Cell Stem Cell
Meeting Report

Stem Cells: Time to Check Our Premises

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A recent meeting titled "Conserved Mechanisms of Stem Cell Control and Regeneration" was held at the Biopharmaceutical Technology Center Institute (BTCI) in Madison, Wisconsin. The diversity of stem cells and biological contexts discussed highlight the field's rapid progress in deciphering the molecular basis of stem cell functions and emphasize the challenges facing the future exploitation of these cells as therapeutic vectors.

The Metaphors of Stems and Niches

The way in which most biologists think about particularly complex phenomena is oftentimes framed by a metaphor. For instance, we refer to DNA as the blueprint of life, to proteins in a cell as the machinery utilized to read the blueprint, and so on. A similar situation applies to the undifferentiated cells found in embryos and adults, capable of proliferating and producing new cells that will eventually differentiate into one or more cell types. We call these highly specialized cells "stem cells" (Ramalho-Santos and Willenbring, 2007), a translation from the word "stammzellen" used by German embryologists of the 1800s (Figure 1) and likely coined from the German word "stamm," meaning a group of people, families, or clans with a shared common ancestor. More recently, the metaphor of a "stem" has been joined by yet another metaphor, i.e., the niche, a concept introduced by ecologists at the beginning of the 20th century to describe a multidimensional space possessing a range of resources needed by a given species (Pidwirny, 2008). Its application to the study of stem cells was first proposed by Schofield as a hypothesis in which "the stem cell is seen in association with other cells which determine its behaviours. It becomes essentially a fixed tissue cell. Its maturation is prevented and, as a result, its continued proliferation as a stem cell is assured" (Schofield, 1978). On the surface, both metaphors are appropriate, as they have brought conceptual order to a rapidly growing field of scientific endeavor. In the past few years, the "stem" concept has helped spur a high degree of molecular resolution for in vitro systems such as mammalian embryonic stem cells (ESCs). Such understanding has culminated in the generation of ES-like cells from adult mouse and human fibroblasts known as induced pluripotent stem cells (iPSCs). The "niche" concept, on the other hand, has provided a conceptual framework that has helped uncover differences between adult and embryonic stem cells. In addition, the idea of niches has offered a platform for work using invertebrate model systems that highlights the complex population dynamics of stem cells and their equally intricate interactions with in vivo microenvironments.

Yet, given all we have learned thus far, do these metaphors promote or hinder the way we think about and experiment with undifferentiated cells? It was this fountain, fed by such diverse sources, from which the organizers of the 3rd annual Wisconsin Stem Cell Meeting filled their cups. In fact, the broad distribution and diversity of functions of stem cells among animals was the *leitmotif* of this thought-provoking conference held in Madison on April 16, 2008, organized by Judith Kimble, Clive Svendsen, the BTCI, and the University of Wisconsin Stem Cell and Regenerative Medicine Center. Given the brisk pace at which the field of stem cells is moving, a meeting such as this provided a unique opportunity not only to discuss the features of various stem cells, but also to attempt an integration of the various types, functions, and malfunctions of stem cells in the animal kingdom. Work on stem cells of both invertebrates (fly, nematode, planarians) and vertebrates (human, mouse, and fish) were discussed by leaders in the field. The range of topics represented in this meeting ultimately led the participants to reflect on the mechanistic relationships that may or may not exist between embryonic, postembryonic (adult), and cancer stem cells. It also highlighted the respective layers of complexity and differences that exist between in vitro and in vivo stem cell biology, while providing a forum to place current knowledge in a broader context not frequently afforded by more specialized stem cell meetings.

The Public In Vitro and Private In Vivo Lives of Stem Cells

Due in part to their ease of cultivation and accessibility to experimental manipulation (Figure 2A), much has been learned in recent years about cultured mammalian ESCs. Given the high level of molecular resolution that has been achieved, it is understandably tempting to extend this knowledge to all other stem cells, particularly those hidden away in the privacy of embryonic and adult animal anatomy. For example, Rick Young (Whitehead, MIT) discussed an impressive body of work aimed at defining and understanding the regulatory interactions between transcription factors, chromatin regulators, and signaling pathways in cultured mammalian ESCs. Experiments involving both human and mouse ESCs and a combination of extensive chromatin immunoprecipitation (ChIP) and high-throughput DNA sequencing were discussed. Among the key findings were the following: (1) the identification of a core, feed-forward, autoregulatory, transcriptional regulatory loop involving OCT4, SOX2, and NANOG (Boyer et al., 2005); (2) the specific co-occupancy of Polycomb Group (PcG) repressor proteins with a subset of gene promoters occupied by OCT4, SOX2, and NANOG to effect net repression rather than activation (Boyer et al., 2006); and (3) that the terminal component of the Wnt pathway (the transcription factor Tcf3) occupies promoters in the genome already populated by OCT4 and NANOG, providing a model of how signaling pathways may

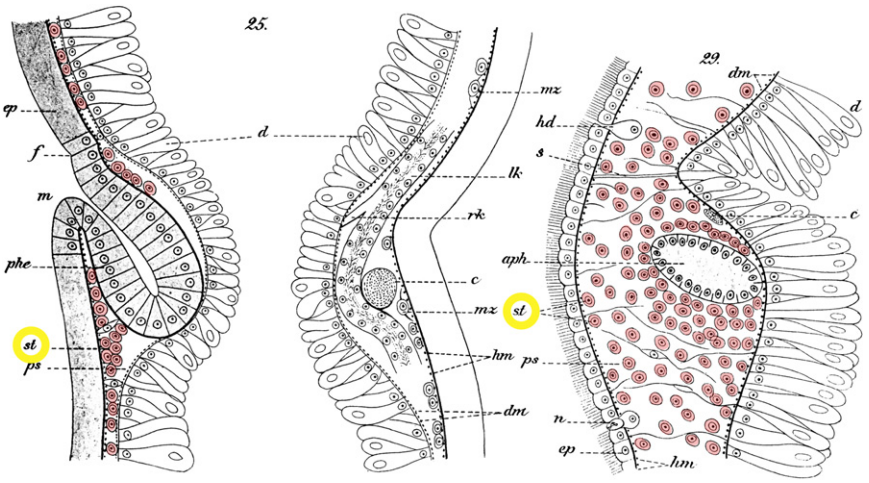

Figure 1. One of the Earliest Descriptions of Undifferentiated Cells in Adult Animals
The plate illustrates longitudinal wax sections of a freshwater turbellarian, and pseudocolored in red are the abundant undifferentiated cells populating the body of the flatworm. Note that these cells are labeled as "st" (yellow circles) or "stammzellen" in the original manuscript by Keller. Modified from Keller (1894).

modulate ESC functions via direct integration in these cells' core transcriptional regulatory circuitry (Cole et al., 2008).

This concept of "circuitry," borrowed from electrical engineering, has now been applied to wet-lab research to synthesize the complex interactions among transcription factors, chromatin regulators, and signaling pathways that regulate the proliferation and differentiation of mammalian ESCs (Jaenisch and Young, 2008). This is, in fact, an efficient and elegant way to visually display complex regulatory interactions. However, like all such representations, it is difficult to model the temporal dimension in two-dimensional diagrams, and yet the transformation of a cell from an undifferentiated to a differentiated state does not occur in a single quantum change. This failing is compounded by the fact that ESCs maintained in vitro likely occupy a narrow, developmental space of sustained proliferation and self-renewal, in which the coordination of multiple autonomous and nonautonomous inputs that drive differentiation in vivo are not easily captured. For instance, what activates the initial expression of OCT4, SOX2, and NANOG? Where does the Wnt ligand come from in the embryo? The answers to these questions require similar studies performed before and after fertilization in order to gain an understanding of when and how the identified core regulatory circuitry is established, briefly maintained, and eventually dismantled in the developing embryo. Because literally millions of cells are required to perform the ChIP-sequencing analyses described, an in vivo analysis will have to resort to more sensitive methods, such as single-cell microfluidics or other technologies aimed at profiling the genomic output of single cells (Zhong et al., 2008).

These limitations are underscored by the significantly more private (i.e., less accessible) lives of in vivo ESCs (Figure 2B). This complexity was eloquently articulated by Janet Rossant (University of Toronto), who presented work on the characterization of the three lineage-specific progenitors present in the early mouse embryo: the trophectoderm and the internal cell mass (ICM)-derived epiblasts and primitive endoderm cells. As she and others have shown previously, stem cells can be readily derived from each: ESCs from the epiblast (EPI), trophoblast stem cells from the trophectoderm, and extraembryonic endoderm (XEN) cells from the primitive endoderm (PE) (Yamanaka et al., 2006). Thus, unambiguous delineation of the originating lineage is absolutely essential if one wishes to correctly interpret the biological activities of cells derived from early embryos. This is particularly apparent in the case of the ICM, from which arise not only the EPI and PE, but also all mammalian pluripotent ESCs derived to date. The segregation of the EPI and PE lineages within the ICM was not considered a problem in ESC derivation because all cells in the ICM were thought to be homogeneous, each with the ability to become either EPI or PE. However, Rossant's work has unambiguously shown that the ICM of the mouse embryo is composed of a heterogeneous population of cells that may have already become lineage restricted prior to the blastocyst stage of mouse embryogenesis (Chazaud et al., 2006). So why are only ESCs and not XEN cells derived from the ICM in vitro? The most likely explanation is that the derivation of ESCs is performed under conditions that inhibit FGF signaling, which is a prerequisite for PE development. These findings have direct implications to the characterization of human ESCs, which are derived from the ICM of the human blastocyst. Unlike mouse ESCs, human

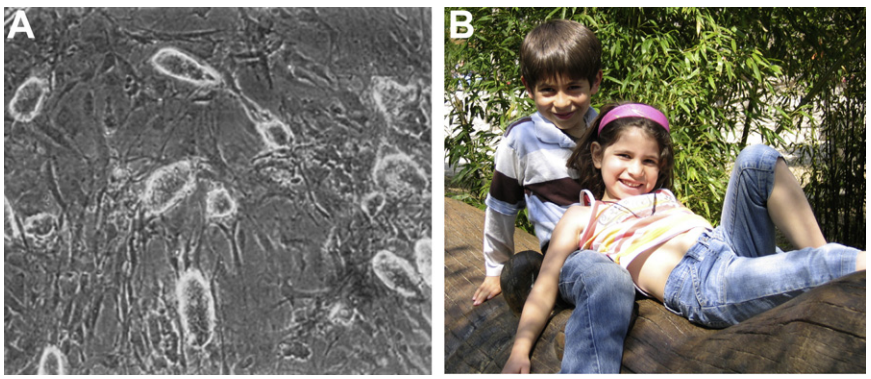

Figure 2. The Biological Scale of the Problem: In Vitro to In Vivo Stem Cells
Stem cells can be studied easily at the micro scale (A), due to their relative accessibility in vitro. However, the layers of complexity that also need to be resolved in order to understand and influence stem cell function at the macroscopic level (B) are much harder to assay. These challenges were a central theme to this symposium. Mouse embryonic stem cells in (A) are at ×35 magnification. The idea for this figure is based on a slide presented by Judith Kimble at the symposium.

ESCs are routinely maintained and expanded in conditions that promote PE and trophectoderm stem cell renewal in the mouse (presence of FGF, activin, nodal). Moreover, Rossant also pointed out that human ESCs express markers of the trophectoderm lineage (Xu et al., 2001). Because the lineages of the early human embryo remain uncharacterized, the intriguing possibility exists that the observed differences between human and mouse ESCs may arise not from inherent differences in functionality between the two species, but rather from differences in the developmental stages of the human and mouse blastocyst from which the lines were derived. Future work must consider the possibility of generating a detailed lineage map of the early primate embryo to address these important issues.

Memo to iPSCs: Don't Forget to Call on Adult Stem Cells

The findings in cultured and in vivo mammalian stem cells argue that a combination of these two primary modes of research is necessary to understand the biology of undifferentiated cells and should provide a robust platform to develop rational strategies to utilize iPSCs either as therapeutic agents or as human disease models. To this end, James Thomson (University of Wisconsin, Madison) discussed both technical limitations and gaps in our understanding that each need to be overcome if iPSCs are to become clinically relevant. The current method of producing iPSCs relies on the use of viral integration vectors, which could potentially introduce mutations at the insertion site. Moreover, expression of the exogenous genes introduced by the virus persists at different levels even after the endogenous genes are reactivated. Given the interest these cells have generated, one would be taking little to no risk in predicting that these limitations will be overcome in short order. More difficult to resolve will be the barriers facing the use of iPSCs in cell-based transplantation therapies. It remains to be seen whether iPSCs can functionally integrate in diseased or injured tissues in a physiologically useful way, or if the conditions that led to the death or degeneration of the original cells can be overcome to allow the transplanted cells to survive.

Addressing these issues will need at least as thorough an understanding of the adult conditions in which stem cells operate as to what thus far has been achieved for ESCs. If an adult neuron is required, for example, why not induce differentiated cells to become adult rather than embryonic stem cells? If the core transcriptional regulators of adult stem cells were to be elucidated, could these be used instead of OCT4, NANOG, and SOX2 to revert differentiated cells to the appropriate postembryonic developmental stage? In this regard, work from my own laboratory on the planarian *Schmidtea mediterranea* aimed at identifying genes involved in adult stem cell function and regeneration may inform this particular aspect of stem cell biology. RNAi-based screening for genes that affect adult stem cell function in planarians, tissue homeostasis, regeneration, and the functional integration of newly regenerated body part tissues into pre-existing tissues (Reddien et al., 2005) offers an experimental approach that broadly informs the search for regulatory pathways and mechanisms that may be specific to the adult rather than the embryonic condition. That such differences exist between embryonic and adult stem cells was illustrated by Sean Morrison (University of Michigan). Studies from his laboratory have demonstrated a requirement for the PcG transcriptional repressor *Bmi-1* in the postnatal maintenance of stem cells in multiple tissues, including the central and peripheral nervous systems. *Bmi-1* is undetectable in ESCs but is expressed in the stem cells of young and old adult mice (Molofsky et al., 2003) and has been shown to modulate the senescence-associated genes Ink4a and Arf (Molofsky et al., 2006). The temporally regulated overlapping transcriptional program that exists between *Bmi-1*, *Ink4a*, and *Arf* suggests the existence of age-dependent, stem cell regulatory pathways absent in embryos and capable of regulating lifelong physiological changes in populations of adult stem cells. Hence, studies aimed at understanding the developmental origin and age of stem cells, and the function of genes involved in adult stem cell function and regeneration, will likely inform attempts to induce stage-appropriate multipotent stem cells, and to drive the therapeutic differentiation of both ES and iPSCs.

A Wnt-Wnt Situation

It is difficult to imagine how the complexity of multicellular organisms could possibly arise without robust cell-cell communication. Signaling pathways are essential in transmitting intercellular information, and this point was independently and frequently emphasized at the meeting by the recurrence of key roles for the Wnt/β-catenin pathway in the autonomous and nonautonomous regulation of both embryonic and adult stem cells. In ESCs, Young reported how the Wnt/β-catenin pathway transduces developmental signals directly to the core regulatory circuitry of ESCs to influence the balance between pluripotency and differentiation. Judith Kimble (University of Wisconsin, Madison) discussed genetic studies in which perturbation of this pathway in non-stem cells negatively affects the development of germ stem cells in *Caenorhabditis elegans.* Two of the three genes identified code for genes associated with the Wnt/β-catenin pathway (Sys-2/TCF and Sys-3/Nkx 2.5), while the remaining gene (Sys-1) codes for a novel protein. Missing from this picture was β-catenin, which is essential for mediating Wnt-activated gene transcription. Although the SYS-1 amino acid sequence was novel, in genetic and biochemical studies this molecule functions as if it were β-catenin: SYS-1 was shown to bind to the β-catenin-binding domain of TCF, and to coactivate TCF-dependent transcription (Kidd et al., 2005). In fact, when SYS-1 was crystallized on its own, or bound to TCF, its structural similarities to human β-catenin were, simply put, astonishing (Liu et al., 2008). The important discovery that SYS-1 is related to β-catenin not by sequence but by structure suggests that additional divergent β-catenins with potentially important cell signaling modulation roles await discovery.

In planarians, β-catenin plays key roles in regulating anteroposterior polarity of stem cell progeny in both intact and amputated animals. We discovered that abrogation of β-catenin by RNAi results in animals that regenerate heads at posterior ends, while abrogation of its inhibitor, APC, results in the regeneration of tails at anterior ends. Remarkably, even in unamputated animals, silencing β-catenin also disrupted the anteroposterior axis of the adult animal, resulting in a transformation of the tail into a head and the eventual appearance of multiple heads along the edges of the organism. Because planarian stem cells give rise to all cell types in this organism, our discoveries suggest that the evolutionarily ancient β-catenin protein

acts as a molecular switch in adult animals to determine whether the stem cell daughter cells will assume anterior or posterior identity (Gurley et al., 2008). Altogether, the Wnt/β-catenin findings from different embryonic and adult stem cell systems illustrate how a single signaling pathway can induce widely varying outcomes, depending on the developmental context in which the signal is received.

Rethinking Stem Cell Biology

Allan Spradling (Carnegie Institution of Washington and HHMI) challenged the audience by stating that "the field of stem cells has been too focused on stem cells." Of course, he was referring to the fact that an equally important aspect of stem cell function is dictated not by the stem cells proper, but rather by the niches, or microenvironments, in which they reside. In recent years, many such microenvironments have been carefully defined in the gonads of both the fruit fly *Drosophila melanogaster* and the nematode *C. elegans* and also studied in the mammalian central nervous system and the hematopoietic compartment, among others (Morrison and Spradling, 2008). Based on observations of the dynamic interactions of stem cells (germline and somatic) with their niches in *Drosophila*, Spradling defined two general categories of niches: stromal and epithelial. The stromal niche is represented by the interaction between the dividing germ cells with the nondividing stromal cells of the ovary (Cap cells) and the testis (Hub cells). The epithelial niche is a stroma-free microenvironment and is populated by the follicle cell stem cell (FSC). In this case, unique combinations of signals provided by moving neighbors may yield a niche that is dynamic rather than static.

Clearly, the dynamic, highly regulated interactions of stem cells and their microenvironment will not be easily recapitulated in vitro. For example, in stromal niches that harbor multiple adjacent stem cells such as those that maintain *Drosophila* germ cells, replacement of lost stem cells is readily provided either by the division of neighboring stem cells or by the developmental reversion of transit-amplifying progeny back to a germ stem cell state. In the case of the epithelial niche in which nonneighboring FSCs reside in opposite sides of the *Drosophila* ovariole, FSC daughters migrate across the ovariole to the other niche before proliferating and contributing to the follicle cell monolayer. In fact, the crossmigrating FSC progeny compete with the resident FSC for niche occupancy and eventually become the source of FSC replacement (Nystul and Spradling, 2007). Common to both of these types of niches is the observation that individual stem cells are often replaced and that such replacement may be driven by competition. Under normal conditions such competition-driven stem cell replacement would favor the fittest stem cell, ensuring the overall quality of the population. However, an interesting corollary emerging from this dynamic model is the possibility that precancerous mutations may spread by stem cell competition, as well as by generating dysplastic lesions (Nystul and Spradling, 2007). One likely place where such a mechanism may operate is the mammalian intestine, in which during development a crypt progenitor cell and its descendants normally displace all other cells from the neonatal crypt, eventually reaching monoclonality (Schmidt et al., 1988). Under pathological conditions, cancerous cells appear to outcompete normal stem cells, resulting in the replacement of normal cells by cancerous ones (Barker et al., 2007). Thus, it is likely that the ontogeny of complex diseases such as cancer may depend on similar dynamic interactions that exist between stem cells and their microenvironments. If such dynamics are not elucidated, the prospect of introducing stem cells grown in vitro into an adult organism without causing disease will be severely hampered.

The dynamic nature of stem cells and their microenvironments was also discussed by Ken Poss (Duke University) using the zebrafish heart as an experimental paradigm. Using transgenic reporters to trace both differentiated and undifferentiated cells, Poss observed sustained proliferation of undifferentiated cardiac progenitor cells after resection of the cardiac apex. These cells also expressed the earliest markers of the embryonic zebrafish heart field (*nkx 2.5*, *tbx 20*, and *hand 2*). In contrast to the myocardium, in which changes in gene activation were restricted to the plane of amputation, the entire adult epicardium responded to injury by expressing the normally absent embryonic genes *raldh2* and *tbx18*. Such reactivation of developmental gene expression was followed by the epithelial-to-mesenchymal transition of a subpopulation of epicardial cells, which then invaded the wounded myocardium and subsequently differentiated to provide new vasculature to regenerating muscle (Lepilina et al., 2006). Interestingly, aspects of these regenerative events appear to be recapitulated during normal cardiac homeostasis. Pulse-chase labeling experiments revealed that the epicardium recurrently contributes cells to the ventricular wall, and that inhibition of FGF signaling depressed this epicardial supplementation of the ventricular wall, eventually leading to spontaneous ventricular scarring (Wills et al., 2008). Combined with the anteroposterior phenotypes observed in planarians after abrogation of β-catenin, the data demonstrate that cells can be encouraged to alter their fates dramatically depending on what signals they receive.

Prospects

It is clear that much remains unknown about the pluripotent state, the cells that effect this activity, and the environments in which they operate. It is also clear that the stem and niche metaphors have shaped the way in which biologists think about these phenomena. Although these models have had a generally positive impact on the field thus far, such metaphors have also imparted a relatively linear rather than dialectic view onto stem cell biology. For instance, in real life, the progeny of a group of people cannot go back developmentally and replace their ancestors, nor can a sibling transition itself into another sibling. Thus, the discovery that stem cell progeny can actually revert developmentally and reenter a stem cell state was immediately considered remarkable by the field. Likewise, it was also unexpected to find that epicardial cells can undergo an epithelial-to-mesenchymal transition in the adult heart. Such is the grip of metaphors once they transmogrify from a descriptive technique to almost literal truths. Extending (and often overextending) such metaphors has brought a series of assumptions to the field of stem cell biology that need to be constantly examined. As Arturo Rosenblueth and Norbert Wiener once wrote: "the price of metaphor is eternal vigilance" (Rosenblueth and Wiener, 1945). The data discussed in this meeting demonstrate that our understanding of stem cells both in vivo and in vitro now approaches a level that demands re-evaluation of the

explanatory system that frames our understanding of stem cell biology. How profound the coming conceptual changes will be to the way we think about undifferentiated cells and their microenvironments may depend entirely on how vigilant we remain, and how frequently and rigorously we are willing to check our premises.

REFERENCES

Barker, N., van Es, J.H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P.J., et al. (2007). Nature *449*, 1003–1007.

Boyer, L.A., Lee, T.I., Cole, M.F., Johnstone, S.E., Levine, S.S., Zucker, J.P., Guenther, M.G., Kumar, R.M., Murray, H.L., Jenner, R.G., et al. (2005). Cell *122*, 947–956.

Boyer, L.A., Plath, K., Zeitlinger, J., Brambrink, T., Medeiros, L.A., Lee, T.I., Levine, S.S., Wernig, M., Tajonar, A., Ray, M.K., et al. (2006). Nature *441*, 349–353.

Chazaud, C., Yamanaka, Y., Pawson, T., and Rossant, J. (2006). Dev. Cell *10*, 615–624.

Cole, M.F., Johnstone, S.E., Newman, J.J., Kagey, M.H., and Young, R.A. (2008). Genes Dev. *22*, 746–755.

Gurley, K.A., Rink, J.C., and Sánchez Alvarado, A. (2008). Science *319*, 323–327.

Jaenisch, R., and Young, R. (2008). Cell *132*, 567–582.

Keller, J. (1894). Jen. Zeit. Naturw. *28*, 370–407.

Kidd, A.R., III, Miskowski, J.A., Siegfried, K.R., Sawa, H., and Kimble, J. (2005). Cell *121*, 761–772.

Lepilina, A., Coon, A.N., Kikuchi, K., Holdway, J.E., Roberts, R.W., Burns, C.G., and Poss, K.D. (2006). Cell *127*, 607–619.

Liu, J., Phillips, B.T., Amaya, M.F., Kimble, J., and Xu, W. (2008). Dev. Cell *14*, 751–761.

Molofsky, A.V., Pardal, R., Iwashita, T., Park, I.K., Clarke, M.F., and Morrison, S.J. (2003). Nature *425*, 962–967.

Molofsky, A.V., Slutsky, S.G., Joseph, N.M., He, S., Pardal, R., Krishnamurthy, J., Sharpless, N.E., and Morrison, S.J. (2006). Nature *443*, 448–452.

Morrison, S.J., and Spradling, A.C. (2008). Cell *132*, 598–611.

Nystul, T., and Spradling, A. (2007). Cell Stem Cell *1*, 277–285.

Pidwirny, M. (2008). Fundamentals of Physical Geography, http://www.physicalgeography.net.

Ramalho-Santos, M., and Willenbring, H. (2007). Cell Stem Cell *1*, 35–38.

Reddien, P.W., Bermange, A.L., Murfitt, K.J., Jennings, J.R., and Sánchez Alvarado, A. (2005). Dev. Cell *8*, 635–649.

Rosenblueth, A., and Wiener, N. (1945). Philos. Sci. *12*, 316–321.

Schmidt, G.H., Winton, D.J., and Ponder, B.A. (1988). Development *103*, 785–790.

Schofield, R. (1978). Blood Cells *4*, 7–25.

Wills, A.A., Holdway, J.E., Major, R.J., and Poss, K.D. (2008). Development *135*, 183–192.

Xu, C., Inokuma, M.S., Denham, J., Golds, K., Kundu, P., Gold, J.D., and Carpenter, M.K. (2001). Nat. Biotechnol. *19*, 971–974.

Yamanaka, Y., Ralston, A., Stephenson, R.O., and Rossant, J. (2006). Dev. Dyn. *235*, 2301–2314.

Zhong, J.F., Chen, Y., Marcus, J.S., Scherer, A., Quake, S.R., Taylor, C.R., and Weiner, L.P. (2008). Lab Chip *8*, 68–74.