

Review

# Stem cells and aging: expanding the possibilities

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Received 22 November 2000; accepted 22 November 2000

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**Abstract**

In the very early stages of embryonic development, cells have the capability of dividing indefinitely and then differentiating into any type of cell in the body. Recent studies have revealed that much of this remarkable developmental potential of embryonic stem cells is retained by small populations of cells within most tissues in the adult. Intercellular signals that control the proliferation, differentiation and survival of stem cells are being identified and include a diverse array of growth factors, cytokines and cell adhesion molecules. Intracellular mechanisms that regulate stem cell fate are also emerging and include established second messenger pathways, novel transcription factors and telomerase. The possibility that a decline in the numbers or plasticity of stem cell populations contributes to aging and age-related disease is suggested by recent findings. The remarkable plasticity of stem cells suggests that endogenous or transplanted stem cells can be ‘tweaked’ in ways that will allow them to replace lost or dysfunctional cell populations in diseases ranging from neurodegenerative and hematopoietic disorders to diabetes and cardiovascular disease. © 2001 Published by Elsevier Science Ireland Ltd.

*Keywords:* Alzheimer’s disease; Apoptosis; Brain; Differentiation; Endothelial; Hematopoietic; Neurons; Notch; Telomerase

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## 1. Stem cell biology: what, when, where?

Early development in the embryo is characterized by a progressive restriction in developmental fate (Fig. 1). The fertilized egg through its early stages of development is capable of generating an entire organism as well as contributing to the

### Stem Cells in Development

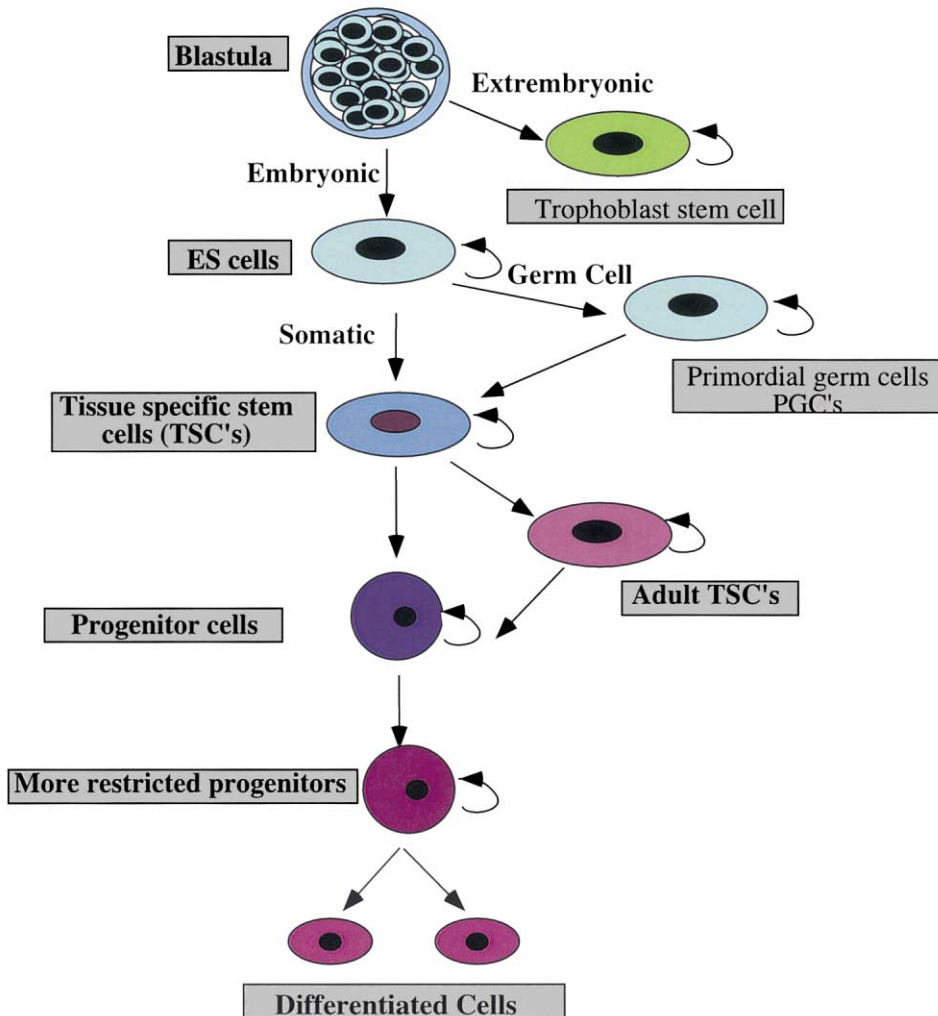


Fig. 1. Outline of the progressive restriction of stem cell potentials during development. Some progeny of totipotent stem cells in the developing blastula (embryonic stem cells) become restricted in the cell types they can give rise to as different types of tissues develop (TSCs).

trophoectoderm and the germ line. These so-called embryonic stem (ES) cells are thus defined by their potential to generate the entire organism. As development proceeds through the blastula stage cells become more restricted and, in general, cells from the inner cell mass do not contribute to extraembryonic structures in chimeras. The inner cell mass however is pluripotent and can contribute to all germ layers. ES cells from the epiblast have been isolated from the blastula and cultured indefinitely *in vitro*; they are characterized by the expression of specific markers (Pesce and Scholer, 2000), high telomerase levels (Armstrong et al., 2000a) and specific growth factor responses (De Felici and Pesce, 1994; Pedersen, 1994). Restricted precursor cells capable of acquiring neuronal and glial phenotypes (Okabe et al., 1996; McDonald et al., 1999; Mujtaba et al., 1999) as well as non-neural phenotypes (Kataoka et al., 1997; Weiss, 1997; Suwabe et al., 1998) can be isolated from mouse ES cells. Cells similar to ES cells have been isolated from the primordial germ layer, but the self renewal abilities of these cells remains to be determined (Shamblott et al., 1998). ES cells can differentiate *in vitro* to generate a multitude of cell types and *in vivo* can contribute to all germ layers as well as to gonadal tissue. Seminal work by a number of laboratories has led to rapid advances in our understanding of phenotypic specification. *In vitro* as *in vivo* differentiation proceeds through several intermediate stages of cellular differentiation. A complex terminology that is still evolving has developed to distinguish between these multiple stages of differentiation. For the purposes of this review we will define a stem cell is any cell that is capable of self renewal and can undergo asymmetric division to generate differentiated cells. Totipotent stem cell is a term we will reserve for early blastula stage cells that can contribute to both ectodermal and extraectodermal tissue. The term pluripotent will be used to describe stem cells which can differentiate into germinal and somatic tissue. Cells which have a wide repertoire of differentiation including most cells in a particular tissue or organ will be termed multipotent. The terms 'bipotent' and 'unipotent' will be used to describe stem cells that generate two or one class of differentiated progeny, respectively.

Progenitor/blast and transit amplifying cells are, in our opinion, equivalent terms and denote cells with a limited self renewing capacity and a relatively restricted repertoire of differentiation (Morrison et al., 1997; Rao, 1999; Temple, 2000). Using these definitions, multipotent stem cells have been isolated from most tissues, and several stages of stem cell to progenitor cell differentiation have been identified. A schematic of such differentiation in the developing nervous system is shown in Fig. 2. It is important to emphasize that stem cells are not simply a feature of embryonic development but are also present in adult tissue and may play an important role in the normal homeostasis of the organism. For example, hematopoietic stem cells provide a continuous supply of blood cells which, depending upon the particular type of blood cell, may turnover very rapidly (days) or more slowly (months to years). Likewise, precursor cells in the liver likely contribute to hepatic regeneration and adult liver stem cells have been isolated. Similarly, in the adult brain populations of neural stem cells located in the subventricular zone form olfactory bulb cells and stem cells are also located in the dentate gyrus of the hippocampus where they may give rise to neurons or astrocytes. Adult stem cells have now been

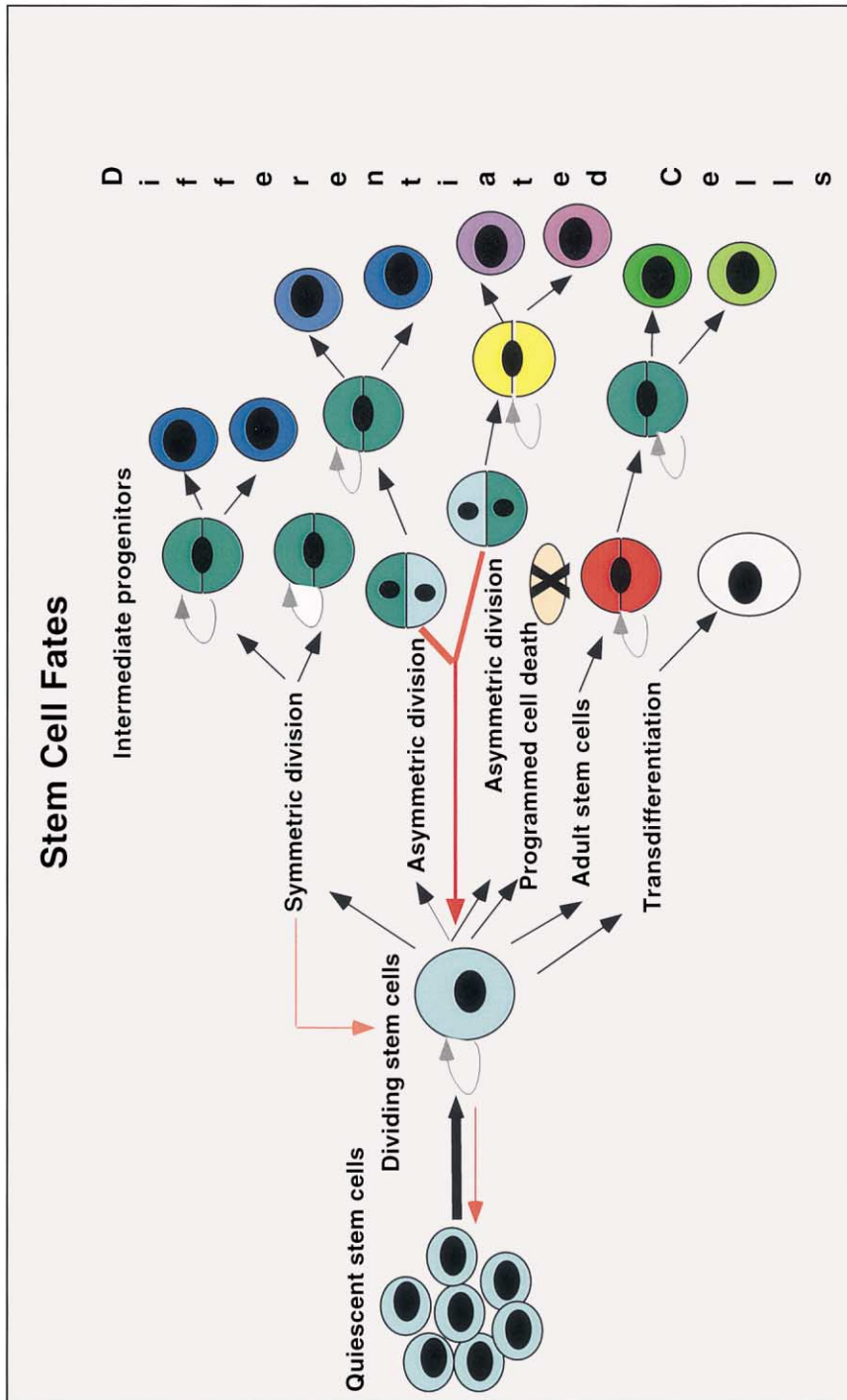


Fig. 2. Asymmetric cell divisions provide a mechanism for formation of highly specialized differentiated cells, with the maintenance of populations of self-renewing stem cells within a tissue.

identified or isolated from multiple tissues including mesoderm, skin, intestinal epithelium, myocardium, and so on.

The importance of adult stem cells in normal tissue homeostasis, and in aging and disease, is being recognized. Studies in which mitotic cells are labeled with bromodeoxyuridine and other DNA precursors have not only been instrumental in establishing the presence of stem cells in various tissues throughout the body, they have also provided insight into localization, the phenotypes of their differentiated progeny, and their turnover rates. Such studies have revealed a tremendous heterogeneity in the kinetics of stem cell production, differentiation and survival among tissues. For example, hematopoietic stem cells have a very high proliferation rate and most of their progeny have a relatively short lifespan on the order of days. In contrast, neural progenitor cells in the brain have a slower proliferation rate and a longer lifespan (weeks to years). The present article is focussed on the impact of aging on stem cells, on the one hand, and the involvement of abnormalities in the regulation of stem cells (proliferation, differentiation and survival) in age-related diseases on the other hand. In order to study stem cells in the context of aging, it is important to understand the molecular mechanisms that regulate stem cell behaviors.

## **2. Regulation of stem cell proliferation, differentiation and survival**

The number of stem cells and the process of stem cell differentiation is carefully regulated to meet the demands of the developing tissue; accordingly, complex feedback cues have developed to maintain appropriate pools of undifferentiated precursor cells and differentiated progeny. The normal fate of the stem cells is summarized in Fig. 2. A stem cell may remain quiescent and simply not enter the cell cycle. This may be important in sequestering a reserve pool of cells for use in times of stress or at later stages of development. A stem cell may undergo apoptosis and not contribute to further development; this scenario may be the norm in tissues such as the brain where turnover of differentiated cells (neurons and glial cells) is very low. Stem cells may undergo symmetric cell divisions to self renew or undergo terminal differentiation, or they may undergo asymmetric cell divisions to generate differentiated progeny as well as maintain a pool of stem cells. A dynamic balance between proliferation, survival and differentiation signals ensures that an appropriate balance between stem cells, precursor cells, and differentiated cells is maintained throughout development and adult life.

Two additional aspects of stem cell biology are important in understanding or manipulating stem cells, namely, 'transdifferentiation' and 'transformation'. Transdifferentiation is a term used for stem cells that do not go through their normal process of progressively restricted differentiation, but rather transit to another kind of stem cell or transdifferentiate to acquire a distinct set of properties. This process will both dilute the pool of stem cells and will increase aberrant differentiation. This process may however be beneficial as well. For example, it was recently shown that oligodendrocyte precursor cells can transdifferentiate to become pluripotent stem

cells capable of forming neurons (Kondo and Raff, 2000); this type of cellular plasticity might be of value in conditions where neurons degenerate. Transformation is the loss of normal cellular control and is an initial aspect of cancerous tumor formation. It is important to note that most tumors arise from dividing populations of stem or precursor cells. Indeed, in the hematopoietic system each stage of stem cell to blast cell to differentiated cell is associated with a leukemia or lymphoma (Ballen et al., 2000). The relative infrequency of transformation however suggests that the ability to self renew, survive, proliferate, differentiate, transdifferentiate or transform is closely regulated such that the overall stem cell status in any self renewing tissue is a dynamic balance between cell intrinsic and cell extrinsic factors. Furthermore, abnormalities in any of these stages will alter normal development or will affect cellular response to the normal aging process. Factors regulating each of these fates have been characterized *in vitro* and *in vivo* and several examples are presented below.

It is clear that the behaviors of stem cells are carefully regulated to meet the demands of the tissue for which they may supply new cells. For example, in the adult brain populations of neural stem cells located in the subventricular zone and the dentate gyrus of the hippocampus turn over continuously such that most, if not all, newly generated cells die within a certain time period in the postmitotic state. Most subventricular zone neural progenitor cells form olfactory bulb cells which subserve their function and then are replaced by newly-generated cells. Hematopoietic stem cells provide a continuous supply of blood cells which, depending upon the particular type of blood cell, may turnover very rapidly (days) or more slowly (months to years). Stem cells are highly sensitive to environmental demands. Thus, the proliferation and/or survival of neural stem cells is increased in response to brain injury (Gould and Tanapat, 1997; Parent et al., 1997; Tzeng and Wu, 1999), and intellectual (Kempermann et al., 1997; Young et al., 1999) and physical (Neeper et al., 1996) activity. While multiple signaling pathways can influence stem cell fate, there appear to be a few mechanisms that are widely employed across a range of tissues and in an array of multicellular organisms. Prominent intrinsic regulators include telomerase and Bcl-2 family members, while extrinsic regulators include the Notch signaling pathway, growth factors/cytokines and cell adhesion molecules. In simple terms, such signals control two major cellular ‘decisions’, namely, to proliferate or differentiate and to live or to die. Thus, stem cells must often ‘guide’ themselves down roads that are bordered by a cancerous state on one side and mortality on the other. For many stem cells this road becomes increasingly difficult to navigate during aging, with an all too common consequence being disease.

### **3. Molecular mechanisms that control stem cell proliferation, differentiation and survival**

The power of invertebrate molecular genetics, and extension of this work to mouse and man, has revealed an intriguing signaling pathway that controls stem

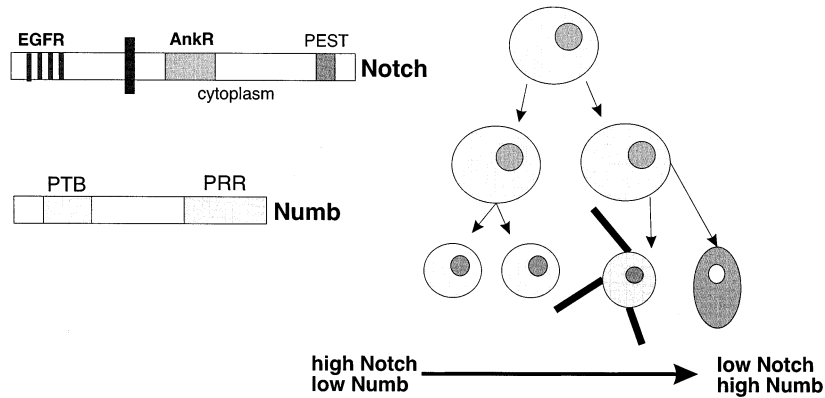


Fig. 3. Roles of Notch and Numb in controlling stem cell proliferation, differentiation and survival. See text for discussion.

cell fate. At the heart of this pathway is a cell surface receptor called Notch. Notch was discovered in *Drosophila* where it is essential for the proper specification of many different cell fates during the processes of oogenesis, myogenesis, neurogenesis and wing and eye development (Artavanis-Tsakonis et al., 1995). Four mammalian homologues of Notch have been identified (Notch1–4), with Notch1 playing a widespread role in determination of cell fate during development. Mice lacking Notch1 die during early embryonic development with major abnormalities including defects in somite formation, neurogenesis and hematopoiesis (Conlon et al., 1995). Notch is an integral membrane protein with a single membrane-spanning domain and is located primarily in the plasma membrane (Fig. 3). The extracellular (N-terminal) domain contains a series of tandem epidermal growth factor-like repeats and three Lin/Notch repeats (LNR) that function in ligand binding and Notch activation; the major ligand for Notch1 in mammals is called Delta. The intracellular (C-terminal) region of Notch contains three ankyrin repeats which mediate interactions with other (cytoplasmic) proteins, a ‘PEST’ sequence that regulates protein turnover, and a nuclear localization sequence. Activation of Notch by binding of ligand results in proteolytic cleavage of Notch at a site located at the cytoplasmic face of the membrane, resulting in release of a Notch C-terminal fragment (CTF). The CTF has been shown to translocate to the nucleus where it is thought to modulate transcription. Extranuclear mechanisms of Notch CTF action have also been proposed based upon interactions with proteins such as NF- $\kappa$ B subunits (Guan et al., 1996).

Notch has been shown to mediate both homotypic and heterotypic cell–cell interactions (Delta, the ligand for Notch, is a cell surface protein), with both the level and subcellular localization of Notch appearing to play a role in determining cell fate. Among a group of equipotent cells, those with higher levels of Notch will continue to divide, whereas those with lower levels of Notch will differentiate (Fig. 3). For example, during development of the nervous system in *Drosophila* cells

expressing low levels of Notch become neurons, while cells with higher levels of Notch become epidermal cells (Heitzler and Simpson, 1991). However, the outcome of Notch signaling (proliferation or differentiation) is subject to modification by growth factors and cell adhesion molecule signaling pathways. Several cytoplasmic proteins have been shown to modify Notch signaling and one such protein that appears to be particularly important in mammalian stem cells is called Numb. By interacting with a cytoplasmic domain of Notch, Numb plays a role in cell fate determination that is antagonistic to that of Notch (Guo et al., 1996) (Fig. 3). During development of the nervous system, Numb plays a pivotal role in controlling asymmetric division of neural progenitor cells by segregating to the daughter cell that remains a progenitor cell (Zhong et al., 2000). Mice lacking functional Numb die during early development at embryonic day 11.5 and exhibit profound defects in cranial neural tube closure and premature neuron production (Zhong et al., 2000). Two protein–protein interaction domains have been shown to influence Numb function; a phosphotyrosine-binding (PTB) domain and a proline-rich region (PRR) that functions as a SH3-binding domain (Guo et al., 1996; Verdi et al., 1996, 1999). Four different isoforms of Numb that differ in their PTB (lacking or containing an 11 amino acid insert) and PRR (lacking or containing a 48 amino acid insert) domains (Verdi et al., 1999) have been identified. When expressed in neural progenitor cells Numb isoforms containing a long PRR promote proliferation whereas isoforms containing a truncated PRR promote cell differentiation (Verdi et al., 1999). When taken together with emerging evidence that Notch signaling can promote cell survival, the phenotype of the Numb-deficient mice suggests a possible role for Numb in modulating cell survival.

An array of growth factors and cytokines have been identified that can promote the proliferation, differentiation and/or survival of one or more populations of stem cells. For example, epidermal growth factor and basic fibroblast growth factor can maintain brain neural stem cells in a proliferative state (Gritti et al., 1999), while brain-derived neurotrophic factor can facilitate the survival and differentiation of neuronal precursors (Eaton and Whitemore, 1996). Hematopoietic stem cells, and the differentiation of their various progeny, are regulated by several cytokines including members of the transforming growth factor- $\beta$  and colony stimulating factor families (Dickson et al., 1995; Bigas et al., 1998). Insulin-like growth factor-1 is a survival and differentiation factor for several types of progenitor cells including precursors of neurons (Arsenijevic and Weiss, 1998) and hematopoietic cells (Muta et al., 1994). Cell adhesion molecules are another class of signaling proteins that play important roles in regulating stem cell behaviors. One very important adhesion signaling system involves integrin receptors which transduce cell–cell and cell–extracellular matrix interactions into various cellular responses including changes in cell proliferation, differentiation and survival. Integrins are activated by binding to extracellular matrix proteins such as laminin or to integrins on the surface of other cells, resulting in an intracellular signaling pathway involving PI3 kinase and Akt kinase. Integrin signaling promotes survival of many different cell types including and neurons (Gary and Mattson, 2001). Hematopoietic stem cells bind to extracellular matrix components through  $\beta$ 1 integrins, and many other integrin and

non-integrin cell adhesion receptors are expressed in hematopoietic progenitor cells and their expression is often regulated in ways that suggest important roles in cell differentiation (Coulombel et al., 1997). Hematopoietic stem cells deficient in  $\beta$ 1-integrin, although exhibiting the ability to differentiate, are defective in their ability to colonize hematopoietic tissues in irradiated recipient mice (Potocnik et al., 2000). Neural progenitor cells express several different integrins which appear to be differentially involved in the regulation of proliferation and cell migration/differentiation. For example, proliferation requires activation of  $\alpha$ 5 $\beta$ 1 integrins whereas migration requires activation of  $\alpha$ 6 $\beta$ 1 integrins (Jacques et al., 1998).

A final example of molecular control of stem cell fate concerns an intrinsic mechanism for maintaining genomic instability and responding to DNA damage. Telomeres, the ends of chromosomes, consist of repeats of six-base DNA sequence (TTAGGG) that preserve chromosome integrity and prevent end-to-end fusions. A reverse transcriptase called telomerase is responsible for adding the TTAGGG sequence to the chromosome ends (Klapper et al., 2001). Telomerase consists of a catalytic subunit (TERT) and an RNA template (TR). Telomerase is expressed in highly proliferative cells throughout the developing embryo and is then dramatically down-regulated as cells differentiate, and is not detectable in many somatic cells in the adult. However, stem cells retain telomerase activity. Studies of cancer cells and somatic cells overexpressing TERT have shown that telomerase can confer upon cells an immortal phenotype. Recent studies have provided evidence that telomerase can promote cell proliferation by protecting telomeres and thereby preventing cell cycle arrest. Moreover, telomerase can prevent apoptosis (programmed cell death) by suppressing DNA damage-induced death cascades involving the tumor suppressor protein p53 (Fu et al., 1999, 2000). Studies of brain development in mice have correlated a decrease in telomerase activity with decreased neuroblast proliferation, and a decrease in TERT expression with cell differentiation and natural cell death (Klapper et al., 2001). DNA damage is a trigger for cell cycle arrest and apoptosis, and telomerase is one important mechanism for suppressing such DNA damage. Additional DNA damage response and repair mechanisms are likely to play important roles in the regulation of stem cell proliferation and survival including poly (ADP-ribose) polymerase, TRF2, Ku80 and tankyrase (Evans et al., 1998; d'Adda di Fagagna et al., 1999).

#### **4. Age-related alterations in stem cell populations**

What are the changes that occur in stem cell populations during aging? Might changes in stem cells control the rate of aging of various organs? How might stem cells be used to replace dysfunctional or dead cells and thereby restore vigor to failing organs? Remarkably little information is available concerning molecular and cellular changes that occur in stem cell populations during aging. This dearth of information is partly the result of the fact that stem cells comprise only a very small percentage of all cells within a tissue, and it is therefore very difficult to obtain pure populations of stem cells in quantities sufficient to perform many different bio-

chemical or even molecular analyses. Moreover, in many cases the stem cells can not be unambiguously identified *in situ*, making it difficult to perform immunohistochemical or *in situ* hybridization analyses, for example. Nevertheless, the ‘immortal’ nature of stem cells makes their study an imperative in the field of aging research.

Several studies have provided evidence that the ability of stem cells to respond to environmental demands may be diminished during aging. Most of the studies on stem cells and aging have been performed on hematopoietic stem cells (Globerson, 1999). Measurements of the number of osteoprogenitor cells in bone marrow from adult and aged rats revealed a significant decrease in numbers with aging, as well as a decreased capacity of the progenitor cells to form bone *in vivo* (Quarto et al., 1995). Analyses of clonogenic myeloid progenitors from bone marrow suggest that the proliferative capacity of myeloid progenitors decreases progressively with increasing age (Marley et al., 1999). Similar results were obtained in studies in which the ability of bone marrow stromal cells to support the production of B-lymphocytes was examined (Stephan et al., 1998). Chen and coworkers (Chen et al., 1999) used a competitive dilution assay to measure both the functional ability of hematopoietic stem cells and their concentration in BALB/cBy mice. Their data suggest that their functional ability declines during development and aging. However, the stem cells in old mice exhibit an ability to repopulate engrafted host mice that is not different than stem cells from young mice. These findings suggest that, at least in the case of bone marrow, stem cell populations retain their potential to repopulate an organ throughout life. Although the proliferative potential of hematopoietic stem cells can be maintained during aging in a given species or strain of rodent, cross-species analyses suggest that the proliferative potential of hematopoietic stem cells is related to maximum lifespan (Van Zant, 2000). While the proliferative potential of hematopoietic stem cells may be maintained during aging under basal conditions, the ability of stem cells to respond to injury and disease may be compromised during aging.

Incorporation of BrdU by neuronal progenitor cells in the rat hippocampus is decreased during aging in rats (Kuhn et al., 1996), an alteration which could conceivably account for age-related deficits in hippocampus-mediated brain functions such as learning and memory. Interestingly, adrenalectomy can restore neurogenesis in the hippocampus of old rats (Montaron et al., 1999), demonstrating that neural stem cells in the hippocampus retain the ability for proliferation even in very old animals and suggesting a role for increased activation of the hypothalamic–pituitary–adrenal axis in age-related compromise of neural stem cell populations. The declining ability of the nervous system to recover from injury and disease with advancing age might result, at least in part, from a reduced capacity of stem cells to respond to environmental demands. It has been shown by several laboratories that neural stem cells are mobilized in response to injury (Fig. 4). For example, cerebral ischemia increases neurogenesis in the dentate gyrus of gerbils (Liu et al., 1998) and severe seizures increase proliferation of neural stem cells in the same brain region of rats (Parent et al., 1997). In addition, increased functional demands on the brain may increase the proliferation, differentiation and/or survival of neural

stem cells. Thus, the numbers of newly generated neural cells that survive are increased in the hippocampus of rodents maintained in an enriched environment (Young et al., 1999), as well as in rats that exercise regularly (van Praag et al., 1999). It will be important to determine whether aging affects the ability of neural stem cells to respond to such environmental demands.

The mechanisms that underlie changes in the ability of pluripotent stem cells to proliferate, differentiate and/or survive during aging are not known. Possible players include telomerase, growth factor and cytokine signaling pathways and oxidative stress. Studies have shown that levels of telomerase activity increase in hematopoietic progenitor cells upon their proliferation and differentiation, and decrease with aging (Hiyama et al., 1995). A contribution of reduced telomerase activity to the adverse effects of aging on hematopoietic stem cells is supported by data showing that telomere length decreases in these cells during aging (Vaziri et al., 1994). Changes in the levels of expression of some growth factors and cytokines have been documented in several tissues during normal aging and in association with age-related diseases (Mattson and Lindvall, 1997; Baraldi-Junkins et al., 2000), but it is not known whether these changes contribute to age-related alterations in stem cells. Increased cellular oxidative stress is a widespread occurrence during aging and manifests as oxyradical-mediated damage to proteins, lipids and DNA. There is considerable evidence that such oxidative stress plays a central role in the aging process itself, as well as in age-related diseases. For example, oxidative damage to DNA promotes genomic instability and cancer formation (Bohr and Dianov, 1999), oxidative damage to membranes of neurons may be central to the pathogenesis of Alzheimer's disease (Mattson, 1998), and oxidative stress related to protein glycation is believed to be a pivotal event in many of the complications of

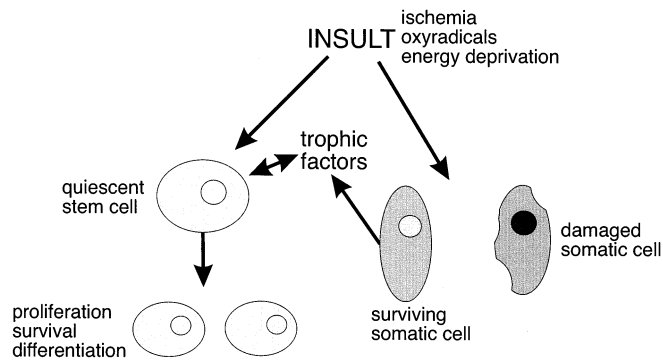


Fig. 4. Effects of cellular stress on stem cell behaviors. Cellular stress (e.g. ischemia, oxyradical production, energy deficits) stimulates production of growth factor and cytokines by somatic cells (and possibly by stem cells). The growth factors and cytokines then stimulate proliferation, differentiation and/or survival of the stem cells, which may then replace somatic cells damaged or killed by the tissue injury.

diabetes (Ceriello, 1999). There is surprisingly little information available on the impact of oxidative stress on stem cells.

## **5. Stem cells and age-related diseases: from bench to bedside**

Three major questions to be addressed in the remainder of this article focus on the important issue of the roles of stem cells in age-related disease: (1) Do alterations in stem cells play a role in disease initiation and/or pathogenesis?; (2) How do genetic and environmental factors impact on stem cells in ways that either promote or prevent age-related disease?; (3) Can manipulations that affect endogenous stem cells, or stem cell transplantation, be used to treat patients? Alterations in stem cell proliferative potential have been documented in studies of bone marrow and brain, but virtually no information on the molecular and cellular mechanisms underlying such alterations have been obtained. However, clues are arising from the combined efforts of molecular geneticists, developmental biologists, and molecular and cellular gerontologists. An example of one specific stem cell-regulating signaling pathway linked to several age-related diseases comes from work on Notch. In addition to regulating cell proliferation and differentiation, recent findings link Notch signaling to cell death, and suggest that alterations in Notch signaling may contribute to several age-related diseases. Overexpression of Notch can protect T lymphocytes against apoptosis induced by T-cell receptor crosslinking and exposure to glucocorticoids (Jehn et al., 1999). Many cancer cells exhibit enhanced Notch activation (Jeffries and Capobianco, 2000) and Notch can prevent drug-induced death of erythroleukemia cells (Shelly et al., 1999). A link between Notch and Alzheimer's disease (AD) is suggested by data demonstrating that mutations in presenilin-1 can cause Alzheimer's disease (Mattson, 2000) and that presenilin-1 may play a role in Notch processing and/or signaling (Shen et al., 1997; Berechid et al., 1999; Song et al., 1999; Handler et al., 2000). Interestingly, mutations in presenilin-1 that cause Alzheimer's disease promote neuronal apoptosis (Guo et al., 1997, 1999) and inhibit Notch signaling (Song et al., 1999), although it remains to be determined whether inhibition of Notch signaling is required for the proapoptotic action of the mutant presenilin-1.

Much of the excitement in stem cell research arises from the expectation that the potential of these cells to form entire tissues and organs can be harnessed and used to replace cells that have been damaged by disease. There is now considerable experimental evidence to back up the therapeutic value of stem cells. The use of stem cells for therapeutic purposes has been proposed for many different diseases, and in several cases data from animal studies have demonstrated efficacy. In addition, bone marrow transplantation (which can be considered as a form of stem cell therapy) has been used to successfully treat patients with certain forms of leukemia as well as patients with autoimmune disorders such as lupus erythematosus and rheumatoid arthritis. However, the translation of basic research to clinical trials has always been slow, and this is also likely to be the case with stem cell therapy.

## 6. Cancer

The strongest evidence that alterations in stem cells may cause age-related disease comes from studies of various cancers. Because of their high proliferative capacity and immortal characteristics, stem cells are ideally suited to become cancerous and, indeed, the vast majority of cancers are believed to arise from stem cells. The risk for most types of cancer increases with advancing age, presumably because of increased accumulation of (oxidative) damage to DNA which increases the probability for abnormal responses to such damage. Most cancers appear to arise from abnormalities associated with genomic instability, and loss of molecular controls on cell cycle checkpoint and apoptosis. This is evident from the observations that many cancers are defective in mechanisms for cell cycle arrest and apoptosis, with prominent examples being mutations in the tumor suppressor proteins p53 and PTEN (Bruckheimer et al., 1999). In addition, many cancer cells overexpress the *anti*-apoptotic proteins Bcl-2 and the catalytic subunit of telomerase (MacCarthy-Morrogh et al., 1999; Klapper et al., 2001). Moreover, enhancement of the activity of cell survival-promoting transcription factors such as NF- $\kappa$ B is associated with many cancers (de Martin et al., 1999).

How might advances in our understanding of stem cells be used to prevent cancer or treat cancer patients? The risk of many types of cancers can be decreased by diet and lifestyle changes that apparently decrease the probability that DNA damage to stem cells. Two notable examples are a decreased calorie intake and consumption of a diet rich in *anti*-oxidants; both of these dietary manipulations have been shown to reduce levels of oxidative stress and associated DNA damage (Weindruch and Sohal, 1997). The major treatment strategy for cancer patients has been to kill the tumor cells by radiation or chemotherapy, an approach that is effective for some types of cancer, but not for others. One stem cell-based therapeutic approach that has already proven effective in treating some forms of blood cell cancers (particularly leukemias) involves bone marrow transplantation, a procedure that replaces cancerous hematopoietic cells with normal hematopoietic donor cells (Anasetti, 2000).

## 7. Nervous system disorders

Death of specific populations of neurons in the brain and spinal cord is responsible for many of the most devastating human disorders including Alzheimer's and Parkinson's diseases, stroke and traumatic brain and spinal cord injury. Although it had been generally accepted that such lost neurons are not replaceable, recent findings strongly suggest that this may not be true. It has been shown that the brain contains populations of neural progenitor cells which are particularly concentrated in the subventricular zone (most of these cells become olfactory sensory cells, but some may become cortical neurons) and the dentate gyrus of the hippocampus (Gage, 2000). The stem cells in the hippocampus can differentiate into neurons and astrocytes, although it has yet to be demonstrated that the newly generated neurons integrate into circuits.

The hippocampus is a brain structure that plays a critical role in learning and memory processes and is a site of neuronal degeneration in Alzheimer's disease, and is also vulnerable in stroke and epilepsy. Studies in animal models have shown that brain injury can induce proliferation of neural stem cells in the hippocampus. Increased production and/or survival of newly generated neurons may also occur in response to physiological demands. For example, rearing of rats and mice in enriched environments results in an increase in neurogenesis (Young et al., 1999). Similarly, maintenance of rats on a reduced calorie diet results in an increase in the survival of newly-generated hippocampal cells (Lee et al., 2000).

Transplantation studies suggests provide further evidence that neuronal progenitor cells can restore function in damaged brain regions. Transplantation of mesencephalic neural progenitor cells into the striatum of rats that had received 6-hydroxydopamine lesions of the substantia nigra differentiated into dopamine-producing neurons and ameliorated motor dysfunction in this rat model of Parkinson's disease (Nishino et al., 2000). Transplantation of mouse embryonic stem cells into the spinal cord of rats that had been subjected to traumatic spinal cord injury resulted in survival, and differentiation into neurons and glial cells, of the transplanted cells (McDonald et al., 1999). Moreover, the transplanted animals showed improved hindlimb function compared to control spinal cord-injured rats. In a model of stroke in which the middle cerebral artery of rats is transiently occluded, transplantation of embryonic neural progenitor cells can ameliorate deficits in learning and memory caused by the ischemic brain injury (Fukunaga et al., 1999) suggesting the possible application of stem cell therapy to human stroke patients. Similar evidence that transplanted neural progenitor cells can differentiate into neurons and perhaps integrate into neuronal circuits has been obtained in animals models of Huntington's disease (Armstrong et al., 2000b).

## 8. Diabetes and autoimmune diseases

There are two types of diabetes, both of which are characterized by hyperglycemia and consequent vascular complications. Type I (insulin-dependent) diabetes results from destruction of the cells that produce insulin (pancreatic  $\beta$ -cells), which may result from autoimmune attack on those cells. Type II diabetes, is characterized by increased insulin resistance and is often associated with obesity; it is a prominent cause of disability and death in industrialized societies (Kahn and Flier, 2000). The latter form of diabetes is mimicked in mice in which specific components of the insulin signaling pathway are rendered (through gene inactivation) defective (Kadowaki, 2000). Insulin-producing pancreatic islet cells can be generated from multipotent ductal stem cells under the influence of growth factors (Rosenberg, 1995), but these cells are apparently not mobilized in response to dysfunction and death of  $\beta$ -cells in patients with diabetes. An array of other age-related chronic diseases are caused by immune attack on specific tissues. Prominent among such autoimmune disorders are rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, and Crohn's disease (Van Noort and

Amor, 1998). Rheumatoid arthritis results from attack of connective tissue proteins such as collagen in joints resulting in reduced joint mobility and chronic pain. In multiple sclerosis, myelinating cells in the central nervous system (oligodendrocytes) are viewed as non-self by the immune system resulting in their death and consequent neurological dysfunction. Systemic lupus erythematosus occurs predominantly in women and may result from production of autoantibodies against several connective tissue antigens resulting in an array of symptoms that are most prominent in the skin and joints. Crohn's disease is characterized by chronic inflammation of the bowel and appears to result from autoimmune attack on gastrointestinal cells. Because bone marrow stem cells are the source of the lymphocytes and macrophages that attack the specific antigens and associated cells in autoimmune disorders, they are required for the disease process.

The therapeutic potential of stem cell therapy for patients with diabetes and several autoimmune disorders has already been demonstrated in animal studies and human patients. Allogenic bone marrow transplantation has proven effective in halting the disease process and restoring function in the following animal models: collagen-induced arthritis in mice as model for rheumatoid arthritis (Kamiya et al., 1993); experimental allergic encephalomyelitis as model for multiple sclerosis (van Gelder et al., 1993); autoimmune-prone mice as models of systemic lupus erythematosus (Levite et al., 1995). Studies of rodent and cat models of lysosomal storage disorders such as Tay-Sachs and Gaucher's diseases have shown that bone marrow transplantation can, in many cases, halt progression of the disease process or even restore function of the compromised organs. Not only are hematopoietic stem cells the source for all blood cell lineages, they are the source for immune cells that reside in many different organs including the liver (Kupfer cells) and brain (microglia).

Human patients with multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus have undergone bone marrow transplantation and, although the number of patients are low, there appears to be a clear stabilization or improvement in most of the patients (Burt et al., 1998). Beneficial effects of bone marrow transplantation have been reported in patients with lupus erythematosus, rheumatoid arthritis, multiple sclerosis and Crohn's disease (Marmont, 2000). These are striking examples of the successful use of stem cell therapy in humans.

## **9. Cardiovascular diseases**

The heart and blood vessels are major targets for the ravages of aging and age-related disease. Atherosclerosis and related vascular lesions accumulate in arteries during aging, and this process is exacerbated by a variety of genetic (mutations and polymorphisms in lipoproteins and their receptors, for example) and environmental (overeating, smoking and lack of exercise) factors. These vascular lesions are central to the cause of most cases of myocardial infarction and stroke. During development cardiac myocytes and vascular endothelial cells arise from mesoderm; their proliferation and differentiation may be controlled by a

collection of growth factors and cytokines (Eisenberg and Markwald, 1997; Choi, 1998). In the adult, there are populations of stem cells located in the heart, blood vessels and blood itself that are capable of differentiating into cardiac myocytes or vascular endothelial cells.

Experimental studies in cell culture and animal models have demonstrated the potential for various types of progenitor cells, including embryonic stem cells and hematopoietic cells, to differentiate into functional cardiac myocytes with the ability to repair damaged myocardium (Kessler and Byrne, 1999). Vascular endothelial cells are damaged during the process of atherosclerosis, and entire vessels are often destroyed as the result of a myocardial infarction or stroke. Vascular endothelial cell precursors located in the vessels or circulating in the blood can be induced to proliferate and form new blood vessels in response to vascular endothelial cell growth factor (Kalka et al., 2000). Interestingly, vascular endothelial cell growth factor may promote the 'homing' of circulating endothelial precursors to the site of an ischemic focus. This homing phenomenon has also been described in the nervous system. For example, neural stem cell in the subventricular zone can migrate to the site of a brain tumor located at a considerable distance (5–10 mm) away (Aboody et al., 2000).

#### **10. Impact of environment and diet on stem cells: implications for retardation of aging and disease**

The ability of a low calorie and vitamin-rich diet to reduce risk for cancer is perhaps the first clue that stem cells are responsive to environmental factors. While there is increasing evidence for environmental regulation of stem cells in various tissues, perhaps the most intriguing findings in this area have come from recent studies of neural stem cells in the brains of rodents. In particular, it has been shown that neural progenitor cells located in the dentate gyrus of the hippocampus are remarkably responsive to environmental factors. The hippocampus is a region of the brain that plays a critical role in learning and memory processes, and neurons in the hippocampus are particularly prone to dysfunction and degeneration in several age-related disorders including Alzheimer's disease and stroke. As in other tissues, the proliferation rate of hippocampal neural stem cells increases in response to injury. For example, numbers of Brdu-labeled cells dramatically increases following severe epileptic seizures (Parent et al., 1997) and physical trauma (Gould and Tanapat, 1997). More intriguing are responses of the neural stem cells to more subtle environmental manipulations. Three different environmental changes that have been shown to increase the numbers of newly generated neural cells in the dentate gyrus are environmental enrichment, physical exercise and dietary restriction (reduced calorie intake) (Kempermann et al., 1997; Young et al., 1999; Lee et al., 2000). In the cases of environmental enrichment and dietary restriction, it was shown that the major effect of these environmental factors is to increase the survival of the newly-generated cells. The mechanism whereby environmental enrichment and dietary restriction promote survival of newly produced neural cells

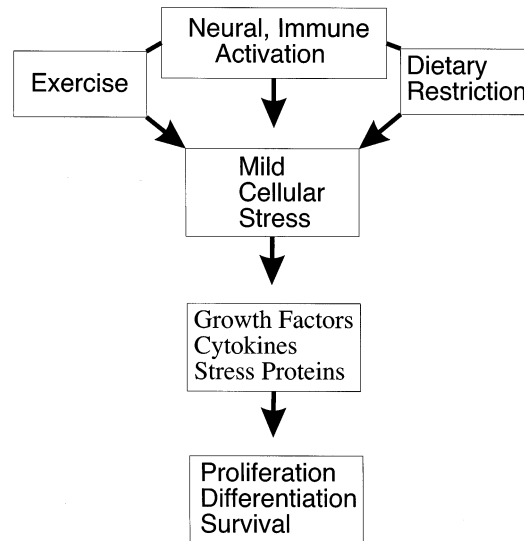


Fig. 5. Flow diagram showing the proposed mechanisms whereby environmental stimuli such as exercise, intellectual activity and dietary restriction can stimulate stem cell activity and thereby enhance the function of various tissues and increase their resistance to age-related dysfunction and deterioration.

may involve stimulation of production of neurotrophic factors such as brain-derived neurotrophic factor (Lee et al., 2000; Duan et al., 2001), and stress proteins such as HSP-70 and GRP-78 (Duan and Mattson, 1999; Lee et al., 1999; Yu and Mattson, 1999). The kinds of data just described suggest a homeopathic mechanism for environmental enhancement of stem cell plasticity during aging (Fig. 5), and identify novel strategies for promoting successful aging and preventing certain age-related diseases.

### Acknowledgements

We thank our those who performed research in our laboratories for their important contributions to the field of stem cell research. We are also grateful for the continued support of the NIH.

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