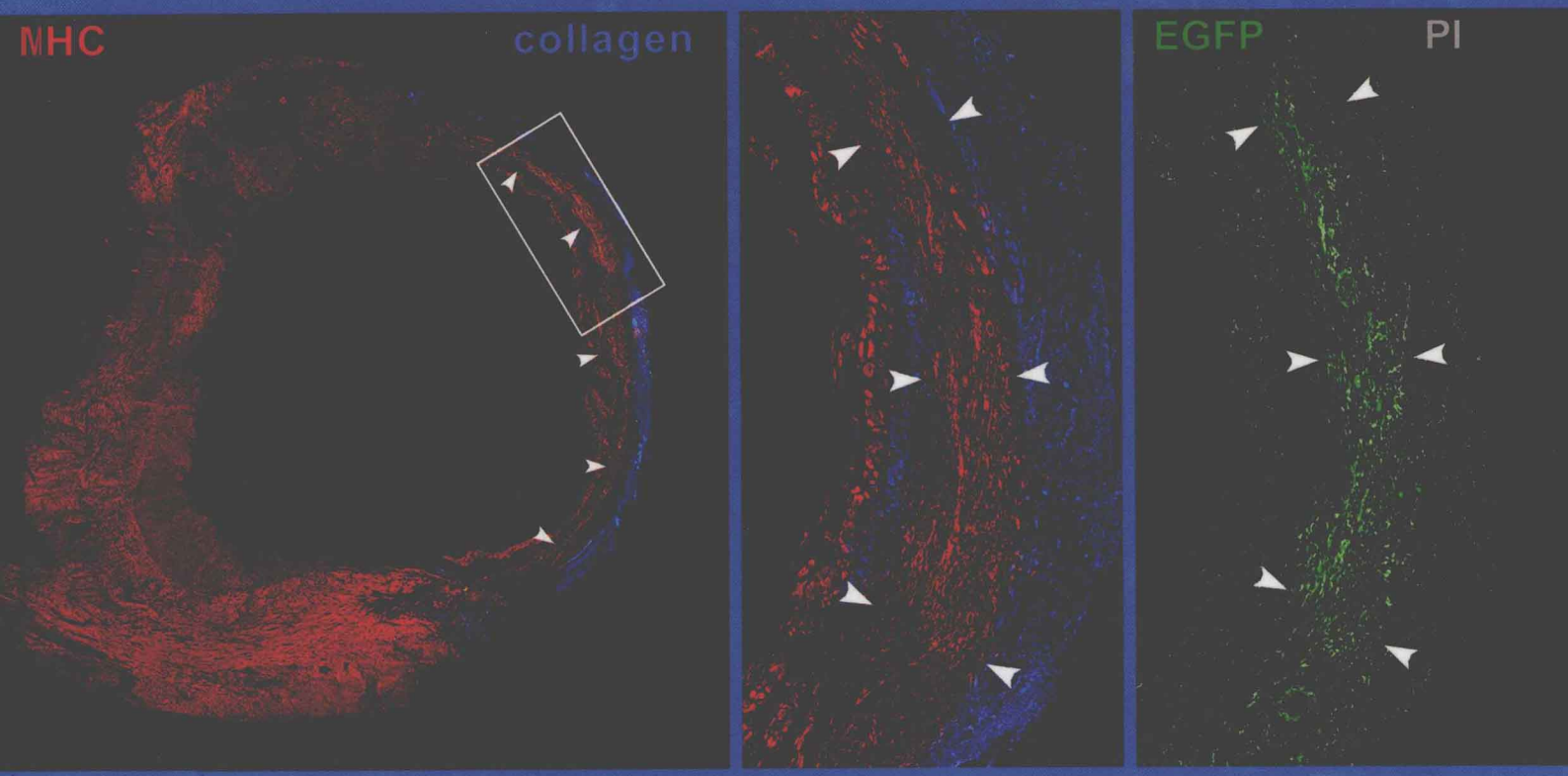
STEM CELL ANTHOLOGY

STEM CELL BIOLOGY, TISSUE ENGINEERING, CLONING, REGENERATIVE MEDICINE AND BIOLOGY



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Stem Cell Anthology

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Introduction

Over the past decade, stem cell biology has been a dominant theme in biomedical research. Its prominence is due not only to the potential of employing stem cells in the treatment of many otherwise intractable diseases, but also to the public furor that has arisen because of the use of human embryos as the source of many lines of stem cells. The debate over the source and use of stem cells has been especially acrimonious in some political and religious circles, but even as the debate has continued, new recent technological developments have altered its character. Beneath the public aspect of the debate and discussion about stem cells, intense research activity has been taking place in laboratories throughout the world. The pace of progress in the field of stem cell biology has been breath-taking, in part because it is a new field, but also because of the relatively recent development of tools in the fields of molecular and cell biology that have direct application to stem cell research.

Despite the fact that in the field of hematology the existence of stem cells has been widely accepted for decades, the biomedical research community was for many years quite resistant to the notion. Since the major breakthroughs of the 1990s and early years of this century, however, the pendulum has swung the other way, and currently optimism about the therapeutic applications of stem cells, and even their existence in certain tissues and organs, has led to often unrealistic expectations in the eyes of both the public and the scientific community about the potential application of stem cell therapy for the treatment of human disease in the near future.

Within the past decade a new field, called regenerative medicine, has been created. It is now represented by research institutes or large interdisciplinary groups in almost all major medical centers throughout the world. A prime goal of regenerative medicine is to restore damaged tissues and organs through the applications of stem cell therapy, usually in combination with techniques of tissue engineering, especially in situations where natural biological regenerative processes are not sufficiently well developed to produce effective repair. The application of techniques of regenerative medicine typically involves the production of an artificial substrate or tissue matrix by tissue engineers and then the seeding of that matrix by stem cells before it is introduced into a pathological site within the body. In a number of cases, this is followed by the reconstruction within the body of a regenerated tissue that bears a remarkable similarity in both structure and function to the original tissue that had been damaged. In other cases, however, such faithful restitution does not occur, but yet, e.g. in the heart, some functional improvement may take place. Much yet remains to be learned about what happens after stem cells have been introduced into the body.

A major consideration in stem cell therapy is the source of stem cells. Although embryonic stem cells have the potential to differentiate into a large variety of specialized cell types, many biological issues, such as controlling the pathway of differentiation both before and after implantation into the body, face researchers. Naturally occurring stem cells have been found in an increasing number of adult tissues and organs, but other problems face those who wish to use these as sources of stem cell therapy. These include obtaining sufficient numbers of adult stem cells, as well as a relatively limited repertoire for differentiation in most types of adult stem cells.

Considerable research effort has gone into defining at both a cellular and molecular level what makes a cell behave as a stem cell. The results of this research have not only allowed better definitions of stem cell characteristics and better control over their behavior, but this information has provided the basis for the recent reports of conversion of certain types of adult cells into cells with many of the characteristics of embryonic stem cells.

This volume is a collection of chapters by leaders in the stem cell field. They place in perspective both the promise and the potential limitations of stem cell biology and regenerative medicine. It is designed to provide both an introduction to the field of stem cell biology for the non-specialist and an up-to-date summary of progress for those working in the field. An introductory section (I) introduces the reader to basic properties of stem cells and then describes in greater detail the major types of stem cells in both embryos and adults. This is followed by a practically-oriented section (II) that provides technical laboratory detail for those who are actually planning or carrying out stem cell research. Section III comprises a collection of chapters that, in addition to reviewing characteristics of a generic stem cell, discuss specific types of stem cells, their locations in the body and their biological properties. Section IV concentrates on stem cell biology as it applies to specific tissues and organs that seem at present to be most amenable to the application of regenerative medicine to specific types of pathology. These chapters are heavily oriented toward laboratory studies, because in very few cases are the techniques sufficiently well developed for application to humans, but they do give a good sense of perspective concerning the state of development of the field overall. The final section (V) is a collection of

chapters that outline major ethical and religious concerns, as well as important regulatory considerations. Because of the rapidly changing legal environment as of this writing, chapters on the legal status of stem cells have not been included in this anthology.

Few aspects of biomedical science over the past half-century have captured the public imagination as much as stem cells. It is my hope that the chapters in this volume will provide the reader an accurate and accessible overview of the present status of the field, as well as a realistic depiction of the potential for the eventual clinical application of regenerative medicine.

Bruce M. Carlson

Foreword

Stem cells raise the prospect of regenerating failing body parts and treating a wide range of disorders that result from tissue loss or dysfunction. Over 16 million patients worldwide suffer from neurodegenerative disorders such as Parkinson's and Alzheimer's disease, over 200 million people suffer from diabetes, and millions more from cardiovascular disease, blindness, and other disorders that may one day be treatable using stem cell technology. With the advent of induced pluripotent stem (iPS) cells, we may even have a way to overcome the problem of immune rejection. In addition to being a promising—and potentially unlimited—source of cells for regenerative medicine and drug discovery, stem cells serve as an excellent model for studying both normal and abnormal vertebrate development.

The rapid progress and interest in stem cells has led to an avalanche of new knowledge and research tools that span multiple areas of scientific inquiry, ranging from cellular reprogramming to cloning to tissue engineering. Although books on various stem cell topics abound, *Stem Cell Anthology* combines in a single volume a collection of definitive chapters and essential subject matter from five leading stem-cell related volumes, including *Essentials of Stem Cell Biology, Principles of Tissue Engineering, Principles of Regenerative Medicine, Human Stem Cell Manual*, and *Principles of Cloning*.

Stem Cell Anthology successfully integrates this exciting area of biology, combining the prerequisites for a general understanding of adult, embryonic, and induced pluripotent stem cells. No topic in the field of stem cells is left uncovered, including methods for preparing, maintaining, and genetically manipulating stem cells and progenitor populations; the types, properties, and characteristics of stem cells; application of stem cells to specific organ systems and diseases, including neural and pancreatic stem cells; cancer; burns and ulcers, and cardiovascular and musculoskeletal repair; as well as a section on ethical, religious, and FDA product and pre-clinical regulatory considerations. The result is a comprehensive, easy to follow reference by the world's experts that will be useful to students, scientists, and clinicians alike.

Robert Lanza, M.D.

"Stemness": Definitions, Criteria, and Standards

INTRODUCTION

Stem cells have recently generated more public and professional interest than almost any other topic in biology. One reason stem cells capture the imagination of so many is the promise that understanding their unique properties may provide deep insights into the biology of cells as well as a path toward treatments for a variety of degenerative illnesses. And although the field of stem cell biology has grown rapidly, there exists considerable confusion and disagreement as to the nature of stem cells. This confusion can be partly attributed to the sometimes idiosyncratic terms and definitions used to describe stem cells. Although definitions can be restrictive, they are useful when they provide a basis for mutual understanding and experimental standardization. With this intention, I present explanations of definitions, criteria, and standards for stem cells. Moreover, I highlight a central question in stem cell biology, namely the origin of these cells. I also suggest criteria or standards for identifying, isolating, and characterizing stem cells. Finally, I summarize the notion of "stemness" and describe its possible application in understanding stem cells and their biology.

WHAT IS A STEM CELL?

Stem cells are defined functionally as cells that have the capacity to self-renew as well as the ability to generate differentiated cells (Weissman *et al.*, 2001; Smith, 2001). More explicitly, stem cells can generate daughter cells identical to their mother (self-renewal) as well as produce progeny with more restricted potential (differentiated cells). This simple and broad definition may be satisfactory for embryonic or fetal stem cells that do not perdure for the lifetime of an organism. But this definition breaks down in trying to discriminate between transient adult progenitor cells that have a reduced capacity for self-renewal and adult stem cells. It is therefore important when describing adult stem cells to further restrict this definition to cells that self-renew throughout the life span of the animal (van der Kooy and Weiss, 2000). Another parameter that should be considered is potency: Does the stem cell generate to multiple differentiated cell types (multipotent), or is it only capable of producing one type of differentiated cell (unipotent)? Thus, a more complete description of a stem cell includes a consideration of replication capacity, clonality, and potency. Some theoretical as well as practical considerations surrounding these concepts are considered in this chapter.

Self-renewal

Stem cell literature is replete with terms such as "immortal", "unlimited", "continuous", and "capable of extensive proliferation", all used to describe the cell's replicative capacity. These rather extreme and vague terms are not very helpful, as it can be noted that experiments designed to test the "immortality" of a stem cell would by necessity outlast authors and readers alike. Most somatic cells cultured *in vitro* display a finite number of (less than 80) population doublings prior to replicative arrest or senescence, and this can be contrasted with the seemingly unlimited proliferative capacity of stem cells in culture (Houck *et al.*, 1971; Hayflick, 1973; Hayflick, 1974; Sherr and DePinho, 2000; Shay and Wright, 2000). Therefore, it is reasonable to say that a cell that can undergo more than twice this number of population doublings (160) without oncogenic transformation can be termed "capable of extensive proliferation". In a few cases, this criteria has been met, most notably with embryonic stem (ES) cells derived from either humans or mice as well as with adult neural stem cells (NSCs) (Smith, 2001; Morrison *et al.*, 1997). An incomplete understanding of the factors required for self-renewal *ex vivo* for many adult stem cells precludes establishing similar proliferative limits *in vitro*. In some cases, a rigorous assessment of the capacity for self-renewal of certain adult stem cells can be obtained by single-cell or serial transfer into acceptable hosts, an excellent example of which is adult hematopoietic stem cells (HSCs) (Allsopp and Weissman, 2002; Iscove and Nawa, 1997). Adult stem cells are probably still best defined *in vivo*, where they must display sufficient proliferative capacity to last the lifetime of the animal. Terms such as "immortal" and "unlimited" are probably best used sparingly if at all.

Clonality

A second parameter, perhaps the most important, is the idea that stem cells are clonogenic entities: single cells with the capacity to create more stem cells. This issue has been exhaustively dealt with elsewhere and is essential for any definitive characterization of self-renewal, potential, and lineage. Methods for tracing the lineage of stem cells are described in subsequent chapters. Although the clonal "gold standard" is well understood, there remain several confusing practical issues. For instance, what constitutes a cell line? The lowest standard would include any population of cells

that can be grown in culture, frozen, thawed, and subsequently repassaged *in vitro*. A higher standard would be a clonal or apparently homogenous population of cells with these characteristics, but it must be recognized that cellular preparations that do not derive from a single cell may be a mixed population containing stem cells and a separate population of "supportive" cells required for the propagation of the purported stem cells. Hence, any reference to a stem cell line should be made with an explanation of their derivation. For example, it can be misleading to report on stem cells or "stem cell lines" from a tissue if they are cellular preparations containing of a mixed population, possibly contaminated by stem cells from another tissue.

Potency

The issue of potency maybe the most contentious part of a widely accepted definition for stem cells. A multipotent stem cell sits atop a lineage hierarchy and can generate multiple types of differentiated cells, the latter being cells with distinct morphologies and gene expression patterns. At the same time, many would argue that a self-renewing cell that can only produce one type of differentiated descendant is none-the-less a stem cell (Slack, 2000). A case can be made, for clarity, that a unipotent cell is probably best described as a progenitor. Progenitors are typically the descendants of stem cells, only they are more constrained in their differentiation potential or capacity for self-renewal and are often more limited in both senses.

Definition

In conclusion, a working definition of a stem cell is a clonal, self-renewing entity that is multipotent and thus can generate several differentiated cell types. Admittedly, this definition is not applicable in all instances and is best used as a guide to help describe cellular attributes.

WHERE DO STEM CELLS COME FROM?

The origin or lineage of stem cells is well understood for ES cells; their origin in adults is less clear and in some cases controversial. It may be significant that ES cells originate before germ layer commitment, raising the intriguing possibility that this may be a mechanism for the development of multipotent stem cells, including some adult stem cells. The paucity of information on the developmental origins of adult stems cells leaves open the possibility that they too escape lineage restriction in the early embryo and subsequently colonize specialized niches, which function to both maintain their potency as well as restrict their lineage potential. Alternatively, the more widely believed, though still unsubstantiated, model for the origin of adult stem cells assumes that they are derived after somatic lineage specification, whereupon multipotent stem cells–progenitors arise and colonize their respective cellular niches. In this section, I briefly summarize the origin of stem cells from the early embryo and explain what is known about the ontogeny of adult stem cells focusing attention on HSCs and NSCs.

Stem Cells of the Early Embryo

Mouse and human ES cells are derived directly from the inner cell mass of preimplantation embryos after the formation of a cystic blastocyst (Papaioannou, 2001). This population of cells would normally produce the epiblast and eventually all adult tissues, which may help to explain the developmental plasticity exhibited by ES cells. In fact, ES cells appear to be the *in vitro* equivalent of the epiblast, as they have the capacity to contribute to all somatic lineages and in mice to produce germ line chimeras. By the time the zygote has reached the blastocyst stage, the developmental potential of certain cells has been restricted. The outer cells of the embryo have begun to differentiate to form trophectoderm, from which a population of embryonic trophoblast stem cells has also been derived in mice (Tanaka *et al.*, 1998). These specialized cells can generate all cell types of the trophectoderm lineage, including differentiated giant trophoblast cells. At the egg cylinder stage of embryonic development (embryonic day (E) 6.5 in mice), a population of cells near the epiblast can be identified as primordial germ cells (PGCs), which are subsequently excluded from somatic specification or restriction (Saitou *et al.*, 2002). PGCs migrate to and colonize the genital ridges, where they produce mature germ cells and generate functional adult gametes. PGCs can be isolated either prior or subsequent to their arrival in the genital ridges and, when cultured with appropriate factors *in vitro*, can generate embryonic germ (EG) cells (Matsui *et al.*, 1992; Resnick *et al.*, 1992). EG cells have many of the characteristics of ES cells with respect to their differentiation potential and their contribution to the germ line of chimeric mice (Labosky *et al.*, 1994; Stewart *et al.*, 1994). The most notable difference between ES and EG cells is that the latter may display (depending upon the developmental stage of their derivation) considerable imprinting of specific genes (Surani, 1998; Sorani, 2001; Howell *et al.*, 2001). Consequently, certain EG cell lines are incapable of producing normal chimeric mice.

Importantly, no totipotent stem cell has been isolated from the early embryo. ES and EG cells generate all somatic lineages as well as germ cells but rarely if ever contribute to the trophectoderm, extraembryonic endoderm, or extraembryonic mesoderm. Trophectoderm stem (TS) cells have been isolated, and these only generate cells of the trophectoderm lineage. It remains to be seen whether cells can be derived and maintained from totipotent

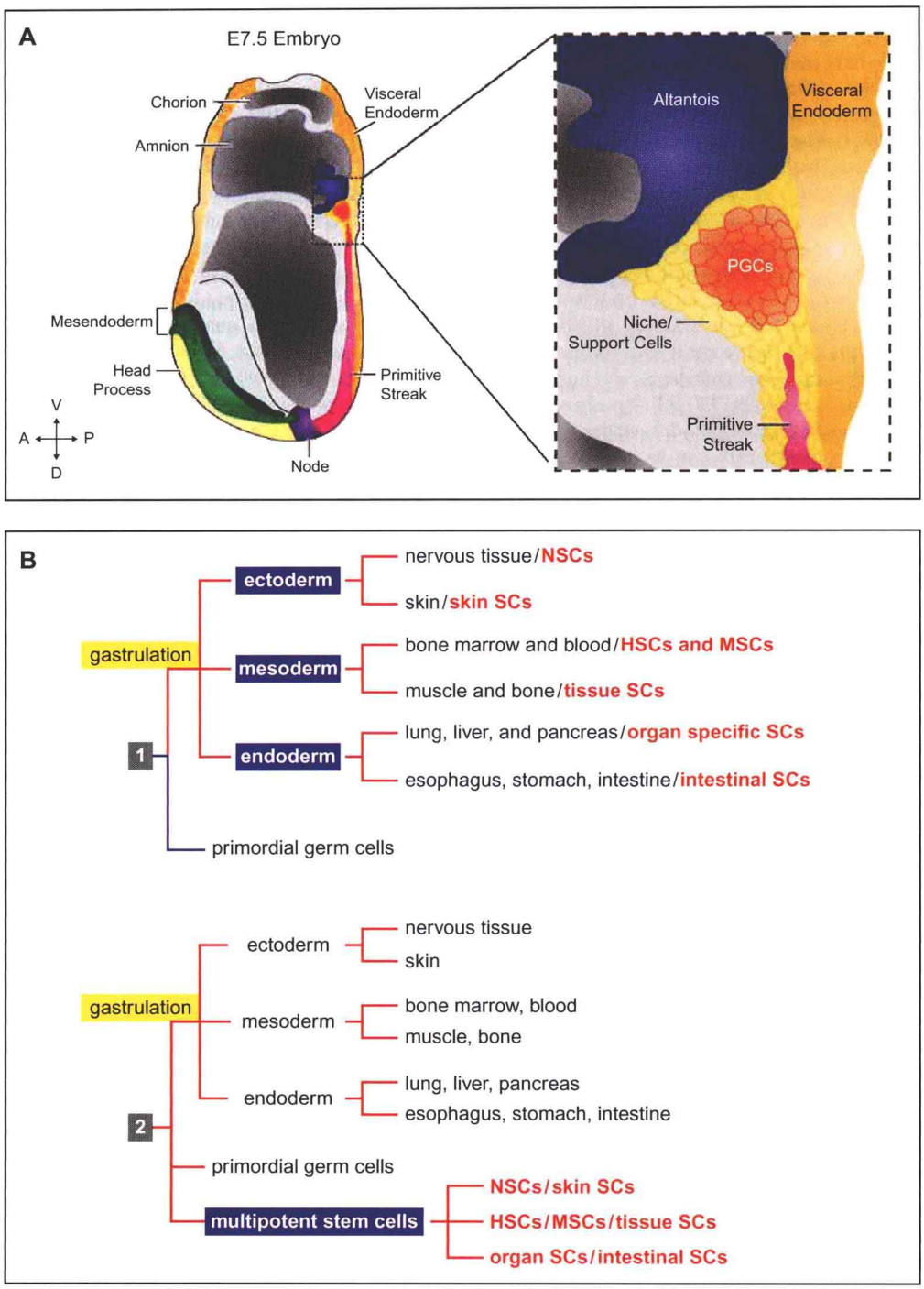

FIGURE 1 (A) Development of primordial germ cells. A schematic of an embryonic day 7.5 mouse embryo highlights the position of the developing primordial germ cells (PGCs) proximal to the epiblast. The expanded view on the right serves to illustrate the point that PGCs escape lineage commitment/restriction by avoiding the morphogenetic effects of migrating through the primitive streak during gastrulation. (B) Putative developmental ontogeny of stem cells. In lineage tree 1, the development of stem cells occurs after the formation of germ layers. These stem cells are thus restricted by germ layer commitment to their respective lineage (e.g., mesoderm is formed, giving rise to hematopoietic progenitors that become hematopoietic stem cells). Lineage tree 2 illustrates the idea that stem cells might develop similarly to PGCs, in that they avoid the lineage commitments during gastrulation and subsequently migrate to specific tissue and organ niches.

embryonic stages. Although our understanding of cell fates in the early embryo is incomplete, it appears that the only pluripotent stem cells found after gastrulation are PGCs (with the possible exceptions of multipotential adult progenitor cells (Jiang *et al.*, 2002) and teratocarcinomas). It may be that PGCs escape germ layer commitment during gastrulation by developing near the epiblast and subsequently migrate to positions inside the embryo proper. This developmental strategy

may not be unique to PGCs, and it raises the interesting possibility that other stem cells might have similar developmental origins. Alternatively, it may be the case that adult stem cells are derived from PGCs. Although intriguing, it is important to stress that this idea lacks experimental evidence.

Ontogeny of Adult Stem Cells

The origin of most adult stem cells is poorly understood. With the issue of adult stem cell plasticity at the forefront, as described in this section, studies designed to elucidate the ontogeny of adult stem cells may help to reveal their specific lineage relationships and shed light on their plasticity and potential. Information on the origins of adult stem cells would also help to define the molecular programs involved in lineage determination, which may in turn provide insights into methods for manipulating their differentiation. To this end, I summarize what is known about the development of adult stem cells within the context of the hematopoietic and neural systems.

The development of hematopoietic cells in mice occurs soon after gastrulation (E7.5), although HSCs with the same activities as those in the adult have only been observed and isolated at midgestational stages (E10.5) (Orkin, 1996; Dzierzak, 2002; Weissman, 2000). These observations suggest that the embryo has a unique hematopoietic lineage hierarchy, which may not be founded by an adult-type HSC. Thus, hematopoiesis appears to occur at multiple times or in successive waves within the embryo, and the emergence of an HSC may not precede or be concomitant with the appearance of differentiated hematopoietic cells.

The first site of hematopoiesis in the mouse is the extraembryonic yolk sac, soon followed by the intraembryonic aorta–gonad–mesonephros (AGM) region. Which of these sites leads to the generation of the adult hematopoietic system and, importantly, HSCs is still unclear. Results from nonmammalian embryo-grafting experiments, with various findings in the mouse, suggest that the mammalian embryo, specifically the AGM, generates the adult hematopoietic system and HSCs (Kau and Turpen, 1983; Medvinsky *et al.*, 1993; Medvinsky and Dzierzak, 1996). Interestingly, the midgestational AGM is also the region that harbors migrating PGCs and is thought to produce populations of mesenchymal stem cells, vascular progenitors, and perhaps hemangioblasts (Molyneaux *et al.*, 2001; Minasi *et al.*, 2002; Alessandri *et al.*, 2001; Hara *et al.*, 1999; Munoz-Chapuli *et al.*, 1999). In the absence of studies designed to clonally evaluate the lineage potential of cells from the AGM, and without similarly accurate fate mapping of this region, it remains possible that all of the adult stem cell types thought to emerge within the AGM arise from a common unrestricted precursor. This hypothetical precursor could help to explain reports of nonfusion-based adult stem cell plasticity. The observed lineage specificity of most adult stem cells could likewise be attributed to the high-fidelity lineage restriction imposed on them by the specific niche they colonize or are derived from. Simple ideas such as these have not been ruled out by experimental evidence, underscoring both the opportunity and the necessity for further study of the developmental origins of adult stem cells.

A key lesson from studies of the developing hematopoietic system is that the appearance of differentiated cells does not tell us where or when the corresponding adult stem cells originate. Definitive lineage tracing, with assays of clonogenic potential, remains the method of choice for identifying the origin of stem cells. Another potential pitfall revealed by these studies is that the definition of the stem cell can make all the difference in its identification.

The development of NSCs begins with the formation of nervous tissue from embryonic ectoderm following gastrulation. Induction of the neural plate is thought to coincide with the appearance of NSCs as well as restricted progenitor types (Temple, 2001). The exact frequency and location of stem cells within the developing neuroepithelium remains unknown; specific markers must be discovered to fully unravel this question. An emerging view in the field is that embryonic neuroepithelia generate radial glial that subsequently develop into periventricular astrocytes and that these cells are the embryonic and adult NSCs within the central nervous system (Alvarez-Buylla *et al.*, 2001; Tramontin, 2003; Doetsch *et al.*, 1999; Gaiano and Fishell, 2002). Developing and adult NSCs also appear to acquire positional and temporal information. For example, stem cells isolated from different neural regions generate region-appropriate progeny (Kalyani *et al.*, 1998; He *et al.*, 2001; Anderson *et al.*, 1997). In addition, several studies suggest that temporal information is encoded within NSCs, that earlier stem cells give rise more frequently to neurons, and that more mature stem cells preferentially differentiate into glia (Temple, 2001; Qian *et al.*, 2000; White *et al.*, 2001). Moreover, more mature NSCs appear incapable of making cells appropriate for younger stages when transplanted into the early cerebral cortex (Desai and McConnell, 2000). Thus, the nervous system appears to follow a classical lineage hierarchy, with a common progenitor cell generating most if not all differentiated cell types in a regional- and temporal-specific manner. There may also be rare stem cells in the nervous system, perhaps not of neural origin, that have greater plasticity in terms of producing diverse somatic cell types and lacking temporal and spatial constraints (Weissman, 2000; Temple, 2001). There are several caveats that must be considered when describing the developmental origins of NSCs. First, disrupting the neuroepithelia to purify NSCs may have the undesirable effect of dysregulating spatial patterning acquired by these cells. Second, growth of purified NSCs in culture may reprogram the stem cells through exposure to nonphysiological *in vitro* culture conditions. Both of these problems can be addressed either by *in vivo* lineage tracing or by prospectively isolating NSCs and transplanting them into acceptable hosts without intervening culture.

Carefully designed experiments promise to answer questions important not only for stem cell biology but also for neuroembryology and development. These include which features of the developmental program are intrinsic to individual cells, which differentiation or patterning signals act exclusively to instruct specific cell fates, and how developmental changes in cell-intrinsic programs restrict the responses of progenitors to cell-extrinsic signals.

HOW ARE STEM CELLS IDENTIFIED, ISOLATED, AND CHARACTERIZED?

How stem cells are identified, isolated, and characterized are the key methodological questions in stem cell biology, so much so that subsequent chapters are devoted to addressing these problems in detail. Here, I briefly outline standards and criteria that may be employed when approaching the challenge of identifying, isolating, and characterizing a stem cell.

Embryonic Stem Cells

The basic characteristics of an ES cell include self-renewal, multilineage differentiation *in vitro* and *in vivo*, clonogenicity, a normal karyotype, extensive proliferation *in vitro* under well-defined culture conditions, and the ability to be frozen and thawed. In animal species, *in vivo* differentiation can be assessed rigorously by the ability of ES cells to contribute to all somatic lineages and produce germ line chimerism. These criteria are not appropriate for human ES cells; consequently, these cells must generate embryoid bodies and teratomas containing differentiated cells of all three germ layers. Moreover, as a stringent *in vivo* assessment of pluripotency is impossible, human ES cells must be shown to be positive for well-known molecular markers of pluripotent cells. These markers are defined as factors expressed consistently, and enriched, in human ES cells (Brivanlou *et al.*, 2003). As a substitute for whole-animal chimerism, human ES cells could be tested for their contributions to specific tissues when transplanted in discrete regions of nonhuman adults or embryos. A complementary analysis might include transplanting human ES cells into nonhuman blastocysts and evaluating their contribution to various organs and tissues, though this experiment has raised ethical concerns in some quarters. Finally, a practical consideration is the passage number of ES cells. Although it is important to establish the capacity of ES cells to proliferate extensively, it is equally important that low-passage cells are evaluated experimentally to guard against any artifacts introduced through *in vitro* manipulation.

Adult Stem Cells

The basic characteristics of an adult stem cell are a single cell (clonal) that self-renews and generates differentiated cells. The most rigorous assessment of these characteristics is to prospectively purify a population of cells (usually by cell surface markers), transplant a single cell into an acceptable host without any intervening *in vitro* culture, and observe self-renewal and tissue, organ, or lineage reconstitution. Admittedly, this type of *in vivo* reconstitution assay is not well-defined for many types of adult stem cells. Thus, it is important to arrive at an accurate functional definition for cells whose developmental potential is assessed *in vitro* only. Above all, clonal assays should be the standard by which fetal and adult stem cells are evaluated because this assay removes doubts about contamination with other cell types.

Two concepts about the fate or potential of stem cells have moved to the forefront of adult stem cell research. The first is plasticity, the idea that restrictions in cell fates are not permanent but are flexible and reversible. The most obvious and extreme example of reversing a committed cell fate comes from experiments in which a terminally differentiated somatic cell generates to another animal following nuclear transfer or cloning (Solter, 2000; Rideout *et al.*, 2001). Nuclear transfer experiments show that differentiated cells, given the appropriate conditions, can be returned to their most primal state. Thus, it may not be surprising if conditions are found for more committed or specified cells to dedifferentiate and gain a broader potential. A related concept is that of transdifferentiation. Transdifferentiation is the generation of functional cells of a tissue, organ, or lineage that is distinct from that of the founding stem cell (Liu and Rao, 2003; Blau *et al.*, 2001). Important issues here are whether the cells proposed to transdifferentiate are clonal and whether the mechanism by which they form the functional cell requires fusion (Medvinsky and Smith, 2003; Terada *et al.*, 2002; Wang *et al.*, 2003; Ying *et al.*, 2002). Experiments designed to carefully evaluate these possibilities will yield insight into the nature of stem cells.

STEMNESS: PROGRESS TOWARD A MOLECULAR DEFINITION OF STEM CELLS

Stemness refers to the common molecular processes underlying the core stem cell properties of self-renewal and the generation of differentiated progeny. Although stems cells in different cellular microenvironments or niches will by necessity have different physiological demands and therefore distinct molecular programs, there are likely certain genetic characteristics specific to and shared by all stem cells. Through transcriptional profiling, many of the genes enriched in ES cell, TS cell, HSC, and NSC populations have been identified (Ivanova *et al.*, 2002; Ramalho-Santos *et al.*, 2002; Tanaka *et al.*, 2002; Anisimov *et al.*, 2002; Luo *et al.*, 2002; Park *et al.*, 2002). By extending this approach to other stem cells and more organisms, it may be possible to develop a molecular fingerprint for stem cells. This fingerprint could be used as the basis for a molecular definition of stem cells that, when combined with their functional definition, would provide a more

comprehensive set of criteria for understanding their unique biology. Perhaps more importantly, these types of studies could be used to help identify and isolate new stem cells. This goal is far from being accomplished, but the preliminary findings for specific stem cells have been described. The transcriptional profiling of stem cells has suggested that they share several distinct molecular characteristics. Stem cells appear to have the capacity to sense a broad range of growth factors and signaling molecules and to express many of the downstream signaling components involved in the transduction of these signals. Signal transduction pathways present and perhaps active in stem cells include TGF®, Notch, Wnt, and Jak/Stat family members. Stem cells also express many components involved in establishing their specialized cell cycles, either related to maintaining cell cycle arrest in G1 (for most quiescent adult stem cells) or connected to progression through cell cycle checkpoints promoting rapid cycling (as is the case for ES cells and mobilized adult stem cells) (Burdon *et al.*, 1999; Savatier *et al.*, 2002). Most stem cells also express molecules involved in telomere maintenance and display elevated levels of telomerase activity. There is also considerable evidence that stem cells have significantly remodeled chromatin acted upon by DNA methylases or transcriptional repressors of histone deacetylase and Groucho family members. Another common molecular feature is the expression of specialized posttranscriptional regulatory machinery regulated by RNA helicases of the Vasa type. Finally, a shared molecular and functional characteristic of stem cells appears to be their resistance to stress, mediated by multidrug resistance transporters, protein-folding machinery, ubiquitin, and detoxifier systems.

Although in its infancy, the search for a molecular signature to define stem cells continues. We have begun to understand in general terms what molecular components are most often associated with stem cells. In the future, it may be possible to precisely define stem cells as a whole and individually by their telltale molecular identities. Until that time, stemness remains a concept of limited utility with tremendous potential.

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