Theoretical concepts of tissue stem cell organization

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Summary

Many recent experimental findings on heterogeneity, flexibility, and plasticity of tissue stem cells are challenging the classical stem cell concept of a pre-defined, cell-intrinsic developmental program. Moreover, a number of these results are not consistent with the paradigm of a hierarchically structured stem cell population with a uni-directional development. Non-hierarchical, self-organizing systems provide a more elegant and comprehensive alternative to explain the experimental data.
Within the last decade, our modeling attempts in stem cell biology have considerably evolved and are now encompassing a broad spectrum of phenomena, ranging from the cellular to the tissue level. Based on our results we advocate to abandon the classical assumption of a strict developmental hierarchy and to understand stem cell organization as a dynamic, self-organizing process. Such a concept, which makes the capabilities for flexible and regulated tissue function based on cell – cell and cell – environment interactions the new paradigm, would permit to incorporate the context-dependent lineage plasticity, within-lineage plasticity, and generation of stem cell heterogeneity as a result of a dynamically regulated process. This perspective has implications for a prospective characterization of tissue stem cells, e.g. regarding gene expression profiles and genetic regulation patterns.
To be validated, such concepts need a rigorous examination by quantitative and predictive modeling of specific biologically relevant tissues. Within the following chapter, we provide some general ideas on how to proceed with such theories and illustrate this with a worked model of hematopoietic stem cells applied to clonal competition processes. Furthermore, we give an example of how to include possible effects of a spatial arrangement of cells into the proposed new stem cell paradigm.

Defining tissue stem cells

"Is this cell a stem cell?" This frequently posed question implies the idea that one can decide about the capabilities of a selected cell without relating it to other cells and without testing the capabilities functionally. We argue that this is a very naive and unrealistic point of view. To explain this perspective, let us start by having a look at the definition of tissue stem cells, which has been extensively discussed elsewhere (1, 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. Within this definition, stem cells are defined by virtue of their functional potential and not by an explicit directly observable characteristic.

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. This kind of definition, however, imposes difficulties since in order to identify whether or not a cell is a stem cell its function has to be tested. This inevitably demands that the cell must be manipulated experimentally in subjecting it to a functional bioassay. This, however, alters its properties. Here, we find ourselves in a circular situation. In order to answer the question, whether a cell is a stem cell, we have to modify it. In doing so, we unavoidably lose the original cell, and in addition we may only see a limited spectrum of responses. In analogy to the *Heisenberg's uncertainty principle* in quantum physics we call this the *uncertainty principle of stem cell biology*. In simple terms, this principle states that the very act of measuring the functional properties of a certain system always changes the characteristics of that system, hence, giving rise to a certain degree of uncertainty in the evaluation of its properties. We believe that this analogy holds true for the functional tissue stem cells in a very fundamental sense. Therefore, all statements that we can make about stem cells will be necessarily probabilistic statements about the future behavior under particular conditions.

Conceptual challenges in tissue stem cell biology

One essential aspect of the discussed definition of tissue stem cells is the flexibility criterion. There is an accumulating experimental evidence for flexibility and reversibility. We like to highlight a few of these, preferably related to the hematopoietic system.

It is now widely accepted that 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, recent experimental evidence is accumulating that these properties are able to reversibly change (3-12). 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. I.e. there is no pool of permanently dormant stem cells (13, 14). 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 (6, 15). There is increasing evidence that 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 (5, 16). Other groups investigated hemoglobin switching of hematopoietic stem cells in the blastocyste growth environment. Geiger et al. (17) showed that the switch from embryonic/fetal-type to adult-type globin is reversible. 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 transplantation maneuvers in cats and mice has been shown to fluctuate with time (18-22) indicating variations in the composition of active and inactive tissue stem cells. For the intestinal crypt there is good evidence for a competition process of tissue stem cells within the individual crypts. This competition leads to a fluctuation of the clonal composition with a dynamic instability leading to crypt fission (23, 24). 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 (25-29). 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 (4, 30). The present concept to explain the fluctuations observed in lineage specification is based on a dynamic network of interacting transcription factors (31-37). Cross and Enver put forward the concept of fluctuating levels of transcription factors with threshold dependent commitment (38).

Moreover, there is a rapidly growing literature that 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 (e.g. neuronal, myogenic) (39-43). As suggested by experimental observations on these tissue plasticity phenomena, microenviromental effects seem to play an essential role in directing cellular development. Very clearly this tissue plasticity represents a particular degree of flexibility consistent with the above definition. On the other hand this phenomenon explains the necessity to include the homing to a specific growth environment into the stem cell definition.

Motivated specifically by these experimental results on stem cell plasticity, a debate, whether the view of a strict, unidirectional developmental hierarchy within tissue stem cell populations is still appropriate, has been initiated (8, 44-50). Although the general existence of tissue plasticity properties is widely accepted, the underlying mechanisms (e.g. trans-/ de-differentiation or cell-fusion) and the relevance of these plasticity potential in normal *in vivo* systems or even in clinical setting is still unclear. Furthermore, high throughput analysis of genomic data (e.g. gene expression profiling) and signaling studies offer the chance to extend our knowledge on tissue stem cells to the molecular level (32, 51-53). Because classical stem cell concepts are not able to explain all these experimental findings consistently, new conceptual approaches and theoretical models are required.

Predictive theories and quantitative models

Within the natural sciences a *model* is understood as a simplifying abstraction of a more complex construct or process. In contrast to *experimental models* (e.g. animal or in vitro models), we will focus in the following on *theoretical models*. Theoretical models in biology include *qualitative concepts*, i.e. descriptive representations, and *quantitative model*s, 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. We believe that 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: The 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 common construction and regulation principles. Furthermore, they contribute to the understanding of latent mechanisms or crucial parameters of biological processes and may predict new phenomena. Subsequently, we give a list of general requirements which quantitative models should fulfill in order to be suitable to serve a the bases 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:

- 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.
- They must consider growth environments and the interactions between the cells.
- The system has to be dynamic in time and possibly space.
- The system requires assumptions on mechanism to regulate proliferation, cellular differentiation, and cell - cell / cell - growth environment interactions.
- 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 procedure. This criterion requests that system – measurement interactions must be consistently considered.

A new perspective on stem cell systems

The basic concept of a functional definition of tissue stem cells (see above) has proven useful. This 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 tissue stem cells 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. The leitmotifs here are the aspect of capabilities (i.e. actual and potential expression of cellular properties), of flexibility, and of reversibility. Apparently these aspects are controlled by the genetic and epigenetic status of the cells and by the activity of the signal transduction pathways including the transcription factor networks. Clearly, it is presently impossible to describe these processes in any reasonable detail. It will, therefore, be necessary to propose a simplified basic scheme of the cellular dynamics.

One possibility to consistently explain the variety of experimental phenomena without explicitly assuming a predefined *stemness* property of the cells has been developed by our group recently. This approach radically differs from other concepts presented so far in the literature. It strictly avoids assumptions that end up with direct or indirect labeling of particular cells as stem cells *a priori*. We rather attribute to all model cells only 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 fulfils the functional criteria of the stem cell definition.

To explain our conceptual approach, let us consider the activity of genes relevant for the behavior of tissue stem cells. There may be circumstances when sets of genes are insensitive to activation despite the availability of regulatory molecules. This is the case if e.g. epigenetic constellations prevent accessibility or if key regulator molecules such as transcription factor complexes are lacking (54-57). Therefore, we will conceptually distinguish two levels of gene activity control. Level 1 is qualitative and decides whether a gene is accessible for activation or not (sensitive or insensitive). Level 2 is quantitative and describes the degree of gene expression in a sensitive gene. Within this concept of a two level control, 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 there is no or minor activation 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 or down regulated before.

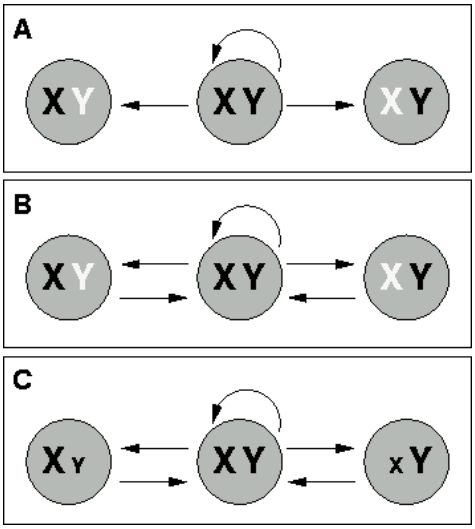

Figure 1: Examples of a simple state transition graphs according to level 1 and 2 dynamics. X and Y illustrate certain genes or functionally related gene clusters. Whereas the color is coding for the level 1 dynamics status (black: sensitive, white: insensitive), the font size illustrates the quantitative 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.

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 would be considerable, but in particular the frequency of transitions between them. For examples, Figure 2 illustrates how signals from different growth environments can influence the cellular fate, i.e. the trajectories of cells within a property (e.g. gene expression) space, with respect to level 2 dynamics. 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.

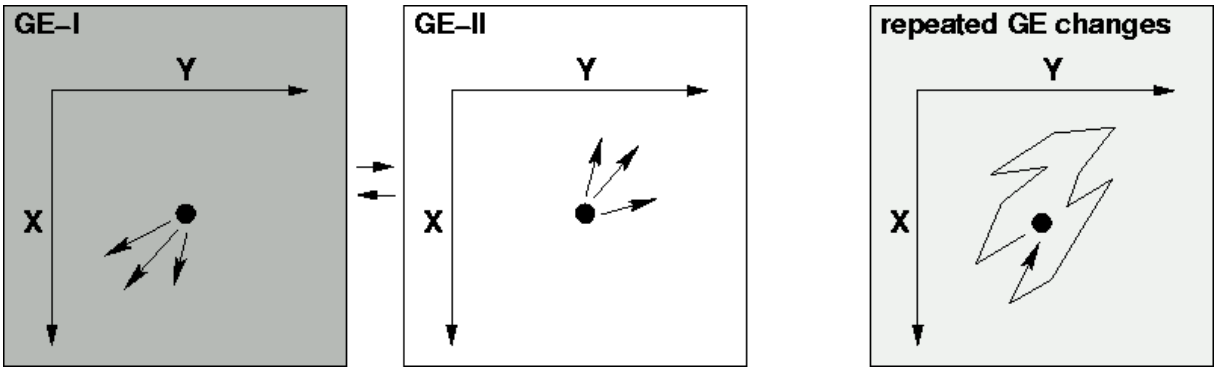

Figure 2: Dependency of cellular development on growth environment. This figure illustrates the actual position of a cell (•) and the preferred developmental directions (arrows) with respect to level 2 dynamics of cellular properties X and Y (e.g. gene expression) depending on the actual growth environment (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.
In the following section, we will demonstrate how this concept, implemented into a quantitative, mathematical model, has been applied to one specific tissue stem cell system to explain dynamical processes of clonal competition in the hematopoietic system.

Modeling of the dynamics of clonal competition in hematopoietic stem cells

Applying the principles described in the last section to the hematopoietic stem cell system leads to the concept of *within-tissue plasticity* (2, 58), which will subsequently be described. Herein, we assume that cellular properties of hematopoietic stem cells can reversibly change within a range of potential options. The direction of cellular development and the decision whether a certain property is actually expressed, depends on the internal state of the cell and on signals from its growth environment. Individual cells are considered to reside in one of two growth environments (GE-A or GE-Ω). The state of each cell is characterized by its actual growth environment, by its position in the cell cycle (G_1, S, G_2, M or G_0), and by a property (a) which describes its affinity to reside in GE-A. Whereas cells in GE-Ω gradually loose this affinity, cells in GE-A are able to gradually regain it (level 2 dynamics). Furthermore, cells in GE-A are assumed to be non-proliferating (i.e. in G_0), while cells in GE-Ω are assumed to proliferate with an average generation time τ_c. The transition of cells between the two GE is modeled as a stochastic process. The corresponding transition intensities (probabilities of growth environment change per time step, α and ω) depend on the current value of the affinity a and on the number of stem cells residing in GE-A and GE-Ω, respectively. If the attachment affinity a of an individual cell has fallen below a certain threshold (a_{min}), the potential to home to GE-A is inactivated (level 1 dynamics). These cells are released from the stem cell compartment and start the formation of clones of differentiated cells. Figure 3 gives a graphical illustration of the model structure and of the cell number dependency described in the model by the transition characteristics f_α and f_ω.

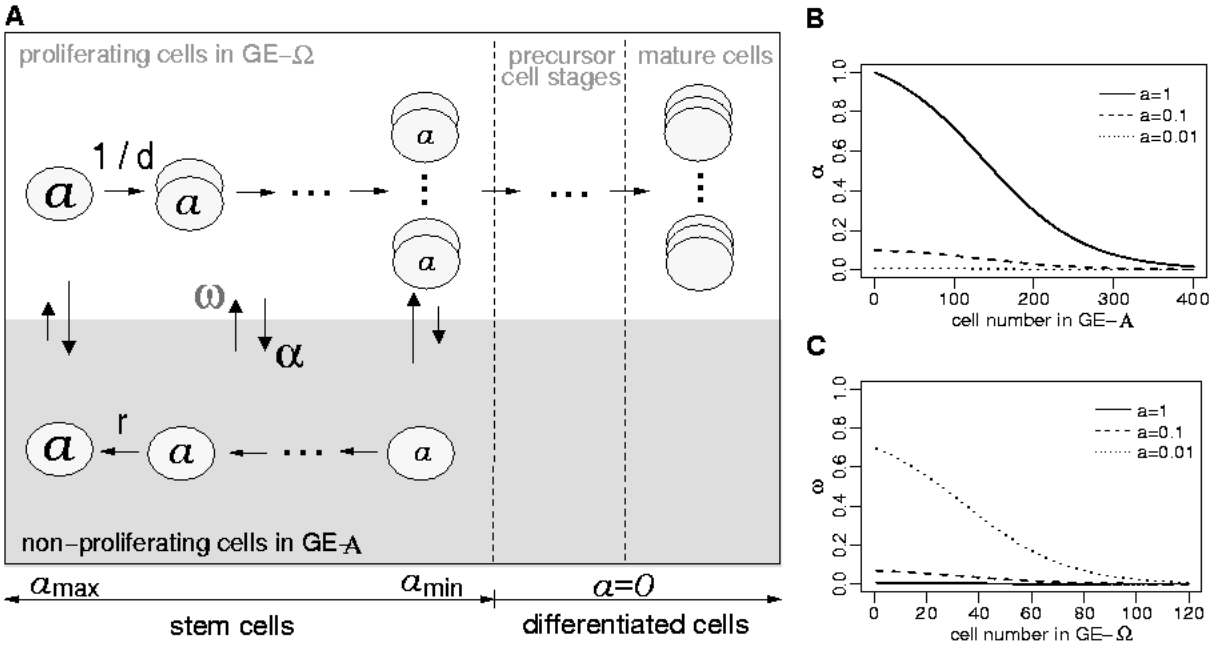

Figure 3. Schematic representation of the model concept. (A) The lower part represents growth environment GE-A and the upper part GE-Ω. Cell amplification due to proliferation in GE-Ω is

illustrated by growing cell numbers (cell groups separated by vertical dots represent large cell numbers). Whereas attachment affinity *a* decreases by factor *1/d* per time step in GE-Ω, it increases by factor *r* per time step in GE-A. The actual quantity of the affinity *a* is sketched by different font sizes. If *a* fell below a critical threshold a_{min}, the cell lost its potential to switch to GE-A and *a* is set to 0 (represented by empty cells). Transition between GE-A and Ω occurs with intensities $\alpha = (a / a_{max}) \cdot f_\alpha$ and $\omega = (a_{min} / a) \cdot f_\omega$, which depend on the value of *a* (represented by the differently scaled vertical arrows) and on the cell numbers in the target GE. Typical profiles of the cell number dependent transition intensities f_α and f_ω for different values of attachment affinity *a* shown in panels B and C.

A mathematical representation of this concept has been implemented in a computer program. Using extensive simulation studies we could demonstrate that this model can describe a large variety of observed phenomena, such as heterogeneity of clonogenic and repopulation potential (demonstrated in different types of colony formation and repopulating assays), fluctuating clonal contribution (observed in chimeric animals or in individual clone tracking experiments), or changing cell cycle activity of primitive progenitors (described by the use of different S-phase labeling studies) (22, 58, 59). One of these phenomena, the competition of different stem cell populations in mouse chimeras, will subsequently be used as an example to illustrate the potential of mathematical modeling in describing and explaining biological observations.

To be able to apply the model to a mouse chimera setting, i.e. to the coexistence of cells from two different mouse strain backgrounds (DBA/2 and C57BL/6) in one common host, we consider two populations of cells within one model system. These populations potentially differ in their model parameters *d*, *r*, τ_c, f_α, or f_ω. This approach allows the analysis of the influence of these model parameters on the competitive behavior of the two cell types and, therefore, on the dynamics of chimerism development.

Simulation studies, lead to two major qualitative predictions for the chimeric situation: Firstly, the model predicts that small differences in model parameters may cause unstable chimerism with a slow but systematic long-term trend in favor of one clone. Secondly, it is predicted that the chimerism development depends on the actual status (i.e. cell numbers) of the entire system. Therefore, system perturbations, e.g. by stem cell transplantation after myeloablative conditioning, cytokine or cytotoxic treatment, are expected to result in significant changes of chimerism levels at a short time scale. These prediction are also supported by previously reported experimental results on the contribution of DBA/2 (D2) cells to peripheral blood production in C57BL/6 (B6) – D2 allophenic mice (18). In these animals, the D2 contribution declines over time, but can be reactivated by a bone marrow transplantation into lethally irradiated B6-D2-F1 (BDF1) mice.

To subject our qualitative model predictions to an experimental test and to investigate whether these phenomena could be explained consistently by one single parameter configuration of the model, a specific set of experiments had been performed. To quantitatively compare experimental data and simulation results, we investigated the chimerism kinetics in primary and secondary B6-D2 radiation chimeras. The detailed experimental procedure has been described elsewhere (22). Shortly, primary irradiation chimeras were constructed by transplantation of fetal liver cells isolated from B6 and D2 mice into lethally irradiated BDF1 mice. To measure chimerism levels, blood samples were drawn from each chimera at various time points after transplantation. The percentage of leukocytes derived from D2, B6 and BDF1 was assessed by flowcytometry. To determine the effect of serial bone marrow transplantation on the chimerism dynamics, secondary transplantations were performed. Herein, bone marrow cells from individual chimeric donors at different time points after primary transplantation of FL cells were transplanted into cohorts of 5 and 12 lethally irradiated female BDF1 mice, respectively. Identically to primary hosts, the chimerism was determined by repeated peripheral blood samples in these secondary chimeras.

To simulate the chimeric development of individual mice, the actual status of each stem cell, characterized by its attachment affinity (*a)*, its position in the cell cycle, and its current growth environment (GE-A, GE-Ω or pool of differentiated cells), is updated at discrete time steps (for details see (22)). Additionally, the actual number of stem cells in GE-A, GE-Ω, and of differentiated cells is recorded at these time points. To determine the number of peripheral blood leukocytes in the simulations, the pool of mature cells (see Figure 3A) is used. Hereby, it is assumed that the number of mature leukocytes is proportional to the number of cells released from the stem cell compartment. Details of amplification, differentiation, and maturation within precursor cell stages are neglected in the current model version. Chimerism levels are obtained by calculating the D2 proportion among model cells within the mature leukocyte compartment.

Due to the assumed stochastic nature of the growth environment transition of stem cells, individual simulation runs produce different chimerism levels even though identical parameter sets are used. Therefore, to determine the mean chimerism levels under a specific parameter set, repeated simulation runs have been performed. To illustrate the average behavior the mean chimerism levels are determined at each time step.

Starting from a parameter configuration which has previously been demonstrated to consistently explain a variety of experimental phenomena in the non-chimeric situation, we fitted the simulation outcome to the observed chimerism development in primary irradiation chimeras initiated with a 1:4 ratio of transplanted D2 and B6 fetal liver cells. Due to the documented difference between D2 and B6 cells with respect to their cycling activity, we assumed different average generation times. However, solely assuming this difference is not sufficient to explain the observed biphasic chimerism development. Therefore, we performed a sensitivity analysis of the model parameters controlling the cellular development, i.e. the differentiation coefficient (*d*), the regeneration coefficient (*r*), and the transition characteristics f_α and f_ω. We found that only differences in the transition characteristics induce the observed biphasic pattern. Whereas the qualitative chimerism development was primarily determined by the transition characteristics, the maximally reached D2 levels are dependent on the ratio of initially engrafting D2 and B6 cells. Optimal parameter values of the initial D2 proportion of engrafting stem cell and of the shape parameters of the transition characteristics f_α and f_ω have been determined by fitting simulation results to experimental data using an evolutionary strategy. For technical details of the fitting procedure and for a description of the specific form of the transition characteristics we refer to (22).

The data points in Figure 4A show the experimentally observed chimerism development in unperturbed radiation chimeras together with an average simulation using the fitted set of model parameters. Without any further change of the model parameters, our simulations demonstrate that the experimentally observed heterogeneity of chimerism development in different experiments can be explained by variations in the initial D2:B6 ratio (Figure 4B). To test, whether these parameter configurations (obtained for the competition situation in chimeric systems) are also able to explain differences in the reconstituting behavior of non-chimeric D2 and B6 systems, we simulated the reconstitution of non-chimeric systems using the D2 and the B6 parameter sets, respectively. It could be shown (22) that the simulations are able to reproduce the differences in the time scales of reconstitution between D2 and B6 which had been observed experimentally.

Furthermore, using the same parameter configuration, simulations predict that a reduction of the total stem cell pool size, as assumed for the transplantation setting, induces an initial elevation of the D2 contribution in the host (compared to donor chimerism prior to transplantation) followed by a gradual D2 decline (Figure 4C). This is consistent with the experimental results obtained by the transplantation of bone marrow cells from a primary radiation chimera at day 133 after first transplantation into secondary cohorts of lethally

irradiated BDF1 mice, which clearly show a reactivation of D2 contribution in the peripheral blood (data points in Figure 4C).

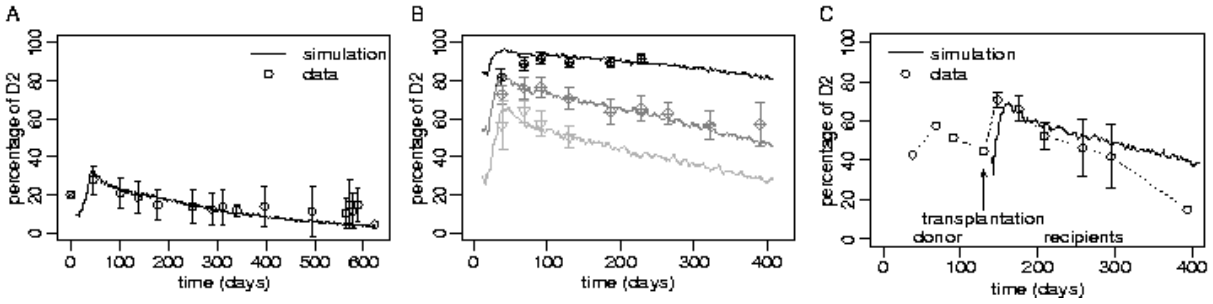

Figure 4. Simulation results on chimerism development. (A) Data points (open circles) represent the observed chimerism levels (mean +/- 1 SD) in primary radiation chimeras with ⊗ illustrating the initial D2:B6 ratio in the transplant. The solid line shows the simulated chimerism of mature model leukocytes (average of 100 simulation runs). (B) Effect of the initial D2:B6 ratio: Data points represent the results (mean ± 1 SD) from three independent experiments using different D2 proportions of the transplant. Solid lines represent corresponding average simulation results using identical parameter sets, but different initial D2 proportions: 85% - black, 50% - dark gray, 30% - light gray. (C) The circles show the experimentally observed peripheral blood leukocyte chimerism in a primary radiation chimera (single values) and in a corresponding cohort of secondary host mice (mean +/- SD). The solid lines show average simulations for the chimerism development in the secondary chimeras.

These results provide an experimental test of our novel concept of tissue stem cell organization based on the within-tissue plasticity idea for the situation of competitive hematopoiesis. Using a parameter configuration obtained by fitting the model to one specific data set, the mathematical model made several predictions for the situation of clonal competition and unstable chimerism. We demonstrated that this single parameter configuration can explain the majority of the presented phenomena in the chimeric situations and is also consistent with the variety of further phenomena analyzed before (22, 58, 59). It should be noted that parameter adjustments for the simulation of each individual data sets would provide even better model fits. However, it was our main goal to validate the model by the application of one parameter configuration to several independent data sets.

Our results suggest that chimerism levels, observed in the peripheral blood, depend on the actual dynamic status of the stem cell system. The simulation studies reveal that variations in strain specific cellular properties of stem cells, which sensitively affect the competitive behavior in a chimeric situation, do not necessarily influence their growth and repopulating potential in a non-chimeric system. These findings point to the relative nature of stem cells and their repopulating potential in general. Therefore, stem cell potential must not be regarded as an isolated cellular property, but has to be understood as a dynamic property taking into account the individual cellular potential, the cell-cell and the cell-microenvironment interactions. This has potentially important implications for the treatment of clonal disorders, gene therapeutic strategies, or tissue engineering processes where it is aimed to control the competitive potential of a specific cell type or clone.

Spatio-temporal stem cell organization

The assumption of different growth environments suggests that a spatial component might also influence tissue stem cell organization. This hypothesis is supported by several experimental findings (60-63), however, it is ignored in the stem cell model discussed so far. In the following, we show that the spatial arrangement of cells in a stem cell compartment and

the related effects on the system behavior can consistently be incorporated into the above described concepts.

First of all, an extension of the described model to incorporate spatio-temporal dynamics requires an explicit physical representation of the cells. As real cells, the model cells need to have a shape, a volume, and specific biomechanical properties. Furthermore, they need to be able to detect shape and stress changes within their local environment by sensing the degree of their own extension or compression. Thereby, these models need to describe a link between shape changes and functional processes such as proliferation, differentiation, and apoptosis. As a consequence, basic effects of tissue organization can be attributed to cell contact formation between individual cells and their local growth environment.

Due to recent experimental advances (64-66), the possibilities to collect new information on biophysical parameters of cells and tissues are rapidly improving. Utilizing this information, a specific class of so called 'individual cell-based biomechanical models (ICBM)', is now available. Recently, we have shown that this model class is capable of explaining the complex spatial growth and pattern formation processes of epithelial stem cell populations growing *in vitro* (67). ICBM permit to model the growth and pattern formation of large multi-cellular systems since they tie properties averaged on the length scale of a cell to the macroscopic behavior on the cell population and tissue level. Consequently, they allow for an efficient simulation and, therefore, permit the analysis of spatial arrangements of large cell populations on large time scales. Thus, ICBM enable approaches to cell differentiation, maturation, and lineage specification accounting for tissue formation and regeneration (68, 69). A number of different individual based models of cell populations have been studied so far ((70)and the ref. therein).

In the following we describe basic properties of a lattice–free ICBM, which has been introduced to extend our concepts on stem cell organization to more general spatio-temporal dynamics.

- In the spatial model we assume that an isolated cell adopts a spherical shape. As the cell gets into contact with other cells or with the substrate its shape changes. Cells in contact form adhesive bonds. With decreasing distance their contact areas increase and so does the number of the adhesive contacts.
- The attractive cell-cell and cell-substrate interaction is assumed to be dominated by receptor-ligand interactions. We assume homogeneously distributed receptors/ligands on the cell surfaces and the substrates. Accordingly, the strength of attraction is proportional to the product of the size of the contact area A_C, the number of receptor-ligand complexes, and the strength of a single bond.
- Contact formation is accompanied by cell deformations. These deformations lead to stress in the cell membranes and cytoskeletons resulting in repulsive interactions. In our model we approximate a cell by a homogeneous, isotropic, elastic object.
- Furthermore, we consider a subdivision of the cell cycle into two phases, the interphase, and the mitotic phase. During the interphase, a proliferating cell doubles its mass and its volume. We model the cell growth process by increasing an intrinsic (target) volume V_T of the cell by stochastic increments. After the V_T reached twice a standard volume V_0, the cell enters the mitotic phase and is split into two daughter cells of equal target volume V_0.

In order to enable the model cells to couple shape changes to processes such as proliferation, differentiation, and apoptosis, we consider a hierarchy of different regulation mechanisms (Figure 5), namely, (i) a biomechanical-mediated form of growth inhibition (contact inhibition), (ii) an anchorage dependent growth inhibition (anchorage dependent growth), (iii) and an anchorage dependent programmed cell death (anoikis).

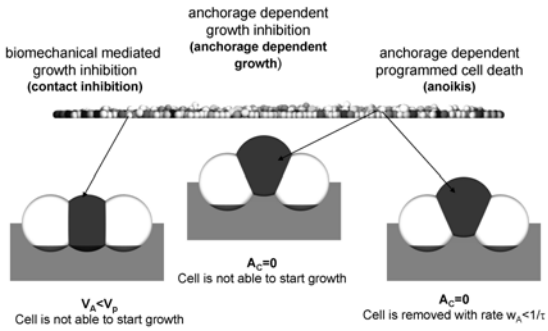

Figure 5: Cellular regulation mechanisms controlled via cell-cell and cell-substrate contacts and cell deformation/compression. A_C is the contact area to the substrate, V_A is the actual cell volume, V_p a threshold volume.

In simulation studies we have investigated the consequences of modifying the parameters for cell-substrate adhesion, the cell cycle time, and have studied how this affects the morphology, biomechanics, and kinetics of the growing cell population (67). We found that in particular the cell-substrate anchorage has a significant impact on the population morphology (Figure 6). For instance, cells within a monolayer undergo contact inhibition of growth only for strong cell-substrate anchorage. Thus, anoikis (anchorage dependent programmed cell death) only substantially contributes to growth control in case of low cell-substrate anchorage, or if contact inhibition is deficient. Whether a variation of the substrate anchorage can initialize the formation of self-organized and spatially structured clonogenic units (cell niches), which are able to reproduce themselves, remains an open question.
Our model analyze on epithelial cell layers predicts that weak substrate anchorage is accompanied by a continuous cell shedding out of the basal layer and consequently by an ongoing self-renewal of the population (Figure 6A). In contrast, strong anchorage results in stable growth and an ageing population (Figure 6B). However, the property of self-renewal is also conserved in the latter case and perturbations, e.g. emanating from induced death of cells, would be followed by an immediate re-growth of the population.

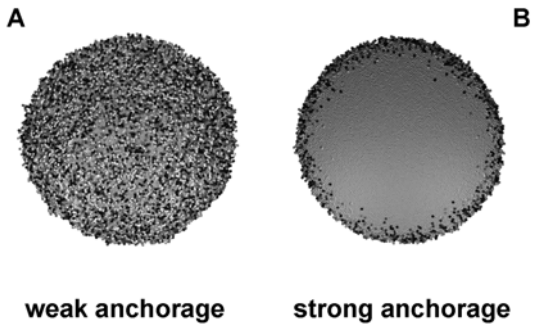

Figure 6: Top views of the macroscopic morphology of growing cell populations with N=10.000 cells. Cell anchorage strength: (A) 200 mN/m, (B) 600 mN/m. The shaded value of the cells is a marker of the cell target volume V_T. Dark shaded cells indicate imminent cell division.

The proposed ICBM links properties of individual cells and the substrate on a small spatial scale to the macroscopic spatio-temporal dynamics of a cell population. All cells were assumed to be capable of proliferation and able to produce an unlimited number of progeny. Thus, each of the cells has the potential to self-maintain the population and to regenerate (self-renew) it after injury. In this respect, the cells comply with the stem cell criteria introduced above. However, the capabilities to differentiate and to undergo lineage

specification are not yet included in our model representation at the moment. The challenge is to develop a generic theoretical framework of cell-environment interaction, which is controlling these processes. For that purpose, one may allow for cell specific parameters, which fluctuate due to varying interactions of the cells with their local environment. In other words, one may consider reversibly changing biophysical properties of the cells, combining the general concept of within-tissue plasticity and the concept of spatial effects of tissue stem cell organization.

But, how does the cell microenvironment actually influences the cell properties? Experimental studies demonstrate that cells adapt their shape to micro-patterned structures (71, 72) and sense their stiffness (73, 74) and composition (60, 75), thereby changing their growth and differentiation properties. This may include changes of their own specific gene expression. Models of tissues with spatio-temporal organized stem cell compartments, as e.g. the intestinal mucosa, might have to consider all these effects and will be a considerable challenge.

Conceptual novelty and achievements

The concepts proposed above change the paradigm of thinking about stem cells. Rather than to assume these cells as being specialized in the first place, we suggest that they are selected and modified due to interactions with the growth environment. Their properties are considered to permanently fluctuate so that some cells meet a situation of expansion and growth. Therefore, tissue stem cells are conceived as cells capable of behaving in a variety of ways and hence, it is their potential and the flexibility to use this potential that matters.

We argue that it is conceptually misleading to consider *stemness* as a specific property that can be determined at one point in time without putting the cells to functional tests. The potential of stem cells rather relates to the complexity of the state-transition graphs describing the potential dynamics of gene/protein activation than to the actual activity status in one of these states. This has implications for attempts to define tissue stem cells e.g. by gene- or protein-profiling (76-81). There are several problems that we envisage. First, molecular profiles obtained by high-throughput technologies (e.g. micro-arrays) are mostly measured on cells obtained from negative selection procedures leading to a heterogeneous mixture of cells. Second, the assays typically represent snapshots at one point in time. However, such snapshots give little insight into the potentials and the dynamic responses of a (stem) cell population. It would be essential to track the molecular profiles over time in various experimental settings putting the system under various modes of stress. Such an approach is necessary to sketch the topology of gene/protein activity networks and to identify (potentially reversible) developmental and regulatory pathways. Thirdly, to conform with the functional definition of tissue stem cells, it will be crucial to correlate the molecular activity network to the functional capabilities of the cells in functional assays. Hence, all techniques based on snapshot measurements of some surface markers or gene activity patterns must be considered as surrogate techniques. At present we cannot see the possibility for a molecular definition of tissue stem cells disregarding functional aspects as a reference point. Thus, we are reluctant to believe that tissue stem cells can be defined by a "tissue stem cell chip". Such an approach would basically ignore the two basic aspects of stem cell potentiality and of cell – growth environment interaction. Furthermore, the discussed uncertainty principle would still apply and all statements could only be made in a probabilistic sense. However, gene-/protein-profiling approaches are still a possibility to select cells with properties required for (potential) stem cells and one can expect a more detailed insight into the mode of stem cell operation by investigating the underlying mechanisms. In particular one can hope for test procedures to screen functional capabilities of tissue stem cells.

There are a number of further predictions arising from the proposed mathematical models. One basic prediction is that two twin cells originating from the same mother cell put into different growth environments will take different developments paths. This is, however, also predicted if they are placed into identical growth environments. The ongoing fluctuations will eventually lead to different fates. Another prediction concerns clonal evolution. All our presented model simulations are based on a simultaneous activity of several coexisting tissue stem cells. They generate several clones and the situation is polyclonal at any given point in time. This should be evident always shortly after introducing some genetic markers (e.g. retro- or lentiviral marking). However, there are fluctuations and some active stem cells become silent (or get lost) and others are activated. Thus the clones contributing to tissue formation change with time. Actually, in the long run the pattern is predicted to change. If one could label all cells in a tissue with a unique marker our simulations would predict that coexistence is impossible in the long run and that decedents from one clone will eventually generate all active stem cells in the tissue. This conversion to long-term mono-clonality is a consequence of fluctuations. It would, however, not be possible to know in advance which clone will be the winner. Hence, we predict that depending on the time scale of measurement it is equally valid to argue that stem cells systems are polyclonal (actual activity) and monoclonal (descendent status) at the same time. A detailed understanding of the long-term dynamic features will be important in gene therapy based on random insertion of genes into tissue stem cells. A third important model prediction concerns the role of self-renewal. If one has a stem cell system with a homogenous population of cells, self-renewal and self-maintenance are actually equivalent. In stem cell systems with heterogeneity the distinction is very important. One can prove that systems which are only capable of self-maintenance can live for a long time but will with certainty die out at some point in the future. The reason is that once a sub-population at the root of the network is lost it cannot be recovered. Self-renewal is a mandatory prerequisite for a system that is structurally robust against repeated damage and extensive stress. We, therefore, predict that *self-renewal* is an essential property of stem cell systems, but it may be a very slow and selective process and, therefore, difficult to detect.
Our reasoning has emphasized the role of cell – cell and cell – microenvironment interactions. This implies that specific attention needs to be paid to the role of the microenvironment, which is a complex subject itself. Growth environments encompass an element of spatial neighborhood to other stem cells and matrix cells, ways to adhere to them, and ways to receive signals (growth factors, direct cell contacts, gap junctions, pseudopods). Growth environments may home a cell for a certain while and can then be called a niche. However, such niches may have limited life times, and currently little is known about the dynamic changes of growth environments. Any kinetic changes present will, however, increase the fluctuations in the stem cell population. Our approach to include biomechanical properties of cells and, therefore, to include a spatial component into the control of cellular fates is one possible way to get more insight into the underlying mechanisms of cellular interaction.

In summary, our modeling approaches prove that one can conceive regenerative tissue systems fully consistent with the functional definition of stem cells, without assumptions on uni-directional hierarchies, preprogrammed asymmetric divisions or other assumptions implying *a priory* the entity of predetermined tissue stem cells. It has been shown by our modeling works that functional, self-organizing systems with stochastic components (sources for generation and for elimination of variance) are powerful alternative concepts to consistently explain tissue stem cell organization. We, therefore, propos a revised conceptual view on tissue stem cell organization, replacing the classical perspective of a predefined stem cell entity by considering stem cell potential as a system property resulting from dynamically controlled cell-cell and cell-microenvironment interactions (Figure 7).

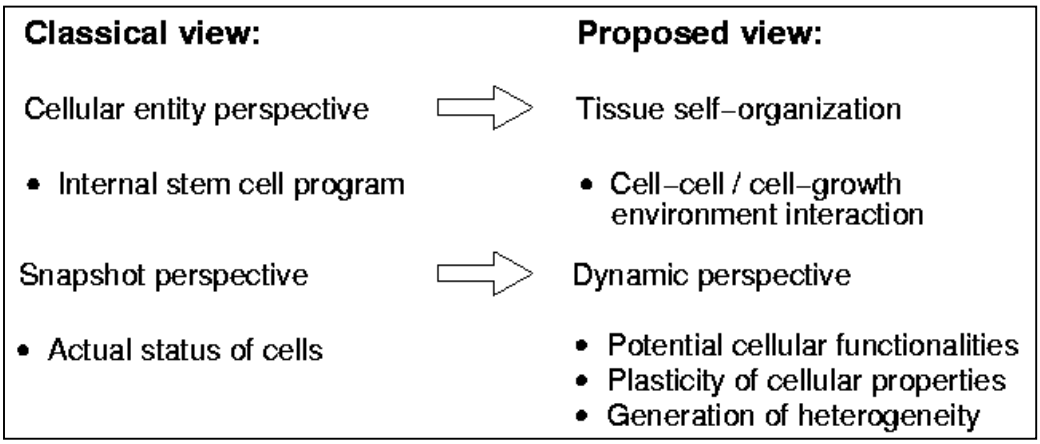

Figure 7. Classical versus proposed view on tissue stem cell systems.

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, i.e. to focus on the scope of skills rather than on selected individual abilities. Also modeling approaches need to be extended in several regards. Firstly, 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 theoretical modeling, however, is the design of predictive models which can bridge the different levels of description (i.e. tissue, cells, molecules) and, hence, link a molecular description of tissue stem cells to the functional definition. It is evident, that modeling, beside new bioinformatic methods in data analysis, will be important to link data from all these three description levels into one comprehensive framework.

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