Commitment and Promiscuity in the Twenty-First Century

Hematopoiesis: A Developmental Approach
Edited by Leonard I. Zon
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Scientifically, medically, and politically, no topics in biomedicine are being scrutinized more intensely than the biology, clinical potential, and ethical ramifications of stem cell research. From the classic experiments in the 1960s demonstrating that an adult hematopoietic stem cell (HSC) could reconstitute an animal or human following lethal ablation, to the later development of systems to study primitive hematopoietic cells in vitro, the study of hematopoiesis, or the process of formation of all the blood elements, has been central to the broader field of stem cell biology.

Recently, a wide variety of scientists and clinicians have turned their attention to hematopoiesis, following the tantalizing though still preliminary reports that adult HSCs can contribute to or even regenerate diverse tissues (Orkin and Zon, Nat. Immunol. *3*, 323–328, 2002). Following marrow transplantation, donor-derived cells with phenotypic characteristics of myocytes were detected in damaged murine muscle (Ferrari et al., Science *279*, 1528–1530, 1998). Many reports have followed, with the most convincing indicating that purified murine HSC could contribute functionally to both liver and cardiac regeneration following biochemical or ischemic injury (Lagasse et al., Nat. Med. *6*, 1229–1234, 2000; Orlic et al., Nature *410*, 701–705, 2001). Conversely, neural stem cells and muscle satellite cells have been used to reconstitute hematopoiesis following lethal irradiation, although these experiments have been more difficult to replicate, and in some cases, hematopoietic repopulating activity appears to reside in $CD45^+$ primitive hematopoietic cells lodged in these tissues (Bjornson et al., Science *283*, 534–537, 1999; Jackson et al., Proc. Natl. Acad. Sci. USA *96*, 14482–14486, 1999).

This entire field of "plasticity" is only several years old. There is no consensus and little data regarding mechanisms. Do truly uncommitted multipotent stem cells persist in all tissues from fetal through adult life, awaiting physiologic signals to be recruited to a lineage or tissue? Or do cells with a phenotype and transcriptosome of one primitive cell type, such as HSCs, actually completely change their commitment program under certain circumstances? Alternatively, are primitive cells committed to one tissue or organ simply lodged in multiple diverse sites, where they can be recruited back to the appropriate location following injury? Most recently, the possibility that at least some of the findings are artifactual was suggested by a report that coculture of embryonic stem (ES) cells and adult marrow cells resulted in tetraploid cells with both fetal characteristics and adult marrow cell genetic material (Terada et al., Nature *416*, 542–545, 2002). Despite this confusion, the potential clinical applications of these observations are enormous, and investigators have begun clinical trials using HSCs or marrow mesenchymal stem cell therapies for diseases as diverse as coronary artery disease and amyotrophic lateral sclerosis. Political pressure has intensified, as the potential for adult stem cell reprogramming and thus diverse regenerative potential has been seized upon by those opposed to any sort of embryonic stem cell research or utilization as a "scientific" reason to ban further federal funding and generation of new human ES cell lines.

A related and more ethically complex field involves therapeutic cloning. Since Dolly the sheep was generated by somatic cell nuclear transfer, the race to understand the process of developmental reprogramming and bring it to the clinic has progressed simultaneously with the societal controversies raised by this new technology. A paper published recently in *Cell* demonstrated that transplantation of a somatic cell nucleus from an immunodeficient mouse into an oocyte, followed by ES cell line generation, corrective homologous recombination of the aberrant RAG allele, differentiation of the cells toward the hematopoietic lineage, and transplantation back into the original immunodeficient strain, could result in correction of the disease phenotype (Rideout et al., Cell *109*, 17–27, 2002). Our practical progress has jumped far beyond our understanding of the basic biologic principles involved, and has also outstripped any political consensus on the appropriate applications of these technologies.

Thus, the timing of this new comprehensive book, edited by Leonard I. Zon and entitled *Hematopoiesis: A Developmental Approach*, could not be better. No other monograph or textbook has meshed knowledge from the fields of hematopoietic stem cell biology and developmental hematopoiesis. A newly expanded audience of hematologists, transplantation clinicians, and basic scientists need an education in the historical context and current concepts of self renewal and lineage commitment. All of us need to understand the elegant model systems and experiments that have elucidated central aspects of embryonic and postnatal hematopoiesis before attempting to understand what basic mechanisms could underlie the processes of nuclear transfer or findings of HSC "plasticity." Advances in HSC gene therapy and the expansion of indications for human allogeneic HSC transplantation have also generated more intense interest in hematopoiesis, and the use of cord blood as an HSC source requires knowledge of aspects of developmental hematopoiesis.

Dr. Zon, an accomplished stem cell biologist and zebrafish geneticist, has assembled an impressive set of contributors. In his preface, Zon explicitly states that he intended to be inclusive, and to give the reader the benefit of many different but clearly influential perspectives, even when this resulted in conflicting hypotheses and redundancy in content. Practically, if the editor had attempted to edit the individual contributions extensively to reduce redundancy and increase coherence, publication would have been delayed significantly; the time-sensitive nature of the topics clearly led Zon to move ahead quickly. The resulting book is monumental, over 800 pages in sections, and very comprehensive. There is significant redundancy, but in most cases, the topics covered by more than one author relate to the most central issues, such as transcriptional control of

lineage commitment, impact of cytokine signaling on lineage decisions, relationships between fetal and adult hematopoiesis, and dual roles of certain proteins in basic developmental processes and adult hematopoiesis.

The first section includes chapters on the basic tenets of postnatal HSC biology. High points include a focused summary by Akashi and Weissman of their contributions to phenotypic and functional definition of stem cells and lineage-restricted progenitors, and several chapters on various in vitro and in vivo progenitor and HSC assays by their developers. May and Enver contribute what I believe to be the central chapter in the entire book, integrating general principles of the development of the hematopoietic system with lineage commitment in the embryo, summed up by the question "how do you get the ball rolling, and how does it roll in the right direction?" Chapters on gene therapy and ex vivo stem cell expansion round out this section. Lacking are chapters addressing the knowledge gained from preclinical and clinical HSC transplantation studies.

The next section includes chapters on the ontogeny of hematopoiesis during embryogenesis, drawing from a wide variety of model organisms such as *Drosophila*, *Xenopus*, zebrafish, chickens, and mice. The progression of these chapters is confusing for readers with limited backgrounds in developmental biology, but a very clear chapter by Yoder and Polis covering early hematopoiesis in the mouse embryo would be helpful to read first. The third section, confusingly entitled "Important Factors in Hematopoiesis" includes many of the most valuable chapters in the volume. The first several describe hematopoietic cytokines and the signal transduction pathways of cytokine receptors. The chapter by Rhodes and Druker on Jak-STAT signal transduction is particularly well written and comprehensive. Next come several chapters on the roles of transcription factors in the control of hematopoiesis in both embryos and adults. An essential synthesis of the current paradigms regarding transcriptional control of self renewal and lineage commitment should not be missed and is provided in one chapter by Orkin, and in the two following chapters by Graf and coauthors. Finally, the section concludes with an excellent description from Lawrence and coauthors regarding the impact of homeobox gene expression on development, normal hematopoiesis, and leukemogenesis.

The next two sections are focused on the myeloid and lymphoid lineages, respectively. Ever since Metcalf first developed myeloid in vitro colony assays, the myeloid pathways, especially the decision point between granulocyte and monocyte progeny, have been productively studied, and Tenen provides a current overview. Several chapters on megakaryocytopoiesis and on mast cell development demonstrate that the progress in these myeloid lineages has been slower, but the central questions are well presented. The chapters on lymphoid development are in some ways the most disappointing in the volume, not all individually weak, but unconnected and selective in their topics.

Instead of a separate section on erythropoiesis, the editor chose to include many of the relevant topics in the next section, entitled "Genetics of Blood Formation." It was unfortunate to separate the two excellent chapters on hemoglobin switching, by Broyles and Stamatoyanopoulos, from Orkin's chapter on the transcriptional control of erythropoiesis. This section of the book also includes chapters on genetic techniques such as large-insert genomic cloning, a discussion of the genes involved in aberrant leukemic hematopoiesis, and summaries of specific informative hematopoietic mutants in the murine and zebrafish models. The book concludes with two sections on topics not always considered part of hematopoiesis, but clearly appropriate additions to a global view of the field. First, several chapters on the hematopoietic microenvironment are grouped together in the "Stroma" section. Finally, five chapters on vascular system development are included because of close associations between hematopoietic and endothelial cells throughout development, and evidence that adult endothelium may also be derived in an ongoing manner from marrow progenitors or even HSCs.

There are difficulties with utilizing this enormous book effectively. One stumbles on the gems of chapters and the information one is seeking not by design but stochastically! Assignment of some chapters to their sections seems almost random. Why would "Mouse Genetics for the Analysis of Stem Cell Behavior" be included in Part VII: "Stroma"? The chapter with the most relevance to the role of the microenvironment in homing of primitive hematopoietic cells is located in Part III, "Important Factors in Hematopoiesis" instead of in Part VII, "Stroma." Dr. Zon's own chapter on "Zebrafish Hematopoietic Mutants" is separated from his other chapter on "Zebrafish Hematopoietic Development." It is always difficult, of course, to assign chapters to only one heading when their content may relate to a number of different topics. Chapter assignment would matter less if the titles were more informative or if a brief summary of the content preceded each chapter. Worse, the index provides little assistance. Generated by a standard indexing program, it seems never to have been checked by a human. For example, multiple entries for the same entity, "SCF/c-kit," "stem cell factor (SCF)," and "kit ligand," all refer to different sets of page numbers. Since this is not a book that any person would or could sit down and read cover-to-cover, these navigation difficulties are a significant drawback.

Is this comprehensive hybrid between a textbook and a series of monographs of practical utility in the internet era, with almost universal access to PubMed and online journals? I believe the positives outweigh the negatives. This collection of authors represents both a broad and deep array of the most active scientists in hematopoiesis, translational clinical stem cell applications, and hematologic aspects of developmental biology. Many chapters place their topic in a detailed historical context, and not only catalog central experiments and findings, but provide approaches for applying the knowledge to the "big" issues central to stem cell biology, such as mechanisms of lineage commitment, maintenance of pluripotentiality, and differences between fetal and adult hematopoiesis. As hematologists, stem cell biologists, and clinical scientists working on many organ systems and tissues are attracted by the siren call of "plasticity," they will find specific knowledge and overall perspectives on these topics invaluable, especially since the

contributors include both developmental and adult stem cell biologists.

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Nuclear Transport: The Bottom Line

Nuclear Transport
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$104.75

Nucleocytoplasmic transport plays a pivotal role in eukaryotic cell function and is relevant to many areas of cell biology. As such, it is somewhat surprising that books on this subject are in short supply. *Nuclear Transport*, edited by Karsten Weis, is a welcome addition.

To understand the organization of the book, a brief overview of nuclear transport is helpful. Nuclear localization signals (NLSs) and nuclear export signals (NESs) direct proteins to enter or leave the nucleus. Most types of nuclear transport use a set of cytoplasmic receptors derived from a single family of proteins, the importin β (or karyopherin β) nuclear transport receptor family. These receptors are regulated by the binding of a small GTPase, Ran. Ran exists predominantly in its GTP form inside the nucleus (where its GDP-GTP exchange factor, RCC1, resides), and in its GDP form in the cytoplasm (where its GTPase activating protein, RanGAP, is found). This nuclear/cytoplasmic RanGTP gradient was recently confirmed visually using novel fluorescent biosensors (Kalab et al., Science *295*, 2452–2456, 2002).

Getting back to the basics of nuclear transport, an eager import receptor arrives in the nucleus carrying its cargo and is then approached by RanGTP. The import receptor binds to RanGTP, which causes the receptor to undergo a conformational change and release its cargo, completing import. Export receptors show the opposite behavior. They strongly bind to their export cargo (for example, a nuclear protein containing an NES) only when simultaneously bound to RanGTP. The heterotrimeric complex of export receptor/RanGTP/NES-bearing protein translocates through the nuclear pore, a structure ~400 times the size of the export complex. Immediately upon reaching the opposite side of the pore, the export complex dissociates due to the RanGAP tethered to the cytoplasmic filaments of the pore.

Individual organisms contain at least 10–20 members of the importin β receptor family. Some are import receptors, specialized for ferrying different nuclear proteins or snRNP cargo into the nucleus. Other receptors are specific for exporting nuclear proteins, 5S RNA, tRNA, or ribosomal subunit precursors to the cytoplasm. Although most nuclear give-and-take uses importin β-like receptors, the export of mRNA apparently does not. During the splicing process, mature mRNA, a very large cargo, appears to form a complex with a variety of distinct non-importin β-type proteins that together mark the mRNA for export and participate in its egress from the nucleus.

Nuclear Transport provides an overview of much of our current knowledge in the field, summarized in nine chapters by different contributors. Despite variations in style and depth of coverage, the authors have made a largely successful effort to provide an overview of the basic mechanism of transport, as well as comprehensive snapshots of our understanding of specific areas of nuclear transport. The breadth and the highlighting of controversial issues and unanswered questions are strengths of the book.

Strambio-de-Castilia and Rout begin by reviewing many aspects of the *Saccharomyces cerevisiae* nuclear pore complex, revolving around the impressive proteomics work recently published by Rout and collaborators (Rout et al., J. Cell Biol. *148*, 635–651, 2000). The yeast nuclear pore is compared and contrasted to the larger and more complex vertebrate nuclear pore in the next chapter by Fahrenkrog and Aebi. This chapter puts greater emphasis on structural methods, including recent work involving cryo-electron tomography and atomic force microscopy, techniques which are hoped will improve our understanding of the three-dimensional architecture of the nuclear pore.

Also included in the review by Rout is his model for transport, which is presented in a measured and interesting way. Missing from the book, however, is a rendition of a competing model for the mechanism of translocation by Ribbeck and Görlich, EMBO J. *20*, 1320–1330, 2001). It should be noted that, although each of the models has its strong points, experimental proof of such models is hard to come by. Indeed, it has been very difficult to confirm a specific translocation mechanism for the nuclear pore, which contains multiples of 30–50 different proteins in the final 500–1000 protein nuclear pore complex.

In the third chapter of *Nuclear Transport*, Bischoff et al. focus on the small GTPase Ran, a molecule that is appropriately crowned the "king" of nuclear transport elsewhere in the book. This review provides a wealth of structural and biochemical information on the function and regulation of Ran.

Four chapters describe different export receptors and pathways. In the first of these, Fornerod and Ohno focus on the receptor that mediates the export of NES-bearing nuclear proteins and certain ribonucleoprotein complexes, exportin 1 or Crm1. This accessible chapter not only provides a review of the subject and some of the historical background leading to the discovery of exportins, but also discusses general principles relevant to other types of transport receptors. Simos et al. review all aspects of exportin-t, a β-like receptor that is unique in that it directly recognizes an RNA molecule, i.e., tRNA, and exports it to the cytoplasm. Izaurralde next describes the exciting developments in the area of mRNA export. Particularly informative to these reviewers was a final export review by Cullen, detailing the lessons retroviruses teach us on nuclear export.

The comprehensive set of export chapters is punctu-