Adult Stem Cells

Edited by

Kursad Turksen

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Preface

Studies on stem cells have been attracting intense scientific and public attention, not only because of controversies surrounding the use of embryonic stem cells but also because of very provocative data that have been emerging on adult stem cells. Much of the public attention and debate has been focused on the possibility that adult stem cells may be used as a substitute for human embryonic stem cells or as a justification for stopping work on them. This has somewhat diminished attention on very heated scientific debates that take us to the very heart of how the concept of stem cells is perceived. To this author, the latter debates have not been unlike certain philosophical debates of the last century.

Since the seminal studies of Till and McCulloch in the 1960s, the popular paradigm on adult stem cells has been that lineage-restricted stem cells are derived from pluripotent stem cells very early during development. To many, and consistent with much data, the restriction to particular lineages was considered absolute. In other words, there was a sense of determinism in the stem quality of particular stem cells: once they were allocated, they were programmed to specific roles in a given tissue. Furthermore, some adult tissues were considered devoid of detectable stem cell presence or activity. During the last decade, new challenges to our previous notions about stem cells have arisen, one example being the demonstration of stem cells in adult neuronal tissue where they had been said not to exist. Our certainty about stem cell biology has been challenged even further by recent reports that previously designated tissue-restricted adult stem cells might not only be multipotent but also pluripotent. In essence, the debate has become similar to the that between Cartesian and Existentialist philosophers many decades ago. Are stem cells fated to be particular stem cells determined to particular lineage(s) or do they have they the capacity to actualize diverse potentials in diverse environments? In other words, do stem cells exercise "free will"? In a sense, we are debating in a cellular context whether "essence precedes existence" or "existence precedes essence" of stem cells.

In *Adult Stem Cells*, the authors have made an effort, if not to enter the philosophical debate, at least to contribute to current understanding of the potential of several adult stem cell types and their regulation. The debate is certainly still heated and ongoing, and we are confronting new challenges to our understanding of stem cell biology on a weekly basis. Nevertheless, it is hoped that this volume will challenge all of us interested in stem cells to dream about, and to discriminate between, the "essence" and the "existence" of stem cells.

I would like to express my appreciation to all contributors for their unique contributions to this volume. I would also like to thank Elyse O'Grady for supporting this project from its inception during a brief conversation that we had at an ASCB meeting. I also acknowledge the Humana Press staff for doing such an excellent job in publishing this volume.

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Kursad Turksen

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Color Plates

Color plates 1–10 appear in an insert following p. 82.

1

Adult Stem Cell Plasticity

William B. Slayton and Gerald J. Spangrude

1. INTRODUCTION

Although modern medicine has provided the ability to cure infections and malignancy, the ability to repair damaged organs is less advanced. Solid organ transplantation has been performed successfully, but is fraught with problems such as rejection, infection, and secondary malignancy from immunosuppression. Organ shortages create ethical issues with respect to the equitable distribution of donated tissues. Regenerative medicine, the field devoted to rebuilding damaged organs from stem cells, may provide alternatives to solid organ transplantation. However, the field of regenerative medicine is in its infancy. The potential sources of the tissues to regenerate organs include cloned cells, embryonic or fetal stem cells, or adult stem cells. Although each of these sources of stem cells has potential biological advantages and disadvantages, ethical and legal concerns have been raised by cloning *(1–4)* and the use of embryonic and fetal stem cells *(5)*.

Adult stem cells might provide medical solutions that avoid the ethical and legal problems of cloning and fetal stem cell approaches. Until recently, stem cells from adult tissues were believed restricted in their capacity to produce tissues other than the tissue from which they arose. A number of studies have challenged this view. Specifically, these studies have suggested that adult stem cells from various organs are *plastic*, meaning that they can differentiate not only into their original source tissue, but also into cells of unrelated tissue.

Bone marrow transplant has been used to treat nonhematopoietic disorders such as osteogenesis imperfecta *(6)* and metachromatic leukodystrophy. However, in the case of osteogenesis imperfecta, transplanted mesenchymal stem cells are believed to be the source of reparative osteocytes. In metachromatic leukodystrophy, the mechanism of improvement is unknown, but is thought to be related to a bystander effect of cells, with normal aryl sulfatase circulating past diseased neurons *(7,8)*. The role of the hematopoietic stem cell as a replacement for diseased osteocytes or neurons

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has not been advocated as a possible mechanism for transplant-induced improvement in these disorders.

Adult stem cell plasticity might allow, for instance, use of bone marrow stem cells to replace damaged myocardial cells following ischemic damage, pancreatic islet cells to cure insulin-dependent diabetes, or cells from the substantia nigra to cure Parkinson's disease. However, as exciting as the prospect is for adult stem cells to solve some of our most daunting medical challenges, newer studies have challenged the interpretation of some of the pioneer studies that generated this excitement. This chapter is an overview of the current controversies in adult stem cell biology.

2. EVOLUTIONARY PERSPECTIVE

Limb and organ regeneration is common among organisms. Alvarado wrote an excellent review of the evolutionary aspects of regeneration *(9)*. Stem cell activity and regeneration can be studied at the most basic level in simple organisms such as the planarian *(10)* (Fig. 1). In the planarian, the molecular mechanisms underlying asexual modes of reproduction are indistinguishable from mechanisms of regeneration following injury. In the hydra, similar molecular messages that stimulate asexual reproduction are triggered by injury. Primitive organisms capable of regenerating damaged body parts include hydra, planarian, mollusks, insects, crustaceans, and echinoderms (starfish). Chordates that can regenerate include amphibians such as frogs and salamanders.

Almost every phylum has species that are able to regenerate lost body parts *(9)*. Regeneration in these organisms requires the ability of cells in the injured tissue to "dedifferentiate," which requires the ability of these organisms to regulate pluripotentiality. Sites of injury in chordates that regenerate form an area of dedifferentiated cells called the "regeneration blastema," and the cells within this structure recapitulate molecular developmental processes that occur during embryogenesis *(9)*.

In summary, the ability to regenerate is a common trait shared by many species. The reason some classes of animals have lost the ability to regenerate is unclear. Alvarado hypothesized that the ability to regenerate confers neither a positive nor negative evolutionary bias, allowing this trait to disappear in many classes of animals, including mammals *(9)*. However, reversal of cell fates and stem cell plasticity may be vestiges of these evolutionarily ancient processes.

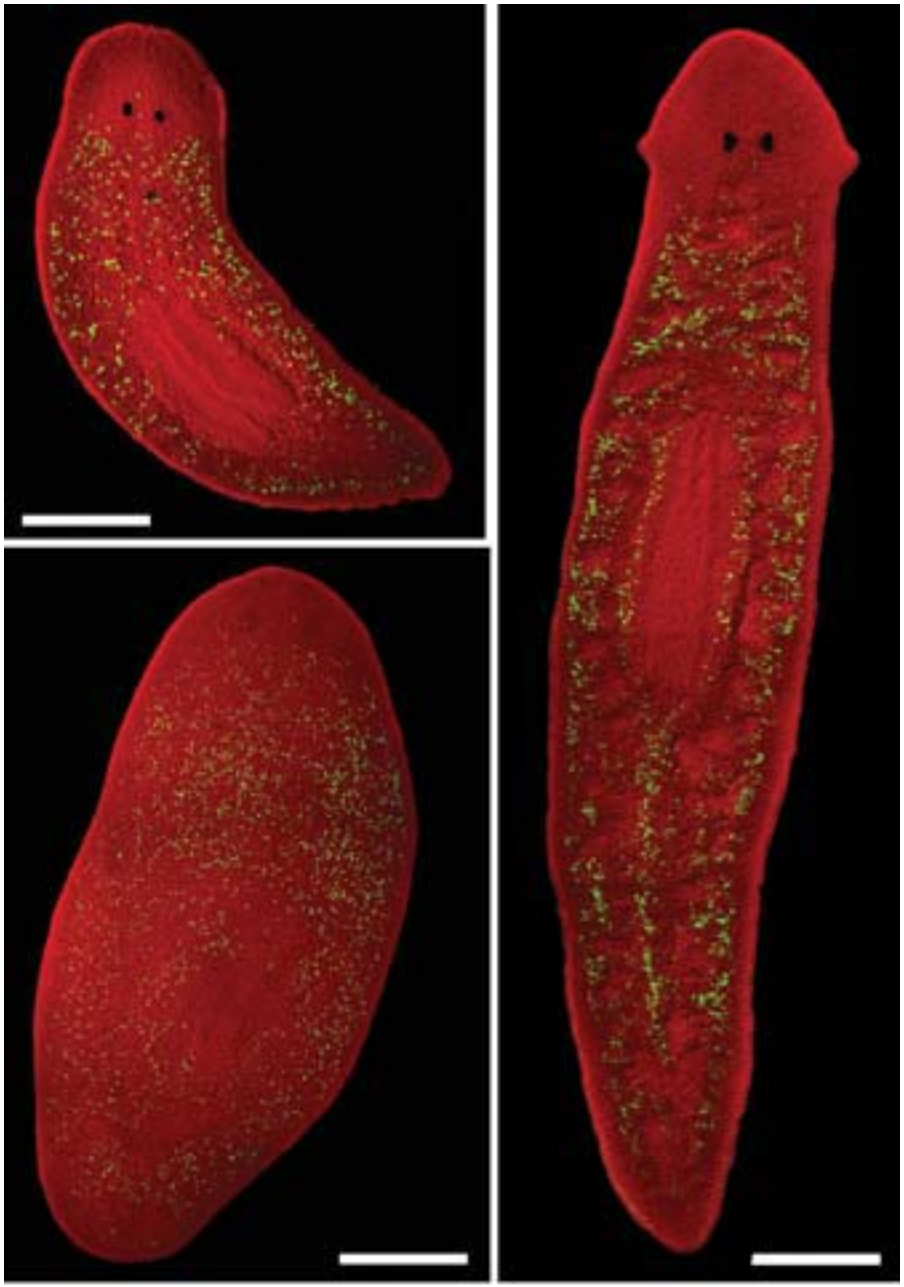

Fig. 1. Bromodeoxyuridine labeling of regenerative stem cells in planarians *Phagocata* sp. (*upper left*); *Girardia dorotocephala* (*lower left*); and *Schmidtea mediterranea* (*right*). Scale bars: A, 150 microns; B, 300; C, 450. (From ref. *10*. Photo courtesy Dr. Alejandro Alvarado. Used with permission of Academic Press.) (*See* color plate 1 in the insert following p. 82.)

3. MODELS OF PLASTICITY

One way that stem cells might achieve plasticity prior to generating heterologous cell types is by reversion to a state similar to the embryonic stem cell *(11)*. The fact that the differentiated state is reversible was first inferred from heterokaryon experiments *(12)*. In these studies, cells from mature muscle tissue were fused with cells of various mature phenotypes. Muscle-specific genes were induced from nuclei of hepatocytes, keratinocytes, and fibroblasts *(13,14)*. The process of cell culture prior to transplant, as performed in many of the studies involving brain or muscle, may allow for a "dedifferentiation" process.

The concept of transdetermination that developed through experiments performed in *Drosophila melanogaster* fits this model of plasticity. Imaginal disks are areas of tissue within the fly larva that eventually develop into adult cuticular structures such as antennae, legs, and wings. During metamorphosis, these cells synthesize pigment and secrete cuticle for specific fly structures. When transplanted prior to metamorphosis, these disks still make the part they would have made if not transplanted. However, imaginal disks can be broken apart and transplanted into the abdominal cavity of adult flies, where regenerative growth can occur. When subsequently transplanted into the body cavity of a host larva, these cells will enter metamorphosis synchronously with the host larva and produce the appendage that is appropriate to the location of migration *(11)*. The primary mechanism by which neural stem cells (NSCs) acquire the ability to produce hematopoietic cells seems to require a period in culture when such dedifferentiation may take place.

Another model of plasticity contends that stem cells are common to all tissues, but are limited in their ability to differentiate based on aspects of the microenvironment *(15)*. Supportive of this model is the fact that stem cells from various tissues express numerous common genes. Common expression of subsets of genes found in cDNA (complementary deoxyribonucleic acid) libraries generated from hematopoietic stem cells and neurospheres has been reported *(16)*. However, it is unclear why hematopoietic stem cells lose their ability to self-renew when cultured, whereas NSCs grow well in culture and maintain stem cell function. If all stem cells were equal, this would not be the case.

In summary, the mechanisms by which stem cells from one tissue can produce mature cells of another tissue have not been clearly established and may vary depending on the particular conditions of the experimental system.

4. PROVING PLASTICITY

A number of recent reviews have outlined the current controversies in stem cell plasticity *(12,17–19)*. Problems with initial studies in this field are numerous. First, plasticity has primarily been inferred from the behavior of undefined mixtures of cells. It is therefore unclear which cells in these mixtures produce the cells that give rise to the original and new phenotypes and whether separate cell lineages arise from the same cell. Second, cell populations have been transplanted following time in tissue culture, and it is unclear whether the stem cells as originally isolated had the ability to produce heterologous tissue or whether epigenetic modification occurred because of the culture period. Third, most studies have not demonstrated the ability of transdifferentiating stem cells to self-renew. Finally, most studies have not demonstrated functionality of the progeny of transdifferentiated stem cells.

These criteria are the measure by which all further studies that claim to demonstrate stem cell plasticity should be evaluated. Hematopoietic stem cell biologists have developed a number of approaches to identify and characterize the behavior of putative stem cells, and by using modifications of these approaches, many of the controversial questions in this field will be resolved. In summary, the science that has been performed to date suggesting stem cell plasticity has not clearly established that adult stem cells are plastic.

Technical limitations to each approach used to measure the presence of donor-derived cells in recipient tissue following transplantation can also lead to misleading results. For instance, two recent studies have demonstrated that embryonic stem cells will fuse with hematopoietic cells or with NSCs when cultured together, and that these chimeric cells will display the phenotype of both original cell types *(20,21)*. The possibility of such fusion events would call into question plastic behavior observed following a culture period, especially when different cell types were mixed. When using the Y chromosome or β-galactosidase (β-Gal) staining to detect donor-derived cells, controls that measure background staining within specific tissue types are crucial *(18)*. Autofluorescence can be mistaken for the fluorescence of green fluorescent protein. When evaluating studies that argue that stem cells are plastic, a careful review of the model system, discovery whether prior culturing of the cells was performed, and a review of experimental controls are essential.