Quantitative stem cell biology: computational studies in the hematopoietic system

Ingo Roeder

Purpose of review

This review is intended to provide an overview of recently published computational methods, including bioinformatic algorithms, mathematical models and simulation studies, applied to stem cell biology, with particular reference to the hematopoietic system.

Recent findings

The analysis of molecular data is making an increased contribution to identify dynamic system responses. Specifically, there are promising computational approaches to characterizing the functional interrelation of network components regulating the process of differentiation and lineage specification of hematopoietic stem cells. Furthermore, evidence is accumulating that stem cell organization should be regarded as a flexible, self-organizing process rather than as a predetermined sequence of events. A number of mathematical models relevant to the hematopoietic (stem cell) system are applied successfully to clinical situations, demonstrating the predictive power of theoretical methods.

Summary

Based on the advances in measurement technology, an increasing amount of cellular and molecular data is being generated within the field of stem cell biology. The complexity of the underlying systems, however, most often limits a direct interpretation of the data and makes computational methods indispensable. Mathematical models and simulation techniques are contributing considerably to the discovery of general regulatory principles of stem cell organization and are providing clinically relevant predictions.

Keywords

computational methods, hematopoietic stem cells, mathematical model, regulatory network, simulation

Curr Opin Hematol 13:222–228. © 2006 Lippincott Williams & Wilkins.

Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany

Correspondence to Ingo Roeder, PhD, University Leipzig, Institute for Medical Informatics, Statistics and Epidemiology, Haertelstr. 16–18, D-04107 Leipzig, Germany
Tel: +49 341 97 16 111; fax: +49 341 97 16 109;
e-mail: ingo.roeder@imise.uni-leipzig.de

Current Opinion in Hematology 2006, 13:222–228

Abbreviations

ESC	embryonic stem cell
G-CSF	granulocyte colony stimulating factor
HSC	hematopoietic stem cell
ODE	ordinary differential equations
PCML	periodic chronic myeloid leukemia
QTL	quantitative trait locus
WBC	white blood cell

1065-6251

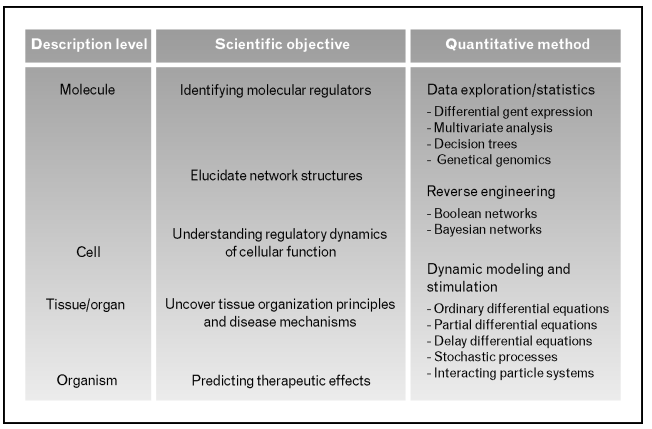
Introduction

The clinical application of hematopoietic stem cells (HSCs) is gaining more and more importance. Besides classical fields, such as bone marrow or stem cell transplantation in the treatment of clonal disorders, the immense regenerative potential of HSCs suggests an ability to support the reconstitution of injured or malfunctioned tissues, outside the hematopoietic system. A prerequisite for the application of hematopoietic and other stem cells in the optimization and development of treatment strategies is the possibility of making quantitative predictions concerning their functional behavior and the parameters which influence it. Although experimental studies have provided a lot of new insights into the properties and the functional potential of HSCs, the underlying mechanisms are still poorly understood.

As is clear from the fields of physics, chemistry and engineering, the availability of a theoretical framework is an extremely important aid to understanding and controlling both natural and artificial processes. The complexity of biological systems and the enormous amount of emerging data clearly call for the application of mathematical and computational approaches to avoid the danger of getting lost in the overwhelming amount of information. The spectrum of quantitative, computational methods extends from exploratory and statistical tools, through reverse engineering methods and dynamical modeling approaches, to simulation techniques. Figure 1 illustrates the scope of relevant biological description levels and corresponding computational approaches, focusing on methods covered by this review

In the following, I will give an overview of computational studies from different methodological backgrounds that have been applied to problems related to HSCs, focusing on publications from the last two years. To view these

Figure 1 Biological description levels and corresponding fields of computational methods depicted in this review



recent results in a broader historical context, the reader is referred to previous reviews, such as those by Loeffler and Roeder [1] or Viswanathan and Zandstra [2].

Identifying molecular regulators

A definite statement of whether or not a particular cell will give rise to a sustained repopulation of the hematopoietic system can only be made by an explicit test of its function; that is, the benchmark for characterizing hematopoietic and other tissue stem cells is still the use of functional assays. As cellular function is determined ultimately by gene-dependent protein activity, the availability of high-throughput methods, such as micro-array technology, raises hopes of identifying a molecular profile uniquely characteristic of stem cells. It is not yet clear, however, whether such a molecular stem cell profile really exists and whether it would help to predict cellular fate prospectively. Still, high-throughput strategies, such as those described in [3–6], are important tools for the identification of functional complexes (genes or gene clusters) as a prerequisite to the description and understanding of organizational principles, such as self-renewal potential or pluripotency.

As it is becoming increasingly evident that stem cell organization can only be understood as an interplay between stem cells and their local growth environment, the identification of molecular regulators has to include both stem cells and their micro-environment. In this context, Charbord and Moore [7] concluded from their gene expression profiling that stem cells supporting stroma cells exhibit a molecular phenotype which is immature, sessile and contractile. Additionally, these cells are able to explore their surroundings and are particularly reactive after binding to integrin ligands, which might indicate an important aspect of stem cell–niche communication.

So far, most of the gene expression analyses provide snapshots of the molecular activity of cells only. Such static pictures, however, are not sufficient to identify key regulators within processes of cellular control. As these processes are 'constructed' to enable cells to react flexibly to systemic signals, it is necessary to analyze the dynamic response of genes to system disturbances, as proposed by Bruno *et al.* [8••]. These authors analyzed gene expression time courses of the stem cell-like cell line FDCP-mix [9] in response to different combinations of cytokines which induce self-renewal or lineage-specific differentiation. Using micro-array analysis and real-time quantitative PCR at eight subsequent time points, the authors were able to determine culture-specific dynamic patterns of gene expression.

In addition to gene expression itself, the identification of components controlling gene expression is necessary to understand stem cell regulation. Donaldson *et al.* [10•] describe a strategy for a genome-wide identification of gene-regulatory regions – so-called *cis*-regulatory elements. Using a computational method, which combines identification of conserved transcription factor binding sites [11] and genome-wide identification of transcription factor binding site clusters [12], these authors identified new potential regulatory components and derived a nascent transcriptional network for early embryonic blood and endothelial development in mammalians.

The control of gene expression, however, is not the only relevant level of regulation. A number of posttranscriptional mechanisms – one of which is alternative splicing – modulate the availability of regulatory proteins in eucaryotic systems. Pritsker *et al.* [13•] describe a strategy for a genome-wide identification of different splice variants of genes in embryonic and hematopoietic stem cells. Applying a computational algorithm which uses the BLAST program [14] to align stem cell-specific expressed sequence tags (EST) and full-length gene transcripts, Pritsker *et al.* showed that alternative splicing modifies components of signaling pathways involved in stem cell function.

I would also like to draw attention to another innovative computational approach which is combining gene expression analysis with computational genetics, namely quantitative trait locus (QTL) analysis [15]. Interpreting expression levels of individual genes as quantitative traits, this method allows identification of genomic regions that are expected to modulate the expression either of genes within the region itself (*cis*-acting QTL) or of groups of transcripts mapping throughout the whole genome (*trans*-acting QTL). The determination of co-regulated genes and their key regulators permits significant reduction in the dimensionality of the identification

process of functional components included in regulatory networks. The application of this method made it possible to identify candidate genes involved in the regulation of HSC turnover [16••] and in longevity [17] of mice.

Analysis of network structure and dynamics

Although the identification of individual regulatory components, such as transcription factors, their coding genes and isolated signal transduction pathways, is an important step, it is by no means sufficient to understand the complex processes underlying stem cell regulation. Instead, a more global, multivariate analysis of interactions between the individual components – a network analysis – is required. A variety of computational methods for the reconstruction of networks, among them directed/undirected graphs, Boolean networks, Bayesian networks or rule-based formalisms, is available (see [18] for a review).

To infer the topology and the underlying rules of a gene regulatory network, it is necessary to analyze the network reaction to changes in its components. To characterize these responses, one has to know the state of network components (e.g. the expression of a specific gene in a cell) both prior to and following the disturbance. One possibility to gain insight into two successive network states without explicitly determining the network state prior to challenge is to introduce recombinant genes or inhibitory molecules into a cell [19,20] followed by determination of the network state after the intervention. Missal *et al.* [21•] published a simulation study that analyzes the efficiency and the reliability of reverse engineering techniques to reconstruct gene regulation networks from incomplete data. Using a dynamic Bayesian network approach, Missal *et al.* calculated the number of experiments necessary to infer underlying network structures. Analyzing a putative gene regulatory network controlling lineage specification in HSCs, the authors found that here, in principle, one controllable gene is sufficient to determine the complete network topology. Furthermore, it is shown that prior knowledge as well as a larger number of influenceable and accessible initial states reduces the number of experiments required to achieve a network identification of a certain fidelity.

Although not explicitly linked to HSCs, the group of D. Lauffenburger has presented a number of computational approaches which might also be useful in this context. For the examples of pathways involved in murine embryonic stem cell [22] and human T-cell signaling [23], it has been demonstrated that Bayesian networks are an appropriate tool to reverse engineer protein-level signal transduction pathways. Whereas Bayesian networks have the advantage of being relatively robust to unobserved variables, they are restricted to being acyclic, which poses a rather strong limitation in their application to general regulatory networks. Another approach to identify candidate components of regulatory networks has been analyzed by Hautaniemi *et al.* [24] for the example of intracellular signaling in fibroblasts. The proposed decision graph analysis was shown to facilitate elucidation of signal-response cascade relationships and to provide experimentally testable, quantitative predictions of the effect of signaling proteins. A further approach to identify regulatory complexes in signal transduction pathways has been described by Janes *et al.* [25••] for the situation of cytokine-induced apoptosis. Based on a factorial experimental design, the authors identified two functional complexes which seem to play a leading role in the control of cytokine-induced apoptosis. The key idea behind this approach is the characterization of high-dimensional molecular signals over time by so-called signaling 'metrics'. Subsequently, the dimensionality of the signaling space is reduced significantly by means of a Partial Least Square Regression. For the specific example, the authors demonstrated that only two 'principle components' are required to quantitatively describe the apoptotic events.

If regulatory networks can be described by a number of known functional components, it is possible to analyze the dynamics of their interactions using mathematical models. In this context, Cinquin and Demongeot [26•] used a system of ordinary differential equations (ODE) to investigate generic properties of high-dimensional switches. The authors analyzed three different scenarios of a transcription factor network that is suitable to describe lineage-specification processes, such as in HSCs. Herein, they consider transcription factors which show self-enhancing auto-regulation as well as mutual inhibition. All scenarios show a typical switch-like bifurcation behavior, that is the system shows a parameter-dependent change from stable co-expression of all considered transcription factors to an up-regulation of some transcription factors, whereas others are down-regulated. Two scenarios lead to a combined down-regulation of all but one transcription factor. In contrast, the third scenario is able to generate sequential down-regulation of one transcription factor after the other.

A similar approach, describing lineage specification of HSCs, has been proposed by Roeder and Glauche [27•]. The applied ODE system explicitly translates experimental knowledge of activation and inhibition mechanisms of the transcription factors PU.1 and GATA-1. Using analytical and simulation techniques, it was shown that the experimentally proposed interaction mechanisms are able to generate a molecular switch leading to either myeloid or erythroid differentiation. Furthermore, the mathematical analysis

provides two qualitatively different hypotheses to explain the experimentally proposed low-level co-expression of lineage-specific transcription factors (i.e. priming) in primitive HSCs.

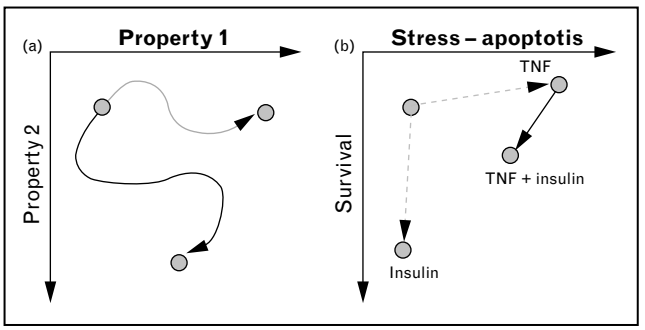
Models of stem cell organization

The identification of mechanisms underlying stem cell self-renewal and differentiation is difficult, particularly because experimental data are most often based on heterogeneous cell populations. One way to tackle this problem has been described by Prudhomme *et al.* [28•] for embryonic stem cells (ESCs). Using a simple ODE model, the authors show that it is possible to decompose the process of ESC self-renewal and differentiation into independent processes of cell amplification and differentiation. Using a factorial design, which exposes ESCs to different combinations of two cytokines (LIF, FGF4) and two extracellular matrix components (laminin and fibronectin), they estimated the different rate coefficients for the above-mentioned processes from cell population data.

The approach of considering amplification and differentiation as two independent processes has already been proposed for tissue stem cells [29,30]. Particularly, it has been used to develop a mathematical model of stem cell organization based on self-organizing principles in the hematopoietic system [31]. Recently, this self-organizing stem cell model, which assumes a reversible development of HSCs within a phase space of different cellular properties, has successfully been applied by Roeder *et al.* [32••] to explain clonal competition phenomena in mouse chimeras consisting of cells from two different strain backgrounds. Comparing simulation results with experimental data, Roeder *et al.* demonstrated that reversible activation and deactivation patterns in the clonal contribution of HSCs can consistently be explained by small strain differences in the response characteristics of HSCs to micro-environmental signals. This approach of interpreting stem cell populations as flexible, self-organizing systems has been proposed independently by Kirkland [33••]. The classical stem cell concept of a hierarchy of different stem cell compartments describing various differentiation states would imply the possibility of definitively separating these stem cell sub-populations experimentally. Motivated by the fact that there always remains a significant overlap of separated sub-populations, Kirkland proposes stem cell differentiation to be a continuous process rather than a sequence of irreversible singular events. This means that individual stem cell fates can be interpreted as trajectories within a (multidimensional) phase space of different cellular properties. In common with the stem cell model proposed by Roeder and Loeffler [31], Kirkland assumes some degree of reversibility in stem cell differentiation. These theoretical models of stem cell organization raise the question of how to relate the theoretical phase space to experimentally measurable parameters. One promising strategy might be the method by Janes *et al.* [25••] which has already been described above. It seems very likely that it is also possible for HSCs to collapse available high-dimensional molecular data into a few relevant functional complexes, which would correspond to the model variables – the phase space dimensions (cf. Fig. 2).

A topic that is tightly linked to stem cell self-renewal is telomere shortening and its impact on the proliferative potential of stem cells. Applying a previously proposed stochastic stem cell model [34], Shepard *et al.* [35•] simulated granulocyte telomere shortening to estimate replication rates of human HSCs *in vivo*. From their results, the authors conclude that human HSCs replicate, on average, about once per 45 weeks, which is substantially slower than in mice (once per 2.5 weeks) and cats (once per 8.3–10 weeks). These results are interpreted as evidence supporting the hypothesis that replication rates decrease with organism size and longevity. Two further computational studies [36•,37] shed light on more general points of telomere shortening. Op den Buijs and colleagues [36•] asked whether the assumption of independence between telomere loss and telomere length is appropriate. Applying a statistical analysis of telomere lengths in fibroblasts in combination with a simple negative feedback model of telomere shortening, the authors conclude that, in contrast to earlier assumptions, telomere shortening in human somatic cells is indeed dependent on telomere lengths. Regarding the mechanisms underlying the telomere-mediated induction of apoptosis, senescence and proliferation, Arkus [37]

Figure 2 Characterization of cellular development



(a) According to the model descriptions proposed by Roeder/Loeffer [31] and by Kirkland [33••], stem cell development is interpreted as a trajectory in a phase space of cellular properties. Depending on the actual context of the cell (e.g. different external stimuli), different trajectories are possible. (b) Internal signaling status of a cell in response to cytokine treatment (TNF or insulin) characterized by the two principle signaling components: stress-apoptosis and survival (sketched after the data published in [25••]). The principle signaling components represent linear combinations of the internal, high-dimensional but measurable signaling status of a cell.

applied a mathematical model to analyze the hypothesis that it is not the telomere length itself, but the binding of regulatory proteins (such as TRF2) to the telomere, that determines cellular fate. Based on the model results, it was concluded to be the inability of telomeres to bind TRF2, rather than telomere length alone, that causes apoptosis or senescence.

Physiological and disease models

A number of recent publications by Mackey and co-workers apply mathematical models to oscillating phenomena within the hematopoietic system in different patho-physiological disorders. Bernard *et al.* [38] studied the dynamics of white blood cell (WBC) production for the situation of cyclical neutropenia. They have demonstrated that alteration in the apoptotic rate of WBC is sufficient to induce the onset of oscillations (with a period of about 20 days) in WBC count. A structurally similar model has been applied by Pujo-Menjouet and Mackey [39] to the situation of periodic chronic myeloid leukemia (PCML). The authors have demonstrated analytically that the system exhibits instabilities, generating periodic solutions, depending on cell cycle lengths of stem cells. Whereas the latter two studies focused on oscillations in WBC counts, it is known that the oscillating behavior is also seen in platelets and erythrocytes. In two joint publications, Colijn and Mackey [40•,41•] investigated coupled oscillations of leukocytes, platelets and erythrocytes in PCML [40•] and cyclical neutropenia [41•]. Applying systems of delay differential equations, the authors show that the clinically observed oscillatory behavior in PCML as well as in cyclical neutropenia can be explained by a destabilization in the stem cell compartment. This is most likely induced by changes in the amplification rate in the leukocyte line (PCML, cyclical neutropenia) and by altered differentiation rates from stem cells into leukocyte precursors as well as by an altered apoptosis rate in the stem cell compartment (PCML).

Another aspect of myeloproliferative disorders is elucidated by Catlin *et al.* [42••]. These authors investigated whether clonal dominance, as observed, for example, in leukemias, can be explained by a loss of micro-environmental control of stem cells. Using simulation studies based on the stochastic stem cell model published by Abkowitz *et al.* [34], Catlin *et al.* show that leukemic overgrowth can be explained by competition of normal and malignant cells for micro-environmental support. In contrast to this hypothesis, Michor *et al.* [43•] assumed a complete growth independence of normal and malignant cells in chronic myeloid leukemia. Applying a mathematical model based on this assumption, these authors explain the clinically observed bi-phasic decline of tumor abundance in CML patients under *imatinib* treatment. From their results, Michor *et al.* conclude that *imatinib* does not act on leukemic stem cells. This conclusion, however, is not compelling. It can be shown that the bi-phasic decline of tumor load can alternatively be explained by a selective effect of *imatinib* on proliferating cells, including leukemic stem cells (Roeder *et al.*, unpublished data).

Model-based predictions of therapeutic strategies

Experimental results in mice have shown that HSCs transduced with vectors that express drug resistance, antiapoptotic or cell cycle-affecting genes/proteins might exhibit a competitive in-vivo growth advantage compared with other HSCs and are, therefore, suitable targets in gene therapeutic settings. To analyze whether such in-vivo selection strategies should also be applied in the human situation, Abkowitz *et al.* [44•] simulated the effect of different selective advantages of stem cells (i.e. differences in stem cell replication and survival rates) on clonal persistence using a previously described stochastic stem cell model [34]. The computational analysis demonstrated that changes in replication rates have a higher impact on clonal persistence than survival, particularly in regenerating systems.

As shown by Foley *et al.* [45•], simulation studies can also be used to optimize treatment protocols for the application of granulocyte colony stimulating factor (G-CSF) in patients with cyclical neutropenia. Based on their computational results, the authors suggest treatment schedules with a reduced total G-CSF dose, which are predicted to achieve essentially the same therapeutic effect as conventional treatments.

I would like to close this review by drawing attention to two mathematical models which are demonstrating the high potential of computational methods for clinical applications. Using an ordinary differential equation model of human granulopoiesis, Engel *et al.* [46••,47] are able to consistently describe the effects of 10 different multicycle poly-chemotherapies on leukocyte numbers in lymphoma patients. Furthermore, the model has been used to provide quantitative predictions for variations in the G-CSF support of different chemotherapy schedules. Recently, this model has also been applied to describe individual therapy effects for different patient risk groups [48]. Another model of human granulopoiesis based on partial differential equations has been proposed by Ostby *et al.* [49]. In a recent publication, Ostby *et al.* [50] applied this model successfully to granulocyte reconstitution after high-dose chemotherapy with stem cell and G-CSF support in breast cancer patients.

Conclusion

Although computational methods are gaining more importance in the life sciences, the number of

computational studies involving HSCs is still quite low. One reason for this might be the lack of quantitative descriptions of stem cell systems. The behavior of stem cells, their interactions with other cells or with micro-environmental components, and the intra-cellular mechanisms involved are still mostly described in a schematic, qualitative and nonstandardized way. A standardized and unmistakable language, however, is the basis for the application of mathematical formalisms.

Such a problem is by no means restricted to the biology of HSCs. Trying to answer the general question of 'whether a biologist can fix a radio', Lazebnik [51] points to a number of issues of broader relevance to understanding stem cell systems. The ever-increasing amount of detailed information must not lead to a situation in which we can no longer 'see the wood for the trees'. In avoiding the paradox that the more facts we learn, the less we understand, mathematical methods and computational tools can provide a considerable contribution by the identification of latent structures, the discovery of generic functional principles or the systematic analysis of system behavior.

A number of approaches are relevant to this aim, as this review clearly shows. As already stated two years ago [1], however, a major challenge in the field of theoretical modeling and computational stem cell biology still is the design of predictive models which bridge the different descriptive levels from molecules through cells to tissues. To achieve this goal, wet lab biology and the different theoretical fields, such as bioinformatic data exploration, statistical analysis and dynamical modeling, must not be regarded as independent approaches, but should be understood as inseparable and indispensable parts of an integrated quantitative (stem cell) biology.

Acknowledgements

This work was supported in part by the Deutsche Forschungsgemeinschaft grant LO 942/1-1,2. The author would like to thank Michael Cross, Ingmar Glauche and Matthias Horn for their helpful comments on the manuscript.

References and recommended reading

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

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 280).

1 Loeffler M, Roeder I. Conceptual models to understand tissue stem cell organization. Curr Opin Hematol 2004; 11:81–87.

2 Viswanathan S, Zandstra PW. Towards predictive models of stem cell fate. Cytotechnology 2003; 4:75–92.

3 Forsberg EC, Prohaska SS, Katzman S, *et al.* Differential expression of novel potential regulators in hematopoietic stem cells. PLoS Genet 2005; 1:e28 [Epub ahead of print].

4 Golan-Mashiach M, Dazard JE, Gerecht-Nir S, *et al.* Design principle of gene expression used by human stem cells: implication for pluripotency. Faseb J 2005; 19:147–149.

5 Palmqvist L, Glover CH, Hsu L, *et al.* Correlation of murine embryonic stem cell gene expression profiles with functional measures of pluripotency. Stem Cells 2005; 23:663–680.

6 Rossi DJ, Bryder D, Zahn JM, *et al.* Cell intrinsic alterations underlie hematopoietic stem cell aging. Proc Natl Acad Sci U S A 2005; 102: 9194–9199.

7 Charbord P, Moore K. Gene expression in stem cell-supporting stromal cell lines. Ann N Y Acad Sci 2005; 1044:159–167.

8 •• 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.

The authors present an innovative approach for a dynamic analysis of transcriptional profiles in multipotent hematopoietic progenitors. The results provide further evidence in support of the multilineage priming hypothesis for hematopoietic stem cells.

9 Spooncer E, Heyworth CM, Dunn A, *et al.* Self-renewal and differentiation of interleukin-3-dependent multipotent stem cells are modulated by stromal cells and serum factors. Differentiation 1986; 31:111–118.

10 • Donaldson IJ, Chapman M, Kinston S, *et al.* Genome-wide identification of cis-regulatory sequences controlling blood and endothelial development. Hum Mol Genet 2005; 14:595–601.

This publication demonstrates the utility of a genome-wide computational approach for the identification of *cis*-regulatory elements.

11 Loots GG, Ovcharenko I, Pachter L, *et al.* rVista for comparative sequence-based discovery of functional transcription factor binding sites. Genome Res 2002; 12:832–839.

12 Markstein M, Markstein P, Markstein V, *et al.* Genome-wide analysis of clustered dorsal binding sites identifies putative target genes in the Drosophila embryo. Proc Natl Acad Sci U S A 2002; 99:763–768.

13 • Pritsker M, Doniger TT, Kramer LC, *et al.* Diversification of stem cell molecular repertoire by alternative splicing. Proc Natl Acad Sci U S A 2005; 102: 14290–14295.

The study describes a combination of experimental and computational methods for a genome-wide identification of slice variants in embryonic and hematopoietic stem cells.

14 Altschul SF, Gish W, Miller W, *et al.* Basic local alignment search tool. J Mol Biol 1990; 215:403–410.

15 Jansen RC, Nap JP. Genetical genomics: the added value from segregation. Trends Genet 2001; 17:388–391.

16 •• Bystrykh L, Weersing E, Dontje B, *et al.* Uncovering regulatory pathways that affect hematopoietic stem cell function using 'genetical genomics'. Nat Genet 2005; 37:225–232.

The authors apply a combination of gene expression analysis with computational genetics. Using this method, they are able to identify groups of candidate genes involved in hematopoietic stem cell turnover.

17 de Haan G, Williams RW. A genetic and genomic approach to identify longevity genes in mice. Mech Ageing Dev 2005; 126:133–138.

18 de Jong H. Modeling and simulation of genetic regulatory systems: a literature review. J Comput Biol 2002; 9:67–103.

19 Hannon GJ. RNA interference. Nature 2002; 418:244–251.

20 McIvor Z, Hein S, Fiegler H, *et al.* Transient expression of PU.1 commits multipotent progenitors to a myeloid fate whereas continued expression favors macrophage over granulocyte differentiation. Exp Hematol 2003; 31:39–47.

21 • Missal K, Cross MA, Drasdo D. Gene network inference from incomplete expression data: transcriptional control of haemopoietic commitment. Bioinformatics 2005; 22:731–738 [Epub ahead of print].

This study analyzes the efficiency of reverse engineering techniques to reconstruct gene regulation networks.

22 Woolf PJ, Prudhomme W, Daheron L, *et al.* Bayesian analysis of signaling networks governing embryonic stem cell fate decisions. Bioinformatics 2005; 21:741–753.

23 Sachs K, Perez O, Pe'er D, *et al.* Causal protein-signaling networks derived from multiparameter single-cell data. Science 2005; 308:523–529.

24 Hautaniemi S, Kharait S, Iwabu A, *et al.* Modeling of signal-response cascades using decision tree analysis. Bioinformatics 2005; 21:2027–2035.

25 •• Janes KA, Albeck JG, Gaudet S, *et al.* A systems model of signaling identifies a molecular basis set for cytokine-induced apoptosis. Science 2005; 310: 1646–1653.

The authors propose a method to elucidate dynamic system responses for cytokine-induced apoptosis. The approach is based on a computational method that considerably reduces the dimensionality of intracellular signals to a few relevant components.

26 Cinquin O, Demongeot J. High-dimensional switches and the modelling of • cellular differentiation. J Theor Biol 2005; 233:391–411.
The authors propose a general mathematical framework that is able to describe high-dimensional molecular switches and apply this methodology to explain cell-fate decisions.

27 Roeder I, Glauche I. Towards an understanding of lineage specification in • hematopoietic stem cells: a mathematical model for the interaction of transcription factors GATA-1 and PU.1. J Theor Biol 2006 [Epub ahead of print].
Using a mathematical model that is able to describe lineage specification processes, the authors derive two different hypotheses to explain the experimentally proposed priming in hematopoietic stem cells.

28 Prudhomme WA, Duggar KH, Lauffenburger DA. Cell population dynamics • model for deconvolution of murine embryonic stem cell self-renewal and differentiation responses to cytokines and extracellular matrix. Biotechnol Bioeng 2004; 88:264–272.
This study describes a simple mathematical model that is able to decompose stem cell self-renewal and differentiation into a basic set of underlying rate processes.

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

30 Loeffler M, Potten CS. Stem cells and cellular pedigrees: a conceptual introduction. In: Potten CS, editor. Stem cells. London: Academic Press; 1997. pp. 1–27.

31 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.

32 Roeder I, Kamminga LM, Braesel K, *et al.* Competitive clonal hematopoiesis in •• mouse chimeras explained by a stochastic model of stem cell organization. Blood 2005; 105:609–616.
The study describes an application of a previously proposed self-organizing concept of stem cell organization. Using a joint experimental and simulation study, it is demonstrated that this model is able to consistently describe dynamic clonal competition phenomena in mouse chimeras.

33 Kirkland MA. A phase space model of hemopoiesis and the concept of stem •• cell renewal. Exp Hematol 2004; 32:511–519.
The author proposes to describe stem cell differentiation as a continuous process rather than as a sequence of irreversible singular events. On this basis, he formulates a mathematical model which describes stem cell fates as trajectories in a phase space of cellular properties.

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

35 Shepherd BE, Guttorp P, Lansdorp PM, *et al.* Estimating human hemato- • poietic stem cell kinetics using granulocyte telomere lengths. Exp Hematol 2004; 32:1040–1050.
Using a stochastic model, the authors estimate replication rates of hematopoietic stem cells on the basis of granulocyte telomere lengths.

36 op den Buijs J, van den Bosch PP, Musters MW, *et al.* Mathematical modeling • confirms the length-dependency of telomere shortening. Mech Ageing Dev 2004; 125:437–444.
Based on a mathematical model, it is concluded that telomere shortening in human somatic cells is dependent on telomere lengths.

37 Arkus N. A mathematical model of cellular apoptosis and senescence through the dynamics of telomere loss. J Theor Biol 2005; 235:13–32.

38 Bernard S, Belair J, Mackey MC. Bifurcations in a white-blood-cell production model. C R Biol 2004; 327:201–210.

39 Pujo-Menjouet L, Mackey MC. Contribution to the study of periodic chronic myelogenous leukemia. C R Biol 2004; 327:235–244.

40 Colijn C, Mackey MC. A mathematical model of hematopoiesis: I – periodic • chronic myelogenous leukemia. J Theor Biol 2005; 237:117–132.
Application of a mathematical model to explain coupled oscillations of leukocytes, platelets and erythrocytes in periodic chronic myeloid leukemia.

41 Colijn C, Mackey MC. A mathematical model of hematopoiesis: II – cyclical • neutropenia. J Theor Biol 2005; 237:133–146.
Application of a mathematical model to explain coupled oscillations of leukocytes, platelets and erythrocytes in cyclical neutropenia.

42 Catlin SN, Guttorp P, Abkowitz JL. The kinetics of clonal dominance in •• myeloproliferative disorders. Blood 2005; 106:2688–2692.
Application of a stochastic model to analyze clonal dynamics in myeloproliferative disorders. It is demonstrated that competition of stem cells for micro-environmental support can lead to the suppression of normal cells at the expense of the malignant clone.

43 Michor F, Hughes TP, Iwasa Y, *et al.* Dynamics of chronic myeloid leukaemia. • Nature 2005; 435:1267–1270.
Application of a simple mathematical model to explain the dynamics of BCR–ABL transcript level in patients treated with imatinib.

44 Abkowitz JL, Golinelli D, Guttorp P. Strategies to expand transduced hema- • topoietic stem cells in vivo. Mol Ther 2004; 9:566–576.
Simulation study to analyze the effect of replication rate and apoptosis on the clonal persistence of transplanted stem cells.

45 Foley, C, Bernard, S, Mackey, MC. Cost-effective G-CSF therapy strategies • for cyclical neutropenia: mathematical modelling based hypotheses. J Theor Biol (in press) 2005 [Epub ahead of print].
The authors propose a model-based optimization of the treatment schedule for G-CSF application in patients with cyclical neutropenia.

46 Engel C, Scholz M, Loeffler M. A computational model of human granulopoi- •• esis to simulate the hematotoxic effects of multicycle polychemotherapy. Blood 2004; 104:2323–2331.
This study describes the application of a mathematical model of human granulopoiesis, demonstrating that the model is able to consistently explain the effect of different treatment options. The authors formulate a number of model predictions suggesting possibilities of treatment optimization.

47 Scholz M, Engel C, Loeffler M. Modelling human granulopoiesis under polychemotherapy with G-CSF support. J Math Biol 2005; 50:397–439.

48 Scholz M, Engel C, Loeffler M. Model based design of chemotherapeutic regimens accounting for heterogeneity in leucopenia. Br J Hematol 2006; 132:723–735.

49 Ostby I, Rusten LS, Kvalheim G, *et al.* A mathematical model for reconstitution of granulopoiesis after high dose chemotherapy with autologous stem cell transplantation. J Math Biol 2003; 47:101–136.

50 Ostby I, Kvalheim G, Rusten LS, *et al.* Mathematical modeling of granulocyte reconstitution after high-dose chemotherapy with stem cell support: effect of posttransplant G-CSF treatment. J Theor Biol 2004; 231: 69–83.

51 Lazebnik Y. Can a biologist fix a radio? Or, what I learned while studying apoptosis. Cancer Cell 2002; 2:179–182.