia in one limb, the MB^{CD34+} cells promoted the formation of new blood vessels [8]. Although the adult MB^{CD34+} and MB^{Flk1+} cells function in some ways like stem cells, they are usually regarded as progenitor cells.

Skeletal Muscle Stem Cells. Skeletal muscle, like the cardiac muscle of the heart and the smooth muscle in the walls of blood vessels, the digestive system, and the respiratory system, is derived from embryonic mesoderm. To date, at least three populations of skeletal muscle stem cells have been identified: satellite cells, cells in the wall of the dorsal aorta, and so-called "side population" cells.

Satellite cells in skeletal muscle were identified 40 years ago in frogs by electron microscopy [62], and thereafter in mammals [84]. Satellite cells occur on the surface of the basal lamina of a mature muscle cell, or myofiber. In adult mammals, satellite cells mediate muscle growth [85]. Although satellite cells are normally non-dividing, they can be triggered to proliferate as a result of injury, or weight-bearing exercise. Under either of these circumstances, muscle satellite cells give rise to myogenic precursor cells, which then differentiate into the myofibrils that typify skeletal muscle. A group of transcription factors called myogenic regulatory factors (MRFs) play important roles in these differentiation events. The so-called primary MRFs, MyoD and Myf5, help regulate myoblast formation during embryogenesis. The secondary MRFs, myogenin and MRF4, regulate the terminal differentiation of myofibrils [86].

With regard to satellite cells, scientists have been addressing two questions. Are skeletal muscle satellite cells true adult stem cells or are they instead precursor cells? Are satellite cells the only cell type that can regenerate skeletal muscle. For example, a recent report indicates that muscle stem cells may also occur in the dorsal aorta of mouse embryos, and constitute a cell type that gives rise both to muscle satellite cells and endothelial cells. Whether the dorsal aorta cells meet the criteria of a self-renewing muscle stem cell is a matter of debate [21].

Another report indicates that a different kind of stem cell, called an SP cell, can also regenerate skeletal muscle may be present in muscle and bone marrow. SP stands for a side population of cells that can be separated by fluorescence-activated cell sorting analysis. Intravenously injecting these muscle-derived stem cells restored the expression of dystrophin in *mdx* mice. Dystrophin is the protein that is defective in people with Duchenne's muscular dystrophy; *mdx* mice provide a model for the human disease. Dystrophin expression in the SP cell-treated mice was lower than would be needed for clinical benefit. Injection of bone marrow- or muscle-derived SP cells into the dystrophic muscle of the mice yielded equivocal results that the transplanted cells had integrated into the host tissue. The authors conclude that a similar population of SP stem cells can be derived from either adult mouse bone marrow or skeletal muscle, and suggest "there may be some direct relationship between bone marrow-derived stem cells and other tissue- or organ-specific cells" [43]. Thus, stem cell or progenitor cell types from various mesodermally-derived tissues may be able to generate skeletal muscle.

Epithelial Cell Precursors in the Skin and Digestive System. Epithelial cells, which constitute 60 percent of the differentiated cells in the body are responsible for covering the internal and external surfaces of the body, including the lining of vessels and other cavities. The epithelial cells in skin and the digestive tract are replaced constantly. Other epithelial cell populations—in the ducts of the liver or pancreas, for example—turn over more slowly. The cell population that renews the epithelium of the small intestine occurs in the intestinal crypts, deep invaginations in the lining of the gut. The crypt cells are often regarded as stem cells; one of them can give rise to an organized cluster of cells called a structural-proliferative unit [93].

The skin of mammals contains at least three populations of epithelial cells: epidermal cells, hair follicle cells, and glandular epithelial cells, such as those that make up the sweat glands. The replacement patterns for epithelial cells in these three compartments differ, and in all the compartments, a stem cell population has been postulated. For example, stem cells in the bulge region of the hair follicle appear to give rise to multiple cell types. Their progeny can migrate down to the base of the follicle where they become matrix cells, which may then give rise to different cell types in the hair follicle, of which there are seven [39]. The

bulge stem cells of the follicle may also give rise to the epidermis of the skin [95].

Another population of stem cells in skin occurs in the basal layer of the epidermis. These stem cells proliferate in the basal region, and then differentiate as they move toward the outer surface of the skin. The keratinocytes in the outermost layer lack nuclei and act as a protective barrier. A dividing skin stem cell can divide asymmetrically to produce two kinds of daughter cells. One is another self-renewing stem cell. The second kind of daughter cell is an intermediate precursor cell which is then committed to replicate a few times before differentiating into keratinocytes. Self-renewing stem cells can be distinguished from this intermediate precusor cell by their higher level of β1 integrin expression, which signals keratinocytes to proliferate via a mitogen-activated protein (MAP) kinase [112]. Other signaling pathways include that triggered by β-catenin, which helps maintain the stem-cell state [111], and the pathway regulated by the oncoprotein c-Myc, which triggers stem cells to give rise to transit amplifying cells [36].

Stem Cells in the Pancreas and Liver. The status of stem cells in the adult pancreas and liver is unclear. During embryonic development, both tissues arise from endoderm. A recent study indicates that a single precursor cell derived from embryonic endoderm may generate both the ventral pancreas and the liver [23]. In adult mammals, however, both the pancreas and the liver contain multiple kinds of differentiated cells that may be repopulated or regenerated by multiple types of stem cells. In the pancreas, endocrine (hormone-producing) cells occur in the islets of Langerhans. They include the beta cells (which produce insulin), the alpha cells (which secrete glucagon), and cells that release the peptide hormones somatostatin and pancreatic polypeptide. Stem cells in the adult pancreas are postulated to occur in the pancreatic ducts or in the islets themselves. Several recent reports indicate that stem cells that express nestin—which is usually regarded as a marker of neural stem cells—can generate all of the cell types in the islets [60, 113] (see Chapter 7. Stem Cells and Diabetes).

The identity of stem cells that can repopulate the liver of adult mammals is also in question. Recent studies in rodents indicate that HSCs (derived from mesoderm) may be able to home to liver after it is damaged, and demonstrate plasticity in becoming into hepatocytes (usually derived from endoderm) [54, 77, 97]. But the question remains as to whether cells from the bone marrow normally generate hepatocytes *in vivo*. It is not known whether this kind of plasticity occurs without severe damage to the liver or whether HSCs from the bone marrow generate oval cells of the liver [18]. Although hepatic oval cells exist in the liver, it is not clear whether they actually generate new hepatocytes [87, 98]. Oval cells may arise from the portal tracts in liver and may give rise to either hepatocytes [19, 55] and to the epithelium of the bile ducts [37, 92]. Indeed, hepatocytes themselves, may be responsible for the well-know regenerative capacity of liver.

SUMMARY

What Do We Know About Adult Stem Cells?

- Adult stem cells can proliferate without differentiating for a long period (a characteristic referred to as long-term self-renewal), and they can give rise to mature cell types that have characteristic shapes and specialized functions.
- Some adult stem cells have the capability to differentiate into tissues other than the ones from which they originated; this is referred to as plasticity.
- Adult stem cells are rare. Often they are difficult to identify and their origins are not known. Current methods for characterizing adult stem cells are dependent on determining cell surface markers and observations about their differentiation patterns in test tubes and culture dishes.
- To date, published scientific literature indicates that adult stem cells have been derived from brain, bone marrow, peripheral blood, dental pulp, spinal cord, blood vessels, skeletal muscle, epithelia of the skin and digestive system, cornea, retina, liver, and pancreas; thus, adult stem cells have been found in tissues that develop from all three embryonic germ layers.
- Hematopoietic stem cells from bone marrow are the most studied and used for clinical applications in restoring various blood and immune components to the bone marrow via transplantation. There are at least two other populations of adult stem cells that have been identified from bone marrow and blood.

- Several populations of adult stem cells have been identified in the brain, particularly the hippocampus. Their function is unknown. Proliferation and differentiation of brain stem cells are influenced by various growth factors.
- There are now several reports of adult stem cells in other tissues (muscle, blood, and fat) that demonstrate plasticity. Very few published research reports on plasticity of adult stem cells have, however, included clonality studies. That is, there is limited evidence that a single adult stem cell or genetically identical line of adult stem cells demonstrates plasticity.
- Rarely have experiments that claim plasticity demonstrated that the adult stem cells have generated mature, fully functional cells or that the cells have restored lost function *in vivo*.

What Do We Need to Know About Adult Stem Cells?

- What are the sources of adult stem cells in the body? Are they "leftover" embryonic stem cells, or do they arise in some other way? And if the latter is true—which seems to be the case—exactly how do adult stem cells arise, and why do they remain in an undifferentiated state, when all the cells around them have differentiated?
- Is it possible to manipulate adult stem cells to increase their ability to proliferate *in vitro*, so that adult stem cells can be used as a sufficient source of tissue for transplants?
- How many kinds of adult stem cells exist, and in which tissues do they exist? Evidence is accumulating that, although they occur in small numbers, adult stem cells are present in many differentiated tissues.
- What is the best evidence that adult stem cells show plasticity and generate cell types of other tissues?
- Is it possible to manipulate adult stem cells to increase their ability to proliferate *in vitro* so that adult stem cells can be used as a sufficient source of tissue for transplants?
- Is there a universal stem cell? An emerging concept is that, in adult mammals, there may be a population of "universal" stem cells. Although largely theoretical, the concept has some experimental basis. A candidate, universal adult stem cell may be one that circulates in the blood stream, can escape from the blood, and populate various adult tissues. In more than one experimental system, researchers have noted that dividing cells in adult tissues often appear near a blood vessel, such as candidate stem cells in the hippocampus, a region of the brain [75].
- Do adult stem cells exhibit plasticity as a normal event *in vivo*? If so, is this true of all adult stem cells? What are the signals that regulate the proliferation and differentiation of stem cells that demonstrate plasticity?

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