9. Article 3 of the UK Human Fertilisation and Embryology Act (Her Majesty's Stationery Office, London, 1990).
10. The Council of Europe is an international organization established in the wake of the Second World War, whose main role is to strengthen democracy, human rights, and the rule of law throughout its member states. For further information, see (*8*).
11. The Convention has been ratified by, and is thus applicable in, six countries: Denmark, Greece, San Marino, Slovakia, Slovenia, and Spain.
12. Directive 98/44/EC of the European Parliament and of the Council of 6 July 1998 on the legal protection of biotechnological inventions (Official Journal of the European Communities, L 213, vol. 41, 30 July 1998).
13. Decision No.182/1999/EC of the European Parliament and of the Council, of 22 December 1998, concerning the Fifth Framework Program of the European Community for "Research, Technological Development and Demonstration Activities" (1998 to 2002) (Official Journal of the European Communities, L 26/1, 1 February 1999).
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REVIEW

Out of Eden: Stem Cells and Their Niches

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Stem cells are currently in the news for two reasons: the successful cultivation of human embryonic stem cell lines and reports that adult stem cells can differentiate into developmentally unrelated cell types, such as nerve cells into blood cells. Both intrinsic and extrinsic signals regulate stem cell fate and some of these signals have now been identified. Certain aspects of the stem cell microenvironment, or niche, are conserved between tissues, and this can be exploited in the application of stem cells to tissue replacement therapy.

Introduction

Stem cells are very much in the news. The announcement that pluripotent stem cells can be cultured from aborted human fetuses or from spare embryos from in vitro fertilization procedures (*1*) has been greeted with both enthusiasm and opprobrium. The potential medical use for tissue replacement therapy is very exciting, but commentators are understandably cautious given the unresolved ethical questions. Less controversial, but equally newsworthy, is the spate of reports that stem cells derived from adult tissues have much wider differentiation potential than was previously thought (*2*). The hope is that this hitherto unrecognized plasticity can be exploited to generate cells for autologous tissue grafts.

The spotlight on stem cells has revealed gaps in our knowledge that must be filled if we are to take advantage of their full potential for treating devastating degenerative diseases such as Parkinson's disease and muscular dystrophy. We need to know more about the intrinsic controls that keep stem cells as stem cells or direct them along particular differentiation pathways. Such intrinsic regulators are, in turn, sensitive to the influences of the microenvironment, or niche, where stem cells normally reside: What is this Garden of Eden from which stem cell descendants are evicted to face differentiation and death?

What Exactly Is a Stem Cell?

Although this question remains contentious after 30 years of debate (*3*) the prevailing view is that stem cells are cells with the capacity for unlimited or prolonged self-renewal that can produce at least one type of highly differentiated descendant. Usually, between the stem cell and its terminally differentiated progeny there is an intermediate population of committed progenitors with limited proliferative capacity and restricted differentiation potential, sometimes known as transit amplifying cells (Fig. 1). In situations that involve a single differentiation pathway, such as interfollicular epidermis, the primary function of this transit population is to increase the number of differentiated cells produced by each stem cell division. This means that, although a stem cell has high self-renewal capacity, it may actually divide relatively infrequently.

Classically, mammalian stem cells have been studied in tissues such as blood and epidermis, where the differentiated cells do not divide and have a short life-span. However, stem cells are also present in tissues that normally undergo very limited regeneration or turnover, such as the brain and liver. In early embryos, stem cell self-renewal is less important than the ability to found specific lineages and, paradoxically, it is as a result of differentiation that embryonic stem (ES) cells give rise to the stem cells of adult tissues.

Stem cells can sometimes be identified quite precisely by their morphology or location. In the *Drosophila* gonad and peripheral nervous system (PNS), for example, stem and nonstem daughters have a well-defined orientation with respect to the surrounding cells (*4*) (Fig. 2). However, in many other tissues, the position of the stem cells is known only approximately, and panels of molecular markers have been developed to define the stem cell compartment or pool. Such markers may provide important clues about how the stem cell phenotype is controlled. Epidermal stem cells, for example, express high levels of β1 integrins, and β1 integrin–mediated adhesion to extracellular matrix suppresses the onset of terminal differentiation (*5*, *6*) (Fig. 3).

Strategies for Stem Cell Self-Renewal and Differentiation

There are two general strategies by which stem cells generate differentiated progeny (*3*). At one extreme, there are mechanisms that might be described as invariant, in which a stem cell gives rise, through an asymmetric cell division, to one stem daughter and one daughter that undergoes differentiation (Fig. 1A). Examples abound in unicellular organisms and invertebrates (for example, *Drosophila* ovary) (Fig. 2).

At the other extreme (Fig. 1B; Fig. 3) are highly regulative mechanisms in which a stem cell gives rise to daughter cells that have a finite probability of being either stem cells or committed progenitors. Most mammalian self-renewing tissues fall into this category. At steady state, each stem cell division gives rise, on average, to one stem and one committed daughter, but asymmetry is achieved on a population basis rather than at the level of individual cell divisions. Furthermore, in some tissues there may be a continuum of cell behavior, with stem and progenitor cells at opposite ends of a spectrum, instead of discrete stem and progenitor populations (*5*, *6*).

Although the two strategies are mechanistically very different, both involve multiple feedback controls and reciprocal intercellular interactions. Populational asymmetry facilitates the response to variable physiological need, as when increased production of blood or epidermal cells is required after an injury. Nevertheless, potential flexibility in the invariant strategy has been revealed experimentally by ectopically expressing regulatory factors in non–stem cell daughters (*4*).

Intrinsic Controls of Stem Cell Fate

Maintenance of the stem cell compartment ultimately depends on cell autonomous regulators modulated by external signals. Such intrinsic regulators include the proteins re-

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sponsible for setting up asymmetric cell divisions, nuclear factors controlling gene expression and chromosomal modifications in stem and nonstem daughters, and clocks that may set the number of rounds of division within the transit amplifying population.

Asymmetric Partitioning of Cellular Determinants

During asymmetric cell division the two daughters may acquire different developmental potentials, either by unequal segregation of cell fate determinants or because of differential influences from their surroundings. Structural proteins, in particular cytoskeletal components, are important for partitioning of cell fate determinants.

In the *Drosophila* PNS, asymmetric divisions of the sensory organ precursor cell are controlled by a hierarchy of genes, one of which is *inscuteable* (*insc*) (*4*). The Insc protein coordinates at least three aspects of asymmetric division: asymmetric localization of membrane-associated cell fate determinants, including Numb, asymmetric mRNA localization, and orientation of mitotic spindles. The central region of the Insc protein has some homology to ankyrin repeats and is required to orient the mitotic spindle and to localize Numb. Asymmetric localization of Insc in dividing neural precursors depends on the microfilament cytoskeleton.

In the *Drosophila* ovary, each stem cell undergoes asymmetric divisions to produce a stem cell that remains associated with the somatic cells of the basal terminal filament and a differentiated daughter that becomes displaced from its niche and eventually develops into a mature egg (Fig. 2). The intracellular mechanism for controlling the orientation of the asymmetric stem cell division involves a cytoplasmic organelle called the spectrosome, which contains a variety of membrane skeletal and regulatory proteins, such as spectrins and cyclin A, respectively. The spectrosome anchors the mitotic spindle to define the orientation of each stem cell division with respect to the position of the terminal filament and may also localize molecules that are important for stem cell fate to selectively retain them in the stem daughter (*4*).

Transcription factors. Although vertebrate homologs of genes regulating asymmetric stem cell divisions in *Drosophila* have been identified, it is unclear whether they have similar roles in controlling stem cell fate. However, there is abundant evidence that transcription factors control stem cell fate. In hematopoiesis, for example, a large number of evolutionarily conserved transcription factors have been implicated (*7*). One of these is SCL/Tal-1, which is ectopically activated in many acute T cell lymphoblastic leukemias and is essential for formation of all the blood cell lineages in the mouse. Other transcription factors have been identified with functions restricted to particular differentiated lineages. Every lineage is controlled by unique combinations of transcription factors, each of which may be expressed individually in several lineages; in some cases these combinations involve formation of physical complexes (*7*).

In epidermis and intestinal epithelium, recent data highlight the importance of the Tcf/Lef family of transcription factors. Homozygous null *Tcf4* mice lack stem cells in the small intestine, whereas *Lef1* homozygous mutants have defects in hair and whisker formation (*8*). β-Catenin activates Tcf/Lef-mediated transcription and is more abundant in epidermal stem cells than in transit amplifying cells; overexpression of a stabilized form of the protein increases the proportion of stem cells in vitro, and in vivo it causes keratinocytes to revert to a pluripotent state in which they can differentiate into hair follicles or interfollicular epidermis, with some of the follicles going on to develop tumors (*9*). By contrast, overexpression of β-catenin in the intestinal epithelium of transgenic mice stimulates proliferation but there is no net increase in

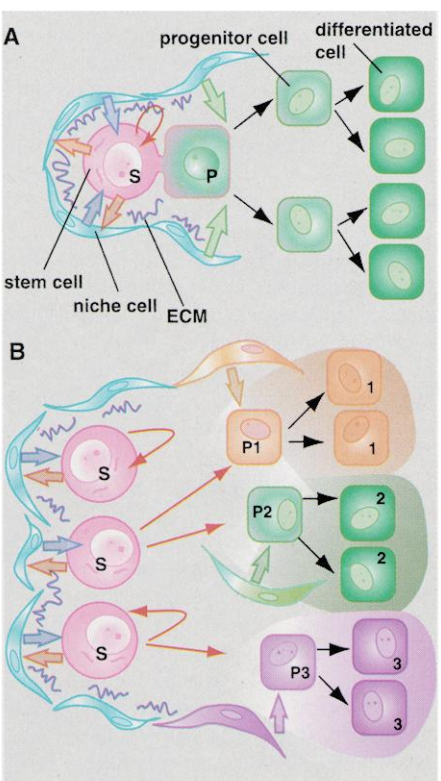

Fig. 1. Alternative models for stem cell deployment. (**A**) Invariant asymmetry. A stem cell (S) gives rise by asymmetric division to a progenitor (P) with a more restricted proliferation potential, which differentiates in response to extrinsic cues. The stem cell phenotype is regulated by reciprocal short- and/or long-range signaling (thick colored arrows). (**B**) Populational asymmetry. Stem cells give rise to daughter cells that can be either stem cells or else progenitors that differentiate along different pathways (1, 2, and 3) depending on the combination of extrinsic factors to which they are exposed. ECM, extracellular matrix.

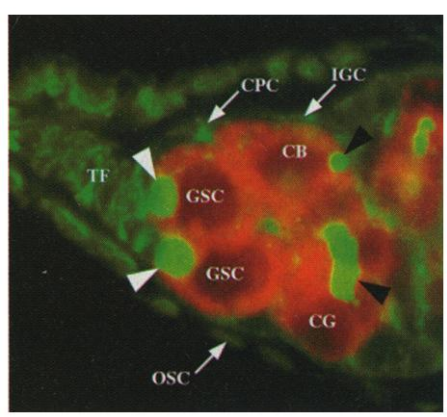

Fig. 2. Invariant asymmetric division in the anterior part of the *Drosophila* germarium, the niche for germ line stem cells (GSC) in the ovary. Ovary is stained with anti-Hulitashao (green) and anti-Vasa (red) antibodies. The germarium is wrapped by outside sheath cells (OSC, green). Two GSCs are identified by the round spectrosome at their anterior (white arrowheads, yellow) and also express the Vasa protein (red). GSCs are surrounded by three groups of differentiated somatic cells—namely, terminal filament (TF, green), cap cells (CPC, green), and inner germarial sheath cells (IGC, green). The differentiated GSC daughter, called a cystoblast (CB), is located posterior to the GSCs and also contains a round spectrosome (upper black arrowhead, yellow). A cystoblast further develops into a cyst (CG), which contains a branched fusome (lower black arrowhead, yellow) (*4*).

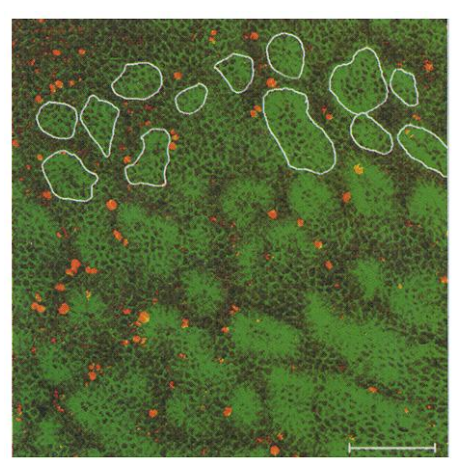

Fig. 3. Populational asymmetry in human epidermis. Epidermal whole mount stained for β1 integrins (green). Stem cells are enriched in the clusters of cells with the highest integrin levels (some of which are delineated in white); these clusters are separated by actively proliferating (Ki67-positive nuclei in red), transit amplifying cells with lower integrin levels. Scale bar, 100 μm. [From U. B. Jensen *et al.* (*5*)]

cell number, because there is a corresponding increase in apoptosis (*10*).

Clocks. Once a cell has left the stem compartment, what determines the number of rounds of division that it undergoes before terminal differentiation? Interest has focused on the possible role of intracellular clocks that control changes in the level of cell cycle promoters or inhibitors (*11*). An example of the first is the cul-1 protein in *Caenorhabditis elegans*, which is involved in destruction of cell cycle proteins such as G1 cyclins. An example of the second is the CDK inhibitor p27/Kip1 (p27), which accumulates as part of a timing mechanism that limits proliferation and promotes differentiation of rat oligodendrocyte precursor cells.

A third potential clock mechanism is telomere length. In most human tissues, telomerase activity is low or undetectable; progressive shortening of telomeres could act as a mitotic clock, counting off divisions before senescence. Stem cells may not be subject to senescence because of constitutive telomerase activity (*12*). Elderly first-generation telomerase null mice have normal hematopoiesis and no detectable abnormalities in the testis, intestine, or epidermis. However, by the sixth generation, long-term renewal of hematopoietic stem cells is compromised, male mice are infertile, and there is hair loss and delayed reepithelialization of skin wounds (*12*). Thus, although a telomerase-based clock may not control progenitor populations during the normal life span of the mouse, such a role may be important in longer-living mammals such as humans.

External Controls: The Stem Cell Niche

The external signals that control stem cell fate collectively make up the stem cell microenvironment, or niche (*3*). This niche is important both in tissues with populational asymmetry and in those with invariant asymmetry, involving a complex interplay of short- and long-range signals between stem cells, their differentiating daughters, and neighboring cells (Fig. 1).

Secreted factors. The concept of the niche was first developed in hematopoiesis, where in vitro systems that support proliferation, differentiation, and survival of distinct progenitor populations were found to depend on factors secreted by other cell types (*3, 13*). In this system, the primary function of secreted factors appears to be selective—to prevent the death of lineage-committed progenitors that have been generated stochastically (*3, 7, 14*). By contrast, secreted factors play an instructive role in the differentiation of neural crest stem cells (*15*).

A wide range of secreted factors regulate stem cell proliferation and fate. Two families, the TGFβs and Wnts, show remarkable functional conservation between species and between tissues that self-renew through asymmetric divisions or populational asymmetry. Wnts activate transcription by a complex pathway involving β-catenin (*16*); although, as described above, the importance of this pathway has been established in mammalian epidermis and intestinal epithelium, the source of Wnts in these tissues remains largely obscure (*9, 10*). In *C. elegans* the asymmetric division of the EMS blastomere requires an inductive Wnt signal from its sister cell, which controls both spindle orientation and endoderm specification (*4*). At least two members of the TGFβ family of signaling proteins are important in regulating differentiation of neural crest stem cells (*15*); in *Drosophila*, the Bmp2/4 homolog Decapentaplegic (Dpp) is required to maintain female germ line stem cells and promote their division (*4*).

Cell-cell interactions mediated by integral membrane proteins. Although secreted factors can potentially act over many cell diameters, other signals that control stem cell fate require direct cell-cell contact. Even though β-catenin is a structural component of adherens junctions (*9, 10, 16*), surprisingly little is known about intercellular adhesion as a stem cell regulatory mechanism. However, an excellent example of local signaling that requires cell-cell contact is provided by the receptor Notch and its ligand Delta, both of which are transmembrane proteins (*17*). In *Drosophila*, Notch activity is required for the progeny of the sensory organ precursor cell to assume their correct fate. During each cell division within the sensory organ lineage, Numb appears to bias Notch-mediated cell-cell interaction by inhibiting Notch activity so that the cell-cell interaction becomes asymmetric. Thus, an intrinsic mechanism (involving Numb) and an extrinsic mechanism (involving Notch) are integrated to control cell fate (*4*). Notch signaling also turns out to be important in embryonic and adult tissues of vertebrates; examples include retinal neuroepithelium, skeletal muscle, and blood (*17*).

Integrins and extracellular matrix. Adhesion to the extracellular matrix is mediated by several classes of receptor, the most extensively characterized being integrins. High expression of β1 integrins is required for maintenance of epidermal stem cells, and β1 integrins regulate differentiation of keratinocytes and other cell types through MAP kinase signaling (*5, 6*). Integrins hold cells in the right place in a tissue, and loss or alteration of integrin expression ensures departure from the stem cell niche through differentiation or apoptosis (*5, 6, 13*). They are also signaling receptors in their own right and can directly activate growth factor receptors (*18*). Extracellular matrix proteins can modulate expression and activation of β1 integrins, and local variation in the composition of the basement membrane could play a role in establishing and maintaining the distribution of epithelial stem cells, as in the crypt villus axis of the small intestine (*19*). Finally, the extracellular matrix can potentially sequester and modulate the local concentration of secreted factors available within the stem cell niche (*3, 13, 16*).

Homeostatic Controls

In its original formulation (*3*), the niche model envisioned that when a stem cell divides, only one daughter remains in the niche and the other must differentiate unless another niche is available. In tissues with invariant asymmetric divisions, it is easy to see how departure from the niche is engineered by the orientation of the mitotic spindle. However, in stem cell populations of the regulatory type, the idea of competition for the stem cell niche undoubtedly is an oversimplification. For example, when hematopoietic stem cells are transplanted from one mouse to another the final engraftment phenotype is determined simply by the ratio of host to donor stem cells, which suggests that there is no need to deplete the host stem compartment to make space for the newly added stem cells (*13*).

There are numerous examples of stem cells or their differentiated progeny regulating stem cell number (*3, 5, 6, 13*) by mechanisms depending, at least in part, on positive and negative feedback loops that have been conserved during evolution (*4, 20*). Feedback mechanisms can involve the specific transcription factors induced in response to an external signal or the antagonistic effects of different external signals.

Plasticity

There is increasing evidence that some stem cell populations isolated from adult tissues can show remarkable plasticity upon transplantation into recipients (*2*). Quite often, the tissues to which the donor cells contribute are embryologically related to the tissues from which the cells were derived. This result resembles the well-documented, if rare, phenomenon known as "transdifferentiation" or "transdetermination" (*21*). In some cases, however, such as neuronal stem cells giving rise to blood cells, this relationship does not hold. Currently, we can only speculate about the mechanisms involved in such dramatic changes in cell fate. One idea derives from two observations in the hematopoietic system. First, single-cell reverse transcription–polymerase chain reaction studies show that

individual stem and progenitor cells coexpress different lineage-associated genes before commitment, which suggests that commitment to a specific lineage is prefaced by a promiscuous phase in which the cell is activating genes associated with several different lineages (*7, 14*). Second, when B lymphoid differentiation is blocked by ablation of Pax5, B cell progenitors are able to differentiate into a wide range of other hematopoietic cell types (*22*). Thus, after stem cell transplantation, rare uncommitted progenitors may be able to undergo transdifferentiation or reprogramming if they find themselves in a new stem cell niche. Several factors might enhance this probability. For example, the ability of a cell to respond to differentiation signals may be stimulated by, and in some cases dependent on, neighboring cells that are differentiating at the same time (*23*). In addition, removing a stem cell or progenitor from the community of neighbors that are telling it what to do may lead to the up-regulation, reexpression, or de novo activation of surface receptors for a wide range of signaling factors, some of which are present in the new niche.

ES Cells: Present and Future

Pluripotential stem cell lines, also known as ES cells, are routinely derived from mouse blastocysts and, when reintroduced into host blastocysts, contribute to all adult tissues, including germ cells (*24*). In spite of their widespread use, surprisingly little is known about their origin—in particular, whether all cells of the inner cell mass (ICM) and/or epiblast have the potential to found ES lines (*25*). Initial formation of the ICM depends on asymmetric division of polarized cells of the compacted morula (*25*), but the genes that control these divisions are unknown. It is clear, however, that the POU domain transcription factor Oct4 is essential for generation of ICM cells. *Oct4* null embryos develop only as far as the blastocyst stage, and the inside cells, instead of differentiating into ICM, express trophoblast markers (*26*).

The in vivo niche of the pluripotent epiblast is provided by the enveloping extraembryonic tissues—the trophoblast-derived extraembryonic ectoderm and the primitive or visceral endoderm (*27*). The anterior visceral endoderm secretes a TGFβ-related signal, nodal, that controls differentiation of the most anterior embryonic lineages, whereas ventral mesodermal and primordial germ cell lineages are induced by Bmp4, which is produced by the trophoblast. Such findings provide important clues about how to drive ES cells down different lineage pathways in a controlled way in culture, an essential element in their therapeutic use (*28*).

Although the self-renewal of mouse ES cells in culture is promoted by leukemia-inhibiting factor or related cytokines (*29*), self-renewal of human pluripotential stem cells is not (*1*), which illustrates just one of the reasons why it is important to compare the properties of human and mouse ES cells. If relatively few signaling pathways regulate the self-renewing, pluripotent phenotype, could they be transiently activated in adult stem cells with more restricted potential to trigger pluripotency as a stable state? Fusion of adult cells with cytoplasts of ICM or ES cells might be another means to this end, providing an alternative to making human pluripotential stem cell lines from blastocysts (*30*). Reactivation of the self-renewing, pluripotent state is already possible in some differentiated early embryo cells. For example, endodermal cells of the rat and mouse yolk sac can be induced to transdifferentiate into pluripotential cells in vivo (*31*), and mouse and human primordial germ cells give rise to self-renewing pluripotential cells when cultured with a cocktail of signaling factors (*1*, *32*).

Pluripotential stem cells are not the only cell lines that can be derived from early mammalian embryos. Trophoblast stem cell lines have been derived from early mouse embryos, and their human counterparts would provide invaluable information relevant to implantation and maternal-fetal interactions (*33*). Mesoderm of gastrulation stage mouse embryos can give rise to long-term cultures of intraembryonic endothelial progenitor cells (*34*), a potentially important finding because some major blood vessels are sites of hematopoiesis in early human and mouse embryos. Human embryos (3 to 8 weeks) have been used to identify and culture hematopoietic cells associated with intraembryonic blood vessels. The histological simplicity of these regions compared with the bone marrow makes them excellent models for understanding the niche in which hematopoietic stem cells are engendered.

For some critics, acquiring such knowledge and deriving human pluripotential stem cells is tantamount to society leaving the original Garden of Eden. For others, such studies, carried out under appropriate guidelines, hold great promise not only for unexpected insights into biology but ultimately for the alleviation of human suffering.

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