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Stem Cells: A New Lease on Life

Review

Elaine Fuchs* and Julia A. Segre
Howard Hughes Medical Institute
Department of Molecular Genetics and Cell Biology
Department of Biochemistry and Molecular Biology
The University of Chicago
Chicago, Illinois 60637

During embryogenesis, a single fertilized oocyte gives rise to a multicellular organism whose cells and tissues have adopted differentiated characteristics or fates to perform the specified functions of each organ of the body. As embryos develop, cells that have acquired their particular fate proliferate, enabling tissues and organs to grow. Even after an animal is fully grown, however, many tissues and organs maintain a process known as homeostasis, where as cells die, either by natural death or by injury, they are replenished. This remarkable feature has ancient origins, dating back to the most primitive animals, such as sponges and hydrozoans. Throughout evolution, nature has exerted considerable fun and fancy in elaborating on this theme. Some amphibians, for instance, can regenerate a limb or tail when severed, and the neurons of bird brains can readily regenerate. While mammals seem to have lost at least some of this wonderful plasticity, their liver can partially regenerate providing that the injury is not too severe, and the epidermis and hair of their skin can readily repair when wounded or cut. Additionally, the epidermis, hair, small intestine, and hematopoietic system are all examples of adult tissues that are naturally in a state of dynamic flux: even in the absence of injury, these structures continually give rise to new cells, able to transiently divide, terminally differentiate and die.

The fabulous ability of an embryo to diversify and of certain adult tissues to regenerate throughout life is a direct result of stem cells, nature's gift to multicellular organisms. Stem cells have both the capacity to self-renew, that is, to divide and create additional stem cells, and also to differentiate along a specified molecular pathway. Embryonic stem cells are very nearly totipotent, reserving the elite privileges of choosing among most if not all of the differentiation pathways that specify the animal. In contrast, stem cells that reside within an adult organ or tissue have more restricted options, often able to select a differentiation program from only a few possible pathways. Or so it seemed, until very recently. In the last year, some spectacular fireworks have exploded many long-standing dogmas in the stem cell world, giving adult stem cells a new lease on life, and enabling them to be what researchers previously thought they were not.

Embryonic Stem Cells

Emanating from the pioneering mouse research of Martin Evans in the 1970s and culminating with the recent successful parallels with human tissue, cells from the inner cell mass (ICM) of mammalian blastocysts can be maintained in tissue culture under conditions where they can be propagated indefinitely as pluripotent embryonic stem (ES) cells (Thomson et al., 1998 and references therein). If injected back into a recipient blastocyst that is then carried to term in a female host, these cells can contribute to virtually all the tissues of the chimeric offspring, including the germ cell compartment. To maintain cultured ES cells in their relatively undifferentiated, pluripotent state, they must both express the intrinsic transcription factor Oct4, and constitutively receive the extrinsic signal from the cytokine leukemia inhibitory factor (LIF) (Nichols et al., 1998; Niwa et al., 1998 and references therein).

Upon LIF withdrawal, cultured ES cells spontaneously aggregate into embryo-like bodies, where they differentiate and spawn many cell lineages, including beating heart muscle cells, blood islands, neurons, pigmented cells, macrophages, epithelia, and fat-producing adipocytes (Figure 1; for review, see Bradley, 1990). Similarly, when ES cells are injected into nude mice, they differentiate into multicellular masses, called teratocarcinomas. Although the programs of gene expression in these structures often bear strong resemblance to the differentiation pathways typical of developing animals, the triggering of these programs is chaotic, yielding a jumbled grab bag of tissue types. These examples graphically illustrate the importance of intercellular interactions and cellular organization in orchestrating development and embryo shape.

During development, intercellular cross-talk results in the generation and transmission of specific signals from a cell to its neighbor, altering in some key way the subsequent behavior of the neighbor. Of prime importance is sifting through the galaxy of environmental signals to determine which constellations of cues can selectively coax ES cells down a specific cell lineage pathway at the expense of all others. To this end, Brüstle et al. (1999) were recently able to obtain pure populations of multipotent progenitor cells expressing glial precursor markers. They achieved this goal by taking aggregates of cultured mouse ES cells and propagating them sequentially in medium containing first fibroblast growth factor (FGF) 2 alone, then a mixture of FGF2 and epidermal growth factor (EGF), and finally a mix of FGF2 and platelet-derived growth factor (PDGF). Bathed in this last broth of growth factors, these pluripotent cells could be maintained for many generations in culture. Upon growth factor withdrawal, they subsequently differentiated into either of two specific lineages, oligodendrocytes or astrocytes (Brüstle et al., 1999).

Illustrating the enormous potential of this type of research for clinical application, McKay and coworkers transplanted these cloned glial precursor cells into the ventricle of myelin-deficient rats. Myelin sheaths formed around host axons in various brain regions, including cortex, hippocampus, and hypothalamus (Figure 2; Brüstle et al., 1999). No signs of nonneuronal tissue were detected in the transplants. The use of pure populations

*To whom correspondence should be addressed (e-mail: liptack@midway.uchicago.edu).

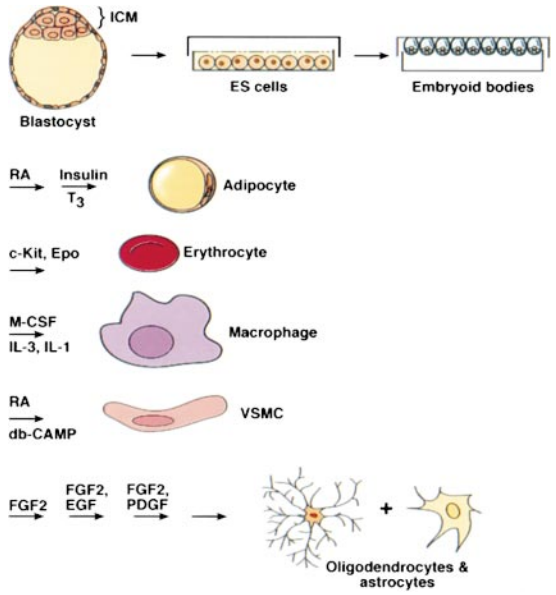

Figure 1. Differentiation Potential of Embryonic Stem (ES) Cells

ES cells are isolated from the ICM (inner cell mass) of a blastocyst, cultured, and then differentiated as embryoid bodies. Given the proper combination of growth factors, these embryoid bodies can develop into such diverse cell types as adipocytes, erythrocytes, macrophages, vascular smooth muscle cells (VSMC), oligodendrocytes, or astrocytes (see Keller et al., 1993; Dani et al., 1997; Drab et al., 1997; Brüstle et al., 1999). RA, retinoic acid; T_3, triiodothyronine; Epo, erythroprotein; M-CSF, macrophage colony stimulating factor; IL, interleukin; db-CAMP, dibutyryl cyctic AMP; FGF, fibroblast growth factor; EGF, epidermal growth factor; PDGF, platelet-derived growth factor.

Figure 2. Developmental Potential of ES Cells In Vivo

Frame shows myelin repair by ES cell transplantation. ES cell–derived glial precursors grafted into the ventricle of myelin-deficient rats generate oligodendrocytes and astrocytes in multiple host brain regions. Donor cells are identified by mouse-specific DNA in situ hybridization (purple nuclear labeling) and double labeling with antibodies to myelin proteolipid protein (green), produced by the ES-derived cells. (Courtesy of Oliver Brüstle; see also Brüstle et al., 1999.)

of ES-derived glial precursor cells to repair the nervous system of a genetically defective rodent has direct implications for the treatment of Pelizaeus-Marzbacher disease (PMD), a hereditary human myelin disorder. More broadly, the research holds promise for the future use of other ES-derived, lineage-restricted precursor populations in the treatment of various human disorders where the loss or degeneration of a particular cell type threatens body function.

With more than 2,000 growth factors purified and/or cloned at the close of the millenium, the ability to employ growth factor supplements to direct ES cells down specific lineage pathways seems on the one hand endless and on the other, hopelessly complex. While the nuances of obtaining pure ES-derived clonal precursor cells are still poorly understood, headway has been made in determining which extrinsic factors govern certain other lineages. As new clues are obtained from additional developmental and cell culture studies, an understanding of how human ES cells choose other specific pathways of differentiation will continue to provide pivotal groundwork for future clinical applications of stem cell technology.

The Existence of Adult Stem Cells and Their Niches

Identifying the signals that regulate stem cell differentiation is fundamental to understanding cellular diversity. In recent years, tremendous advances have been made in our understanding of how various cell fates become determined as embryos develop. The identification of intrinsic markers—e.g., transcription factors, essential for the specification of a differentiation pathway and/or organ—has provided molecular tools for further exploring how cell fate commitments are systematically made (for reviews, see Firulli and Olson, 1997; Orkin and Zon, 1997; Orkin, 1998; Edlund and Jessell, 1999).

While diversification of cell types is largely complete at or shortly after birth, many tissues in the adult undergo self renewal and accordingly must establish a life-long population of relatively pliable stem cells. Adult stem cells are often relatively slow-cycling cells able to respond to specific environmental signals and either generate new stem cells or select a particular differentiation program (Figure 3). When a stem cell undergoes a commitment to differentiate, it often first enters a transient state of rapid proliferation. Upon exhaustion of its proliferative potential, the transiently amplifying cell withdraws from its cycle and executes its terminal differentiation program (Potten et al., 1979). Adult stem cells are often localized to specific niches, where they utilize many but not necessarily all, of the external and intrinsic cues used by their embryonic counterparts in selecting a specific fate. Locating and analyzing stem cell niches and elucidating the molecules that orchestrate specific developmental programs are important steps in determining the key components of the environment that legislate differentiation commitments and stem cell regulation.

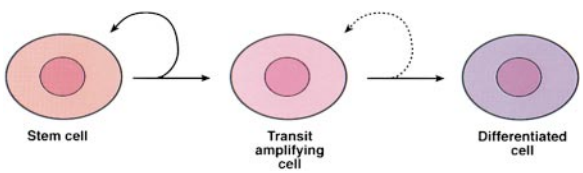

Figure 3. Stem Cells Self-Renew and Differentiate to Give Rise to Transit Amplifying and Fully Differentiated Cells

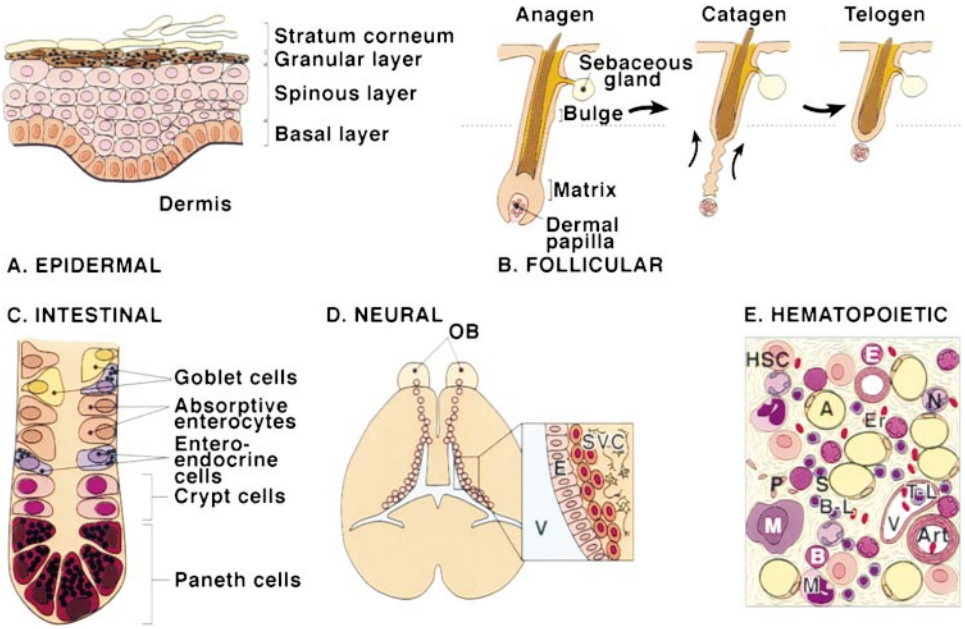

Figure 4. Stem Cells Develop and Maintain Their Ability to Self-Renew within a Specific Niche

Shown are the stem cell niches for (A) epidermis in nonhaired skin (the tip of the undulations within the basal layer); (B) hair follicle and epidermis (the bulge); (C) small intestine (the crypt cells); (D) neural cell precursors (either the ependymal or the subventricular zone cells); (E) the hematopoietic system (HSCs; in the bone marrow). V, ventricular zone; SVC, subventricular zone; E, ependymal; OB, olfactory bulb; HSC, hematopoietic stem cell; A, adipocyte; M, megakayocyte; T-L, T lymphocyte; B-L, B lymphocyte; B, basophil; E, eosinophil (in panel [E]); N, neutrophil; Er, erythrocyte; S, stromal cell; V, vein; Art, artery.

Some stem cells of adult mammals don't seem to have a specified niche within their respective tissue. Thus, for example, skeletal muscles are renewed primarily upon injury, when quiescent satellite myoblasts attached to muscle fiber bundles become reactivated, proliferating transiently and then fusing to form the differentiated myotubes necessary to repair damaged fibers (for review, see Miller et al., 1999). In other cases, however, a stem cell compartment is established within a developing tissue, and cells within this niche are then activated in response to specific environmental cues. Figure 4 illustrates five different examples of stem cell compartments that reside within adult tissues.

Self-Renewing Epithelial Stem Cell Niches

Epidermis and Hair Follicles. A prime example of a tissue that must undergo continual and rapid self-renewal is the epidermis and its notable appendage, the hair follicle. Derived from a common embryonic origin and located at the skin surface, mammalian epidermis and hair follicles are naturally exposed to ultraviolet radiation and other physical and chemical assaults, necessitating a mechanism for self-renewal as well as for protecting the stem cell compartment(s). The epidermis does so by maintaining a single inner (basal) layer of dividing cells, which periodically withdraw from the cell cycle, commit to differentiate terminally, and move outward toward the skin surface (Figure 4A). Terminally differentiated cells that reach the skin surface are sloughed, continually being replaced by inner cells differentiating and moving outward. Since human epidermis turns over every two weeks, and since each transiently amplifying basal cell divides only 3–6 times before it differentiates, the self-renewing capacity of epidermal stem cells is enormous.

The hair follicle is composed of an outer root sheath (ORS) that is contiguous with the epidermis, an inner root sheath (IRS), and the hair shaft itself (Figure 4B). The actively dividing cells that give rise to the IRS and hair shaft are called matrix cells, a transiently dividing population of epithelial cells in the follicle bulb that engulf a pocket of specialized mesenchymal cells, called the dermal papilla. In the adult hair follicle, the lower segment undergoes periods of active growth (anagen) and destruction (catagen/telogen) (Figure 4B; for review, see Hardy, 1992). As matrix cells exhaust their proliferative capacity, the follicle regresses, dragging the pocket of dermal papilla cells up to the permanent epithelial portion of the follicle, called the bulge, which is the putative home of follicle stem cells (for review, see Lavker et al., 1991). In response to a stimulus from the dermal papilla, one or more stem cells in the bulge commit to regenerating the follicle. These stem cells can also generate epidermis, and upon wounding or burn injury, cells from this region of the follicle reepithelialize the damaged epidermis (Green, 1991).

Given nature's desire to tuck stem cells away from harm's way, the bulge would seem to be the ideal niche to harbor both epidermal and follicle stem cells, and experimental evidence in favor of this hypothesis continues to accumulate. Thus for example, bulge cells have a long cell cycle, as illustrated by the fact that this compartment is the residence of the majority of label-retaining skin cells (>65%) in mice given a pulse of tritiated thymidine (Cotsarelis et al., 1990; Kobayashi et al., 1993; Morris and Potten, 1994). The bulge also yields the best outgrowth of hair follicle keratinocytes in culture (Kobayashi et al., 1993; Yang et al., 1993), and when bulge cells are combined with dermal papilla cells, they can

reconstitute a viable hair follicle (Oliver, 1966). All of these data lend further support for the bulge as a prime stem cell niche. This said, at least in human skin, some stem cells are also likely to reside in the epidermal basal layer (Jones et al., 1995).

Intestinal Epithelium. The small intestine is composed of ciliated villi, each surrounded by crypts, embedded in the intestinal wall for protection (Figure 4C). Each crypt is composed of about 250 simple epithelial cells that include the stem cell compartment for replenishing the villi. The multipotent stem cells are located near or at the base of each crypt (Loeffler et al., 1993). To maintain homeostasis, slow cycling stem cells are converted to rapidly but transiently proliferating cells that move to the midsegment and subsequently differentiate into either the absorptive brush-border enterocytes, mucus-secreting goblet cells, or enteroendocrine cells of the villi. The differentiated cells eventually die and are shed from the villi into the lumen of the gut. Crypt stem cells also produce Paneth cells at the base of the crypt, which synthesize and secrete antimicrobial peptides, digestive enzymes, and growth factors. These cells are eventually cleared from the crypt by phagocytosis.

The ability to select among multiple terminal differentiation pathways and to generate differentiating cells that can either migrate upward or downward are characteristics that parallel those of the hair follicle bulge. Moreover, like the hair follicle stem cells, stem cells of the small intestine rely upon mesenchymal cues for their survival and differentiation.

Multipotent Neural Stem Cells in the Brain. The diversification of cell types in biology reaches its extreme with the vertebrate nervous system. During embryogenesis, neural progenitor cells develop in the neural crest (Le Douarin, 1986). These multipotent versatile cells detach from the dorsolateral margins of the neural tube and migrate to specified sites throughout the developing embryo, where they differentiate into neurons and glia of the peripheral nervous system, as well as melanocytes, smooth muscle cells, facial bones, and cartilage. The particular fate of a neural crest cell is influenced by local cues, which are likely to be transmitted in part by the dorsal neural tube and in part by the migration path fated by the cell (for review, see Le Douarin et al., 1993; Edlund and Jessell, 1999).

To assess the multipotency of individual neuronal cells and their potential for self-renewal, cultures have been made from embryonic neural crest or fetal neuronal tissue (for review, see Anderson et al., 1997). Cultured quail neural crest cells permit the preferential expansion of melanocytic and glial lineages when exposed to the growth factor endothelin 3 (Lahav et al., 1998), and clonal populations derived from neural crest or embryonic rat sciatic nerve give rise to neurons, Schwann cells and smooth muscle-like fibroblasts in culture (Morrison et al., 1999). If cultured rat neural crest cells are then injected into early host chick embryos, they migrate correctly and colonize the neural crest derivatives appropriately (Morrison et al., 1999; White and Anderson, 1999; Figure 5). While these neural progenitor cells have a rather modest ability to proliferate (6–10 generations), their multipotency confirms the existence of "stem cell–like" progenitors in the developing nervous system. Multipotent neural progenitors have also been found in the embryonic CNS and forebrain, and similar grafting studies indicate that even human embryonic brain cells can divide, migrate, and populate the major areas of recipient rodent brains (Johe et al., 1996; Brüstle et al., 1998; Flax et al., 1998).

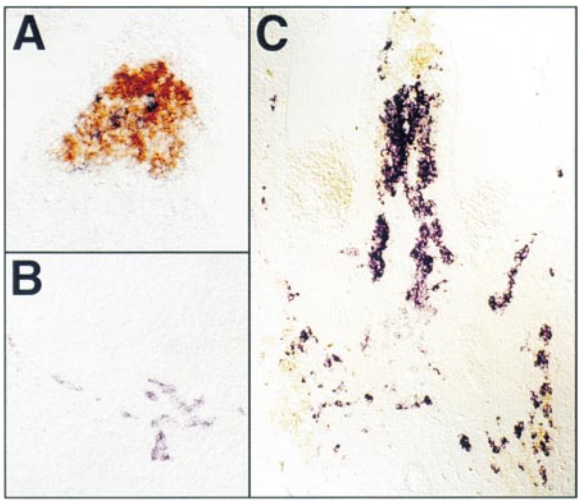

Figure 5. Cultured Rat Neural Crest Cells Injected into Early Chick Embryos Migrate Correctly and Colonize the Neural Crest Derivatives

Stained cells are positive for the panneuronal marker SCG10; rat neurons are in purple and chicken neurons are in orange.

(A) Remak's ganglia from a chicken that as an embryo was injected at the sacral axial level with sciatic nerve stem cells.

(B) Peripheral nerve of a chicken injected with sciatic nerve stem cells.

(C) Pelvis parasympathetic plexus of a chicken injected with migratory neural crest cells. (Courtesy of Pamela White and David Anderson; see also Morrison et al., 1999; White and Anderson, 1999.)

Mammalian brain develops as a tube containing a ventricular compartment filled with cerebral fluid. Dividing, uncommitted neural crest–derived cells reside initially in the luminal cell layer, or ventricular zone, and then as development proceeds, in the "subventricular zone" that forms beneath it. In development, these cells give rise to astrocytes, oligodendrocytes, and the neurons that populate the olfactory bulb. Building upon these developmental clues, Reynolds and Weiss (1992) isolated the nerve tissue beneath the lateral ventricles of adult mouse brain, and after enzymatic dissociation, cultured the cells from the tissue. When induced to proliferate in vitro by epidermal growth factor, some of the cultured cells initially expressed nestin, composing the intermediate filaments (IFs) of neuroepithelial stem cells (Fuchs and Weber, 1994). These cultures subsequently aggregated into structures referred to as neurospheres, which spontaneously differentiated into a mixture of astrocytes, oligodendrocytes, and neurotransmitter-expressing neurons.

The clonogenicity of neurospheres, and their ability to give rise to multiple neural cell types provides strong evidence that somewhere within the ventricular/subventricular zone of the adult mammalian brain lurks a multipotent stem cell compartment (Figure 4D). Researchers have continued to hone in on this location as a niche for multipotent, adult brain stem cells. Since the

identification of these stem cells is dependent upon biochemistry, we will discuss this in more detail in the next section.

Finding a Niche for Hematopoietic Stem Cells. Self-renewal is essential in the hematopoietic system as we lose and replace over a billion red blood cells every day. Many of the concepts and generalizations about stem cells come from studies of multipotent hematopoietic stem cells (HSCs). Through a step-wise series of extrinsic and intrinsic cues, many of them elucidated, HSCs differentiate to give rise to progenitor cells with progressively more limited options (see Weissman, 2000 [this issue of *Cell*]). HSCs give rise to lymphoid progenitor cells that can in turn differentiate into T and B lymphocytes, and HSCs can also give rise to myeloid progenitor cells that can produce basophils, eosinophils, neutrophils, macrophages, platelets, and erythrocytes (Figure 4E).

During development in mammals, the first blood cells, called embryonic nucleated erythrocytes, appear in the extraembryonic yolk sac and express certain transcription factors that specify these cells to their hematopoietic fate (Robb et al., 1995; Shivdasani et al., 1995). In the developing embryo itself, mesodermally derived precursor cells migrate into an environment permissive for hematopoiesis that is located in the aorta, genital ridge (gonad), and mesonephros (AGM) region (Medvinsky and Dzierzak, 1996). Based on (a) the phenotype of mice lacking vascular endothelial growth factor (VEGF) or its tyrosine kinase receptor, Flk1, and (b) complementary in vitro studies with Flk-1 null ES cells, the VEGF-Flk-1 (and VEGFR2) signal transduction pathway appears to be essential in regulating the migration of embryonic blood cell and endothelial progenitors to the AGM (Asahara et al., 1997; Hidaka et al., 1999; Ziegler et al., 1999; Schuh et al., 1999 and references therein).

As development progresses, HSCs migrate to the fetal liver, a process that relies heavily on β1 integrin. This integrin partners with several α subunits on the HSC surface, reacts to environmental cues and governs the expression of new intrinsic factors (Hirsch et al., 1996; for review, see Orkin and Zon, 1997). While in residence in the fetal liver, some HSCs can differentiate to give rise to more restricted progenitor cells for both the myeloid and lymphoid lineages. Just before birth, HSCs relocate to the bone marrow, where blood formation is maintained throughout the lifetime of the animal (Figure 4E).

HSCs differ from epithelial and embryonic stem cells in that they do not adhere tightly to one another. This may explain why they seem to have a hard time settling down to a particular niche. Even in the adult, populations of stem cells and their progeny with more restricted potential are detectable in peripheral blood, spleen, and liver, as well as in bone marrow. Tracking experiments have shown that HSCs are mobile, and like young adults, periodically come home after their travels away from the niche. Like epithelial and embryonic stem cells, the hematopoietic system relies heavily on homeostasis in the adult. In this case, however, HSC homing as well as the conversion of HSCs to more limited multiprogenitor cells become key, and as discussed later, these processes depend upon external cues provided by the bone marrow niche.

The Biochemical Properties of Stem Cells: Just How Primitive Is a Stem Cell?

A classic perception of stem cells is that they are undifferentiated cells, making them pliable to adopt various different cell fates. Perhaps more than any other feature attributed to stem cells, this has misled biologists into peering into the wrong niches and ignoring the right niches in search of stem cells. The realization that stem cells can masquerade behind morphological and biochemical features normally attributed to differentiated cell types has revolutionized our view of what stem cells look like and where to find them.

Skin Stem Cells: Really a Keratinocyte After All, but One with Some Unique Biochemistry

The biochemical hallmark of keratinocytes is expression of K5 and K14, keratins that produce elaborate intermediate filament (IF) networks in these cells (for review, see Fuchs and Weber, 1994). Whereas transiently amplifying matrix cells are largely devoid of keratin IFs, bulge stem cells possess a K5 and K14 IF network; these keratins are even more abundant in basal epidermal and upper ORS cells (Figure 6A; Coulombe et al., 1989; Vasioukhin et al., 1999). If a single population of bulge stem cells gives rise to matrix, epidermis, and ORS, as is presently thought, then these stem cells must downregulate, upregulate, and not change keratin expression, respectively, in choosing among these pathways.

Expression of keratins 5 and 14 defines bulge stem cells as keratinocytes. In vitro, a single human follicle stem cell can generate as many as 1.7×10^{38} progeny in culture, far more than necessary to cover an adult human (Rheinwald and Green, 1975; Rochat et al., 1994). By studying those cultured keratinocytes that have high proliferative capacity, researchers have been able to define additional biochemical properties of these cells (Jones et al., 1995; for review see Watt, 1998; Li et al., 1998 and references therein). These include elevated levels of cell surface integrins, and the ability to adhere rapidly to type IV collagen ($\alpha2\beta1$ receptor) and fibronectin ($\alpha5\beta1$ receptor). As judged by immunofluorescence, these cells also appear to have reduced levels of the intercellular junction protein, E-cadherin (Moles and Watt, 1997). Taken together, these findings predict that keratinocyte stem cells in vivo are likely to adhere strongly to their extracellular matrix-rich basement membrane, perhaps keeping them in their niche, and yet be less adhesive to one another, enabling an individual stem cell to be rapidly mobilized and exit from its niche. The integrins and cadherins on the surface of keratinocytes not only play a role in cell adhesion, but in addition activate signal transduction pathways essential for their proliferation and survival (see Watt, 1998).

Still in its infancy is the topic of how keratinocyte stem cells respond to external cues and commit to their various lineages. Recent evidence suggests that Wnt or equivalent signals may be involved. The product of Wnt signaling is the accumulation of β-catenin, a protein that normally interacts with E-cadherin to promote intercellular adhesion (for reviews, see Gumbiner, 1997; Nusse, 1999). When rapid turnover of β-catenin is prevented by Wnt signaling, excess β-catenin is free to interact with available members of the Lef/Tcf family of DNA-binding proteins, producing a transcription complex able to activate downstream target genes, including c-*myc* and

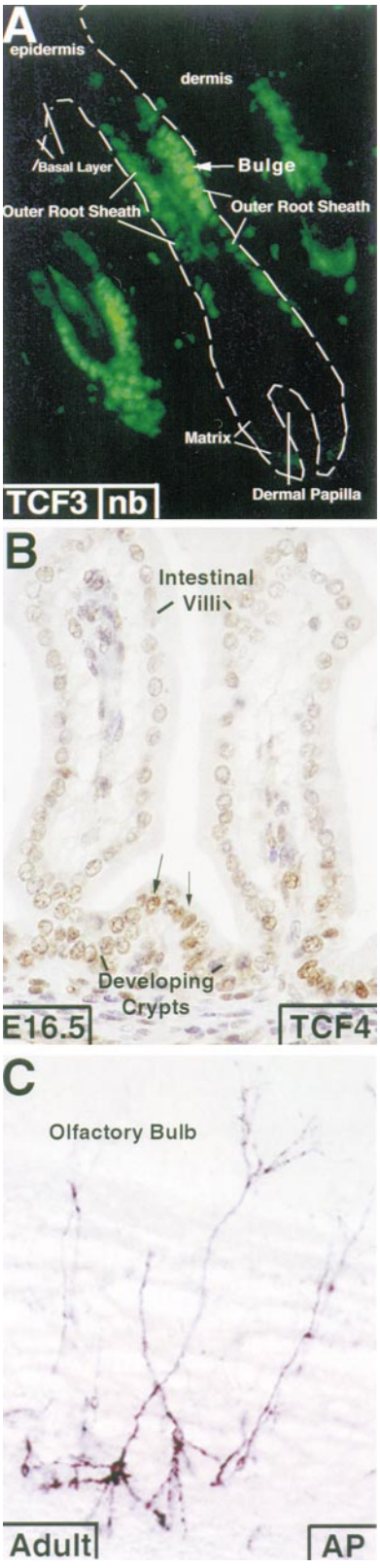

Figure 6. Stem Cells Display Characteristic Biochemical Markers

(A) Stem cells of a mouse hair follicle bulge show expression of Tcf3 (green). (Courtesy of Ramanuj DasGupta and Elaine Fuchs; see also DasGupta and Fuchs, 1999.)

(B) Crypt stem cells of mouse embryonic intestine shows expression of Tcf4 (brown); higher intensity in the crypt, denoted by arrows. (Courtesy of Hans Clevers; see also Korinek et al., 1998.)

(C) Glial-derived alkaline phosphatase–positive (AP, purple) neurons (see text) migrating en route to the olfactory bulb. (Courtesy of F. Doetch and A. Alvarez-Buylla; see also Doetsch et al., 1999.)

cyclin D1 (He et al., 1998; Tetsu and McCormick, 1999). In this way, β-catenin could be a key intermediary between weakening the niches' hold on a stem cell and enabling it to activate proliferation and differentiation.

Cultured keratinocyte stem cells have elevated levels of activated β-catenin (Zhu et al., 1999), and follicle bulge cells express Tcf3, a member of the Lef/Tcf family (Figure 6A; DasGupta and Fuchs, 1999). Moreover, when stabilized β-catenin is expressed in transgenic mice under the control of the K14 promoter, follicle cells in the bulge can be activated to express Tcf/Lef/β-catenin target genes, and interfollicular epidermis can be induced to form de novo hair follicles, normally a property of pluripotent embryonic ectoderm (Gat et al., 1998; DasGupta and Fuchs, 1999). Interestingly, a cousin of Tcf3, Lef1 seems to be important for early follicle develoment and for the later stages of hair follicle differentiation (van Genderen et al., 1994; Zhou et al., 1995). The precise molecular pathways involved and the external cues regulating these processes are complex and remain largely unknown.

Intestinal Stem Cells of the Crypt: Characteristics of Epithelial Cells and Expression of Tcf Family Members

Despite their well-defined niche, intestinal stem cells have been difficult to culture, and hence, much of what has been learned about the biochemistry of intestinal stem cells comes from studying tumorigenesis (for review, see Stappenbeck et al., 1998). This field began with the cloning of the gene whose defect is responsible for familial ademato us polyposis coli (APC), or colon polyps, and expanded with the realization that in its functional form, APC physically interacts with β-catenin and accelerates its degradation through the ubiquitin-mediated proteosome system (for reviews, see Gumbiner, 1997; Nusse, 1999). This led soon to the discovery of the intestinal-specific Lef/Tcf family member, Tcf4, which resides within the crypt (Figure 6B). Subsequent functional analysis revealed that Tcf4 null mutations in mice obliterate the stem cell compartment of the intestinal crypt (Korinek et al., 1998). These findings are reminiscent of those more recently obtained for skin, and provide the best evidence to date that Lef/Tcf-β-catenin complexes may be important intrinsic factors that control maintenance of and/or exit from epithelial stem cell compartments.

While many of the features that govern the behavior of intestinal stem cells remain unknown, common themes continue to surface among epithelial stem cell compartments. Thus, integrins and their extracellular matrix ligands appear to be important for stem cell maintenance and/or homeostasis in the crypt, and crypt stem cells express IFs, in this case, the simple epithelial keratins, K8, K18, and K19 (Beaulieu, 1992; Potten et al., 1997). Intriguingly, colon hyperplasia is seen in the large intestine of mice lacking K8 (Baribault et al., 1994), suggesting that changes in keratin levels may influence proliferation

and/or migration, properties that are important for controlling homeostasis in the intestinal crypt. Intercellular adhesion is also involved in crypt homeostasis, and overexpression of intestinal E−cadherin in mice suppresses proliferation and induces apoptosis in the crypt, slowing cell movement up the villus (Hermiston et al., 1996). One feature of the intestine, seemingly less important in the epidermis and hair follicle, is the regulation of intestinal cell number by apoptosis (for review, see Potten et al., 1997). Overall, however, the stem cells of the intestine, like their skin counterparts, possess quite marked biochemical features that classify them as epithelial cells, rather than primitive cells bearing little or no resemblance to their neighbors.

Multipotent Neuronal Stem Cells: A Glial Cell After All?

Two groups have recently purified the multipotential cells in nerve tissue from adult mouse brain (Doetsch et al., 1999; Johansson et al., 1999). The groups differ in their view of whether these progenitors are ependymal cells that line the luminal surface of the adult ventricular zone (Johansson et al., 1999), or astrocytes that reside in the subventricular zone just beneath it (Doetsch et al., 1999). However, both groups seem to have isolated similar cells, which are relatively well-differentiated, ciliated cells able to push the cerebral spinal fluid through the ventricles, and also able to divide and give rise to both neurons and glial cells in culture. To provide direct evidence that a glial-like stem cell can give rise to neurons, Doetsch et al. (1999) used transgenic mice expressing the avian leukosis viral (ALV) receptor driven by the promoter/enhancer of the gene encoding the glial-specific IF protein (GFAP). When these mice were infected with ALV encoding alkaline phosphatase, they generated alkaline phosphatase–expressing subventricular zone astrocytes (glial cells) that gave rise to neurons which subsequently migrated to the olfactory bulb (Figure 6C).

It remains unresolved whether ependymal cells are slow cycling stem cells that give rise to more rapidly proliferating subventricular astrocytes (Johansson et al., 1999), whether ependymal cells transition to subventricular cells (and vice versa) (see e.g., Birgbauer and Fraser, 1994) or whether the adjacent subventricular astrocytes are truly the multipotent stem cells, as implied by the findings of Doetsch et al. (1999). Irrespective of the outcome of this controversy, it is intriguing that this multipotent stem cell displays a number of biochemical features of differentiated astrocytes. In this regard, it is also relevant that many astrocytes express EGF and FGF-2 receptors, and these and other cell surface markers have been used to isolate adult neural stem cells from various CNS regions, including spinal cord (Weiss et al., 1996) and hippocampus (Palmer et al., 1997; Eriksson et al., 1998).

The emerging view that adult stem cells can display quite conspicuous differentiated characteristics evokes the debate of whether multipotent stem cells in adult niches might dedifferentiate prior to selecting certain cell fates. A priori, since GFAP IFs are produced uniquely by glia and since neurofilaments (NFs) are the hallmarks of neurons, it is tempting to speculate that some type of reprogramming must take place in the conversion of a glial-like stem cell to a neuron. While this may be the case, ependymal cells are unusual glial cells in that they express Notch 1 receptors, nestin, and other neuronal cell markers, as well as markers such as GFAP, typical of glial cells. Thus, it could be that these neural stem cells are Jekyll and Hydes, possessing multiple differentiation markers that they can suppress or enhance depending upon the fate they become committed to.

Properties of HSCs and Their Progeny.

How Primitive Are HSCs After All?

By an order of magnitude, the molecular pathways of hematopoiesis are the most thoroughly explored of all stem cell systems. The cytokine requirements, transcriptional regulators, and cell surface features necessary for hematopoietic stem cells to interact with their niches are quite well defined, as are the molecular distinctions between hematopoietic stem cells and their progenitors (for reviews, see Orkin, 1998; Glimcher and Singh, 1999; Weissman, 2000). Until recently, these studies had pointed to a model whereby biochemically naive pluripotent stem cells, exposed to various cytokines, progressively acquire specific intrinsic factors and differentiate to generate a hierarchy of progenitors. In the early stages of differentiation, these progenitors are themselves stem cells, but are more restricted in their options than their parent stem cells (Figure 7).

To some extent, this deterministic model, paralleling models of myogenesis (Firulli and Olson, 1997), works well to explain many of the key features of hematopoietic stem cell maintenance, proliferation, and differentiation. Thus, for example, the GATA-2 transcription factor is essential for proliferation and survival of hematopoietic progenitors (Tsai et al., 1994), and GATA-1 regulates the survival, proliferation, and maturation of erythroid and megakaryocyte (basophil/mast cell) lineages (Weiss and Orkin, 1995). Expression of the transcription factor PU.1 then seems to push HSC progeny stem cells further down differentiation pathways to produce multipotential cells of the lymphoid and myeloid lineage, and subsequent appearance of additional factors completes the specification of T and B cell progenitors (for review, see Glimcher and Singh, 1999).

Despite the attractiveness of a linear model for hematopoietic lineage selection, recent evidence disputes the extent to which the underlying mechanisms can be so neatly packaged. Gene knockout and transgenic studies, in combination with more detailed analyses of purified HSCs and their progeny, have led to the realization that mere expression of key transcription factors does not necessitate commitment to a lineage. Rather, HSCs coexpress many of the so-called lineage-restricted factors (Hu et al., 1997; Enver and Greaves, 1998), making lineage selection considerably more complex than initially anticipated. Additional complexities such as the need for combinatorial action of negative and positive acting transcription cofactors, as well as the continued impact of environmental signals (see next section), modify the prevailing view to encompass a more flexible model that portrays lineage commitment as the stabilization and maintenance of certain intrinsic features at the expense or inhibition of others.

Overall, the hematopoietic stem cell is not so primitive as once supposed, and researchers can still look with confidence to the principles learned from the HSC in seeking to understand the features of other adult stem

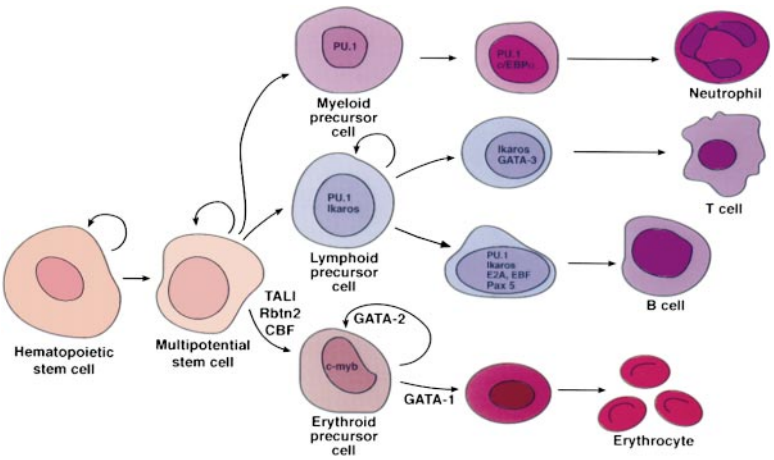

Figure 7. Transcription Factors that Regulate Development of the Myeloid, Lymphoid, and Erythroid Lineages

For description of these factors, see Weiss and Orkin, 1995; Glimcher and Singh, 1999.

cells. Moreover, even though the cells of the hematopoietic system may at first glance seem very different from those of adherent tissues, they still rely heavily on interactions with their niches and often employ similar molecules in doing so. In this regard, HSCs utilize integrins not only for migration and mobility, but also for maintaining their residence in the bone marrow (for review, see Lasky, 1996). As seen with other adult stem cells, integrin-mediated interactions of HSCs with extracellular matrix are not only employed for adhesiveness, but also for proliferation and survival in the bone marrow. Thus, integrins may act as a universal bridge keeping stem cells in their place and maintaining their proliferative potential. In performing these tasks, the flexibility of integrins becomes especially desirable. They can both respond to ECM ligands and transmit internal signals, as well as receive internal signals from activated cytokine or growth factor pathways and respond by organizing ECM on the stem cell surface (for review, see Giancotti and Ruoslahti, 1999).

The Microenvironment of the Niche: What Does It Provide and How Important Is It?

A major challenge in stem cell biology is defining the key components of the niche that impart to stem cells many of the properties they display while in residence there. A prime example is the bone marrow, which provides hematopoietic stem cells with a rich, but complex, milieu composed of many cell types (macrophages, adipocytes, fibroblasts) and the vast array of extrinsic molecules that they produce. Some of these factors, such as soluble and membrane-bound stem cell factor (SCF), are key not only for interacting with the c-Kit receptor on the stem cell surface and adhering it to its niche, but also for stem cell maintenance. Thus, while genetic lesions in SCF and c-Kit do not affect primitive hematopoiesis, they do severely impair hematopoiesis from the fetal liver stage onward (Nocka et al., 1990; Toksoz et al., 1992; see also Porter et al., 1997 and Pinto do O et al., 1998). Additional stromal factors that impact on stem cell maintenance, propagation, homing, and homeostasis include the fibroblast growth factors FGF-1 and FGF-2, the α-chemokine stromal cell derived factor-1 (SDF-1) and a slew of ECM molecules, all primed for interacting with various receptors on the HSC surface (for review, see Whetton and Graham, 1999). Despite these major efforts to characterize the bone marrow niche, however, in vitro studies indicate that HCSs still survive better when cultured with bone marrow stroma than when placed in a defined medium supplemented with characterized bone marrow components. This is also true for epidermal keratinocytes, which require coculturing with fibroblasts to display their optimal survival and proliferative capacity (Rheinwald and Green, 1975).

Defining the components of stem cell niches becomes even more complex when it is considered that in some instances, more than one stem cell may reside within a niche. This appears to be the case for the bone marrow, which houses not only HSCs, but also mesenchymal stem cells. These cells can replicate as undifferentiated cells, but possess the capacity to differentiate into lineages of mesenchymal tissues, including bone, cartilage, fat, tendon, muscle, and marrow stroma (Pittenger et al., 1999). This raises the intriguing possibility that different stem cell populations can respond differentially to cues within a niche, and perhaps even influence each other. Overall, this finding adds additional challenges to defining the molecular characteristics of stem cell niches.

Given the relatively dormant state of most stem cells, it is not surprising that in vitro systems have led primarily to identifying factors that either stimulate the proliferation of stem cells and their progeny, or instruct them to select particular lineages. While gene targeting technology has unveiled an array of transcription factors, including GATA-2, Tcf4, Oct4, Mash1, LH2, and SCL/tal-1, which appear to maintain different progenitor cells, we now face the challenge of uncovering the next molecular layer, namely how these transcription factors are themselves regulated and how they act to keep stem cells in their pluripotent state.

You Can't Judge a Stem Cell by Its Cover

The stem cell keratinocyte of the follicle bulge looks very different from the ciliated glial-like neural stem cell of the ependymal or subventricular zone, which in turn looks very different from the hematopoietic stem cell burrowed within the bone marrow; moreover, they each give rise to their own subset of lineages. Are these differences permanent, i.e., intrinsic ones, or ones that depend largely upon the niche in which the stem cell is

located? A major issue emerging in stem cell biology is the extent to which the niche imposes on its stem cells certain irreversible characteristics that make these cells able to adopt only specific lineages and not others. Recent evidence suggests that stem cells are considerably more pliable than other cells when plucked from their niches and transferred to a new residence.

The cloning of the ewe named Dolly (Wilmut et al., 1997) revealed that the nucleus from an adult cell could be reprogrammed in the confines of an enucleated, fertilized oocyte to ultimately create a sheep nearly genetically identical to its "parent". While still not optimal in efficiency, cloning animals by nuclear transfer has now been demonstrated for other species (Wakayama et al., 1998). Additionally, researchers soon showed that at least for some somatic cells, it may be possible to strip the nucleus of its memory and genetically reprogram it without removing it from its cytoplasmic environment.

In one recent study designed to test the role of the microenvironment on stem cells, HSCs from an adult mouse bone marrow were injected into the inner cell mass of mouse blastocysts and then traced through subsequent stages of embryonic hematopoietic development (Geiger et al., 1998). Interestingly, these adult cells were reprogrammed to express fetal globin genes. Conversely, when fetal HSCs were implanted into an adult spleen, they behaved as adult progenitor cells, and switched to expressing adult globin genes. Taken together, these findings reveal the dominance of the microenvironment on the development stage–specific gene expression program of the HSC.

In another remarkable study, Bjornson et al. (1999) isolated stem cells from the brain of adult transgenic mice and systemically injected them into recipient mice that had been sublethally irradiated to destroy the preexisting HSCs. Some months later, most of the multipotent cells within the host bone marrow scored positive for β-galactosidase activity, the biochemical marker of the transgenic donor cells. Colonies generated from the chimeric bone marrow included β-galactosidase-expressing granulocytes, macrophages, and B cells. In this experimental system, even clonally derived, cultured donor neural stem cells gave rise to multipotent HSCs in the host animal (Figure 8A). While it is difficult to unequivocally rule out the possibility of HSC contamination in the isolated brain stem cell population, it seems that brain stem cells normally fated to adopt a neural or glial cell fate can be redirected into hematopoietic fates if placed in the proper environment. Thus, amazingly, when extracted from their niches and forced to take up residence at new body sites, it appears that some stem cells can adopt lineages previously not thought possible.

Lending support to this extraordinary conclusion are several additional studies that focus on the plasticity of bone marrow cells and challenge their ability to select atypical lineages when placed in nonhematopoietic environments. When implanted into the brain of rats, human bone marrow stromal cells did not differentiate into neural cells, but they did lose some of their stromal cell characteristics and migrated along well-established neuronal migratory pathways into successive layers of the brain (Azizi et al., 1998). Additionally, bone marrow stromal cells from male mice could populate the bone,

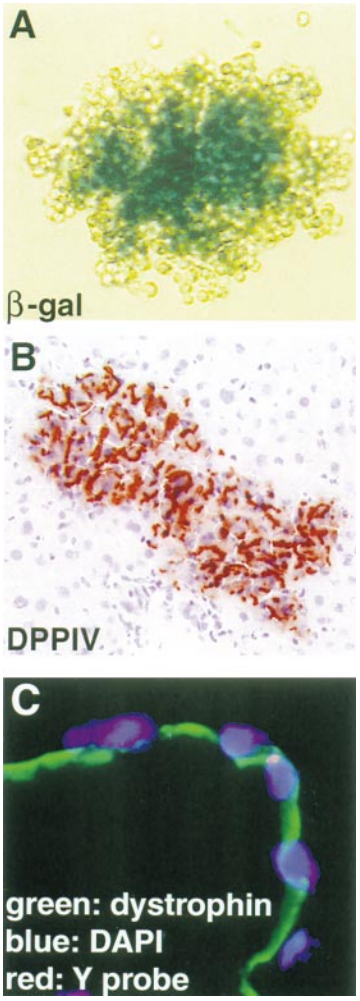

Figure 8. Evidence that Stem Cells Can Reprogram in a New In Vivo Niche

(A) Putative neural stem cells (NSCs) were isolated from a β-galactosidase-expressing, transgenic donor mouse and transplated into irradiated recipients. Shown is an in vitro clonogenic assay to analyze hematopoietic precursors in the recipient mouse. The blue hematopoietic cells indicate that they were derived from the donor NSCs. (Courtesy of Christopher Bjornson; see also Bjornson et al., 1999.)

(B) The bone marrow of a dipeptidyl peptidase IV (DPPIV) mutant rat was lethally irradiated and transplanted with bone marrow from a donor wild-type rat. Following recovery, the rat was subjected to hepatic injury and after repair, the liver was examined for DPPIV-positive cells (stained in red) derived from the donor marrow. (Courtesy of Bryon Petersen; see also Petersen et al., 1999.)

(C) Twelve weeks after transplanting highly purified hematopoietic stem cells (male) into the bone marrow of a lethally irradiated female dystrophin-deficient mouse, dystrophin expression and Y chromosome–positive nuclei are found in the tibialis anterior muscle. (Example shows red dot depicting a Y chromosome clearly in one of the nuclei.) (Courtesy of Richard C. Mulligan; see also Gussoni et al., 1999.)

cartilage, and lung of lethally irradiated female recipient mice (Pereira et al., 1998), and in similar assays, bone marrow–derived myogenic progenitors populated the regenerating muscle of a wounded recipient animal (Ferrari et al., 1998) and the muscle of dystrophin-defective mice (Gussoni et al., 1999). Finally, when exchanged by

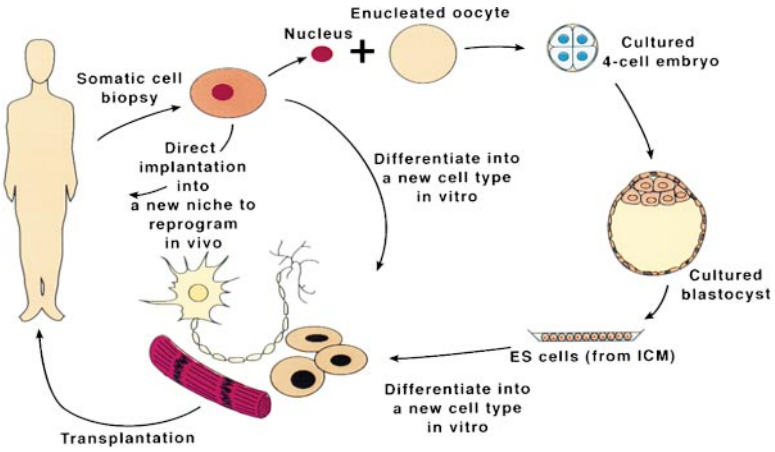

Figure 9. Three Pathways to Possibly Reprogram Multipotent Stem Cells for Treatment of Human Disorders

A biopsy of one somatic tissue is directly reprogrammed in vivo by direct implantation of the biopsy cells into a new somatic tissue (shortest route). Stem cells isolated from somatic tissue are cultivated and differentiated into a new stem cell type of choice in vitro. Following this procedure, the cells are then transplanted to a new in vivo niche where they can repopulate the cells from a damaged or defective somatic tissue (intermediate route). The nucleus of a somatic cell is transferred into an enucleated oocyte. After culturing to the blastocyst stage, embryonic stem cells are isolated from the inner cell mass (ICM) and ES cells are differentiated in vitro along the cell lineage of choice. Following differentiation, cells are transplanted to the appropriate in vivo niche (longest route).

transplant for lethally irradiated female bone marrow, male rat bone marrow repopulated the host liver following hepatic injury (Figure 8B; Petersen et al., 1999). Importantly, some of the differentiated cells within the peripheral tissues of these recipient animals were positive for the Y chromosome. In these experiments, the percentage of donor bone marrow cells that migrated to various nonhematopoietic tissues was generally small, leaving the biological importance of these phenomena still unclear. However, in the study testing HSC transplantation as a possible therapy for treating muscular dystrophy, as many as 10% of the muscle fibers within a dystrophin-deficient mouse expressed dystrophin (Figure 8C; Gussoni et al., 1999). Collectively, these findings suggest a significantly greater pluripotency of the hematopoietic and/or stromal stem cell than was previously imagined.

We are now faced with the perception that higher vertebrates harbor many niches that can support the maintenance and self-renewal of stem cells, and that the environment or niche provides a crucial but not necessarily irreversible impact on the ability of a stem cell to select particular fates. The niche also influences the biochemical and morphological properties of stem cells, and in many cases, the bearing is so great that the outward appearance of some stem cells has fooled investigators into thinking that these cells were not sufficiently primitive to be considered a stem cell at all. If we cannot judge a stem cell by its cover, what then can we say about stem cells and their differentiative properties? Perhaps the only infallible traits of stem cells are their robust proliferative capacity and their ability to self-renew. Within this framework, stem cells seem to adjust their properties according to their surroundings, and select specific lineages according to the cues they receive from their niche.

Whether a dedifferentiation step is necessary before stem cells from one niche can adapt to another is not yet clear, but seems likely. In the blastocyst, for instance, the near totipotent embryonic stem cells express E-cadherin and form intercellular adherens junctions typical of epithelial cells. Yet in the course of embryonic development, these cells naturally give rise to many cell types that neither express E-cadherin nor adhere tightly to one another. When taken together with the partially differentiated characteristics of many somatic stem cells and with the realization that even lineage commitments may be dramatically altered by environmental cues, the view of stem cells as primitive, undifferentiated cells would seem close to extinction. This said, it was recently discovered that stem cells with similar phenotypic and functional properties can be isolated from bone marrow and muscle by the same procedure (Gussoni et al., 1999), lending further mystique to the face and character of the elusive stem cell.

Stem Cells and the New Millenium: Therapies and Beyond

The ultimate goal for molecular medicine is to channel multipotent human cells with high proliferative capacity into specified differentiation programs within the body. If this becomes possible early in the next millenium, and all indications are that it will, a multitude of therapeutic uses can be envisioned. Among these are the generation of different types of neurons for treatment of Alzheimer's disease, spinal cord injuries, or Parkinson's disease, the production of heart muscle cells for congenital heart disorders or for heart attack victims, the generation of insulin-secreting pancreatic islet cells for the treatment of certain types of diabetes, or even the generation of dermal papilla or hair follicle stem cells for treatment of certain types of baldness.

What about the use of stem cells for making organs, perhaps a kidney or an eye or even a part of the brain? Clearly, this represents a considerably greater challenge than the mere generation of specialized cell types. This said, for nearly twenty years now, scientists have cultivated skin epidermis in vitro, and this has been used effectively and routinely in the treatment of badly burned patients (Green, 1991). While the creation of other organs or tissues in vitro is likely to be considerably more complicated, the ability to efficiently culture various somatic stem cell types and to direct multipotent stem cells along defined lineages are important first steps in making these possibilities a reality for the future.

What will be the source of human stem cells for such

valuable therapies? The successful culturing and propagation of human embryonic stem cells and primordial germ cells was first reported in 1998. Shamblott and coworkers isolated and cultured the gonadal ridges of 5- to 9-week-old aborted human fetuses (germ cells) (Shamblott et al., 1998), while Thompson and coworkers cultured inner cell masses of human blastocysts (ES cells). From the moment these reports appeared, the generation and use of these human stem cells has come under serious ethical scrutiny, with no clear resolution in sight. At the crux of the issue is how the human stem cells were derived, and in the cases reported, human embryonic or fetal tissue was needed. By adjusting the technology to employ a "Dolly" approach, the need would be reduced to an enuceated fertilized human egg rather than an embryo (Figure 9), but even this modified procedure has serious moral considerations for society.

One possible way to circumvent this issue is to begin with multipotent stem cells isolated from adult human tissues, and direct them in vitro along specified lineages while still maintaining their proliferative potential. If, as recent studies have suggested, the plasticity of stem cells is truly greater than previously imagined, then it may be feasible in the future to reroute easily procurable stem cells to stem cells such as neuronal or pancreatic that are more difficult to obtain. Among the most readily accessible adult stem cells with the greatest proliferative potential are skin keratinocyte stem cells, which can be easily propagated in culture. Taking speculation to the extreme, the "reprogramming" of a keratinocyte stem cell to other somatic stem cell types might make it possible one day to treat a particular disorder by starting with a skin biopsy from the patient and reprogram keratinocyte cultures into the needed stem cell type in vitro (Figure 9). While such notions may still border on the unlikely and perhaps even preposterous, the newly discovered versatility of at least some somatic stem cell types is provocative and may have enormous ramifications for stem cell therapeutics of the future.

In closing, the potential uses for stem cells seem endless. The ability to isolate and in some cases culture adult stem cells leads to future hope in genetically correcting abnormal stem cells to treat various human genetic disorders, and to employ hematopoietic stem cell therapy to treat not only autoimmune diseases (Burt and Traynor, 1998), but also a variety of different genetic disorders extending beyond those of the hematopoietic system (Gussoni et al., 1999). The generation of perfect "Dollys" for agricultural livestock represents another promising avenue of scientific research. When this technology is taken just one small step further, however, most of the world still shudders at the potential moral and ethical dangers of opening the pandora's box of human reproductive cloning. The efficiency of reproductive cloning is presently too low to be feasible for regenerating a lost child or loved one. Yet stem cell biology is advancing at an incredibly rapid pace, and with the powerful potential for this technology in the treatment and cure of various hitherto life-threatening disorders, the feasibility will soon be at our doorstep. It is thus imperative as we begin the next millenium, that we scientists sit at the round table with the rest of humanity and together face these very difficult moral and ethical concerns that touch the essence of life itself, the stem cell.

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