Conceptual models to understand tissue stem cell organization

Markus Loeffler[a] and Ingo Roeder[a,b]

Purpose of review

Theoretic and, in particular, mathematic models can help biologists to select and design experiments, to highlight general principles, to discriminate similar and to link different phenomena, and to predict novel features. Specifically, they contribute to an understanding of latent mechanisms and crucial parameters of biologic processes. The following review gives an overview of recent developments in the field of hematopoietic tissue stem cell modeling.

Recent findings

A number of experimental findings on heterogeneity, flexibility, and plasticity of hematopoietic and other tissue stem cells are challenging the classic stem cell concept of a predefined intrinsic stem cell program. Self-organizing systems provide a more elegant and comprehensive alternative to explain experimental data.

Summary

Within the last few decades, modeling approaches in stem cell biology have evolved and now encompass a broad spectrum of phenomena, ranging from the cellular level to the tissue level. The application of theoretic models is currently suggesting that we abandon the classic assumption of a strict developmental hierarchy and understand stem cell organization as a dynamic, functional process. Such a perspective has implications for a prospective characterization of tissue stem cells (*eg*, regarding gene expression profiles and genetic regulation patterns).

Keywords

tissue stem cells, mathematic model, biologic concept, self-organization, plasticity

Curr Opin Hematol 11:81–87. © 2004 Lippincott Williams & Wilkins.

[a]Institute for Medical Informatics, Statistics and Epidemiology; and [b]Interdisciplinary Centre for Bioinformatics, University of Leipzig, Germany

Supported by the Deutsche Forschungsgemeinschaft, grant LO-941/2 and grant BIZ-6/1-2 (I.R.).

Correspondence to Markus Loeffler, University Leipzig, Institute for Medical Informatics, Statistics and Epidemiology, Liebigstr. 27, D 04103 Leipzig, Germany
Tel: 49 341 9716100; fax: 49 341 9716109;
e-mail: markus.loeffler@imise.uni-leipzig.de

Current Opinion in Hematology 2004, 11:81–87

1065–6251

Introduction

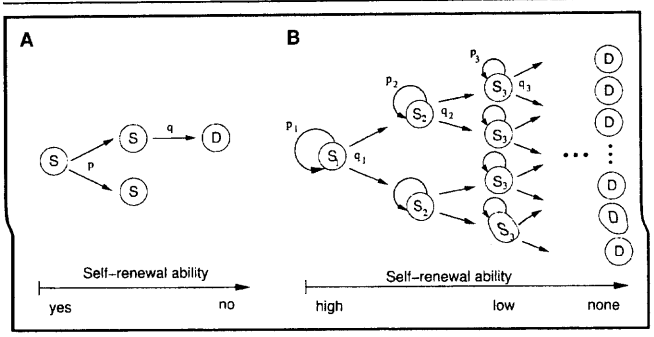
Within the natural sciences, a model is understood as a simplifying abstraction of a more complex construct or process, and examples, such as animal or *in vitro* models, are familiar to all biologists. In contrast to these experimental models, we focus on recent developments among theoretic models. These include qualitative concepts (*ie*, a descriptive representation of a biologic process) and quantitative models (*ie*, mathematic representations). In contrast to qualitative concepts, quantitative models allow for an analytic, numeric, or simulation analysis.

Theoretic models can help biologists in several ways. Model predictions can be used to select and design experimental strategies, and they help to anticipate the impact of manipulations to a system and its response. Modeling is able to discriminate similar and to link different phenomena. Specifically, models originating from the same principles adapted to different systems (*ie*, tissues or cell types) may help to understand common construction and regulation principles. Furthermore, they contribute to the understanding of latent mechanisms or crucial parameters of biologic processes and may predict new phenomena.

Classic entity-based tissue stem cell models

Already the first experimental demonstration of a self-renewing population of hematopoietic progenitor cells using the CFU-S assay [1] was accompanied by a mathematic stem cell model [2]. Using a simple stochastic approach, the authors explained the experimentally observed heterogeneity of secondary CFU-S colonies. The production of secondary CFU-S from a primary cell was used to define functionally the stem cell (self-renewing) property. Till *et al.* [2] assumed that all hematopoietic stem cells (*ie*, CFU-S cells) are alike. To describe the generation of the frequency distribution of secondary CFU-S (0 to 200 CFU-S were obtained from the transplantation of one primary CFU-S colony), they used a single-cell-based model in which stem cells can either divide symmetrically (*ie*, self-renew) or differentiate. These options are realized with fixed probabilities p and q respectively. Flexibility, feedback regulation, or micro-

Figure 1. Stem cell models



(A) Classic p–q model introduced by Till et al. [2]. Stem cells have the ability to divide symmetrically into two stem cells or to differentiate. These two options are realized with probabilities p and q respectively. (B) The pedigree model assumes a heterogeneity of stem cells with an irreversibly declining probability of self-renewing divisions ($p_1 < p_2 < p_3 < \ldots$).

environmental effects were not considered. This type of a stochastic *p-q* model (Fig. 1A) was extended by many other authors for hematopoietic stem cells [3–5] and for other tissues [6–9]. A population-based (deterministic) version, which averaged the individual (stochastic) decisions of self-renewal or differentiation, was proposed by different groups [10,11]. In contrast to the stochastic representations using fixed self-renewal/differentiation probabilities, these approaches included a feedback regulation of the self-renewal rate and the proliferative fraction of stem cells. Additionally, to the steady-state situation, this approach allowed the analysis of system perturbations, like the recovery after damage. Another extension of the classic self-renewing/differentiation view includes pedigree models [9,12], which assume a heterogeneity of the stem cell population with declining self-renewal capacity of the cells (Fig. 1B). This implies an irreversible, unidirectional process with gradual decreasing stem cell (self-renewing) potential.

The homogenous *p–q* concept and the heterogeneous pedigree concept imply that the stem cells somehow "know" that they are stem cells (*ie*, have an imprinted stem cell entity), which can only be lost, but not regained.

The functional tissue stem cell definition

To achieve criteria to validate stem cell models, it is helpful to check whether they comply with the functional definition of tissue stem cell (Table 1 [13,14]).

We like to emphasize that this definition does not require the existence of predefined stem cell entities. Rather, it focuses on the potential of a population of cells as a part of a living system (*eg*, a tissue or organ). This functional view has a number of implications. It uses a relative exclusion criteria (undifferentiated cells; *ie*, lack of differentiation markers) to describe potential stem cells. These have certain capabilities that, however, are not necessarily used in all circumstances. The capabilities can only be checked in specific assay systems (*eg*, transplantation assays, colony-forming assays), which themselves inevitably alter the cells tested. This fact is sometimes addressed as the "uncertainty principle of stem cell biology" [14].

Conceptual challenges based on recent experimental evidence

It is now generally accepted that tissue stem cells are heterogeneous with regard to function (*eg*, cycling activity, engraftment potential, differentiation status) and to marker expression (*eg*, adhesion molecules, cell surface antigens). However, during the last few years experimental evidence has accumulated that these properties can be reversibly changed [15–20]. As suggested by experiments dealing with tissue plasticity phenomena (for reviews see others, for example [21–23]), microenvironmental effects seem to play an essential role in directing the cellular development. These experimental results also initiated debates, whether the view of a strict unidirectional developmental hierarchy within tissue stem cell populations is appropriate [24–27,28••]. Although the general existence of tissue plasticity properties is widely accepted, the underlying mechanisms (*eg*, trans-/dedifferentiation or cell fusion) and the relevance of these plasticity potential in normal *in vivo* systems or even in the clinical setting is still unclear [23,29]. Clone tracking experiments (*eg*, using retroviral marking of individual clones) [30–33] or chimerism studies [34,35] highlight relative differences of inheritable cellular properties between stem cell clones and their impact on the competitive potential. Furthermore, high throughput analysis of gene expression (see, for example, [36–38]) and signaling studies (see, for example [39–42]) offer the chance to extend our knowledge on tissue stem cells to the molecular level.

Because classic stem cells models are not able to explain all these experimental findings consistently, new conceptual approaches and theoretic models are required. To be able to characterize modeling approaches in a sys-

Table 1. Functional tissue stem cell definition

Stem cells of a particular tissue are a potentially heterogeneous population of functionally undifferentiated cells (relative to a functional tissue), capable of

- homing to an appropriate growth environment,
- proliferation,
- production of a large number of differentiated functional progeny,
- self-renewing or self-maintenance of their population,
- regeneration of the functional tissue after injury,

with a flexibility and reversibility in the use of these options.

These criteria were formulated by Loeffler and Roeder [13] in 2002 as one result of a conceptual discourse on general principles of tissue stem cell organization at the Tissue Stem Cells–Models and Concepts workshop held in Leipzig, Germany, 2001. This definition is an amended version of the original definition by Potten and Loeffler [14] from 1990.

tematic way, Table 2 establishes a general characterization scheme for theoretic models.

Recent developments in conceptual modeling of stem cell organization

To describe and classify models of stem cell organization systematically, we discuss the proposed approaches with respect to their relation to the tissue stem cell criteria (Table 1) as well as to the criteria characterizing theoretic models (Table 2).

A simple quantitative, individual cell-based model has been proposed by Agur *et al.* [43]. This approach focuses on local cell–cell interactions of stem cells. Considering a homogenous stem cell population, Agur *et al.* [43] show that a simple combination of a delayed differentiation onset (controlled by a cell intrinsic process) together with a negative feedback regulation of differentiation (dependent on the local stem cell number) are sufficient to ensure the production of a constant amount of differentiated cells, for the self-maintenance of the stem cell population and the regeneration of the system after disturbances. The simulations have not been explicitly linked to experimental data; however, they underline the possibility of generating a homeostatic, globally stable stem cell system on the basis of local cell–cell interactions.

Based on the stem cell entity approach, assuming a self-renewing population of stem cells that are recruited to differentiation at a certain rate, Mackey [44] proposed a quantitative model on the cell population level. In contrast to the classic *p-q*-model of Till *et al.* [2], he considered the possibility of hematopoietic stem cells to change their cell cycle status reversibly (actively cycling/dormant in G0). Based on the comparison with specific S-phase labeling experiments, he provides estimates of cell cycle time, apoptotic rate, activation (reentry in cell cycle from G0) rate, as well as differentiation rate, and analyzed their impact on system behavior. An extension of this model has been described by Bernard *et al.* [45•]. Additional to the features introduced by Mackey [44], Bernard *et al.* [45•] account for the experimentally observed heterogeneity of the stem cell population by introducing an age–maturity structure of the cell population under consideration. This allows a more detailed investigation of stem cell kinetics, particularly their development during the maturation process (*ie*, division history) of stem cells.

Table 2. Charaterization of models

Criterion	Specification
Granularity level	• Tissue level (*ie*, modeling of averaged cell populations) • Cellular level (*ie*, modeling of single cells or clones of cells) • Molecular level (*ie*, modeling of signal transduction, gene regulation, or epigenetic mechanisms)
Formal specification	• Qualitative (conceptual description) • Quantitative (mathematic model)
Link to data	• No comparison with data • Consistent with data from one type of experiment • Consistent with a variety of data sets from different types of experiments

For example, the stochastic *p–q* model can be assigned as a model on a cellular level, with a quantitative approach related to one single phenomenom (CFU-S growth after transplantation).

To understand recent experimental findings on changing contributions of different stem cell clones in chimeric animals, the quantitative, individual cell-based, stochastic model proposed by Abkowitz *et al.* [5] in 1996 has been analyzed in a series of publications [34,46,47]. Also, this model is basically a variant of the classic entity-based *p–q* model with a homogenous, self-maintaining population of stem cells. However, Abkowitz *et al.* [5] adapted their model specifically to the situation of chimeric hematopoiesis. Very recently, this approach has been used to explain long-term skewing in the chimerism development of cat chimeras [48••]. The authors demonstrate that very small differences in the kinetic properties of different stem cell populations (which might not even be detectable in noncompetitive assays) can explain long-term growth advantages of one clone over another.

A similar conclusion has also been reached in an extensive simulation study based on another quantitative, individual cell-based model of hematopoietic stem cell organization introduced by Roeder and Loeffler [49]. Their results strongly suggest that small differences of inheritable (clonal) regulatory characteristics (*ie*, responsiveness to signals) of the cells are affecting the competitive and, therefore, the engraftment potential of stem cells [50] (Roeder I, Kamminga L, Braesel K, *et al.*, unpublished data). Both model analyses point to the relativity of stem cell potential, which can only be specified with regard to the competitor cells [48••,50] and to the signals of the growth environment [50].

In contrast to the approach used by Abkowitz *et al.* [5], the model proposed by Roeder and Loeffler [49] is able to track the fate trajectory of individually labeled cells and their clonal progeny. It postulates the possibility of progenitor cells to change their actual growth environment, which in turn induces different cycling activities. Furthermore, depending on the actual growth environment, the cells are able to change reversibly their affinity for homing to these local environments. The model is also able to generate a stationary heterogeneity of functional stem cells after perturbations and has been compared with a broad variety of different experimental settings [49]. It should be noted that the model by Roeder and Loeffler [49] is conceptually different from the previous models because it attempts to avoid the idea of a

stem cell entity and, rather, uses principles of self-organizing processes, which permit that cell properties can be altered in a reversible way within some limits.

On the level of a quantitative description, other authors have suggested discarding the classic view of an unidirectional developmental hierarchy of tissue stem cells. Under the impression of experimental reports on tissue plasticity phenomena, Blau *et al.* [27] discussed the qualitative concept of evolving stem cells. The concept introduced the possibility of stem cells being able to reverse their differentiation history in a graded fashion. Furthermore, the authors promote the idea that tissue stem cells are able to change their actual tissue compartment (*ie*, the microenvironment) by a transportation process within the blood stream.

Stem cells show plastic changes of cellular properties not only between, but also within one tissue. Because of the observation of reversibility in the engraftment potential, the homing, or the differentiation ability of hematopoietic progenitor cells [18,19], Quesenberry *et al.* [51,52••] suggested the qualitative concept of a stem cell continuum. The main idea is that the phenotype of primitive marrow stem cells can be shifted from one state to another in a reversible way. The authors list a number of experimental results that provide evidence for a nonhierarchical, but functionally plastic nature of hematopoiesis. Besides the formulation of the qualitative concept, which accounts for these observations, the authors also hypothesize molecular mechanisms, such as chromatin modulation and reversible gene expression, as processes responsible for the flexibility of stem cell behavior.

This flexibility of stem cell behavior was already captured in the functional stem cell definition more than a decade ago (compare with Table 1). The characterization of stem cells by capabilities implies a distinction of potential and actually used properties. Potten and Loeffler [14] explicitly suggested the distinction of potential and actually active stem cells in the intestinal crypt. Because of the spatial restrictions in the crypt environment, only a few cells that have the potential to act as clone-forming cells can occupy the appropriate spatial niche at the bottom of the crypt to become actually tissue forming [53].

Based on observations of neuronal cell development in the retina, Liversey and Cepko [54] proposed the so-called competence model. In their model, neuronal stem cells are expected to pass different competence stages (*ie*, with the ability to receive and interpret specific signals), which can be used, if induced by appropriate stimuli from the (micro)environment.

Without restricting to one specific tissue stem cell system, Loeffler and Roeder [13] discussed a similar concept, denoted as two-level dynamics. Level 1 defines the general accessibility of cellular potential (*eg*, a certain gene or a cluster of genes). Level 2 dynamics deal with the activation or inactivation of these capabilities (*eg*, transcription and translation of genes). Therefore, different potentials of stem cells can be reversibly activated on the basis of level 2. In contrast, level 1 allows a (potentially irreversible) locking of certain functionalities (*eg*, terminal differentiation).

An essential conceptual point that is currently discussed in the literature is the question of whether stem cells should be considered as fixed, predefined entities. Such a view implies that stem cells "know" about their status and act as a result of an intrinsic program (which may be deterministic or stochastic). Alternatively, the concept of self-organization has been suggested to explain tissue stem cell organization. Properties of such systems are elaborately discussed and clearly illustrated by Theise [55••] and Theise and d'Inverno [56]. Instead of focusing purely on cellular properties, the self-organization paradigm suggests considering not just individual cells, but the entire system of cell–cell and cell–environment interactions. The authors point out that an important characteristic that enables self-organizing systems to produce complex behavior (*eg*, flexible responses to disturbances) is a certain degree of stochastic behavior (*eg*, in the interpretation of cellular or microenvironmental signals).

A working example, showing that self-organizing principles can be used to explain tissue stem cell organization, has been presented by Roeder and Loeffler [13,49,57••] for the hematopoietic system. The presented quantitative, individual cell-based model is fully compatible with the functional criteria of tissue stem cells and it consistently explains a broad variety of different experimental situations [49,57••].

A similar idea of understanding stem cell organization as a systemic phenomenon has also been promoted by Flake [58•]. He specifically points to the loss of essential information by dissecting systems into isolated components for their analysis and to the context-dependent interpretation of biologic phenomena (*eg*, different assay type, clinical or experimental background investigation). Consequently, a comprehensive model of stem cell organization should explain *in vivo* as well as *in vitro* situations consistently.

Understanding the molecular level of stem cell organization

All quantitative models described so far consider cell fate decisions on the cellular level. Molecular mechanisms underlying, for example, the interpretation of neighboring information [43], the decision for self-renewal or dif-

Table 3. Conceptual views of stem cell organization

Classic view		Proposed view
Cellular entity perspective • Internal stem cell program	⇨	Tissue self-organization • Cell–cell/cell growth environment interaction
Snapshot perspective • Actual status of cells	⇨	Dynamic perspective • Potentials of cells • Plasticity of cellular properties • Generation of heterogeneity

ferentiation [5], or the growth environment transition or the loss and regaining of cellular properties [49] have been described by abstract deterministic or stochastic rules.

A quantitative approach for extending the modeling process to the intracellular level has been presented by Kaneko and others [59,60,61••]. These authors assign a network of autocatalytic biochemical reactions to the cells in which each chemical is linked to several reactions determining the change of its concentration within the cell. Furthermore, the internal chemical concentrations are influenced by gradients of chemical concentrations in the surrounding medium, which in turn are affected by the chemicals produced within the cells [61••]. Using such an intracellular dynamic, cell–cell interactions, and a simple cell division algorithm, major steps in stem cell development (including reversible differentiation) can be explained. Thus far, Kaneko *et al.* [59,60,61••] have analyzed only the mathematic properties of their model without relating the results directly to biologic parameters. Nevertheless, the proposed class of models might serve as a prototype for a quantitative analysis of molecular networks that produce self-organizing stem cell systems.

Some of the conceptual models discussed earlier suggest molecular candidate mechanisms, such as transcriptional networks [54] or chromatin remodeling [51], which might underlie the flexible function of tissue stem cells. Other qualitative models describing molecular mechanism have been formulated (*eg*, for asymmetries in stem cell divisions [62,63]).

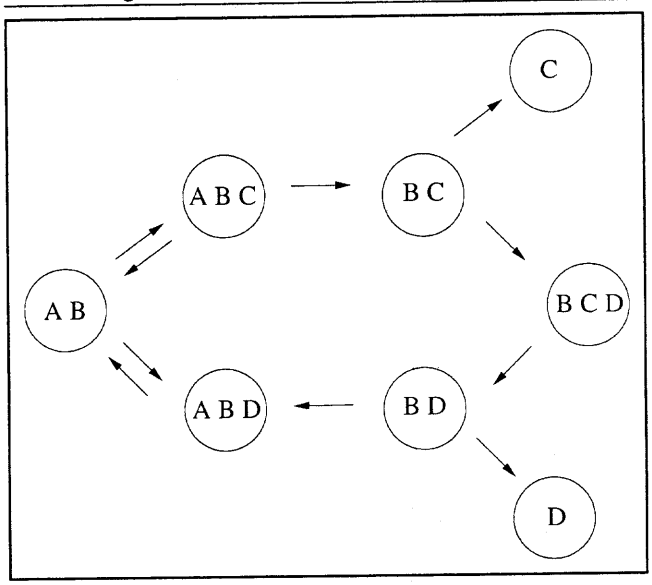
To extend a theoretic stem cell modeling framework to molecular processes, one challenge is to incorporate high throughput data, such as gene expression measurements, into the modeling. However, because of the growing evidence that stem cell populations are highly flexible, self-organized systems, hopes for a simple assessment of one stem cell-specific gene expression pattern (at one time point) may be overoptimistic. To cover the range of options, a complex scanning of regions of potential gene expression patterns (over time and with regard to different modes of perturbation) and their relation to one another (sequence of changes required to migrate from one pattern to another) might be necessary.

Conclusion: conceptual novelty and achievements

One can recognize that the comprehensiveness of models has improved a lot. The scope of the covered phenomenology has extended from a simple description of the heterogeneity in colony-forming assays, to encompass a broad variety of different classes of experimental phenomena on the cellular and tissue levels, ranging from cell kinetic studies to the analysis of fluctuations in clonal contribution.

Actually, we are experiencing a changing view in concepts of stem cell organization (Table 3). Models based on functional, self-organizing systems with stochastic components (sources for generation and for elimination of variance) have been shown to be powerful models and they are challenging the classic entity-based models (compare Figures 1 and 2). It can be shown that self-

Figure 2. Simplified scheme of the self-organizing view on stem cell organization



The current state of a cell is characterized by a set of potential cell functionalities (*eg*, cycling activity, specific homing ability, adhesiveness) coded by capital letters A, B, C, and D. These potentials can be actually expressed and changed according to specific rules that might depend on cell–cell or cell–environment interactions. If defining self-renewal by the return to a previously expressed state, and differentiation by an irreversible loss of some potential, different trajectories of cells realizing these two fates are possible within the illustrated example scenario. For example, self-renewal: AB → ABC → AB or ABC → BC → BCD → BD → ABD → AB → ABC; differentiation: AB → ABC → BC → C or AB → AB → ABC → BC → BCD → BD → D.

organizing principles can generate a heterogeneous population of tissue-forming cells that fulfill all criteria of the functional definition of tissue stem cells, including flexibility of cellular development within a range of permitted options.

Concluding from these conceptual insights, the major experimental challenge is, in our opinion, to explore the potential repertoire of cell populations containing tissue stem cells (*ie*, to focus on the scope of skills rather than on select, individual abilities). Also, modeling approaches need to be extended in several regards. First, more simulation studies are required to demonstrate that the concepts proposed comply with a broad spectrum of data. Furthermore, it will be important to show that the same general model principles hold for tissue stem cells as diverse as the blood-forming stem cells, epithelial stem cells, and other systems. The major challenge in the field of theoretic modeling, however, is the design of predictive models that bridge all three descriptive levels (tissue, cells, molecules), and thus link a molecular description of tissue stem cells to the functional definition. It is evident that modeling, besides new bioinformatic methods in data analysis, will be important in linking data from these three levels into one comprehensive framework.

Acknowledgment

The authors are grateful to the members of the research group Dynamical Modeling of Tissue Stem Cell Organization, Katrin Braesel, Ingmar Glauche, Matthias Horn, and Christian Werner, for their helpful support in the literature screening process.

References and recommended reading

Papers of particular interest, published within the annual period of review, are highlighted as:

- • Of special interest
- •• Of outstanding interest

1 Till JE, McCulloch EA: A direct measurement of radiation sensitivity of normal mouse bone marrow cells. Radiat Res 1961, 14:213–222.

2 Till JE, McCulloch EA, Siminovitch L: A stochastic model of stem cell proliferation, based on the growth of spleen colony-forming cells. Proc Natl Acad Sci U S A 1964, 51:29–36.

3 Vogel H, Niewisch H, Matioli G: Stochastic development of stem cells. J Theor Biol 1969, 22:249–270.

4 Ogawa M, Mosmann TR, ed.: Two stochastic models for stem cell differentiation. In Leukemia: Recent Advances in Biology and Treatment. Alan R. Liss; 1985:391–397.

5 Abkowitz JL, Catlin SN, Guttorp P: Evidence that hematopoiesis may be a stochastic process in vivo. Nat Med 1996, 2:190–197.

6 Loeffler M, Grossmann B: A stochastic branching model with formation of subunits applied to the growth of intestinal crypts. J Theor Biol 1991, 150:175–191.

7 Perez-Losada J, Balmain A: Stem-cell hierarchy in skin cancer. Nat Rev Cancer 2003, 3:434–443.

8 Potten CS: Radiation, the ideal cytotoxic agent for studying the cell biology of tissues such as the small intestine. Radiat Res 2004, 161:123–136.

9 Savill NJ: Mathematical models of hierarchically structured cell populations under equilibrium with application to the epidermis. Cell Prolif 2003, 36:1–26.

10 Lajtha LG, Pozzi LV, Schofield R, et al.: Kinetic properties of haematopoietic stem cells. Cell Tissue Kinet 1969, 2:39.

11 Loeffler M, Wichmann HE: A comprehensive mathematical model of stem cell proliferation which reproduces most of the published experimental results. Cell Tissue Kinet 1980, 13:543–561.

12 Loeffler M, Potten CS: Stem cells and cellular pedigrees–a conceptual introduction. Cambridge: Academic Press; 1997:1–27.

13 Loeffler M, Roeder I: Tissue stem cells: definition, plasticity, heterogeneity, self-organization and models–a conceptual approach. Cell Tissues Organs 2002, 171:8–26.

14 Potten CS, Loeffler M: Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. Development 1990, 110:1001–1020.

15 Quesenberry PJ, Becker PS: Stem cell homing: rolling, crawling, and nesting. Proc Natl Acad Sci U S A 1998, 95:15155–15157.

16 Rolink AG, Nutt SL, Melchers F, et al.: Long-term in vivo reconstitution of T-cell development by Pax5-deficient B-cell progenitors [see comments]. Nature 1999, 401:603–606.

17 Sato T, Laver JH, Ogawa M: Reversible expression of CD34 by murine hematopoietic stem cells. Blood 1999, 94:2548–2554.

18 Frimberger AE, McAuliffe CI, Werme KA, et al.: The fleet feet of haematopoietic stem cells: rapid motility, interaction and proteopodia. Br J Haematol 2001, 112:644–654.

19 Quesenberry P, Habibian H, Dooner M, et al.: Physical and physiological plasticity of hematopoietic stem cells. Blood Cell Mol Dis 2001, 27:934–937.

20 Colvin GA, Lambert JF, Moore BE, et al.: Intrinsic hematopoietic stem cell/progenitor plasticity: inversions. J Cell Physiol 2004, 199:20–31.

21 Quesenberry PJ, Abedi M, Aliotta J, et al.: Stem cell plasticity: an overview. Blood Cell Mol Dis 2004, 32:1–4.

22 Wagers AJ, Weissman IL: Plasticity of adult stem cells. Cell 2004, 116:639–648.

23 Goodell MA: Stem-cell "plasticity": befuddled by the muddle. Curr Opin Hematol 2003, 10:208–213.

24 Lemischka I: Stem cell dogmas in the genomics era. Rev Clin Exp Hematol 2001, 5:15–25.

25 Lemischka I: A few thoughts about the plasticity of stem cells. Exp Hematol 2002, 30:848–852.

26 Wei G, Schubiger G, Harder F, Muller AM: Stem cell plasticity in mammals and transdetermination in Drosophila: common themes? Stem Cells 2000, 18:409–414.

27 Blau HM, Brazelton TR, Weimann JM: The evolving concept of a stem cell: entity or function? Cell 2001, 105:829–841.

28 •• Colvin GA, Lambert JF, Abedi M, et al.: Differentiation hotspots: the deterioration of hierarchy and stochasm. Blood Cell Mol Dis 2004, 32:34–41.
This is a comprehensive overview of new insights in the field of hematopoietic stem cell biology. The article summarizes experimental results, biologic hypothesis, as well as conceptual viewpoints and mathematic modeling approaches.

29 Anderson DJ, Gage FH, Weissman IL: Can stem cells cross lineage boundaries? Nat Med 2001, 7:393–395.

30 Jordan CT, Lemischka IR: Clonal and systemic analysis of long-term hematopoiesis in the mouse. Genes Dev 1990, 4:220–232.

31 Capel B, Hawley RG, Mintz B: Long- and short-lived murine hematopoietic stem cell clones individually identified with retroviral integration markers. Blood 1990, 75:2267–2270.

32 Drize N, Chertkov J, Sadovnikova E, et al.: Long-term maintenance of hematopoiesis in irradiated mice by retrovirally transduced peripheral blood stem cells. Blood 1997, 89:1811–1817.

33 Kim HJ, Tisdale JF, Wu T, et al.: Many multipotential gene-marked progenitor or stem cell clones contribute to hematopoiesis in nonhuman primates. Blood 2000, 96:1–8.

34 Abkowitz JL, Golinelli D, Harrison DE, et al.: In vivo kinetics of murine hemopoietic stem cells. Blood 2000, 96:3399–3405.

35 Kamminga LM, Akkerman I, Weersing E, et al.: Autonomous behavior of hematopoietic stem cells. Exp Hematol 2000, 28:1451–1459.

36 Ivanova NB, Dimos JT, Schaniel C, et al.: A stem cell molecular signature. Science 2002, 298:601–604.

37 Hackney JA, Charbord P, Brunk BP, et al.: A molecular profile of a hematopoietic stem cell niche. Proc Natl Acad Sci U S A 2002, 99:13061–13066.

38 Bruno L, Hoffmann R, McBlane F, et al.: Molecular signatures of self-renewal,

differentiation, and lineage choice in multipotential hemopoietic progenitor cells in vitro. Mol Cell Biol 2004, 24:741–756.

39 Orkin SH: Diversification of haematopoietic stem cells to specific lineages. Nat Rev Genet 2000, 1:57–64.

40 Rothenberg EV: T-lineage specification and commitment: a gene regulation perspective. Semin Immunol 2002, 14:431–440.

41 Ohishi K, Katayama N, Shiku H, et al.: Notch signalling in hematopoiesis. Semin Cell Dev Biol 2003, 14:143–150.

42 Heasley LE, Petersen BE: Signalling in stem cells. EMBO Rep 2004, 5:241–244.

43 Agur Z, Daniel Y, Ginosar Y: The universal properties of stem cells as pinpointed by a simple discrete model. J Math Biol 2002, 44:79–86.

44 Mackey MC: Cell kinetic status of haematopoietic stem cells. Cell Prolif 2001, 34:71–83.

45 • Bernard S, Belair J, Mackey MC: Oscillations in cyclical neutropenia: new evidence based on mathematical modeling. J Theor Biol 2003, 223:283–298.
This paper details the application of a simple mathematic model of stem cell activation and silencing that is able to explain a variety of results on cycling activity of stem cells obtained from different kinds of labeling studies.

46 Abkowitz JL, Catlin SN, McCallie MT, et al.: Evidence that the number of hematopoietic stem cells per animal is conserved in mammals. Blood 2002, 100:2665–2667.

47 Catlin SN, Abkowitz JL, Guttorp P: Statistical inference in a two-compartment model for hematopoiesis. Biometrics 2001, 57:546–553.

48 •• Catlin SN, Guttorp P, McCallie MT, et al.: Hematopoiesis as a competitive exclusion process: estimation of a stem cell selective advantage. *J Agricult Biol Environment Stat* 2004, in press.
This paper delineates the application of the quantitative, individual cell-based model by Abkowitz *et al.* [5]. The authors give a model-based explanation of chimerism skewing in cats resulting from small differences in cell kinetic properties between cells of different genetic background.

49 Roeder I, Loeffler M: A novel dynamic model of hematopoietic stem cell organization based on the concept of within-tissue plasticity. Exp Hematol 2002, 30:853–861.

50 Loeffler M, Roeder I, Braesel K, et al.: Biased and unbiased clonal competition application of a new dynamical model of stem cell organization. Blood 2003, 102:1231a.

51 Quesenberry PJ, Colvin GA, Lambert JF: The chiaroscuro stem cell: a unified stem cell theory. Blood 2002, 100:4266–4271.

52 •• Quesenberry PJ, Colvin GA, Abedi M, et al.: The marrow stem cell: the continuum. Bone Marrow Transplant 2003, 32(suppl 1):19–22.
This article provides a description of a new biologic concept that includes plasticity and flexibility of cellular properties in stem cells. Based on a series of experimental results, the authors proposed molecular processes responsible for reversibly changing stem cell phenotype (see also Quesenberry *et al.* [51]).

53 Meineke FA, Potten CS, Loeffler M: Cell migration and organization in the intestinal crypt using a lattice-free model. Cell Prolif 2001, 34:253–266.

54 Livesey FJ, Cepko CL: Vertebrate neural cell-fate determination: lessons from the retina. Nat Rev Neurosci 2001, 2:109–118.

55 •• Theise ND: Perspective: stem cells react! Cell lineages as complex adaptive systems. Exp Hematol 2004, 32:25–27.
The author discusses evidence that stem cell populations have to be understood as self-organizing systems. He highlights the necessity of new concepts (including quantitative models) to understand current experimental results in stem cell biology.

56 Theise ND, d'Inverno MD: Understanding cell lineages as complex adaptive systems. Blood Cell Mol Dis 2004, 32:17–20.

57 •• Roeder I, Loeffler M, Quesenberry PJ, et al.: Quantitative tissue stem cell modeling. Blood 2003, 102:1143–1144; reply, 1144–1145.
This paper highlights the potential of quantitative modeling approaches. It relates the quantitative stem cell model proposed by Roeder and Loeffler [49] to the qualitative concept suggested by Quesenberry *et al.* [51], and demonstrates how simulation studies can help to explain experimental observations.

58 • Flake AW: The conceptual application of systems theory to stem cell biology: a matter of context. Blood Cell Mol Dis 2004, 32:58–64.
This conceptual discourse focuses on content- and method-specific interpretation of experimental results.

59 Kaneko K, Yomo T: Isologous diversification for robust development of cell society. J Theor Biol 1999, 199:243–256.

60 Furusawa C, Kaneko K: Theory of robustness of irreversible differentiation in a stem cell system: chaos hypothesis. J Theor Biol 2001, 209:395–416.

61 •• Furusawa C, Kaneko K: Robust development as a consequence of generated positional information. J Theor Biol 2003, 224:413–435.
In this important theoretic work, the authors demonstrate how the interaction of intracellular dynamics and extracellular gradients of chemical concentrations is able to generate stabilized cell societies that allow for dynamic regeneration after disturbance as well as emergence of dedifferentiation events.

62 Lin H: Stem cells: to be and not to be. Nature 2003, 425:353–355.

63 Wodarz A, Huttner WB: Asymmetric cell division during neurogenesis in Drosophila and vertebrates. Mech Dev 2003, 120:1297–1309.