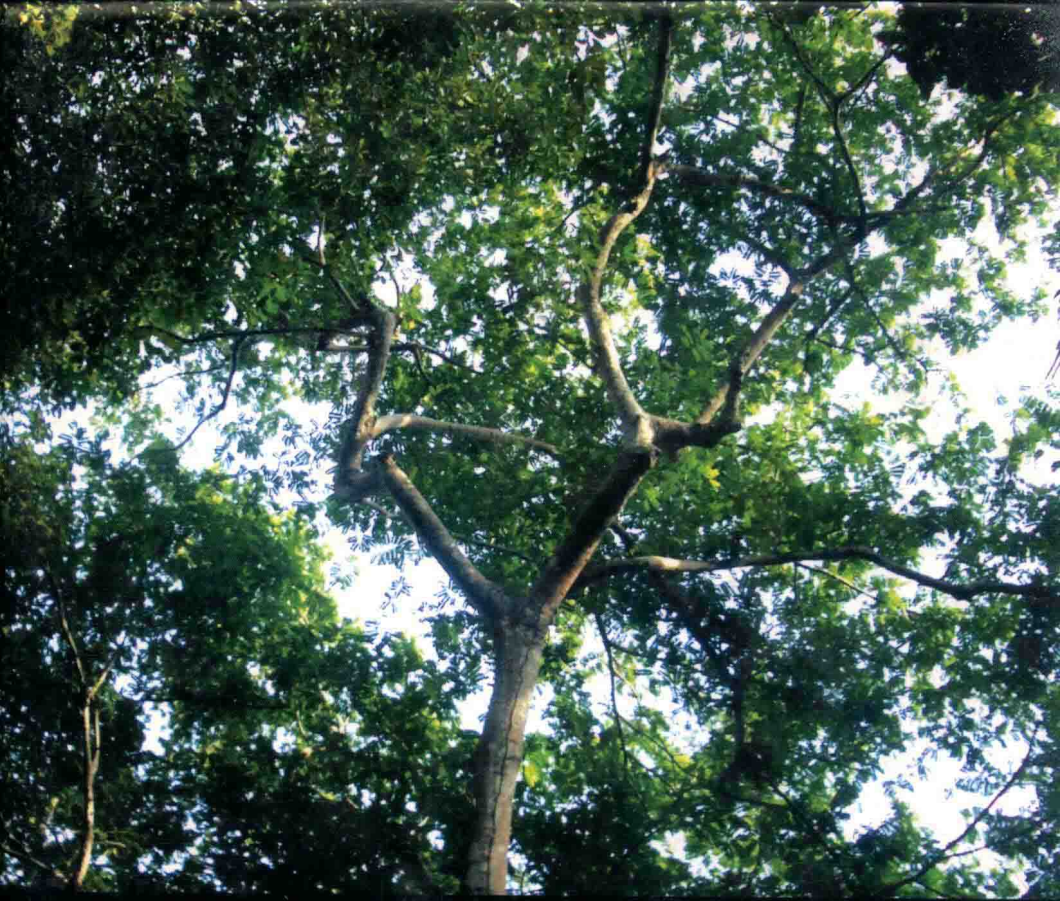

BIOMAT 2006

International Symposium on
Mathematical and Computational Biology

edited by Rubem P Mondaini • Rui Dilão

BIOMAT 2006

International Symposium on
Mathematical and Computational Biology

Manaus, Brazil 27 – 30 November 2006

edited by

Rubem P Mondaini
Universidade Federal do Rio de Janeiro, Brazil

Rui Dilão
Instituto Superior Técnico, Portugal

World Scientific

NEW JERSEY • LONDON • SINGAPORE • BEIJING • SHANGHAI • HONG KONG • TAIPEI • CHENNAI

Published by

World Scientific Publishing Co. Pte. Ltd.

5 Toh Tuck Link, Singapore 596224

USA office: 27 Warren Street, Suite 401-402, Hackensack, NJ 07601

UK office: 57 Shelton Street, Covent Garden, London WC2H 9HE

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library.

BIOMAT 2006

International Symposium on Mathematical and Computational Biology

ISBN-13 978-981-270-768-0

ISBN-10 981-270-768-9

Printed in Singapore by World Scientific Printers (S) Pte Ltd

BIOMAT 2006

International Symposium on
Mathematical and Computational Biology

Preface

This book contains the selected works of the BIOMAT 2006 International Symposium on Mathematical and Computational Biology. This series of symposia started in 2001, in Rio de Janeiro, Brazil, being the oldest interdisciplinary series of conferences in Latin America in the area of biomathematics. A successful realization every year is due to the expertise of the members of the BIOMAT Consortium as well as to the members of the BIOMAT Editorial Board, its Referees, and Scientific Program Committees.

The BIOMAT 2006 Symposium was held in the city of Manaus in the Brazilian Equatorial Rain forest, from November the 25th to December the 1st. We had fifteen Keynote Speakers from Europe and Americas and an impressive number of contributed works presented by scientists and research students from Brazil and abroad. The BIOMAT tutorials, which are already traditional in the BIOMAT symposia, and are lectured on the first two days of these conferences, are a source of motivation for future researchers in these interdisciplinary topics.

The topics of the BIOMAT 2006 Symposium were a combination of state of the art research and review approaches. They range from cell dynamics and surface reaction models of protocells, to the study of collective steady states of cells, to the modelling of infectious diseases like HIV epidemiology, molecular genetic mechanisms of hepatitis B virus, and the dynamics of tuberculosis. Models of physiological disorders like tumor growth and 3D reconstruction of objects were also analyzed. Topics on the modelling of DNA and proteins by using de novo structure prediction, substitution matrices and Steiner trees were discussed. Other subjects covered in the BIOMAT 2006 Symposium were studies in population dynamics like insect sociality, multistability on predator-prey models, and techniques of impulsive differential equations in bio-economics.

We are indebted to the Board of Trustees of two Brazilian Federal sponsoring agencies: Coordination for the Improvement of Higher Education Personnel — CAPES, and National Council for Scientific and Technological Development — CNPq. We thank also PETROBRAS, the Brazilian Oil company and the world leader of oil research on deep sea waters, and the PETROBRAS-CENPES Research Centre. We have received financial support from SUFRAMA — Superintendency of the Manaus Free Trade zone, and from UNINORTE University Centre. The National Institute for Research of the Amazon (INPA) has provided an excursion to its "Science

Park". We thank specially the directors and representatives of these institutions: Prof. José Fernandes de Lima from CAPES, Dr. Mª. de Lourdes Queirós from CNPq, Dr. Gina Vasquez from PETROBRAS-CENPES, Dr. Auxiliadora Tupinambá from SUFRAMA, and Dr. Wanderli Tadei from INPA.

Our warmest thanks are due to the representatives of two host institutions of the BIOMAT 2006 Symposium at Manaus: Prof. Mª. Hercília Tribuzzy, Dr. Isa Leal and Dr. Tania Castelo Branco, from UNINORTE University Centre. Dr. Andrea Waichman, Dr. Marta Gusmão and Dr. José Pedro Cordeiro from UFAM — Federal University of Amazonas.

On behalf of the Editorial Board of the BIOMAT Consortium, we thank all the authors, participants and sponsors of the BIOMAT 2006 Symposium for their continuous support to the scientific activities and administrative tasks of this successful conference.

Rubem P. Mondaini and Rui Dilão

Manaus, December 2006

Editorial Board of the BIOMAT Consortium

Contents

DNA and proteins

Population dynamics

SYSTEMS *STEM CELL* BIOLOGY*

INGO ROEDER

University of Leipzig
Institute for Medical Informatics, Statistics and Epidemiology
Haertelstr 16-18, D-04107 Leipzig, Germany
E-mail: ingo.roeder@imise.uni-leipzig.de

Within the last decade, our modeling attempts in stem cell biology have considerably evolved. Starting from the cellular level, our models now comprise a broad spectrum of phenomena on different scales, ranging from the molecular to the tissue level. Such a scale-bridging description of biological processes does exactly match the intentions of the newly emerging field of systems biology with its central objective to understand biological complexity from molecular scales to ecosystems by a joint application of experimental and theoretical techniques. This work is an attempt to illustrate our systems biological perspective on tissue stem cell organization. Herein, I will describe the general principles of a new concept that understands stem cell organization as a dynamic, self-organizing process rather than as a pre-defined sequence of discrete developmental steps, as classically proposed.

The suitability of these principles to explain a broad variety of experimental results is illustrated for hematopoietic stem cells (HSC). For this system, the general stem cell concept has been translated into a stochastic model that comprises the processes of stem cell self-renewal and differentiation as well as lineage specification on the cellular level. Starting from this model, I will describe one possible way to extend the description towards the intra-cellular level. To do so, we considered a simple transcription factor network as the underlying mechanism controlling lineage specification decisions of HSC and analyzed its dynamical properties applying a system of ordinary differential equations. Finally, a clinical application of the proposed single-cell based model of HSC will shortly be outlined. This application again extends the description level of the model, now also incorporating systemic effects of therapeutic interventions. Based on the assumption that chronic myeloid leukemia can be modeled by a clonal competition process of normal and malignant cells, we analyzed the potential dynamic treatment effects of the tyrosine kinase inhibitor *imatinib*. Our results suggest a selective activity of *imatinib* on proliferating cells which implies the hypothesis that the therapeutic efficiency might benefit from a combination of *imatinib* with drugs promoting the cell cycle activation of primitive stem cells.

*Work partially supported by grants LO942/9-1,2 and RO3500/1-1 of the German Research Foundation DFG.

1. Introduction

Systems biology is an emerging new field of science, covering the complexity of biology from molecular scales to ecosystems. Particularly, it is intended to contribute to a *systemic* understanding of biological processes and regulatory principles applying experimental *and* theoretical (i.e. modeling) techniques. Also with respect to stem cell biology, the enormous amount of emerging data (e.g. by the application of high-throughput techniques in the field of genomics, proteomics or transcriptomics) as well as the recognizable complexity of these systems, apparently call for the application of mathematical and computational methods. This clearly shows that a systems biological approach can and even has to be applied to the field of stem cell biology to achieve a comprehensive understanding, which itself is a prerequisite for the successful application of stem cells in therapeutic setting.

Using the example of hematopoietic stem cells (HSC), this work is illustrating how the application of a simple theoretical concept (approximating biological mechanisms) and its mathematical implementation (providing quantitative results) can be used to understand a broad variety of experimental phenomena. Starting from a description of stem cell self-renewal and differentiation as well as lineage specification on the cellular level, it will be described how the proposed generic regulatory principles might be explained by interactions of molecular regulators. Additionally, the possibility to apply quantitative stem cell modeling to the description of hematologic disorders, including the possibility to predict effects of different treatment options is illustrated.

1.1. *Defining tissue stem cells*

At the begin I would like to point to the fact that there are different types of stem cells. The most prominent classification is into embryonic stem cells (ESC) and adult tissue stem cells (TSC). Whereas ESC are derived by particular culturing methods from the inner cell mass of an blastocyst (i.e. an very early state of an embryo), TSC can be found in different tissues throughout the life of an organism[1]. Although both types are denoted as *stem cells*, ESC and TSC do considerably differ with respect to their potential and their functionality. In the following I will exclusively consider tissue stem cells and whenever the term *stem cell* is used, it refers to this cell type.

To analyze stem cells experimentally it is necessary to characterize and

select these cells for further investigation. Such a prospective selection process imposes the question: *Is this particular cell a stem cell?* This question implies the idea that one can indeed decide about the nature of a selected cell without relating it to other cells and without testing its capabilities. This, however, is a rather unrealistic point of view. To explain this, let us start by looking at the definition of tissue stem cells. Although there are a number of definitions around (see e.g.[1,2,3]), there is a consensus that its *functional* attributes, and not an explicitly observable characteristic, has to be considered as the gold standard for a stem cell characterization.

The following definition, which explicitly refers to the functional capabilities, has been formulated by Loeffler and Roeder[4] on the basis of a definition previously given by Potten and Loeffler[2]: *Stem cells of a particular tissue are a (potentially heterogeneous) population of functionally undifferentiated cells, capable (i) of homing to an appropriate growth environment, (ii) of proliferation, (iii) of production of a large number of differentiated progeny, (iv) of self-renewing their population, (v) of regenerating the functional tissue after injury, and (vi) with a flexibility and reversibility in the use of these options.* This choice of a functional definition is inherently consistent with the biological role of a stem cell, particularly linked to the functional tissue regeneration feature. However, it imposes difficulties since in order to identify whether or not a cell is a stem cell, its function has to be tested. This requires an experimental manipulation, subjecting the cell to a functional bioassay, which inevitably alters its properties. In analogy to the Heisenberg's uncertainty principle in quantum physics[5] this phenomenon is sometimes called *uncertainty principle of stem cell biology*[2]. Simply speaking, this principle states that the very act of measuring the functional system properties always changes the characteristics of the system, therefore, giving rise to a certain degree of uncertainty in the characterization of the system. If this analogy holds true for tissue stem cells - and there is strong evidence for that - all prospective statements that can be made about stem cells will be necessarily probabilistic statements about the future behavior under particular conditions.

1.2. *Conceptual challenges in tissue stem cell biology*

An essential issue of the above given definition of TSC is the flexibility criterion. There is increasing evidence for flexibility and reversibility of stem cells which will be highlighted by a few examples, preferably related to the hematopoietic system. Tissue stem cells are heterogeneous with

respect to functional properties such as cycling activity, engraftment potential or differentiation status, and to the expression of specific markers such as adhesion molecules or cell surface antigens. However, experimental evidence is accumulating that these properties are able to reversibly change[6,7,8,9]. Many authors have described the variability in the proliferative status of hematopoietic stem cells. One important finding in this respect is the fact that primitive cells may leave the cell cycle for many days and even months, but that almost all re-enter cycling activity from time to time[10,11]. Experimental evidence is also provided for reversible changes of the stem cell phenotypes involving differentiation profiles, adhesion protein expression and engraftment/homing behavior associated with the cell cycle status or the point in the circadian rhythm[8]. Also the expression of cell surface markers (e.g. CD34) on hematopoietic stem cells is not constant but may fluctuate. The property can be gained and lost without affecting the stem cell quality[7,12]. Furthermore, there is a lot of indirect evidence for fluctuations in the stem cell population based on the clonal composition of functional cells. Chimerism induced by bone marrow transplantations in animal models has been shown to fluctuate with time[13,14,15] indicating variations in the composition of active and inactive tissue stem cells. Similar observations were made following retroviral marking of individual stem cell clones which highlight the relative differences of inheritable cellular properties between stem cell clones and their impact on the competitive potential[16,17,18]. Another level of flexibility was found for lineage specification within the hematopoietic tissue. It is possible to bias the degree of erythroid, granuloid, or lymphoid lineage commitment by several maneuvers altering the growth-conditions in different culture systems[6,?]. The present concept to explain the fluctuations observed in lineage specification is based on a dynamic network of interacting transcription factors[20,21]. Some authors put forward the concept of fluctuating levels of transcription factors with threshold dependent commitment[22]. Moreover, tissue stem cells specified for one type of tissue (e.g. hematopoiesis) can be manipulated in such a way that they can act as tissue stem cells of another tissue[23,24,25,26]. As suggested by experimental observations on these tissue plasticity phenomena, microenvironmental effects seem to play an essential role in directing cellular development. Clearly, tissue plasticity represents a particular degree of flexibility consistent with the above definition.

Because classical stem cell concepts are not able to explain all these experimental findings consistently, new conceptual approaches are required. To be validated, such concepts need a rigorous examination by quantitative

and predictive modeling. In the following, some general ideas on how to proceed with such theories are presented and illustrated by a worked model of hematopoietic stem cells.

1.3. *Predictive theories and quantitative models*

Within the natural sciences a model is understood as a simplifying abstraction of a more complex construct or process. Theoretical models in biology include qualitative concepts (i.e. descriptive representations) and quantitative models (i.e. mathematical representations) of a biological process. In contrast to qualitative concepts, quantitative models allow for an analytical, numerical, or simulation analysis. The more we realize that we cannot prospectively determine stem cells directly, the more we need theoretical approaches to cope with the complexity. There is a tremendous need for general and specific theoretical concepts of tissue stem cell organization, as well as for related quantitative models to validate the concept by comparison of model predictions and experimental results.

Such a theoretical framework of tissue stem cell functioning will have several advantages: Model predictions can assist biologists 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 (i.e. tissues or cell types) may help to understand generic construction and regulation principles. Furthermore, they contribute to the understanding of latent mechanisms or crucial parameters of biological processes and may predict new phenomena.

The following list represents a summary of general requirements which quantitative models should fulfill in order to be suitable to serve as the basis for a theoretical framework of tissue stem cell organization: The model cells must fulfill the criteria listed in the definition of tissue stem cells consistently. This has the following implications: (i) The models must be based on populations of individual cells to follow clonal development, to conform with the uncertainty principle, and to enable considerations of population fluctuations. (ii) They must consider growth environments and the interactions between the cells. (iii) The system has to be dynamic in time and possibly space. (iv) The system requires assumptions on mechanism to regulate proliferation, cellular differentiation, and cell-cell / cell-growth environment interactions. (v) The model concept must be comprehensive in

the sense of being applicable to the normal unperturbed *in vivo* homeostasis as well as to any *in vivo* or *in vitro* assay/intervention procedure. This criterion requests that system-measurement interactions must be consistently considered.

2. A new perspective on stem cell systems

As discussed above, the basic concept of a functional definition of TSC has widely been accepted. Such a functional definition implies that one does not require *stemness* as an explicit attribute of cells, but rather considers it as a functional endpoint. Therefore, any concept on TSC has to specify assumptions about the mechanisms that potentially control the regenerative and proliferative potential of these cells, such as proliferation, differentiation, maturation, lineage specification, and homing. Hence, the task is to design a dynamic process that drives and controls the cellular attributes. Central points herein are aspects of capabilities (i.e. actual and potential expression of cellular properties), of flexibility, and of reversibility. Apparently, all these aspects are determined by the genetic and epigenetic status of the cells and by the activity of the signal transduction pathways including the transcription factor networks. Presently, it is impossible to describe these processes in any reasonable detail. However, it is possible to propose a simplified basic scheme of the generic principles underlying the cellular dynamics.

2.1. *Phenotypic reversibility as a generic principle of stem cell systems*

Classical concepts of tissue stem cell organization are almost exclusively based on the assumption of a strict unidirectional developmental hierarchy. However, as mentioned above, many current experimental results are challenging these concepts and show the necessity of new conceptual approaches to understand TSC organization. Flexibility and reversibility of tissue/lineage specification and of cellular properties within a tissue (summarized under the term *phenotypic reversibility*) have major implications with regard to concepts of stem cell function. Therefore, our group proposed to give up the view of tissue stem cells as being entities with a pre-programmed development and to replace it by a concept that makes the capabilities for flexible and regulated tissue self-organization the new paradigm[4]. Such a concept permits to incorporate context-dependent phenotypic reversibility and generation of stem cell heterogeneity as the result

of a dynamically regulated process and it strictly avoids assumptions that end up with direct or indirect labeling of particular cells as stem cells *a priori*. All model cells are characterized only by functional properties (e.g. proliferating or not, having an affinity for homing to a particular growth environment, sensitivity to particular growth factors etc.) and request that the system behavior changes these properties such that the population fulfill the functional criteria of the stem cell definition.

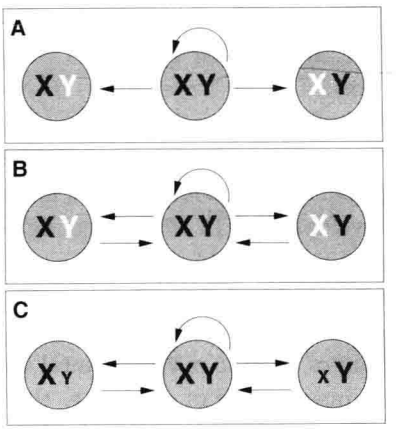

Figure 1. Examples of state transition graphs according to level 1 and 2 dynamics. X and Y illustrate certain genes or functionally related gene clusters. Whereas the color is coding for the level 1 dynamics status (black: sensitive, white: insensitive), the font size illustrates the quantitative expression level according to level 2 dynamics. (A) Shows irreversible loss of cellular properties due to permanent level 1 inactivation. Only self-maintenance of XY state possible. (B) Due to reversible changes (plasticity) with respect to level 1 dynamics (sensitive, insensitive) true self-renewal of XY state possible. (C) Reversibility (plasticity) of XY state due to changes with respect to quantitative level 2 dynamics.

To explain this concept, let us consider the activity of genes[a] determining the behavior of TSC. Because there might be circumstances when particular gene sets are insensitive to activation by regulatory molecules, e.g. if epigenetic constellations prevent accessibility or if key regulator molecules are lacking[23], two levels of gene activity control are conceptually distinguished: Level 1 is qualitative and decides whether a gene is accessible for activation or not (sensitive or insensitive). Level 2 is quantitative

[a]here, genes are used as examples for regulatory units, neglecting any post-transcriptional regulation and identifying genes with their products, e.g. regulator proteins.

and describes the degree of gene expression in a sensitive gene. Within this concept, a gene may not be expressed for two very different reasons. It may either not be sensitive (level 1 dynamics) or it may be sensitive, but it is not activated due to lack of challenge (level 2 dynamics). State-transition graphs can be used to characterize this two level dynamics. If they contain only self-maintaining and irreversible acyclic transitions between states, a population can be self-maintaining but not self-renewing (Figure 1A). In contrast, figures 1B and C illustrate state transition graphs which are characterized by reversible transitions. This would imply the property of true self-renewal, in the sense that cellular properties can be reestablished even if they had been lost/locked (level 1 dynamics) or down regulated (level 2 dynamics) before. We, furthermore, assume that the preferred direction of cellular development is dependent on growth environment specific signals. Therefore, alternating homing to various growth environments would yield a rather fluctuating development. In such a setting not only the influence of the environments, but particularly the frequency of transitions between them would be important. Figure 2 illustrates how signals from different growth environments can influence the cellular fate. Although only explained for level 2 dynamics, growth environmental signals could also affect transient or permanent inactivation of genes, i.e. the level 1 dynamics.

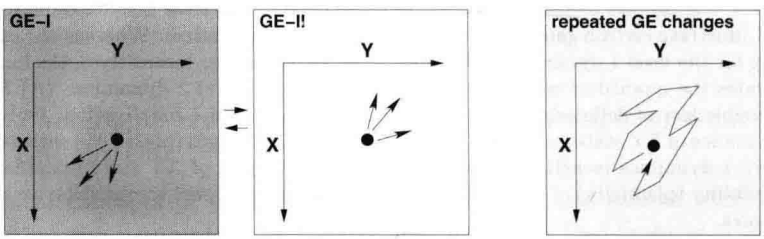

Figure 2. Dependency of cellular development on growth environment. This figure illustrates the actual position of a cell (black dot) and the preferred developmental directions (arrows) with respect to level 2 dynamics of cellular properties X and Y (e.g. gene expression) depending on the actual growth environment (GE). Alternation between different growth environments can induce fluctuating expression of cellular properties (quantitative plasticity), as illustrated in the rightmost panel by one possible example trajectory.

Taken together, such a general concept of growth environment dependent dynamics of reversibly changing cellular properties is a possibility to explain processes of self-renewal and differentiation in tissue stem cell systems. This general framework has been translated into a stochastic, single-