Advances in Experimental Medicine and Biology 844

Seth J. Corey
Marek Kimmel
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A Systems Biology Approach to Blood

Springer

Advances in Experimental Medicine and Biology

VOLUME 844

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ISSN 0065-2598 ISSN 2214-8019 (electronic)
ISBN 978-1-4939-2094-5 ISBN 978-1-4939-2095-2 (eBook)
DOI 10.1007/978-1-4939-2095-2
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2014952025

Printed on acid-free paper

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Part I
Basic Components

The blood system is multi-scale, from the organism to the organs to cells to intracellular signaling pathways to macromolecule interactions. Blood consists of circulating cells, cellular fragments (platelets and microparticles), and plasma macromolecules. Blood cells and their fragments result from a highly-ordered process, hematopoiesis. Definitive hematopoiesis occurs in the bone marrow, where pluripiotential stem cells give rise to multiple lineages of highly specialized cells. Highly-productive and continuously regenerative, hematopoiesis requires a microenvironment of mesenchymal cells and blood vessels.

In this first section, we shall cover the important components of blood: beginning with the microenvironment and then focusing on erythrocytes, megakaryocytes, phagocytic cells, and platelets. In Chap. 1, the editors of this volume provide a multidisciplinary overview of hematopoiesis and systems biology. This should serve to introduce hematology to the quantitative and modeling scientists as well as to introduce basic mathematical principles and Text modeling to the hematologists. In Chap. 2, Krinner and Roeder discuss the interactions among hematopoietic stem cells and the microenvironment. No other tissue undergoes the tremendous amount of regeneration and accurate specialization of diverse tissues as the blood system. Fortunately, defects in production (overproduction or underproduction) are infrequent occurrences. In Chap. 3, Socolovsky and associates focus on how erythropoietin drives production of red blood cells through basal and stress conditions. This highlights an important property of the blood system—the ability to function for the most part within a narrow range of physiological conditions and still retain the dynamic capacity to respond quick to stressful stimuli and other environmental changes. In Chap. 4, the Kaushanskys describe in detail thrombopietin's intracellular signaling that drives the differentiation of megakaryocytes. Interestingly, many of its proximal components are found activated in response to other cytokines. In Chap. 5, Alber and colleagues detail how platelets are formed from megakaryocytes and how they become activated. Platelet production and homeostasis highlights their clinical significance—a sufficient number of platelets must remain quiescent and then be able to respond briskly to bleeding. Too many platelets and too much activation result

in life-threatening clots; whereas too few platelets and too little activation result in life-threatening bleeding. Lastly in this section, in Chap. 6, Corey and colleagues discuss granulocytes and monocytes, two critical components in innate immunity.

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Chapter 1
Systems Hematology: An Introduction

Seth Joel Corey, Marek Kimmel and Joshua N. Leonard

Abstract Hematologists have traditionally studied blood and its components by simplifying it into its components and functions. A variety of new techniques have generated large and complex datasets. Coupled to an appreciation of blood as a dynamic system, a new approach in systems hematology is needed. Systems hematology embraces the multi-scale complexity with a combination of mathematical, engineering, and computational tools for constructing and validating models of biological phenomena. The validity of mathematical modeling in hematopoiesis was established early by the pioneering work of Till and McCulloch. This volume seeks to introduce to the various scientists and physicians to the multi-faceted field of hematology by highlighting recent works in systems biology. Deterministic, stochastic, statistical, and network-based models have been used to better understand a range of topics in hematopoiesis, including blood cell production, the periodicity of cyclical neutropenia, stem cell production in response to cytokine administration, and the emergence of drug resistance. Future advances require technological improvements in computing power, imaging, and proteomics as well as greater collaboration between experimentalists and modelers. Altogether, systems hematology will improve our understanding of normal and abnormal hematopoiesis, better define stem cells and their daughter cells, and potentially lead to more effective therapies.

Keywords Hematology · Models · Reductionist · Systems biology

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S. J. Corey et al. (eds.), *A Systems Biology Approach to Blood,*
Advances in Experimental Medicine and Biology 844, DOI 10.1007/978-1-4939-2095-2_1

Blood, pure and eloquent, wrote Max Wintrobe, one of the pioneers of modern hematology. His description was but a reference to lines written by the seventeenth-century English poet John Donne. Hematology, the study of blood and its components, has undergone dramatic changes over the millennia, since man first recognized its power. The first plague visited upon the Egyptians was blood, "I will strike the water of the Nile, and it will be changed into blood. The fish in the Nile will die, and the river will stink and thus the Egyptians will not be able to drink its water." What we consider so vital to human life was viewed as deleterious. Rabbis later writing commentary warned against circumcising a third son after two had died of bleeding. Contemporaneously, Hippocratic writings described blood as one of the four humors. More appreciative of its vital nature, the Greek physicians equated blood with spring and air. The Greek word for blood, *haima*, has been sustained in all things hematologic and hematopoietic.

Like other branches of medicine, hematology has undergone paradigm shifts. From the ancient Jews' and Greeks' attribution of blood to health and disease through the seventeenth century's rationalists who described its circulation through arteries and veins, to the modern physiologists of the past century, our understanding of blood and its components has advanced. The past 50 years have provided us with more intimate knowledge of its components at the subcellular level. This reductionist approach to science has now been superceded by the awareness of complex, large datasets, made possible by proteomic, flow cytometric, microarray, genomic sequencing, and epigenetics. The complexity of blood and its components is also recognized at multiple levels from the subcellular to the macroscopic, such as the environment and its effect on the organism. While physical and chemical laws have been applied to biology, limitations to their applicability and predictability are frequently encountered. Biology is dynamic.

The biomedical discipline that has been called physiology has evolved to a new approach—a modern synthesis of biochemistry, genetics, mathematics, engineering, and machine-based learning. Complex, large datasets of genes, lipids, metabolites, and proteins have made it impossible for one investigator to intuit the whole. This new, integrative field has been called systems biology. In this volume, we seek to introduce physicians and scientists, qualitative and quantitative, to the different facets of systems hematology. Systems hematology embraces this complexity, utilizing engineering principles and computational methods to build and validate models using experimental data. The approach rests on (i) defining all (or the known knowns) of the components, (ii) systematically perturbing and monitoring the components of the system, (iii) reconcile the experimentally observed responses with those predicted by the model, and (iv) designing and performing new experiments to distinguish between multiple or competing models. The goals are to understand how the system works, identify new systems-based properties, and predict outcomes.

The major obstacle to success in systems biology lies in the disciplines practiced by physicians and scientists. Major differences exist in the methods, jargon, and philosophies between quantitative scientists, the theoretical physicists, the mathematicians, the engineers, computer programmers, and experimentalists. Even within the experimentalists, there is diversity and increasing technologization, as evidenced

by cell biology, molecular biology, and proteomics. Until there is a common vernacular, fundamental concepts in the fields of biology, mathematics, engineering, and computation can be understood and transdisciplinary studies can be successful.

Blood as a System

Biological systems operate at multiple levels (or scales): molecular, cellular, tissue, and organismal, and environmental. Stem cells generate differentiated blood cells through a continuous process of asymmetric stem cell division, yielding daughter cells with different capacities for renewal or differentiation. This process occurs in a specialized microenvironment. The blood system consists of highly specialized cells and plasma containing a range of proteins to regulate different processes. Among the blood cells are erythrocytes that shuttle oxygen or its waste product to and from tissues; white blood cells to fight infection and mediate inflammation; and platelets to stop bleeding. Within the compartment of white blood cells, there is variability: neutrophils to engulf foreign agents, lymphocytes to make antibodies and coordinate immunity, and monocytes to process and regulate host defense. Plasma contains more than 1000 proteins [1]. Homeostatic mechanisms insure that the right number of cells is produced, but they are sufficiently dynamic to meet the needs of environmental changes (e.g., hypoxia, infection, or bleeding). While hematologists diagnose and treat patients with anemias, immune deficiencies, leukemias and lymphomas, and hypercoagulability, it is astonishing that such high level of quality control of blood and its elements exists and that blood diseases are not more common.

Systems Properties in Hematopoiesis

Because of the facility in sampling blood or bone marrow repetitively and quantitatively, the blood system is well suited for modeling and validation. Hematopoiesis and the functioning of specialized blood cells involve complex processes that can be examined at the level of genes [2], signal transduction proteins [3], or the population distribution of diverse cell types [4]. Both deterministic and stochastic processes contribute. By viewing hematopoiesis (cell proliferation and differentiation) as a dynamic system and disease as perturbations of the system, one can learn more about both disease and physiological states.

Proliferation and loss are fundamental properties of hematopoietic stem cells and their progeny. Population dynamics offers a quantitative approach in studying them. Asymmetric division results in a stem cell dividing into either another stem cell or a more committed cell, while symmetric division yields either two stem cells or two differentiated daughter cells. These processes can be combined in a series of short steps [5–8]. Models built around these division (a)symmetries usually result in exponential cell growth, but such growth cannot be realistically sustained in vitro

due to spatial and nutrient limitations. Models based on heterogeneous population account for cell proliferation and loss due to death or differentiation.

Differentiation is the other fundamental property of hematopoietic progenitor cells and requires critical processes of cell fate decision making. Decision making occurs as a result of biochemical signaling and gene regulatory networks within the cell [9], [10]. Ultimately, transcription factors determine cellular differentiation and specialization [11]. The relative contributions of instructive and permissive programming in hematopoiesis have long been debated [6, 12–23]. To describe hematopoietic stem cell renewal and differentiation, deterministic and stochastic models have been constructed. James Till, a biophysicist, and Ernest McCulloch, a physician, pioneered the study of hematopoiesis in the early 1960s through their development of a quantitative spleen colony assay, establishment of a hematopoietic stem cell, and data analysis that yielded a stochastic model of hematopoiesis [24], [25]. In their stochastic model [5], cells have two possible fates: (1) differentiate and leave the proliferative compartment or (2) undergo symmetric division forming two colony-forming cells. Each fate was assigned a probability. Drawing random numbers to determine the fate of each cell, Till and McCullouch calculated the diversity of stem cell populations after the course of several generations. Colony generation appears as a well-defined process even though individual cell-fate decisions are random. Regulation acts at the population, not cellular, level and the population of stem cells can be affected by influencing processes that define the effective probabilities of birth and death.

A cell uses complex intracellular signaling and gene regulatory networks in order to integrate the multiplicity of cues in its environment and to ultimately make a specific decision. In particular, gene regulatory networks have provided great insights into lineage commitment of hematopoietic progenitors.

Types of Mathematical Models

Different methods of modeling have been developed to describe and predict biological processes. Not all models are accurate, but some are more useful than others. Deterministic models describe the state of a system over time in the absence of random events. These always produce the same output for a given input [26]. In contrast, stochastic models describe the effects of randomness and noise on system output [27]. Statistical models use existing data to estimate a functional relationship between system input and output. Network models graph the direction and magnitude of interactions that exist between the various components in a system [28].

Deterministic models typically consist of one or more differential equations, with each equation describing the change in a system state variable over time, as it depends on other system variables and rates. If the state variable of interest is the number of cells in the population, a differential equation modeling the change in the population over time would consist of the difference between rates of cell production and rates of cell loss:

$$\frac{dN_X}{dt} = (\text{rate at which precursor of X differentiates into X}) - (\text{rate at which X differentiates into next cell lineage}) - (\text{rate at which X dies}) \quad (1.1)$$

where N_X is the number of cells of type X.

Each equation describes the rate of change in the number of cells of given type and maturity in the system by including terms for the rates of cell production, death, and differentiation. Once the equations are established, they are solved either analytically or numerically to determine the population's functional dependence on time. In models describing physiological conditions, the equations tend toward a steady-state solution representing system homeostasis; that is, after sufficient time has elapsed, positive and negative contributions to cell number balance and the population attains a constant level (e.g., $dN_X/dt = 0$ in Eq. 1.1). For disease-state cell populations, other types of behavior such as oscillations or uncontrolled growth are frequently modeled.

Stochastic models are employed to examine the effects of intrinsic and extrinsic randomness on a system. Intrinsic randomness arises from interactions of a finite ("small") number of discrete components, e.g., binding of a given gene's promoters (two copies per diploid genome) by transcription factor's molecules (also a limited number). Extrinsic randomness arises either from variability (genetic and phenotypic) among cells or from environmental fluctuations. The most common type of stochastic model is a Markov process, in which the future state of the system depends only on its current state and is independent of its past states. Monte Carlo simulations are an empirical method to investigate dynamics of a stochastic system, by generating repeated random trajectories and computing frequencies that estimate probability distributions.

Statistical models are sometimes confused with stochastic models. Whereas stochastic models reflect the structure of the biological system, statistical models are data driven. Statistical models can be employed even when no knowledge about system's structure exists and can generate predictions, which may be only statistically validated. However, some statistical models such as Bayesian networks may provide insights concerning the structure. Bayesian network models are built from graphs in which the states of and relationships between network elements are probabilistic. While graph theoretical models can be circular, Bayesian networks have a definite, distinct set of termini. These models have a wide range of uses. For example, a Bayesian network model could be used to predict the probabilities of certain cellular mutations based on abnormalities in protein expression levels (assuming, of course, that there is a relationship between the two). Their structure and necessary constants have to be estimated based on data. Though popular, Bayesian networks suffer from the possible reversal of causality [29].

Network models have recently gained popularity in the social, physical, and biological sciences from the widespread application of graph theory**,** an area of mathematics that investigates the relationships between the objects of a group [30]. Graph theory lends itself to visual representations making it an appealing tool for biologists investigating phenomena ranging from the interactions between populations

in an ecosystem to the interactions between molecular species involved in a signaling pathway. At its simplest, a graph is a map of all known system components or system states and their possible interactions or transitions. Circles (nodes) represent components and states, and lines and arrows (branches or edges) represent relationships between nodes. Graphs help portray topological structures such as loops. Complex dynamics can arise from relatively few interacting components [31], and network maps are widely used to help visualize the interactions. Building upon existing graph theoretical notation, an international group has developed Systems Biology Graphical Notation to standardize the visual representations used to describe biological interaction networks [32].

Current Status of Systems Biology

The success of systems analysis of hematopoiesis will depend upon technological breakthroughs and collaborations between the biological and physical sciences that yield accurate predictions and emergent properties. With each discipline using a different language, this is easier said than done. Changes in undergraduate, graduate, and medical curricula must be implemented to train a new generation of biomedical researchers fluent in quantitative or engineering disciplines [33–35]. Systems biology requires a balance between models sufficiently complex to describe a system and yet simple enough to be clinically useful. Understanding large quantities of data well enough to validate a model is especially challenging. The development of Systems Biology Markup Language (SBML) has made it easier to develop biology-oriented software packages, such as COPASI, Simmune, MetaCore, and Cytoscape, which aid model building and data analysis [32, 36–39]. Since 2001, the number of such packages developed for systems biology has grown from 5 to over 170. With computational power becoming ever greater and cheaper, the number and diversity of such software packages will only increase, bringing within their scope models that may not be impossible to validate with current technology. At present, most models of hematopoiesis are built at a single scale, e.g., cellular or molecular. The future lies in building models that span multiple scales, incorporating more of the connections that exist between them and thereby being able to account for some of the complexity that arises from the connections. Among the fundamental questions in normal and leukemic hematopoiesis that systems biology will address are: integration of signaling pathways, circuits, and networks that determine cell fate, multi-scale modeling of stem cell plasticity, synthesis of genetic and epigenetic data, global analysis of phosphoproteins, dynamics of hematopoiesis in the bone marrow microenvironment presented in three-dimensional imaging, and cellular engineering to expand selective blood cell compartments for therapy. The complexity or density of experimental data will demand a systems approach. More in-depth coverage may be found in the few textbooks of systems biology and bioinformatics that have appeared, none solely devoted to hematologic topics [40–43].

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Chapter 2
Quantification and Modeling of Stem Cell–Niche Interaction

Axel Krinner and Ingo Roeder

Abstract Adult stem cells persist lifelong in the organism, where they are responsible for tissue homeostasis and repair. It is commonly assumed that their maintenance and function are facilitated in local environments called "stem cell niches." Although there is convincing evidence that a variety of niche components determine stem cell fate, the regulatory details of stem cell–niche interactions are widely unknown. To pave the way for a substantiated discussion of these interactions, we first focus on the stem cells themselves and describe the stem cell defining criteria and their implications. The fate of the cells that fulfill these criteria is regulated by a broad spectrum of factors and regulatory mechanisms. A summary of established components and their action is given exemplary for the hematopoietic system. The complexity resulting from the interplay of various cell types, signaling molecules, and extracellular structures can be boiled down to important key features as exemplified by the presented model of hematopoietic stem cell organization. Although neglecting many details, we show that this and similar models have the power to yield intriguing results as proven by the agreement of the presented model with experimental data and the predictions derived from model simulations. Finally, we will discuss the paradigm of systems biology and give a summary of the techniques that promise to unveil further details of the organization principles of stem cell niches at different levels. The synergistic effect of the described techniques together with the integration of their results into a unified model that allows quantitative evaluation and predictions may lead to a better and more systematic understanding of the most relevant niche elements and their interactions.

Keywords Stem cell niche · Hematopoiesis · Mathematical modeling · Systems biology

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S. J. Corey et al. (eds.), *A Systems Biology Approach to Blood,*
Advances in Experimental Medicine and Biology 844, DOI 10.1007/978-1-4939-2095-2_2

Introduction

Although it is generally accepted that microenvironmental cues play a key role in regulating stem cell function, and although many individual regulatory mechanisms and pathways of cell–microenvironment interaction have been identified, a systemic understanding of stem cell–microenvironment interaction and its impact on stem cell fate regulation is still missing. This is also the case for hematopoietic stem cells (HSCs), which have been extensively studied for more than 40 years, starting, e.g., with the pioneering work of James Till and Ernest McCulloch in the early 1960s. The two scientists were able to demonstrate the existence of undifferentiated hematopoietic cells in the bone marrow (BM) that are capable of both, self-renewing and differentiating—two features that are classically used to define cells as *stem cells*. Based on serial transplantation experiments, Till and McCulloch showed that these (stem) cells are able to develop into spleen colonies of irradiated mice, which contain cells with an identical potential [1–3]. These were called colony-forming units in spleen (CFU-S cells) and regarded as stem cells. Later, they turned out to be progenitor cells, which are, in contrast to true stem cells, characterized by only a limited self-renewal and repopulation potential.

Clearly, the origin of CFU-S cells was the BM, but it was by no means clear, whether there are specific regions in the BM that functionally support stem and/or progenitor cells. Unlike other stem cell systems, such as the intestinal crypt [4], the BM is lacking an obviously structured spatial arrangement. This absence of clearly visible, stem-cell-supporting areas widely hampered the study of HSCs and their interactions with local microenvironmental components in the in vivo situation. Nevertheless, the perspective of an instructive local microenvironment of HSCs was introduced already in the early 1970s by John Trentin [5, 6] and Raymond Schofield [7]. Schofield proposed a concept that includes a context dependency of stem cell behavior. In this concept, stem cells live in a certain environment, the *niche*, where differentiation and maturation is prevented and thereby continuous proliferation and maintenance of stem cell potential is guaranteed. Therefore, stem cells lose their potential, if they lack this specific environment. This concept is consistent with the results of contemporary coculture experiments. For instance, Dexter and coworkers were able to maintain proliferative CFU-S cells over several months in vitro using a mixture of feeder cells from the BM, whereas these cells differentiated if cultured without feeder cells [8, 9].

Since these days, new ideas and experimental techniques have extended the list of cells and other microenvironmental factors that presumably act in combination to form the stem cell niche. Other factors, such as geometry and biomechanics, nutrient supply, signaling molecules, metabolic conditions, and contact dependent cues have been shown to contribute to the niche environment, too. Later in this chapter, we will give an overview of some important examples of these presumably stem-cell-regulating niche components with a particular focus on the hematopoietic system.

Defining Stem Cells

Before talking about stem cell regulatory components and effects of a niche environment, we need to precisely define what we mean by a stem cell or by stem cell potential. Because the term *stem cell* resulted from the conceptual aftermath of the discovery of a multipotent and self-renewing cell population, its definition almost exclusively contains functional criteria. Only in the case of embryonic stem (ES) cells [10, 11], the functional definition has its counterpart in a definition by origin. When the blastula is formed, this cell population emerges from the first differentiation step, the separation of trophoblast and inner cell mass. While the first forms only extraembryonic structures, all cell types of the embryo itself develop from the cells of the inner cell mass. Therefore, these cells are characterized as *pluripotent*. They are the source for the in vitro derivation of ES cell lines, which are usually denoted as *pluripotent ES cells*, as they preserve the potential to differentiate into cells of all tissue types. In vivo, the development of the embryo involves further differentiation steps beginning with the development of three germ layers. From those, the different tissues are derived and with this specification process the ability of the cells to generate cells from other tissues is lost. Pluripotency, therefore, turns into *multipotency*. Multipotent cells still have the potential to differentiate into various cell types of a particular tissue and are maintained as so-called *(adult) tissue stem cells* lifelong. They preserve their proliferation and self-renewal capacity as well as their multilineage potential in order to guarantee homeostasis and to repair damaged tissues, which represents the core of their functional definition [12].

Whereas the details of the definition of a tissue stem cell depend on its author, functional characteristics such as self-renewal, differentiation, and proliferative potential were always cornerstones of this definition. Tissue stem cells are defined by a number of qualities, which enable them to guarantee a lifelong maintenance and, in case of injury, to reconstitute a fully functional tissue. Over the years and with new experimental results, the definitions have been modified and a more flexible interpretation of this concept of a *functional* definition has been introduced. Flexibility has been included in the sense that stem cell fate decisions depend on the environment. This dependence results in some flexibility or even reversibility of stem cell properties and functionalities [13].

A general problem with the *functional* definition is the fact that it does not allow for a prospective selection of stem cells on an individual cell basis: Any particular assay (e.g., a colony formation assay) that is required for the examination of a particular cellular function (e.g., proliferative potential) will always alter the state of the cell. Therefore, the assessment of one function of a particular cell might impair the assessment of any other of its functions by another assay. In other words, the measurement process itself (to test for stem cell functionality) alters the object of measurement. This perception is the reason why Potten and Loeffler [14] compared this dilemma to Heisenberg's *uncertainty principle of quantum physics*. Although this analogy is certainly not perfect, it points to a very important aspect that applies to both areas: Any prospective statement about the function of a particular object (in

our case a potential stem cell) can only be made in a probabilistic sense. This should be kept in mind if talking about stem cells; we will come back to this aspect later.

To meet this problem of characterization and selection of tissue stem cells, scientists have put large efforts in the development of purification protocols that enrich a cell population for functional stem cells. Fluorescence-activated cell sorting (FACS) applied simultaneously to a large set of cell surface markers has led and still leads to continuously refined selection protocols. Latest protocols allow for very high enrichment rates of HSCs with long-term repopulating ability (LTRA), which are considered as the *true* HSCs. As an example, the *Lin-Sca+c-Kit+* (*LSK*) *CD34-SLAM* (*CD244-CD48-CD150+*) marker combination allows to enrich mouse primary BM cells to a degree of up to one LTRA-HSC in two target cells [15, 16]. Surprisingly, for most of these markers, no functional, mechanistic link to LTRA has been found. However, it should be noted that despite the high enrichment, prospective statements about the purified cells are still only possible in a statistical, probabilistic sense.

Furthermore, there are two other flaws that are inherently connected with this characterization approach. First, for the application of sorting protocols, the cells have to be removed from their natural habitat. As mentioned above, such a treatment might alter cellular properties during this time of in vitro culture due to the dependence of stem cell properties on environment. Second, for assessment of in vivo functionality, the cells have to be reinjected into host animals. Usually lethally irradiated mice provide the environment that guarantees efficient engraftment. Unfortunately, irradiation does significantly damage the niche environment and the physiological structures in the BM [17–19]. Therefore, cellular and microenvironmental effects are inevitably confounded by the application of such assay protocols.

These two remarks bring us back to the role of the local microenvironment. In our opinion, it does not make sense to talk about HSCs without considering these cells as being embedded in a particular environmental context. This is most likely also true for any other tissue type. However, in the following, we will focus on the hematopoietic system and use HSCs as a model system to describe a general approach to systematically analyze the underlying mechanisms of microenvironment-based stem cell regulation. Herein, we will focus on a description of (potential) regulatory components of the stem cell niche and on mathematical modeling approaches to study the systems dynamics of stem cell–niche interactions. These two major paragraphs will be complemented by some thoughts about a potential road map for a more complete understanding of stem cell–niche interactions.

Components of the HSC Niche

Already decades ago, the BM has been identified as the natural environment and, therefore, a “niche” of HSCs. Basically, it is composed of a scaffold of extracellular matrix components, a cell population comprising cells of various lineages, and a fluid filling the rest of the space (Fig. 2.1). In the marrow, two main structures are

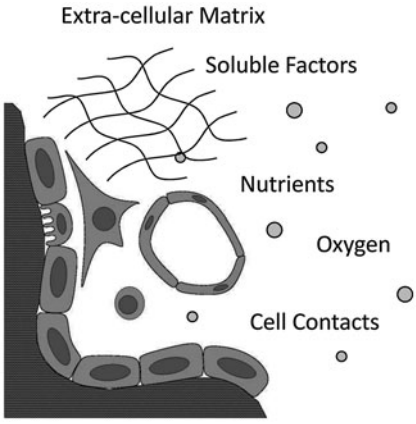

Fig. 2.1 Components of the niche. The niche environment comprises several factors. All of them are dynamically dependent on the cells of the niche environment. They provide growth factors, build and remodel the extracellular matrix and constitute endosteum and vascular network

obvious, mineralized bone and vascularization. Most of the cell types found within the marrow have been attributed to either of these basic structures. Directly associated with the bone is its lining, the endosteum. It is mainly composed of undifferentiated mesenchymal bone-lining cells and the two bone-remodeling cell types, osteoblasts (OBs) and osteoclasts. Also, there is the vascular system connecting the marrow to the rest of the organism by vessels and sinusoids. The walls of these tubular structures are formed by endothelial cells (ECs), which coexist with so-called perivascular cells in their direct vicinity.

There are a number of reports proposing that these two structures represent two distinct local environments in the BM: the endosteal and the perivascular environment, which form two distinguishable stem cell niches fostering different stem cell populations [20]. Whereas the so-called *endosteal niche* is associated with proliferative quiescence (low cell cycle activity), the *vascular niche* has been described to support stem cell proliferation [21, 22]. As a consequence of the two different environments, stem cells with LTRA are found preferentially in the endosteal niche, while the vascular niches hosts stem and progenitor cells with only short-term repopulating ability (STRA) [23]. In this view, the dormant cells form a reserve pool for emergencies, which can be repopulated after a potential emergency operation [23]. In contrast, a more recent study suggests a continuous and frequent exchange of cells between quiescent and proliferative states [24]. The hypothesis inevitably comes up that this exchange happens between the two niche environments. However, these studies only quantify the number of cell divisions in a certain time (using label retaining experiments), but their observations do not link transitions between dormant and proliferative states to translocations between the two niches.

A thorough identification of niche environment and function would require a separation of the two niches. As already shown by early histological studies, the interior vascular system of the bone is connected with the exterior system by a dense system of vessels through mineralized bone, which consequently indicates highly vascularized endosteum [25]. This difficulty of defining a spatial separation

of vasculature and endosteum was recently confirmed by in vivo tracking experiments of HSCs in mice [26]. By fluorescence staining of blood, OBs and injected HSCs, relative positions of HSCs, vasculature, and OBs were measured. In this way, it was shown that sinusoids are abundant in the whole BM, though more dense in the BM cavities [26]. Therefore, a rigorous spatial separation of the two hypothesized niche environments seems impossible. Taking one step beyond, this might suggest integrating the signals emanating from the two presumed "niche environments" into one self-organizing system featuring one continuous niche. This view is supported by the fact that concentrations of various soluble molecules, e.g., chemokine (C-X-C motif) ligand 12 (CXCL12, also known as stromal cell-derived factor, SDF-1), stem cell factor (SCF), or osteopontin (OPN), seem to exhibit continuous rather than step-like gradients. Also, the supply of nutrients and oxygen continuously changes with distance from the bone surface. The latter observation was the origin of yet another idea, the metabolic niche [27, 28]. In such a continuous niche, all components may be present throughout the niche, although with certain tendencies or activities. We will now summarize these components of the niche by describing the cells themselves and their role within the BM, because they represent the active components in the BM that are motile, remodel the bone, and produce HSC-supporting factors.

Hematopoietic Cells

Hematopoietic Stem and Progenitor Cells

An important contribution to the niche organization is made by the HSCs themselves. It is their active migratory behavior that finally determines the niche by bringing them into particular environmental conditions and keeping them there. For example, most dormant HSCs are detected in an isolated position [23]. Also, it has been reported that dormancy and LTRA is associated with cells homing close to the endosteal surface [21]. Furthermore, several properties related to HSC migration, such as membrane fluctuations, cell adhesion, and cell motility, vary with distance to the bone [29]. A prominent cell-adhesion molecule that has been in the focus of discussion in recent years is N-cadherin. Intermediate levels of N-cadherin expression have been reported to indicate a quiescent state, while activated cells express low levels [30]. However, the conditional knockout of N-cadherin in mice illustrates the complexity of the niche system, since it caused no observable change in HSC frequency or repopulation potential [31]. An interesting link to the metabolic niche is given by the observation that reactive oxygen species downregulate N-cadherin in HSCs [32]. Further support of a hypoxic BM niche comes from Parmar and colleagues. They used a perfusion tracer to identify the location of most HSCs in an area of low perfusion [33]. Also consistent with the idea of a hypoxic in vivo niche is the analysis of HSCs in hypoxic culture. In vitro hypoxic conditions induce quiescence in hematopoietic cells [34] and support the Hoechst-stained side population in LSK cells that is commonly accepted as a typical HSC quality [35].

Many different factors have been identified in the context of the stem cell niche, including Angiopoietin-1 (Ang-1) [36], Kit-ligand (Kitl) [37], CXCL12 [38], thrombopoietin (TPO) [39], and OPN [40]. However, in most cases, the identity of their key cellular sources promoting this maintenance remains unclear. Just now conditional knockouts of known factors in hematopoietic cells begin to reveal the cell types most that are most important for a particular signaling route [41].

Macrophages and Monocytes

Recent studies suggest a key role for monocytes in maintenance of HSCs [42–44]. Chow et al. applied four different techniques to induce specific loss of defined subpopulations of monocytes and macrophages [42]. Loss of the addressed cells resulted in HSC mobilization into peripheral blood and spleen. It was accompanied by a 40 % reduction of CXCL12 that is known to critically regulate niche retention of HSCs via activation of its receptor CXCR4 [45, 46]. Addressing the transcription of CXCL12 and other HSC retention factors in stromal cells, it was shown that CXCL12, SCF, Ang-1, and vascular cellular adhesion molecule 1 (VCAM1) mRNAs were not reduced in OBs but in Nestin-positive osteoprogenitors/mesenchymal stem cells (MSCs). Interestingly, total cell numbers of both populations were not affected. These results indicate that the key factors themselves are regulated by further components as in this case the macrophage/monocyte cell numbers. In a similar approach, Winkler et al. [44] depleted phagocytes and also observed mobilization of HSCs. Transcripts of CXCL12, Ang-1, and SCF decreased in total BM and in endosteal stroma, too. Most striking was the simultaneous loss of osteomacs, a particular macrophage subpopulation specifically associated with the endosteal lining [44, 47]. Additionally, a significant reduction of bone remodeling activity was observed. In the depleted system, the proportion of bone surface lined with OBs and the amount of newly formed bone matrix decreased significantly. Thus, both studies nicely illustrate two aspects of the regulation of the stem cell niche: the tight interaction of different cell types, here HSCs, macrophages, and osteoprogenitors, and the complexity resulting from combination of various feedback mechanisms such as bone remodeling, cell numbers, and HSC mobilization.

Osteoclasts

Although osteoclasts take part in the process of bone remodeling, they do not belong to the mesenchymal lineage like OBs and osteocytes, but are derived from hematopoietic cells [48]. They are responsible for bone resorption and, therefore, for Ca2+ blood levels. The calcium-sensitive receptor (CaR) is expressed on various hematopoietic lineages and, in particular, on LSK cells [49, 50]. Ca signaling and its role in niche regulation were investigated by studying a CaR -/- mouse model [49]. In CaR -/- mice, BM cellularity and relative frequency of LSK cells among hematopoietic cells were clearly reduced. The function of fetal liver mononucleated CaR -/-

cells was tested by their transplantation into irradiated mice, and although 100 % survival was observed, homing of these cells in the BM was markedly reduced [49]. Despite no differences in surface expression of many homing related molecules (e.g., CD49d, CD62L and CXCR4) was found, they also showed a remarkably reduced adhesion to one of the main components of bone, collagen I. All together the osteoclasts represent another niche player that intimately connects signaling, extracellular matrix, cell migration, and control via differentiation.

Mesenchymal Cells

Mesenchymal Stem and Progenitor Cells

Like HSCs, MSCs are defined by their functional potential to self-renew, proliferate, and differentiate. As for HSCs, a strictly phenomenological characterization is limited. For MSCs, the multilineage potential comprises three main lineages: the chondrogenic, adipogenic, and osteogenic lineage [51]. In the BM, they directly participate in the regulation of hematopoiesis as adventitial reticular cells (ARCs) in humans [52] or in mice as CXCL12-abundant reticular (CAR) cells [38] or Nestin-positive cells [53]. Additionally, they differentiate into two other cell types that are involved in the control of a HSC niche: OBs [e.g., 54, 55] and adipocytes [56]. Within the BM, they are found in the reticular space as mural or subendothelial cells [57]. Definitely impressive is the variety of cytokines expressed by MSCs that are involved in niche regulation: SCF, leukemia inhibitory factor (LIF), SDF-1, Oncostatin M (OSM), bone morphogenetic protein-4 (BMP-4), Flt-3, and transforming growth factor-β (TGF-β) [57]. MSCs are also capable of producing a variety of interleukins [58], niche related adhesion molecules such as VCAM1 and N-cadherin [52, 53] or even the key hematopoietic growth factors G-CSF and GM-CSF [58]. However, since most of the related experiments have been carried out in vitro, their interpretation regarding the in vivo situation should be done with caution. The role of stromal cells for HSC fate was shown early by their coculture with HSCs where they support proliferation and differentiation in vitro [59]. Another indication of their role as niche keepers is given by subcutaneous transplantation of CD146 + MSCs into immunodeficient mice, where they are able to generate heterotopic BM, trigger its vacularization, and there eventually give rise to hematopoiesis [52].

Osteoblasts

Multiple studies have shown that OBs play a crucial role in supporting HSCs. Genetic data indicate that functional stem cells do need to interact with OBs [16, 20, 55]. In these studies that involved transgenic mice to address the effect of the factors BMP and parathyroid hormone, the number of the stromal pool of OBs was found to correlate with HSC number involving Notch-ligand and N-cadherin interactions

[54, 55]. Coculture with endosteal cells characterized by typical osteogenic markers (such as alkaline phosphatase and OPN) maintains the pluripotent state and hinders HSC proliferation [59] confirming the role of OBs in HSC regulation. Direct communication between HSCs and OBs is given, for example, by Ang-1/Tie2 signaling, which has been reported sustain HSC quiescence [36]. Thus, Ang-1/Tie2 signaling might directly correlate with the long-term repopulation ability of HSCs. However, two details that are mentioned rather rarely have to be considered: (1) OBs are a transient cell state in the osteoblastic lineage finally leading to osteocytes and (2) bone deposition by OBs is a dynamic process restricted to less than 10 % of the bone surface in adults [60]. This leads to the question on the influence of other cells in the osteoblastic lineage and the mechanisms of regulation. If only OBs would enable hematopoiesis and this regulation would act on a purely local scale, hematopoiesis would be limited to the sites of bone deposition. The solution for this conflict might be found in the role of pre- and post-osteoblastic stages. While the role of osteoprogenitors has already been confirmed, it remains elusive whether the abundant osteocytes contribute a regulatory function in the niche.

Adipocytes

The triple differentiation potential of MSCs includes both, osteogenic, and adipogenic lineages. Generally, lineage commitment is an exclusive choice and, therefore, the HSC-supporting OB population competes with the adipocytes for progenitor cells. Interestingly, a study evaluating the occurrence of HSCs in different body regions of wild-type mice and in fat-free transgenic mice has shown that the number of adipocytes in the BM correlates inversely with hematopoietic activity of the BM and suggests a negative regulation of hematopoiesis by adipocytes. Engraftment of HSCs in these fatless mice after irradiation is more efficient than in their wild-type litter mates [56]. Although this effect might be due to an apparent reciprocal correlation of adipocytes and OBs, the control of adipocyte/OB differentiation clearly represents a process that not only regulates HSC number and engraftment but also depends on biomechanics and, thus, introduces biomechanical stress to the set of regulatory mechanisms [61].

Endothelial Cells

Very early hints to a contribution of ECs to hematopoiesis were given in the 1970s when Knospe et al. [62] reported that hematopoietic regeneration in areas of curetted BM in adult mice corresponded with sites of BM sinusoidal vascular regeneration. Further evidence was given by coculture in vitro. Primary human BM ECs supported the proliferation and differentiation of human CD34 + cells (which represent a HSC-enriched subpopulation of BM cells) and produced several hematopoietic cytokines. This stem cell support by ECs is restricted to neither hematopoietic tissues

nor HSCs, but is found in most stem cell systems [63]. Chute and coworkers, therefore, tested the effect of human ECs on self-renewal of human HSCs. Interestingly, noncontact culture of human BM or cord blood HSCs with primary human brain ECs induced a tenfold expansion of human HSCs with the potential to repopulate immunodeficient mice, suggesting that adult brain ECs produced soluble factors, which induce HSC self-renewal [64, 65]. Analysis of several candidate proteins revealed that concerted action of either angiopoietin-like 5, insulin-like growth-factor-binding protein-2 (IGFBP-2) or pleiotrophin together with early acting cytokines (SCF, TPO, Flt3-L) significantly supports the expansion of HSCs in vitro.

Adrenergic Neurons

Circulating HSCs and their progenitors exhibit robust circadian fluctuations in the peripheral blood [66]. They fluctuate in antiphase with the expression of the chemokine CXCL12 in the BM microenvironment. This cyclic release of HSCs follows the oscillations of the circadian clock and is transmitted by the sympathetic nervous system. BM adrenergic nerves secret noradrenaline and this signal leads to the rapid downregulation of CXCL12 via the β_3-adrenergic receptor and subsequent mobilization of HSCs. This interaction with the sympathetic nervous system adds a totally new aspect to the complex control mechanisms of the hematopoietic niche.

Already from the above given overview, it becomes clear that a mechanistic understanding of niche-driven HSC regulation is still a rather "white spot on the map of hematopoiesis." Although there is no doubt about the importance of the local environment in stem cell regulation, and although a number of important components of niche functionality have already been identified, a number of major ingredients for a systemic understanding of stem cell organization and its dependence on the local growth environment (GE) are still missing. These include (i) the spatial organization of niche components, (ii) the general rules of stem cell–niches "communication" (e.g., feedback mechanisms), as well as (iii) a quantification of the functional relationships between the individual components of the stem cell–niche complex.

One way to foster a comprehensive understanding of niche-mediated stem cell regulation is the application of systems biological methods. In particular, the application of mathematical models provides a means for quantitatively studying the effect of different regulatory rules (such as feedback loops or dose–response relations), can help to guide the experimental strategy and to foster a quantitative, mechanistic understanding. However, to be able to mathematically model the dynamics of stem cell systems, it is necessary (a) to derive adequate model assumptions, (b) to estimate model parameters, and (c) to experimentally test model predictions. In the following, we will give an overview on different strategies to measure and quantify stem cell–niche interactions and illustrate a modeling framework that is able to integrate these measurements and to quantitatively study emerging system properties.