Title
Stem cells as probabilistic self-producing entities

Journal
Bioessays, 26(9)

ISSN
0265-9247

Author
Ramalho-Santos, M

Publication Date
2004-09-01

Peer reviewed

Stem cells as probabilistic self-producing entities

Miguel Ramalho-Santos

Program in Developmental and Stem Cell Biology, University of California, San Francisco, San Francisco CA 94143-0520, USA

mrsantos@itsa.ucsf.edu

(415) 502 8543

Abstract

Stem cells have the capacity both to self-renew and to give rise to differentiated progeny, and are vital to the organization of multicellular organisms. Stem cells raise a number of fundamental questions regarding lineage restriction and cellular differentiation, and they hold enormous promise for cell-based therapies. Here I propose a theoretical framework for stem cell biology based on the concepts of autopoiesis (self-production) and complementarity. I discuss how stem cells are pivotal in the self-production of the organism and how we need complementary approaches to understand their probabilistic behavior. I discuss how this framework generates testable hypotheses regarding stem cell functions.

Stem cells as units of autopoiesis

The majority of cells in an organism are specialized to perform essential functions such as sensing the environment, locomotion or digestion. Stem cells are rare cells that contribute to embryonic growth and to replenishing the organism during physiological turnover of differentiated cells or in cases of acute cell loss due to injury. One of the key properties of stem cells is the ability to self-renew, that is, to produce more stem cells. In the 1970's the theoretical biologists Humberto Maturana and Francisco Varela introduced the concept of autopoiesis, meaning 'self-production', as their simple and elegant definition of life[1]. In this section I briefly review the concept of autopoiesis insofar as it applies to stem cells.

According to Maturana and Varela, living organisms are unique in that their components interact in such a way as to continuously produce those components and maintain the relations between them[1,2], and are thus called autopoietic. For example, through a network of relations of production, the components of a living cell, i.e., nucleic acids, proteins and other macromolecules, continuously destroy and re-generate those same macromolecules while preserving the overall organization of the cell. These so-called autopoietic machines are clearly distinct from human-made machines, such as a thermostat, a car or a computer, which cannot through their operation produce their own components, nor realize the networks of relations between those components. The theory of autopoiesis stresses that it is the networks of relations between components (i.e., the organization of the autopoietic system), rather than the components themselves, that should be the central object of biological research. Hence, it is a mechanistic theory in the sense that it rejects vitalism and seeks explanations for the organization of living organisms as physical entities. However, by seeking the principles of autopoietic organization, this theory also rejects the

reduction of living organisms to their components, and as such has many affinities to theories generally known as hierarchy theory[3], or systems biology[4,5].

Maturana and Varela identified the living cell as the universal autopoietic system, but conceived of the notion of composite or higher-order autopoietic systems[1]. Autopoietic systems may become coupled in such a way as to behave as components of a composite system. If in turn the interactions of these components continuously re-generate the components and maintain the relations between them, the composite system is a higher-order autopoietic system. In the authors' own words, "this has actually happened on Earth with the evolution of the multicellular pattern of organization"[1]. Hence, cells can be viewed as first-order autopoietic systems, and multicellular organisms can be viewed as second-order autopoietic systems[2].

Stem cells may be seen as playing a pivotal role between first-order and second-order autopoiesis. The networks of interactions of the molecular components of a stem cell continuously realize its autopoietic organization as a living cell of a particular type. On the other hand, stem cells are essential units of second-order autopoietic systems, that is, multicellular organisms, by appropriately generating the cellular components that realize the organization of such systems. Thus, stem cells represent an essential channel of communication between the two levels of autopoiesis, the cellular and the organismal. Stem cells must be able not simply to self-renew and give rise to differentiated cell types, but to do so in a spatial and temporal coherent fashion so as to maintain the autopoiesis of the organism.

Stem cells and complementarity

An essential question is how stem cells decide to self-renew or to differentiate, and do it according to the needs of the organism. Several researchers have pointed out that the decision between self-renewal and differentiation of stem cells is a probabilistic one. Till and colleagues have proposed a stochastic model for the decision between self-renewal and differentiation to explain the high variability in their pioneering observations of blood stem cells[6]. David Anderson has referred to stem cells as integrators whose inputs affect the probability of outputs[7]. Spradling and colleagues have noted that in several stem cell systems, such as in the nematode gonad and the mammalian intestine, stem cells do not strictly divide asymmetrically to give rise to one stem cell and one differentiating cell. Rather, stem cells may divide symmetrically and give rise either to stem or differentiating cells with a certain probability that is modulated at the cell population level by the local niche [8]. Making an explicit parallel to indeterminism in the field of quantum physics, Christopher Potten has noted that stem cells are defined in terms of their potential capacities (self-renewal or generation of differentiated progeny) and that the techniques available to test those capacities involve perturbation of the system. This "inevitably causes changes in the behavior and characteristics (and possibly even the number) of the stem cells which are to be studied, resulting in a situation analogous to the Heisenberg uncertainty principle"[9].

There is an intrinsic uncertainty about stem cells that may be further compounded by extrinsic factors. The probabilistic choice between two outcomes, self-renewal and differentiation, may be modulated by subtle changes in the stem cell's environment. Furthermore, most stem cells are multipotent, that is, they are able to give rise to several different cell types, and the mechanisms that allow for that versatility of lineage choice may be more sensitive to experimental manipulation. It follows that the influence of the observer may be greater in the analysis of stem cells than differentiated cells. Interestingly, in the

original proposal of the theory of autopoiesis, a critical role was attributed to the observer in defining the entity that is to be considered as autopoietic or not[1]. I next briefly review how the concept of complementarity arose in the field of physics and how it may be useful for stem cell research.

In the late 1920's, the physicist Niels Bohr introduced the concept of 'complementarity' to describe the behavior of atomic particles[10]. Complementarity refers to aspects of atomic phenomena that may only be revealed by experiments employing mutually exclusive conditions. For example, the behavior of photons came to be best understood if they were approached both as particles and as waves, which intuitively seemed to be a contradiction in terms. Towards the end of his career, Bohr discussed extensively how the concept of complementarity could be useful for other sciences. In the case of biology, Bohr proposed a complementarity of reductionist and holistic approaches to each particular problem[11]. With very much the same reasoning, the biologist Conrad Waddington wrote that "like the physicists in their more refined field, biologists have to utilize both atomistic and continuum modes of approach simultaneously"[12].

The standard view of the field, mostly modeled on the hematopoietic system, is that stem cells are clearly distinct entities that can be purified to homogeneity and whose properties define them in opposition to differentiated cells[13]. According to this view, once a stem cell becomes the precursor of a particular cell lineage, it irreversibly loses its self-renewing ability: it may divide a few more times but will eventually give rise only to differentiated cells. However, it has been shown that in some instances precursor cells can re-acquire stem cell status. For example, when exposed to certain growth factors, neural precursors can be converted into self-renewing stem cells[14,15]. In one extreme view, the propensity to behave as stem cells simply decreases from stem to differentiated cells, and

may never be irreversibly lost[16]. In this view, "rather than referring to a discrete cellular entity, a stem cell most accurately refers to a biological function that can be induced in many distinct types of cells, even differentiated cells"[16].

These apparently contradictory views on what a stem cell is have parallels with the physical paradoxes that the theory of complementarity addresses. Making an analogy to quantum physics, one may say that a stem cell should be viewed both as a particle (entity, cell) and as a wave (function, stem). Any single one of these views will give us an incomplete understanding of stem cell biology. Just as in physics complementarity is the best approach to understanding the probabilistic behavior of atomic particles, so in biology complementarity may be the best approach to understanding the probabilistic behavior of stem cells. By this I do not mean to say that any explanation about stem cells is valid at least sometimes. As outlined in the next section, using complementary approaches to the probabilistic behavior of stem cells will only make our explanations more accurate, not less.

A research program for stem cell biology

The theoretical framework for stem cell research that I propose involves:

i) considering stem cells as pivotal units between first-order and second-order autopoiesis;

ii) directing research towards an understanding of the organizational relations of both levels of autopoiesis as they pertain to stem cells; and

iii) considering stem cells as probabilistic autopoietic entities whose behavior may only be understood with complementary approaches.

I conclude by discussing how future avenues of research and testable hypothesis may be conceived within this framework.

The work of several researchers, including my own[17], has focused on the identification of genes expressed in stem cells. Functional studies of individual genes will certainly continue to provide insight into the genetic regulation of stem cells. However, within the framework of autopoiesis, it is the organizational relations between the components, more than the components themselves, that must be identified. An approach to such relations involves the following two general questions. First, what are the intercellular signaling networks active in stem cells and how do they interact to regulate stem cell function? This will be a view from the cell surface and the cytoplasm, and will require the analysis and manipulation of modules of interacting proteins active in stem cells. Second, what are the genetic regulatory networks that activate or repress gene expression specifically in stem cells? This will be a view from the nucleus, and will require the analysis and manipulation of combinations of transcription factor binding sites associated with genes induced or repressed in stem cells. These signaling and genetic regulatory networks to be identified will complement each other in defining the stem cell state. Such are the relations we must seek in order to gain insight into the first-order autopoietic organization of stem cells. The expansion of approaches involving transcriptional profiling and analysis of gene function and regulation to other stem cells and other organisms will reveal how general such relations are.

Furthermore, if one is to study second-order autopoiesis, i.e., the role of stem cells in the context of self-production of the whole organism, it will be important to consider the organism as the autopoietic system in question, the stem or differentiated cells as the components and the identification of the relations between these cells as the central goal of the research. Some critical questions to be addressed are: what are the lineage relationships of stem cells, i.e., what differentiated cell types exactly does each stem cell type contribute

to? What are the intermediate steps between a stem cell and a differentiated cell, and how are these steps modulated? What are the cell cycle and cell death kinetics of stem cells in the body? How is the growth, size, shape and coherence of organs and the organism maintained despite such constant cellular turnover? Just as we can speak of a first-order code for the macromolecular components of a cell, which is embedded in nucleic acid sequences, can there be any second-order code for the cellular components of an organism, embedded in stem cells?

The response of stem cells to injury is likely to be a good experimental setting in which to address the role of stem cells in second-order autopoiesis. Upon injury that imparts loss of differentiated cells or even depletion of the stem cell pool, the remaining stem cells increase their proliferation so as to re-establish the appropriate number of cells. How do stem cells sense the need for more differentiated progeny? And how do they "know" to return to the steady-state? Answers to these questions may provide insight into the broader problem of how the shape and size of organs and organisms is achieved and maintained.

The probabilistic behavior of stem cells may allow them to quickly integrate changing conditions in the organism with growth or injury, and respond accordingly. But how can the probabilities (say, of self-renewal or differentiation) be estimated and what are the factors that may modulate them? If the probability of behaving like a stem cell simply decreases continuously between what we call stem and differentiated cells, is that decrease of linear or exponential kind? The advantage of using complementary approaches to stem cells derives directly from their probabilistic behavior and the fact that this behavior may vary when observed under different experimental conditions. Thus, just like in physics, statements about stem cells should clearly specify the experimental conditions in which they apply. I conclude

by describing two particular cases in which complementary approaches may be useful for stem cell research.

When the whole liver is injured chemically, distinct entities recognized as stem cells in the bile duct drive regeneration of the injured organ[18]. In a different experimental setting, when most of the liver mass is surgically removed, the differentiated cells of the liver (hepatocytes) have been shown to acquire some stem cell properties and regenerate the missing part of the organ[18]. Hence, the liver may regenerate by two very different means depending on how the regeneration is tested. Are there similarities in the genetic regulation of these two modes of regeneration? Can they co-exist? How does the hepatocyte regain stem cell properties? Cases of regeneration like this one are likely to be informative about stem cell function, even if not driven by the entities we are accustomed to recognize as stem cells.

Another case where the complementarity of entity/function approaches to stem cells may be valid is with neural stem cells. These cells, which self-renew and can give rise to both neurons and glia in the adult brain, have been shown to be astrocytes[19]. They have all the morphological and molecular features of astrocytes, a differentiated glial cell type of the brain, and yet they are stem cells. What is it that distinguishes these astrocytes from others that are not thought to be stem cells? In this case, how much of the stem cell behavior is intrinsic to the entity (a special kind of astrocyte) and how much of it is a function imparted by the niche in which the cell resides? How are the specialized functions of a differentiated astrocyte and a stem cell coordinated within the same cell? We stand to learn the most about stem cell behavior by using complementary approaches.

It is important to re-state that the fact that stem cells behave in a probabilistic manner and that they should be studied with complementary approaches does not mean that we are headed towards an unintelligible mess where any explanation is valid at least in some

circumstance. Rigorous assessment of the various probabilities of stem cell behavior measured under a variety of experimental conditions will be required. With my analogy to the field of quantum physics I do not mean to say that the mathematical tools developed in that field will necessarily be the ones to be used in stem cell biology. Nevertheless, mathematical modeling of stem cell behavior will likely become an increasingly useful tool. Although a computer model of autopoiesis has been developed[20] and some models of stem cell differentiation have been proposed[6,21,22], this area remains largely unexplored.

Conclusion

Research on stem cells may have fundamental implications beyond the particular setting or organ system in which they are studied. For example, what can stem cells tell us about the evolution of multicellularity, the allocation of cell lineages during development, or the regulation of body growth, size and regeneration? Ultimately, not only will our explanations be better at describing stem cell behavior, but the new perspective I have outlined here should shed light on the fascinating questions about stem cells that continue to elude us.

Acknowledgements: I thank Boaventura Sousa Santos, Doug Melton, Arturo Alvarez-Buylla, Matthias Hebrok, João Ramalho-Santos, Stephen Haggarty and Ryan Baugh for stimulating discussions, and two reviewers for critical reading of the manuscript.

References

1. Maturana HR, Varela FJ. Autopoiesis. The organization of the living. Boston Studies in the Philosophy of Science 1980;42:59-141.
2. Maturana HR. Autopoiesis: reproduction, heredity and evolution. In: Zeleny M, editor. Autopoiesis, dissipative structures, and spontaneous social orders. Boulder: Westview; 1980. p 45-79.
3. Koestler A. Jannus: A summing up. New York: Random House; 1978.
4. Kitano H. Systems biology: a brief overview. Science 2002;295(5560):1662-4.
5. von Bertalanffy L. Problems of life: an evaluation of modern biological and scientific thought. New York: Harper; 1952.
6. Till JE, McCulloch EA, Siminovitch L. A Stochastic Model of Stem Cell Proliferation, Based on the Growth of Spleen Colony-Forming Cells. Proc Natl Acad Sci U S A 1964;51:29-36.
7. Anderson DJ. Stem cells and pattern formation in the nervous system: the possible versus the actual. Neuron 2001;30(1):19-35.
8. Spradling A, Drummond-Barbosa D, Kai T. Stem cells find their niche. Nature 2001;414(6859):98-104.
9. Potten CS. Preface. In: Potten CS, editor. Stem Cells. San Diego: Academic Press; 1997. p xi-xiii.
10. Bohr N. The quantum postulate and the recent development of atomic theory. Nature 1928;121(sup.):580-590.
11. Bohr NHD. Atomic physics and human knowledge. New York,: Wiley; 1958. viii, 101 p.
12. Waddington CH. The nature of life. London: Allen & Unwin; 1961. 131 p.
13. Weissman IL. Stem cells: units of development, units of regeneration, and units in evolution. Cell 2000;100(1):157-68.
14. Kondo T, Raff M. Oligodendrocyte precursor cells reprogrammed to become multipotential CNS stem cells. Science 2000;289(5485):1754-7.
15. Doetsch F, Petreanu L, Caille I, Garcia-Verdugo JM, Alvarez-Buylla A. EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. Neuron 2002;36(6):1021-34.
16. Blau HM, Brazelton TR, Weimann JM. The evolving concept of a stem cell: entity or function? Cell 2001;105(7):829-41.
17. Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA. "Stemness": transcriptional profiling of embryonic and adult stem cells. Science 2002;298(5593):597-600.
18. Alison M. Liver stem cells: a two compartment system. Curr Opin Cell Biol 1998;10(6):710-5.
19. Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 1999;97(6):703-16.
20. Varela FG, Maturana HR, Uribe R. Autopoiesis: the organization of living systems, its characterization and a model. Curr Mod Biol 1974;5(4):187-96.

21. Loeffler M, Birke A, Winton D, Potten C. Somatic mutation, monoclonality and stochastic models of stem cell organization in the intestinal crypt. J Theor Biol 1993;160(4):471-91.
22. Vogel H, Niewisch H, Matioli G. Stochastic development of stem cells. J Theor Biol 1969;22(2):249-70.