Proc. Indian natn Sci Acad. **B69** No. 2 pp 191-208 (2003)

Stem Cells, Skeletal Muscle and the Mammalian Ageing Process

MOHAN TOORA, DEBORAH MERRICK, ARCHANA RAO and JANET SMITH[1]
School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

(Received on 27 September 2002; Accepted after revision on 3 March 2003)

In adult tissues, stem cells play an important role in tissue renewal and repair, particularly in organs such as skin and blood where turnover is high, but also in less dynamic organ systems such as the reproductive system, skeletal muscle and neural tissues. Stem cell function may thus play a crucial role in the ageing process since aged tissues lose function, undergo atrophy and appear to be refractory to regenerative repair. In this review we focus on the skeletal muscle stem cell system and its role in the ageing of the mammalian skeletal musculature. We discuss recent developments in the field of stem cell biology which could have important implications for our understanding of the ageing process in all tissues. We present some recent work which may shed light on the mechanisms operating in ageing muscles which lead to atrophy. To set this work in context we also review other work on stem cell behaviour in ageing systems and we discuss how the current state of knowledge of replicative cell ageing may relate to the particular case of stem cell ageing. The purpose of understanding such a process is clear, skeletal muscle atrophy and its associated morbidities and mortality are an important cause of distress and loss of independence in the elderly. In recent years there has been a massive expansion in our understanding of stem cell biology and in its potential therapeutic uses. Stem cell replacement therapy has the potential to provide a route by which, 'rejuvenating' aged tissues becomes a reality.

For the sake of simplicity and clarity and because alternative terms (precursor, progenitor, transitory amplifying cell etc) are not used consistently within the literature and refer to different cell populations in different tissue types, in this review we have used the term 'stem cell' throughout to refer to any cell capable of self-renewal and differentiation. Where clearly defined alternative terms are available (for example in the haematopoietic stem cell system) we have used these.

Key Words: **Stem cell, Skeletal muscle ageing, Growth factor, Apoptosis, Differentiation, Proliferation, Stem cell niche**

Introduction

Adult stem cell function in tissue repair is complex. To effect repair of a tissue, a stem cell is required to undergo proliferation and differentiation at an appropriate place and time (see below). In adult tissues these processes are controlled and coordinated by stem cell specific growth factors acting on quiescent stem cells (this is often described as the stem cell niche, reviewed by Watt & Hogan (2000). Stem cells are usually absolutely dependent on specific growth factors for their survival and in their absence undergo death by apoptosis (Ishizaki et al. 1995). Thus age-associated changes in the stem cell niche (particularly in growth factor expression) could lead to impairment of stem cell function at the level of re-entry into cell cycle from the quiescent state, failure of an appropriate proliferative or differentiative response or to excess apoptosis. All of these functions are at least partially genetically regulated (for examples see review by Kenyon 2001). Furthermore, the long periods of quiescence that many adult stem cells undergo makes them vulnerable to environmental insult and damage as they age introducing a stochastic element to stem cell fate similar to that thought to operate in post-mitotic tissues (Souzou & Kirkwood 2001). Stem cell behaviour may thus contribute substantially to

[1]Corresponding Author: *Tel:* +44 (0) 1214145408; *Fax:* 1214145925; *E-mail:* J.Smith.20@bham.ac.uk

both the stochastic and genetic variation seen during the ageing of complex organisms.

A key question is whether these quiescent stem cells are subject to a process of 'quality control' and can be eliminated if defective or whether these damaged stem cells persist and contribute to the ageing process. Evidence exists for both mechanisms (see below) and this may be tissue specific.

The Ageing Process

Organ failure and tissue degeneration are a feature of ageing organisms. This process has been explained in the context of the fully differentiated, post-mitotic tissue as an inevitable result of the wear and tear caused to cellular tissue by extended use, exposure to oxidation and the accumulation of toxins (reviewed by de Grey 2002). Whilst this is undoubtedly an element of the ageing process there is now substantial evidence to implicate a further component in this process, the replicative stem cell. The two are not exclusive as long-term quiescent stem cells may also be subject to ageing-associated damage caused by oxidative stress (see above and Souzou & Kirkwood 2001). Stem cell behaviour is strongly modified by changes in growth factor and hormone availability.

Growth Factor and Hormone Changes

Both longevity and the ageing process are genetically modulated via growth factor or hormone pathways. In *C.Elegans* and Drosophila mutation of genes in the Insulin/IGF signalling pathway affect lifespan (Kenyon 2001). In mammals these genes appear to have an impact on healthspan (they contribute more significantly to the decline in health of the organism rather than its longevity) and have been shown to alter with age. In elderly humans there are age-related increases in osteoporosis and muscle atrophy as well as increased susceptibility to obesity and obesity related diseases such as type II diabetes. These changes have a strong genetic basis and have also been attributed to the decline in serum growth hormone (GH), leptin and sex hormone production with age that occurs in all mammals (Johannsson et al. 2000, Soares et al. 2000, Pescatello et al. 2000, Chong et al. 2000). Age related hormonal changes are accompanied by local and systemic changes in several growth factors. Skeletal muscle and systemic IGF-1 in particular declines with age (Grounds 2000) whereas other growth factors, such as TNF-α increase (Savine & Sonksen 2000).

Treatment of elderly people with GH, oestrogens or androgens can induce a reversal of the depletion of bone and muscle and accumulation of fat with concomittant rise in serum and local IGF-1 levels (Savine & Sonksen 2000). However, these treatments are not without side effects and in some people significantly increase the risk of certain cancers. Physical exercise can also result in increased serum IGF-1 levels and an increase in some cases in bone density and muscle mass as well as a reduction in obesity (Villareal et al. 2001). In the brain there is little evidence for generalised atrophy of neural cell types, although subsets of neuronal types decline with age (Smith et al. 1999). A number of age-related growth factor changes are reported in the mammalian brain and CNS including IGF-1, insulin-signalling pathway molecules, NGF Receptor (p75) and the ErbB-1 EGF-receptor (Smith et al. 1999, Sonntag et al. 2000, Fernandes et al. 2001, Hou et al. 2002). Grafting of NGF-expressing fibroblasts into the brains of Rhesus monkeys results in a reversal of the age-related atrophy of subcortical neurons suggesting that these processes are manipulable (Smith et al. 1999).

Replicative Senescence

To date the ageing of proliferative cells has been largely seen in the context of replicative senescence. Replicative senescence has been proposed as a mechanism by which proliferative cells progressively lose their proliferative capacity with age and thus may contribute to tissue senescence (Faragher & Kipling 1998, Campisi 2001). This is based on three observations. The first of these comes from Hayflick and Moorehead (1961) who showed that cultured human fibroblasts have a fixed average population doubling time in culture. This fixed doubling time of fibroblasts appears to be determined by their species of origin (approximately 20 in mouse and 50 in human) and has been related to longevity. Animals with longer lives often display increased resistance to replicative senescence but there are also a number of exceptions and more recent work demonstrates that fibroblast population doubling times can be manipulated in

culture (reviewed by Campisi 2001). The majority of data on replicative senescence has come from studies on fibroblasts and other non-stem cell types.

A number of recent studies using oligodendrocyte precursors, gut epithelial stem cells and skeletal muscle cells, suggest that stem cell populations may be refractory to replicative senescence and retain their ability to replicate and divide in very old tissues (Tang et al. 2001, Martin et al. 1998b, O'Shea et al. 2001). Also in any single proliferating cell population there will be cells which divide many more or many fewer times than do their neighbours before they reach senescence. This suggests that in these cells the cessation of division and the onset of senescence bears a stochastic element (suggested by the work of Sozou and Kirkwood (2001), which argues against a fixed biological clock mechanism for cell and tissue senescence.

Telomeres and Telomerase

Support for a biological clock mechanism of the ageing of proliferative cells has come from the finding that many somatic cells display telomere shortening when they divide and this relates to the onset of cell senescence. This relationship, first demonstrated for human cells, is much less clear in rodents, which have very long telomeres. (Allsopp et al. 1992, Martens et al. 2000). Both telomere length and senescence are correlated to some extent with the age of the tissue from which fibroblasts derive although exactly how this relates to ageing is controversal (Serra & Von Zglinicki 2002, Miller 2000). The progressive shortening of telomere length can be overcome by the expression of telomerase which acts to re-synthesize the telomere during the replication process. Tumour forming cells often acquire telomerase expression and this is associated with their un-regulated proliferation (Ouellete et al. 2000). Some (but not all) stem cells, early embryonic cells and germ cells express telomerase suggesting that they are not subject to a cell-division driven telomeric clock mechanism. These include Haematopoietic stem cells, oligodendrocyte precursors, embryonic stem cells and cells of the preimplantation embryo (Tang et al. 2001, Wright et al. 2001, Thomson et al. 1998, Miura et al. 2001, Lansdorp et al. 1998). Skeletal muscle satellite cells are reported not to express telomerase although other populations of skeletal muscle stem cells have not yet been tested (Shay & Wright 2002). Telomerase deficient mice show progressive intergenerational genomic instability suggesting that an important function of telomerase is the maintenance of germ line integrity (reviewed in Goytisolo & Blasco 2002).

DNA Replication Defects

It has been demonstrated that defects in genes associated with impaired DNA replication impact negatively on the ageing process. Recent work by de Boer et al. (2002) demonstrates this quite clearly in the mouse. In humans the most dramatic example is a small group of very rare 'progeria' syndromes (Werner's, Bloom's) caused by DNA helicase defects which lead to early death due to multiorgan and tissue failure reminiscent of systemic premature ageing (Martin 1978). Fibroblast cultures from these patients show reduced telomere length and more rapid senescence than normal and can be imortalised by telomerase suggesting that telomere shortening may play a role in their early senescence (Wyllie et al. 2000). Genetic instability in Werner's and Bloom's patients is also thought responsible for their increased susceptibility to tumours (Imamura et al. 2002, Wright 1999).

Many of the triggers described for senescence (genetic instability, telomere shortening, cell cycle and DNA repair defects) can also induce cell apoptosis (Campisi 2001). The relative extent to which each of these contributes to the attrition of tissues during the ageing process is still as yet undetermined and is likely to be tissue and cell type specific.

Pluripotent Adult Stem Cells and Bone Marrow Derived MAPC

A recent flurry of publications has suggested that bone marrow derived stem cells are capable of generating a much wider range of tissue types than the mesenchymal stem cell (MSC). This cell is frequently named the 'Adult stem cell' and much attention has focussed on the 'side-population' (sp) cell isolated using a FACS technique. The evidence comes from a number of grafting experiments carried out mainly using FACS sorted haematopoietic cells. These include brain (Brazelton

et al. 2000, Mezey et al. 2000), skeletal muscle (Bittner et al. 1999, Ferrari et al. 1998, Gussoni et al. 1999), cardiac muscle (Kocher et al. 2001, Orlic et al. 2001) and liver cell (Alison et al. 2000, Lagasse et al. 2000). A recent paper by Krause et al. (2001) suggests that a single donor HSC (achieved by limiting dilution) is capable of regenerating the hematopoietic system, but also contributing to the lung, gut, and skin of the recipient mouse. These cells were sorted on the basis of their expression of CD34, a cell surface marker that is also expressed by skeletal muscle and bone marrow stem cells (Beauchamp et al. 2000). More recently Jiang et al. (2002) have described the isolation of multipotent adult progenitor cells (MAPC) which can be cultured *in vitro* at low cell density. They have demonstrated by limited dilution (see below) that these cells can contribute robustly to a large number of different lineages when injected into mouse blastocysts.

The tissue types reported to be generated by all of these cell types encompass all three germ layers. To reconcile these data with our existing understanding of lineage restriction rules this observation requires the existence of a circulating, embryonic-like pluripotent (or totipotent) adult stem cell. An alternative explanation has been that bone marrow derived cells are able to 'reprogram' or 'transdifferentiate' when taken out of their normal environment.

In addition to the bone marrow many tissues have been reported to contain pluripotent adult stem cells including regions of the nervous system, skeletal muscle, liver, pancreas, epithelium, cornea and retina. Recent evidence however suggests that these cells may originate from contaminating blood cells or by some other means other than transdifferentiation such as cell-cell fusion (Terada et al. 2002, Ying et al. 2002 for details see the neural stem cell (NSC) section below). Cell-cell fusion is clearly a plausible and likely explanation for the data of Clarke et al. (2000) and other experiments relying on the co-fusion of putative adult stem cells with a 'permissive' stem cell such as a skeletal muscle cell or embryonics stem (ES) cell. It is, however, unlikely to account for all of the pluripotency and plasticity demonstrated for bone marrow derived and peripheral stem cells. NSC:- ES fusions arose as rare events (approximately $1/10^5$ cells) under extensive cell selection resulting in pluripotent tetraploid cells. To date extensive karyotyping has not been performed on cells derived from the haematopoietic system thought to generate neural, muscle and other cell types *in vivo*. Some of the reports of stem cell plasticity (for example Gussoni et al. 1999) occur at very low frequency in relation to the number of cells injected and therefore could be accounted for by spontaneous fusion event (s) *in vivo*. Experiments such as those of Krause et al. (2001) and Jiang et al. (2002) however, which show robust, extensive and reproducible contribution of bone marrow derived stem cells to non-haematopoeitic tissues at single cell dilutions are less easily explained in this way and most likely demonstrate the pluripotency of the injected cells.

Whilst the phenomenon of 'transdifferentiation' itself remains unproven, it is clear that there is likely to be a good deal more plasticity in the adult stem cell system than was previously understood. To date all of the work on cross-lineage plasticity relies on the transfer of stem cells out of their normal micro-environment or niche where they appear to respond by differentiating inappropriately. This observation may be of significance to the ageing process where there is evidence that growth factors important to the stem cell niche in a number of tissues alter with age. Such a breakdown of the stem cell microenvironment may lead to abnormal stem cell behaviours that could have a profound effect on the way that we age.

Stem Cells in the Blood System

There is some confusion regarding terminology in the stem cell biology field. The terms 'stem cell', 'progenitor' cell and 'precursor' cell are often used interchangeably, particularly in adult systems. In the haematopoietic system, where these terms were first coined, they have a precise meaning which maps to their position in the blood stem cell differentiation hierarchy and relates to both their proliferative capacity and their potency, generally the name of these cells relates to the cytokines (growth factors) that regulate their differentiation and are required for their survival (Dexter & Spooncer 1987). They follow a strict hierarchy with

the rare, pluripotent Haematopoietic stem cell (HSC) at the top. Derived from the HSC are the more abundant stem cells of the lymphoid and myeloid lineages and from these derive a number of lineage-restricted progenitors and precursor cells (Dexter & Spooncer 1987, see figure 1). The Haematopoietic system is the only system so clearly defined.

HSC are non-adherent cells, which derive from the bone marrow and are present in low numbers in circulating blood in adults and children. HSC enrichment methods based on FACS sorting have existed for a number of years and human HSC are currently the only well-defined stem cells that are routinely in use therapeutically, where they are used to regenerate the haematopoietic system following radiation therapy in childhood leukemia. The success of HSC therapy is due in part to a good understanding of the haematopoietic stem cell system (See figure 1) but also because HSC can be delivered systemically by transfusion into the blood system, where they normally reside. The potential of bone marrow cells to repopulate the blood system of an irradiated host was first demonstrated in mice almost 50 years ago and it is still the most robust demonstration of stem cell regenerative potential in an adult system (Ford et al 1956, Till & McCulloch 1961).

HSC however can not be clonally derived in culture as stem cell lines. Thus the definition of their stem cell status is derived from *in vitro* clonogenic assays and from the demonstration that limiting dilutions (less than 10 cells) of HSC (but not other bone marrow cells) have the capacity to regenerate the haematopoietic system of an irradiated host. A number of markers define HSC and include CD45, Flk-2, c-kit and Sca-1 (Christensen & Weissman 2001). However, FACS sorting is an enrichment and not a purification procedure and HSC are not a single stem cell population, at least two cell types sort to the HSC compartment, long-term HSC and short-term HSC (Spangrude et al. 1988, Christensen & Weissman 2001). It is also likely that other cell types may be present with very low frequency. These may include mesenchymal stem cells (MSC) which are discussed below (Majumdar et al. 1998) and the MAPC discussed in the previous section (Jiang et al. 2002). This potential for contamination should be born in mind in the light of recent data suggesting that HSC may have the ability to generate cell lineages outside of the haematopoietic system. The

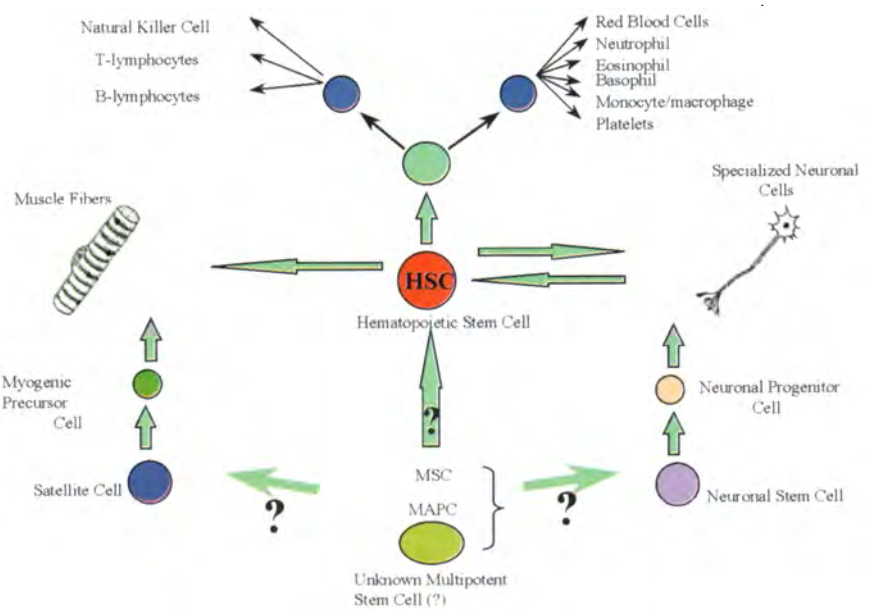

Figure 1 Relationship between different mammalian stem cell types. Cells derived from bone marrow generate HSC which produce cells of the blood and immune system, MSC from which can be derived a number of mesenchymal cell types (cartilage, bone, fibroblast and probably skeletal muscle) and provide stromal support cells for HSC. Tissue specific stem cells repair and renew specific tissues and are produced locally. Pluripotent cells derived from the bone marrow may also contribute to these stem cell populations and include Multipotent adult progenitor cells (MAPC). The precise interrelationships between these different stem cell populations remain to be elucidated (see text).

suggestion comes from the intriguing observation that a number of human recipients of (opposite sex) donor HSC appear to have non-haematopoietic cell types, for example hepatocytes, originating from the donor source (see Alison et al. 2000). It has been suggested that this occurs by a process of 'transdifferentiation'. An alternative, but still novel explanation is that these donor HSC contained small numbers of a pluripotent bone marrow derived cell capable of generating lineages outside the haematopoietic system, the 'Adult Stem Cell'.

Mesenchymal Stem Cells

There are different degrees of plasticity described in the literature for adult stem cells (figure 1). Some such as the mesenchymal stem cell are long established and follow lineage restriction rules laid down in the embryo. Others such as the transition of blood to neural, epithelial or muscle cells require a more novel explanation and are currently the subject of hot debate (see below).

For some years there has been evidence from a number of different sources that suggest the presence of a pluripotent stem cell capable of generating a number of different tissue types of mesodermal origin. This is the mesenchymal stem cell (MSC). The origin and function of MSC is obscure although it is thought to reside in the bone marrow. Fetal mesenchymal cells exisit which form the precursors for a wide range of tissues, most notably tendon, cartilage, bone and skeletal muscle. In the embryo mesenchymal cells (hemangioblast) also form blood and contribute to parenchymal organs such as the liver, lung and kidney. It could be therefore that MSC are an undifferentiated remnant of these fetal cells although they may have a separate adult origin (Minasi et al. 2002 reviewed in Eichmann et al. 2002).

MSC can be derived from bone marrow, where they are best described, but also from a number of other mesodermal tissues including bone and skeletal muscle. In the adult MSC have been shown to generate mesenchymal cell types such as muscle, fat, bone, cartilage, tendon and other connective tissues (Majumdar et al. 1998, Jaiswal et al. 2000, Muraglia et al. 2000). MSC appear to arise in the bone marrow but are probably distinct from the non-adherent haematopoietic cell compartment. The bone marrow contains a number of non-haematopoietic, adherent but mobile cell types including reticular endothelial cells, macrophages, osteoblasts, adipocytes and fibroblasts as well as stromal cells (Beresfords 1989). These cells secrete extracellular matrix and growth factors and are thought to provide the bone marrow micro-environment. Thus the primary function of the MSC may be to maintain the stem cell niche of the HSC and other precursor cells in the bone marrow (Majaumdar et al. 1998). A number of lines of evidence suggest that the MSC is a pluripotent stem cell which can generate some if not all of these cell types (Haynsworth et al. 1992, Johnstone et al. 1996, Pittenger et al. 1999, Tordjman et al. 1999). MSC have been injected into tissue specific sites where they differentiate along the lineage appropriate to their transplantation site eg bone, skeletal muscle, tendon (see for example Awad et al. 2000).

Tissue Specific Stem Cells

There are not always direct equivalents to the HSC hierarchy in other stem cell systems and in many tissues far less is known about the factors that regulate stem cell regeneration. In this review we have taken a functional view and suggest that the term 'Stem cell' can be applied to any single cell capable both of self-renewal and differentiation resulting in ordered tissue generation or repair. To formally demonstrate stem cell status it is necessary to demonstrate *in vivo* both proliferative capacity and the full range of functional differentiated derivatives from a single stem cell or a clonal (derived from one cell) stem cell population (Smith & Schofield 1997). This *in vivo* definition can be applied to some extent to HSC, skeletal muscle stem cells (SMSc), gut stem cells and neural stem cells (NSC) and dermal stem cells. All of these stem cell types have been studied in an ageing context and are implicated in the ageing process of that tissue.

Skeletal Muscle Stem Cells

Skeletal muscle contains a population of undifferentiated stem cells (SMSc) that have the capacity to replace damaged muscle and accommodate post-natal growth (Smith & Schofield 1994, Schultz & Lipton 1982, Smith & Schofield 1997). The ability of a pool of undifferentiated SMSc to repopulate skeletal muscles following irradiation

induced damaged or in immune compromised mice was first demonstrated by Partridge et al. (1989). However the rate of incorporation of these mass cultures was very low (less than 1%). Subsequently it was found that the majority of transplanted cells died within the first 48 hours of transferral (Beauchamp et al. 1999). These data raise the possibility of their being a small number of muscle stem cells within these mass muscle cell (myoblast) cultures. These skeletal muscle stem cells (SMSc) can be cultured *in vitro* and clonally derived (figure 2, Smith & Schofield 1994). A clonally derived, diploid SMSc line (PD50A) is able to reliably and extensively (up to 80% of donor positive fibres) repopulate injected host muscles. The persistence and self-renewal capacity of PD50A SMSc was demonstrated by the re-isolation from host muscles of label positive (b-galactosidase) proliferative cells 12 months after their injection into hosts. These SMSc could be cloned and expandable cell lines were established. Mono-nucleated, b-galactosidase positive cells were identified in muscle satellite cell positions in PD50A recipient muscles suggesting that SMSc may be equivalent to the stem cell component of the satellite cell population (figure 3, Smith & Schofield 1997).

Satellite cells occupy a region between the sarcolemma and basal lamina of the muscle fibres. A large body of work suggests that they contain the skeletal muscle stem cell population, capable of extensive skeletal muscle fibre renewal (Gibson & Schultz 1983). As such they represent an identifiable

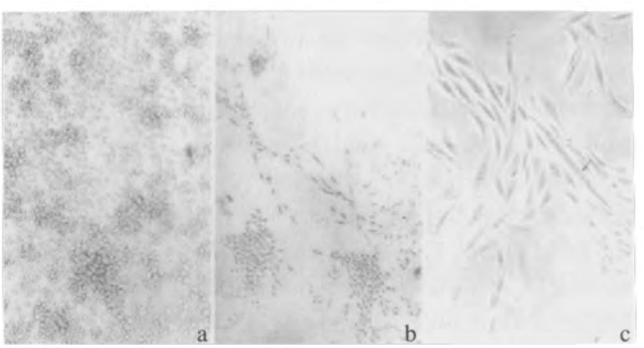

Figure 2 (a) outgrowth of skeletal muscle from a 4-week old mdx mouse, 3 days after explant, showing aggregations of motile, monomorphic, spherical cells growing out from the explanted tissue; (b) "Spindle" morphology of dystrophic myoblasts cultured at low density (passage 3). (c) Spontaneous fusion of dystrophic myoblasts in high serum culture. Arrow indicates a multinucleate myotube (Smith & Schofield 1997). *(Reproduced by permission from the publisher: Academic Press)*

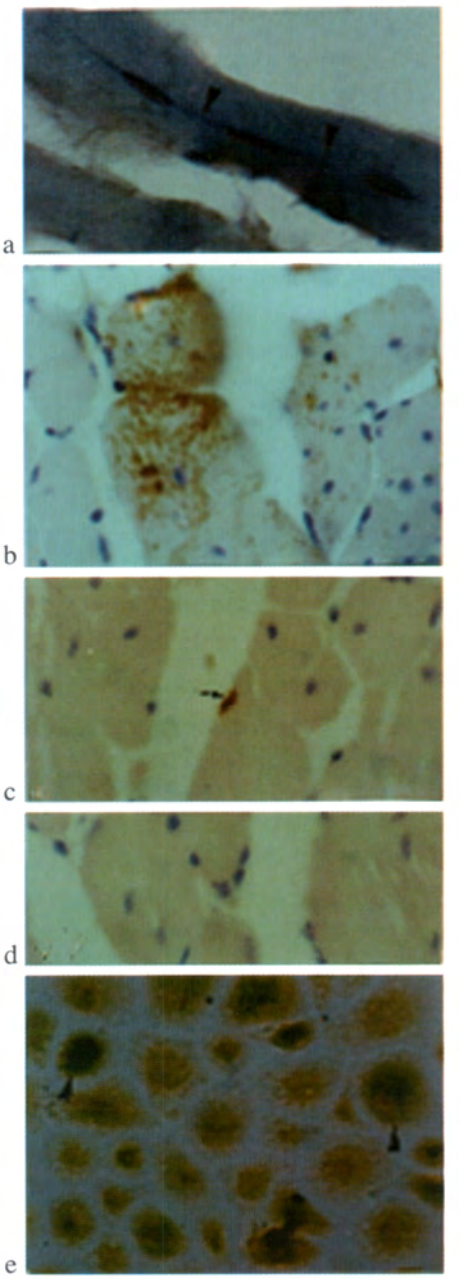

Figure 3 Injection of SMS cell clone PD50A (b-galactosidase expressing) into host mice (A) 3 months and (B-D) 14 months post-injection of 2, 000 PD50A cells into mouse Tibialis Anterior muscle. (A) shows three recently fused (centrally located nuclei) b-gal positive cells (blue histochemistry) in muscle fibre (longitudinal section); (B) Extensive contribution of b-gal positive cells (brown – detected by anti-b-gal antibody) in muscle fibres (transverse section); (C) b-gal positive satellite cell (Brown staining – antibody to b-gal); (D) 2nd antibody control (no staining); (E) b-Galactosidase positive cells (Blue histochemistry) proliferate in culture when isolated from injected host muscles 12 months post-injection (Smith and Schofield, 1997). Proliferation is demonstrated by the isolation of expandable, stably b-gal expressing clonal SMSc lines from explants of host injected muscles.

stem cell population with a defined function (muscle fibre generation). Upon physical stimuli (eg injury, fibre degeneration) cells undergo replication, migration, and finally differentiation into new myofibres (Schultz & Lipton 1982). The local and remote molecular signals thought to orchestrate these processes are still incompletely understood although a number of growth factors have been shown to regulate skeletal muscle differentiation, proliferation and survival. Of these the most important are the Insulin-like growth factor family (IGF) which promote skeletal muscle stem cell differentiation, survival (IGF-2, Smith et al. 1995, Stewart & Rotwein 1996) and proliferation (IGF-1). IGF-2 plays an important role in embryonic muscle formation (Merrick et al. 2002). FGF growth factors promote muscle cell proliferation and also induce skeletal muscle stem cell apoptosis (FGF-1 and FGF-2, Woods et al. 2000). Other growth factors that act on skeletal muscle stem cells included TGF-β, PDGF, HGF and NGF (reviewed in Smith & Schofield 1994, Zorzano et al. 2003). More recently it has been suggested that these cells may have the capacity to differentiate into other mesodermal origin cell types including osteogenic (bone) and adipogenic (fat) lineages as well as the myogenic (muscle) lineage (Asakura et al. 2001, Rao & Smith work presented at Keystone Stem cell meeting, March 2001).

It is been frequently assumed in the literature that SMSc are equivalent to skeletal muscle satellite cells. It seems probable however that only a subset of the satellite cell population are truly pluripotent skeletal muscle stem cells, whilst others have a more restricted proliferative or differentiative role. Since the relationship between the satellite cell and skeletal muscle stem cell has not yet been formally established and myoblast is a non-specific term that includes any dividing skeletal muscle cell, we have used the term SMSc to distinguish those cells in skeletal muscle capable of providing robust regenerative capacity.

Recent work suggests that the satellite cell population, like other stem cell populations, is heterogeneous and may contain a range of stem and precursor cell types. This is based largely on data demonstrating the differential expression of a number of satellite cell markers including CD34, MyoD, Myf5, pax7, m-cadherin, HGF and desmin (Grounds et al. 1992, Cornelison & Wold 1997, Beauchamp et al. 2000, Seale et al. 2000). But is supported by data suggesting that cells derived from fast or slow muscle fibre types generally produce fibres equivalent to those that they are derived from (Feldman & Stockdale 1991). In humans this may not be so since cultured myotubes co-express both fast and slow myosins when isolated from either slow or fast muscle types (Bonavaud et al. 2001).

Pax7 is a particularly important marker for satellite cells. In adult mice Pax7 appears to be expressed exclusively in these cells (based on the morphology of Pax7 cells) and in proliferating cells derived from skeletal muscle. Although skeletal muscle fibres can form normally during myogenesis in the absence of Pax7, EM studies on the Pax7 knockout mouse suggest a loss of satellite cells in Pax7 $^{(-/-)}$ post-natal muscles together with significant reduction of muscle mass (Seale et al. 2000). Skeletal muscle stem cells can still be isolated from these mice however suggesting that there are other sources of stem cell capable of generating the skeletal muscle lineage. Pax7 $^{(-/-)}$ mice do not thrive and so have not yet been studied in an ageing context (Seale et al. 2000).

It was recently reported that cells with both skeletal muscle and haematopoietic cell lineage capacity could be isolated from skeletal muscle. These studies were done using FACS sorted cultured cells derived from skeletal muscle and thus were not clonally derived, although they were subject to limiting dilution (see previous section). Cells were FACS sorted on the basis of two cell surface markers (Sca-1 and c-kit) as well as by the phenomenon of Hoechst 33342 dye exclusion (sp cells, see above). The collagenase digestion method used to generate these cells also does not preclude contamination of muscle cells with blood derived or connective tissue derived cell types (Jackson et al. 1999). A more recent paper acknowledges this fact and demonstrates that the most likely explanation for this observation is that two cell populations were present. When cells were FACS sorted on the basis of a third cell surface marker (CD45 specific in mouse for HSC) it was found that CD45+ cells had haematopoietic potential and the CD45- cells myogenic potential (McKinney-Freeman et al. 2002).

Neural Stem Cells

Neural stem cells (NSC) can be derived from embryonic, neonatal and adult rodent and human

brains and can be maintained for long periods under the influence of EGF and FGF-2 growth factors in culture as suspension cell aggregates known as neurospheres (Gage 2000, Svendsen et al. 1998). Typically, neurospheres contain a mixture of undifferentiated and differentiated cell types and can be induced to differentiate into functional neurons or astroglia by the addition of serum and plating onto a polylysine or laminin substrate (Caldwell et al. 2001, Song et al. 2002). It was once believed that neurones of the adult mammalian brain and spinal cord were incapable of replication. This accepted limitation of the central nervous system (CNS) has been proven not to be the case as it is now established that there are two discrete regions of the brain that can generate new neurons. These are the ventricular subependyma and the subgranular layer of the Dentate Gyrus. Precursors in the subependyma show many characteristics of neural stem cells (NSC) *in vitro* (Morshead et al. 1994, Gritti et al. 1996, Reynolds & Weiss 1992) including self-renewal capacity into senescence (Tropepe et al. 1997). Johansson et al. (1999) show ependymal cells to have NSC properties, but there may be a number of regionally specific subpopulations of NSC with different potencies within this population of cells (Gritti et al. 2002). In the Dentate Gyrus neural cells proliferate, migrate, and then differentiate into hippocampal granule cells (Altman & Das 1965, 1966, Cameron et al. 1993, Kuhn et al. 1996).

As with skeletal muscle, recent reports have suggested that genetically labelled, clonally derived NSC may be able to differentiate into non-neuronal cell types. Contribution of NSC to haematopoietic lineages was achieved by the transfer of NSC into sub-lethally irradiated host mice (Bjornson et al. 1999). The rate of conversion of NSC to haematopoietic cell types was very low (consistent with a rare fusion event) and the result is therefore controversial. More recently Morshead et al. (2002) transplanted over 128 x 10^6 neurosphere cells in to 128 host animals but saw no contribution to the hematopoietic system suggesting that extended passaging of NSC in the work of Bjornson et al. (1999) may have produced genetic or epigenetic alterations resulting in the re-programming of cultured NSC.

NSC co-cultured with pluripotent ES cells prior to injection into mouse blastocyst embryos or chick embryos are reported to generate chimaeric embryos with extensive NSC derived contribution. *In vitro* these cells differentiated to myogenic and other cell lineages (Clarke et al. 2000). The rationale behind the co-culture of NSC with ES cells is that the latter, being pluripotent, may produce instructive signals which could induce NSC to differentiate pluripotently. A more pragmatic explanation was demonstrated recently by two papers in which ES cells were co-cultured with GFP-labelled bone marrow cells (Terada et al. 2002) or with GFP-labelled NSC (Ying et al. 2002) and subject to antibiotic selection. The resulting pluripotent cells were found to be tetraploid hybrids of ES and adult stem cells.

Dermal Stem Cells

Skin is continually replacing its cells. To allow the replenishment of cells, a renewing tissue contains a stem cell population that provides a source of differentiating cells (Hall & Watt 1989). Follicle (keratinocyte) stem cells have been identified to occupy positions along the follicle outer root sheath (Rochart et al. 1994). Follicular stem cells have the ability to make hair follicles and also contribute to the skin epidermis (Taylor et al. 2000). Stem cells derived from the follicle also generate sebaceous gland cells. It is thought that all three lineages may derive from a single multipotent follicle stem cell residing, in adult mice, in the upper region of the vibrissal root sheath (Oshima et al. 2001). Keratinocyte stem cells are thought to give rise to a population of cells with a limited self-renewal capacity called 'transient amplifying cells'. It is these cells which differentiate to generate the mature differentiated keratinocytes which undergo terminal differentiation to form the outer skin layer. Stem cells in skin can be identified in the follicle by their slow cell cycling capacity detected by their retention of a BrdU pulse label (Cotsarelis et al.1990) and other specific biological markers (Brakebusch et al. 2000, Michel et al. 1996, Young et al. 2001). The follicle therefore represents a number of controlled adult stem cell compartments in which stem cell proliferation and differentiation are regulated by their position or niche (Watt & Hogan 2000). Cell lineage choice in skin follicle stem cells is under the regulation of the Tcf/Lef complex and mediated by wnt and β-catenin signalling (Merrill et al. 2000).

Recently Toma et al. (2001) isolated cells from juvenile and adult rodent skin which appear to be pluripotent at least *in vitro*. These cells, termed skin-derived precursors (SKPs), are predominantly positive for a neural precursor cell marker nestin. When plated onto an adherent substrate SKPs are capable of generating mixed cultures of neural and mesodermal progeny including neurons, glia, smooth muscle cells and adipocytes although with relatively low frequency (>1%-25% depending on cell type). Surprisingly dermal and follicle derivatives were not reported although dissection experiments and cell markers suggest that the most likely origin of these cells is the dermis rather than neural crest, neuronal or bone marrow cells (MSC). SKPs are cultured as non-adherent aggregates reminiscent of neurospheres. Cloning of SKP's was achieved by disaggregation of SKP spheres and single cell cloning using conditioned medium to generate clonally derived SKP spheres. Cloned spheres were pluripotent *in vitro*, although the proportion of cell types generated from these clones under differentiation permissive conditions was not reported. Similar findings have also been reported for human skin cells which can differentiate into neural, skeletal muscle, cartilage, bone and adipocyte cells (Young et al. 2001). It is not yet clear how these cells relate to follicular stem cells, it would be interesting to determine whether these cells were able to behave as follicle stem cells if returned to their appropriate (follicle) niche.

Gut Stem Cells

Another stem cell system, which is regionally defined and appears to be self-contained is that of the gut. Stem cells in the gut reside near the base of the crypts of Lieberkuhn (intestinal crypts) are few in number and infrequently dividing. They sit on the Paneth cells at the very base of the crypt. These are differentiated cells that derive from the gut stem cell. Gut stem cells give rise to a larger number of 'clongenic' stem cells which take up positions further up the crypt and which have a function roughly equivalent to the transiently amplifying cells of the dermis. It is the clonogenic cells which normally regenerate the intestinal crypt (Potten & Loeffler 1990, Potten et al. 1997, reviewed in Marshman et al. 2002). Thus like skeletal muscle satellite cells and follicular stem cells, gut stem cells are defined positionally suggesting a precisely regulated niche environment. This system can be investigated intact using an organotypic microcolony culture method for intestinal crypts which enables the *in vitro* analysis of the intact system (Withers et al. 1970)

Stem Cells and Ageing

There is a significant random element to the process of ageing that is evident when genetic background and environment are controlled. This suggests that both rate of ageing and the ageing process itself may be determined by the cummulative effect of a number of factors or by a mechanism that enables stochastic accumulation of error (disposable soma theory). Oxidative stress and mutation accumulation may both play a role and have been extensively studied although largely in post-mitotic and differentiated tissues (Sozou & Kirkwood 2001). Here we discuss the evidence that stem cell behaviours also play an important role in this process.

Replicative senescence is only one of several methods by which a stem cell can be regulated and may not be the most important. Stem cells in a majority of adult tissues (Skeletal muscle, liver, neural tissues) remain non-dividing (quiescent) for long periods of time and many (eg haematopoietic and neural stem cells) express telomerase, suggesting that the impact of telomere shortening could be minimal (Miura et al. 2001, Lansdorp et al. 1998). A key feature of adult stem cell function is the capability for regulated expansion and appropriate differentiation in response to the need for tissue growth or repair. Defects in either of these could lead to regenerative failure. These impairments could arise in ageing tissues by one of two mechanisms (not mutually exclusive). Many adult stem cells are maintained in a quiescent state for long periods of time and thus may become intrinsically damaged. As tissues and organisms age they exhibit substantial systemic hormonal and growth factor changes which probably lead to local changes in the tissue 'environment' (niche) in which stem cells normally reside (see below for references).

Stem Cell Niche and Apoptosis

Stem cell behaviour is largely regulated by niche and particularly by local growth factor action (Novak et al. 2000, Mills & Gordon 2001, Nishimura et al. 2002, Shinohara et al. 2001, Smith & Schofield 1997).

Changes in niche environment are thus likely to extrinsically induce the abnormal behaviour of a stem cell whether or not it is damaged (O'Shea et al. 2001).

In the majority of adult and embryonic stem cell systems there is thought to be an absolute requirement for specific survival factors, in the absence of which a stem cell will die by apoptosis or undergo differentiation (Ishizaki et al. 1995, Woods et al. 2000). Thus a significant factor in the regulation of some stem cell behaviours (eg gut, skeletal muscle) appears to be elimination by apoptosis (Smith et al. 1995, Martin et al. 1998a, Zackenhaus et al. 1996). In the gut apoptosis is thought to be an important quality control mechanism by which, gut stem cell integrity is maintained (Potten & Loeffler 1990). The underlying mechanism for this is not known but is likely to be related to cell damage. In skeletal muscles stem cell apoptosis is elevated in skeletal muscular dystrophy and when cell cycle regulation is perturbed (Zackenhaus et al. 1996, Smith et al. 1995, Baghdiguian et al. 1999). This can be reversed by the growth factor IGF-2 (Smith et al. 1995, Smith et al. 2000).

There is to date only one published systematic study of stem cell apoptosis in aged tissues. This is the work of Martin et al. (1998a, 1998b) in the mouse gut stem cell system. Whilst there is no age-related change in levels of apoptosis in the 'actual' and 'clonogenic' stem cell populations (see below for a description of the gut stem cell system) their response to environmental insult (low level radiation) is altered. Higher numbers of stem cells undergoing apoptosis were found in old guts compared to those of younger animals. The authors suggest that older stem cells were altered and perhaps had accumulated more damage, thus making them more susceptible to apoptosis and less able to sustain environmental damage (Martin et al. 1998a). These stem cells were shown not to be senescent as they were able to regenerate new crypts. However there was an age difference as the regenerated crypts were smaller and fewer despite the clonogenic response being greater in the old mice (Martin et al. 1998b). We observed an age-associated increase in Ki67 staining in skeletal muscle cells (identified as myf-5 positive, immunostaining not shown) which suggests that proliferative index increases rather than declines with age (figure 4). Apoptosis may play an important role in determining whether or not this increased proliferation results in muscle growth. In normal young adults the level of apoptosis in muscles is proportionately much lower than that seen in normal adult muscles (14 months) suggesting that the balance between the two may contribute to regulation of muscle growth during maturation (figure 4).

Stem Cell Depletion and Differentiation

It has been proposed that senile atrophy in skeletal muscle is caused by satellite cell attrition due to senescence and a resultant loss in the proliferative capacity of these cells. A number of studies in the late 70's and early 80's investigated whether or not skeletal muscle satellite cells were indeed depleted with age. These studies showed that although during the post-natal developmental stage (up to 6 months) the relative proportion of satellite cells to myofibre

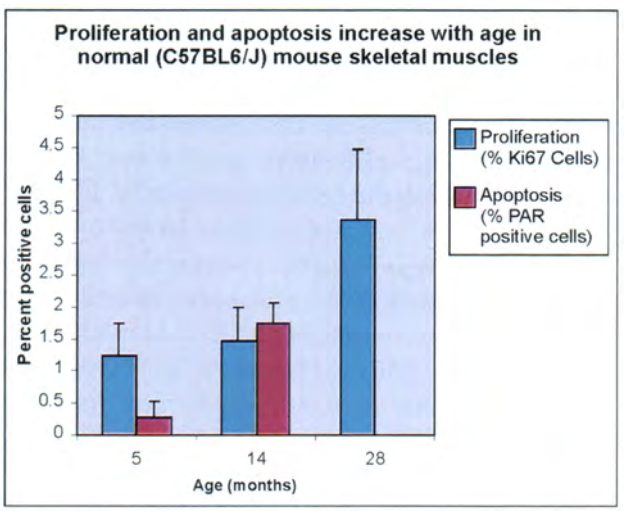

Figure 4 Proliferation index (Ki67 positive cells) was determined in the hindlimb muscles of normal (C57BL6/J) mice at three different time points representing young adult (5 months), adult (14-15 months) and aged adult (28 months). Both proliferation and apoptosis substantially increase during adulthood and this was statistically significant (t-test) at all age points. For each time point a minimum of 5 mid-point sections were counted using a fixed area grid system (2000-3000 nuclei counted per section). Apoptotic index (PAR positive cells) in adult muscles is proportionately increased compared to young adult muscles consistent with the slowing of muscle growth.

Hindlimb skeletal muscles were fixed in 4% Paraformaldehyde in phosphate buffered saline and embedded in paraffin wax and sectioned in transverse orientation (For protocols see Smith et al. 1995 and Westbury et al. 2001). To identify actively proliferating cells, a rabbit polyclonal Ki67 antibody (NCL-Ki67p from Novocastra Labs., UK) was used at a titre of (1/8000) and visualised using a streptavidin/TSA Biotin protocol (protocol according to manufacturers instructions and Merrick et al. 2002). To identify apoptotic cells a mouse monoclonal antibody to poly (ADP-Ribose) known as PAR (10H) antibody (a gift from Dr Alex Burkle, University of Newcastle) was used at a titre of 1/300 and visualised as for Ki67.

nuclei declines that the absolute number of satellite cells in a majority of muscles actually increases substantially. The *apparent* decline in satellite cell number being due to the formation of new myofibres. They also demonstrated substantial differences between the absolute number and proportion of muscle satellite cells present in different muscle fibre types (Gibson & Schultz 1983, Schultz & Lipton 1982). In mice the proportion of satellite to myofibre nuclei (determined by electron microscopy) subsequently remains constant up to at least 24 months of age in the majority of muscle types (Gibson & Schultz 1983).

These data may inform the ageing process in skeletal muscle where, there is variation in the rate at which different muscle fibre types degenerate and are replaced (Lexell et al. 1983, Monemi et al. 1999). The available information on this to date is however sparse. The presence of large numbers of satellite cells in aged and atrophying skeletal muscles raises the possiblity that these stem cells are either deficient in their ability to proliferate or can no longer initiate an appropriate differentiative response. Explant cultures of old muscles are less likely to grow extensively suggesting that there may be some impairment of proliferative capacity in satellite cells derived from old muscles (Di Donna et al. 2000, O'Shea et al. 2001). However we have also demonstrated that SMSc cultures derived from very old muscles (27 months) can be highly and extensively proliferative. We have also observed however that SMSc in such cultures are more likely to differentiate into adipocytes and connective tissue derivatives than are SMSc from young muscles suggesting that the differentiative response of these cells is impaired (O'Shea et al. 2001, A Rao & J Smith, unpublished data). Ageing in skeletal muscle is accompanied by the increased expression of a number of factors associated with adipogenic differentiation including C/EBP and PPARγ (O'Shea et al. 2001, Taylor-Jones et al. 2002, C Johnstone, M Rooney & J Smith unpublished). Similar work published recently adds to these observations and suggests that the pRb family of cell cycle regulators may play a role in this process (Guan et al. 2002).

The Effect of Stem cells on Aging

The nematode worm, *C.elegans* and the fruit fly (*D. Melanogaster*) have been widely used to uncover genetic pathways that play a role in the ageing process. With the exception of the germ line precursor cells, the adult forms of these organisms do not have stem cells. Recent work by Arantes-Oliveira et al. (2002) suggests that in C.Elegans these proliferating germ (stem) cells may influence longevity via a steroid hormone mediated mechanism. When *C. Elegans* germ cells are ablated, life-span increases. Whilst these experiments represent one of the first direct effects of stem cell manipulation on longevity, they may be a special case as reproduction and sterility have long been known to be associated with a lifespan cost. This has been best studied in the fly where the relationship between the two is complex (see Carey et al. 2002). There are currently no data on the effect of stem cell ablation on mammalian ageing.

Conclusion

We conclude that, although there is still much to be learned about stem cell ageing and its impact on the ageing organism, evidence exists that stem cells derived from very different tissue systems behave abnormally when they become old. These changes in behaviour include a possible increase in apoptotic and proliferative response, senescence and inappropriate differentiation. A contributory factor to this may be an intrinsic effect of ageing of the stem cell itself. However, it is also clear that an essential component of normal stem cell function is the integrity of its 'niche' environment. A key component of the 'niche' being the controlled availability or absence of specific hormone and growth factors. There is now a good deal of evidence, which shows dramatic changes in the bio-availabilty of these factors in tissues as they age. This is a particularly important avenue to pursue in the ageing field in the light of recent findings regarding the extensive plasticity of some adult stem cells when transferred into 'inappropriate' or 'unusual' niche environments. The process of ageing appears to induce such changes in local microenvironment and in doing so may thus create precisely the 'right' kind of environment to generate the inappropriate stem cell differentiation that has been described in recent experimental conditions. Similarly, should extensive adult stem cell plasticity be shown to be a normal function of these cells, they are then likely to be exquisitely sensitive to the changes in niche environment brought about by ageing.

References

Alison M R, Poulsom R, Jeffery R, Dhillon A P, Quaglia A, Jacob J, Novelli M, Prentice G, Williamson J and Wright N A 2000 Hepatocytes from non-hepatic stem cells; *Nature* **406** 257

Allsopp R C, Vazir H, Patterson C, Goldstein S, Younglai E V, Futcher A B, Greider C W and Harley C B 1992 Teleomere length predicts replicative capacity of human fibroblasts; *Proc. Natl. Acad. Sci (USA)* **89** 10114-10118

Altman J and Das G D 1965 Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats; *J. Comp. Neurology* **124** 319-335

_____ and _____ 1966 Autoradiographic and histological studies of postnatal neurogenesis. A longitudinal investigation of the kinetics, migration and transformation of cells incorporating tritiated thymidine in neonate rats, with special reference to postnatal neurogenesis in some brain regions; *J. Comp. Neurol.* **127** 337-390

Arantes-Oliveira N, Apfeld J, Dillin A and Kenyon C 2002 Regulation of life-span by germ-line stem cells in Caenorhabditis elegans; *Science* **295** 502-505

Asakura A, Komaki M and Rudnicki M 2001 Muscle satellite cells are multipotential stem cells that exhinit myogenic, osteogenic and adipogenic differentiation; *Differentiation* **68** 245-253

Awad H A, Butler D L and Harris M T et al 2000 *In vitro* characterization of mesenchymal stem cell-seeded collagen scaffolds for tendon repair: efffects of initial seeding density on contraction kinetics; *J. Biomed. Mater. Res.* **51** 233-240

Baghdiguian S, Martin M, Richard I, Pons F, Astier C, Bourg N, Hay RT, Chemaly R, Halaby G, Loiselet J, Anderson L V B, de Munain A L, Fardeau M, Mangeat P, Beckmann J S and Lefranc G 1999 Calpain 3 deficiency is associated with myonuclear apoptosis and profound perturbation of the I kappa B alpha/ NF-kappa B pathway in limb-girdle muscular dystrophy type 2A; *Nat. Med.* **5** 503-511

Beauchamp J, Morgan J, Pagel C and Partridge T 1999 Dynamics of myoblast transplantation reveal a discrete minority of precursors with stem cell-like properties as the myogenic source; *J. Cell Biol.* **144** 1113-1122

Beauchamp J R, Heslop L, Yu D S, Tajbakhsh S, Kelly R G, Wernig A, Buckingham M E, Partridge T A and Zammit P S 2000 Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells; *J. Cell Biol.* **151** 1221-134

Beresfords J N 1989 Osteogenic stem cell and the stromal system of bone and marrow; *Clin. Orthop.* **240** 270-280

Bjornson C R, Rietze R L, Reynolds B A, Magli M C and Vescovi A L 1999 Turning Brain into Blood: A Hematopoietic Fate Adopted by Adult Neural Stem Cells *in vivo; Science* **283** 534-537

Bittner R E, Schofer C, Weipoltshammer K, Ivanova S, Streubel B, Hauser E, Freilinger M, Hoger H, Elbe-Burger A and Wachtler F 1999 Recruitment of bone-marrow-derived stem cells by skeletal muscle and cardiac muscle in adult dystrophic *mdx* mice; *Anatomy Embryol.* **199** 391-396

Bonavaud S, Agbulut O, Nizard R, D'Honneur G, Mouly V and Burtler-Browne G 2001 A discrepancy resolved: human satellite cells are not preprogrammed to fast and slow lineages; *Neuromusc. Disorders* **11** 747-752

Brakebusch C, Grose R, Quondamatteo, Ramirez A, Jorcano J L, Pirro A, Svensson M, Herken R, Sasaki T, Timpl R, Werner S and Fässler R 2000 Skin and hair follicle integrity is crucially dependent on $^{2}1$ integrin expression on keratinocytes; *EMBO J* **19** 3990-4003

Brazelton T R, Rossi F M, Keshet G I and Blau H M 2000 From Marrow to Brain: expression of neuronal phenotypes in adult mice; *Science* **290** 1775-1779

Caldwell M A, He X, Wilkie N, Pollack S, Marshall G, Wafford K A and Svendsen C N 2001 Growth factors regulate the survival and fate of cells derived from human neurospheres; *Nat. Biotechnol.* **19** 475-479

Cameron H A, Woolley C S, McEwen B S and Gould E 1993 Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat; *Neuroscience* **56** 337-344

Campisi J 2001 From cells to organisms: can we learn about aging from cells in culture? *Exp. Gerontol.* **36** 607-618

Carey J R, Liedo P, Harshman L, Zhang Y, Muller H G, Partridge L and Wang J L 2002 A mortality cost of virginity at older ages in female Mediterranean fruit flies; *Exptl. Gerontol.* **37** 507-512

Chong J S W L V, Benbow S, Foy P, Wallymahmed M E, Wile D and MacFarlane I A 2000 Elderly people with hypothalamic-pituitary disease and growth hormone deficiency: lipid profiles, body composition and quality of life compared with control subjects; *Clin. Endocrinol.* **53** 551-559

Christensen J L and Weissman I L 2001 FLK-2 is a marker in hematopoietic stem cell differentiation: A simple method to isolate long-term stem cells; *Proc. Natl. Acad. Sci. (USA)* **98** 1451-1454

Clarke D L, Johansson C B, Wilbertz J, Veress B, Nilsson E, Karlstrom H, Lendahl U and Frisen J 2000 Generalised potential of adult neural stem cells; *Science* **288** 1660-1663

Cornelison D D W and Wold B J 1997 Single-cell analysis of regulatory gene expression in quiescent and activated mouse skeletal muscle satellite cells; *Devel. Biol.* **191** 270-283

Cotsarelis G, Sun T-T and Lavker R M 1990 Label-Retaining Cells Reside in the Bulge Area of Pilosebaceous Unit: Implications for Follicular Stem Cells, Hair Cycle and Skin Carcinogenesis; *Cell* **61** 1329-1337

de Boer J, Olle Andressoo J, de Wit J, Huijmans J, Beems R B, van Steeg H, Weeda G, van der Horst G T J, van Leeuwen W, Themmen A P N, Meradji M and Hoeijmakers J H J 2002 Premature Aging in Mice Deficient in DNA Repair and Transcription; *Science* 296 1276-1279

de Grey A D N J 2002 Three detailed hypotheses implicating oxidative damage to mitochondria as a major driving force in homeotherm aging; *EUR. J. BIOCHEM.* 269 1995

Dexter T M and Spooncer E 1987 Growth and differentiation in the hemopoietic system; *Annu. Rev. Cell Biol.* 3 423-441

Di Donna S, Renault V, Forestier C, Piron-Hamelin G, Thiesson D, Cooper R N, Ponsot E, Decary S, Amouri R, Hentati F, Butler-Browne G S and Mouly V 2000 Regenerative capacity of human satellite cells: the mitotic clock in cell transplantation; *Neurol. Sci.* 21 S943-S951

Eichmann A, Pardanaud L, Yuan L and Moyon D 2002 Vasculogenesis and the search for the hemangioblast; *J. Hematother. Stem Cell Res.* 11 207-214

Faragher R G A and Kipling D 1998 How might replicative senescence contribute to human ageing? *Bioessays* 20 985-991

Feldman J L and Stockdale F E 1991 Skeletal-muscle satellite cell diversity - satellite cells form fibers of different types in cell-culture; *Dev. Biol.* 143 320-334

Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G and Mavilio F 1998 Muscle Regeneration by Bone Marrow-Derived Myogenic Progenitors; *Science* 279 1528-1530

Fernandes M L D A, Saad M J A and Velloso L A 2001 Effects of age on elements of insulin-signalling pathway in central nervous system of rats; *Endocrine* 16 227-234

Ford C E, Hamerton J L, Barnes D W H and Loutit J F 1956 Cytological indentification of radiation chimaeras *Nature* 177 452

Gage F H 2000 Mammalian neural stem cells; *Science* 287 443-448

Gibson M C and Schultz E 1983 Age-related differences in absolute numbers of skeletal-muscle satellite cells. *Muscle Nerve* 6, 574-580

Goytisolo F A and Blasco M A 2002 many ways to telomere dysfunction: *In vivo* studies using mouse models; *Oncogene* 21 584-591

Gritti A, Bonfanti L, Doetsch F, Caille I, Alvarez-Buylla A, Lim D A, Galli R, Verdugo J M G, Herrera D G and Vescovi A L 2002 Multipotent Neural Stem Cells reside into the Rostral Extension and Olfactory Bulb of Adult Rodents; *J. Neurosci.* 22 437-445

_____, Parati E A, Frolichsthal P, Galli R, Wanke E, Faravelli L, Morassutti D J, Roisen F, Nickel D D and Vescovi A L 1996 Multipotential stem cells from the adult mouse brain proliferate and self-renew in response to basic fibroblast growth factor; *J. Neurosci.* 16 1091-1100

Grounds M D and McGeachie J K 1992 Skeletal-muscle regeneration after crush injury in dystrophic mdx mice - an autoradiographic study; *Muscle Nerve* 15 580-586

_____ 2002 Reasons of the degeneration of ageing skeletal muscle: a central role for IGF-1 signalling; *Biogerontology* 19-24

Guan Y, Taylor-Jones J M, Peterson C A and McGehee RE Jr 2002 p130/p107 expression distinguishes adipogenic potential in primary myoblasts based on age; *Biochem. Biophys. Res. Commun.* 296 1340-1345

Gussoni E, Soneoka Y, Strickland C D, Buzney E A, Khan M K, Flint A F, Kunkel L M and Mulligan R C 1999 Dystrophin expression in the *mdx* mouse restored by stem cell transplantation; *Nature* 401 390-394

Hall P A and Watt F M 1989 Stem cells: the generation and maintenance of cellular diversity; *Development* 106 619-633

Hayflick L and Moorehead P S 1961 The serial cultivation of human diploid cell strains; *Exp. Cell. Res.* 25 585-621

Haynsworth S E, Barber M A and Caplan A I 1992 Cell surface antigens on human marrow derived mesenchymal cells are detected by monoclonal antibodies; *Bone* 13 69-80

Hou J W, Li B, Yang Z H, Fager N and Ma M Y I 2002 Altered gene activity of epidermal growth factor receptor (ErbB-1) in the hpothalamus of ageing female rat is linked to abnormal estrous cycles; *Endocrinol.* 143 577-586

Imamura O, Fujita K, Itoh C, Takeda S, Furuichi Y and Matsumoto T 2002 Werner and Bloom helicases are involved in DNA repair in a complementary fashion; *Oncogene* 21 954-963

Ishizaki Y, Cheng I, Mudge A W and Raff M C 1995 Programmed cell-death by default in embryonic cells, fibroblasts, and cancer-cells; *Molec. Biol. Cell* 6 1443-1458

Jackson K A, Mi T and Goodell M A 1999 Hematopoietic potential of stem cells isolated from murine skeletal muscle; *Proc. Natl. Acad. Sci.* 96 14482-14486

Jaiswal R K, Jaiswal N, Bruder S P, Mbalaviele G, Marshak D R and Pittenger M 2000 Adult human mesenchymal stem cell differentiation to the osteogenic or adipogenic lineage is regulated by mitogen-activated protein kinase; *J. Biol. Chem.* 275 9645-9652

Jiang Y, Jahagirdar B N, Reinhardt R L, Schwartz R E, Keene C D, Xilma R. Ortiz-Gonzalez Reyes M, Lenvik T, Lund T, Blackstad M, Jingbo Du, Aldrich S, Lisberg A, Low W C, Largaespada D A and Verfaillie C M 2002 Pluripotency of mesenchymal stem cells derived from adult marrow; *Nature* 418 41–49

Johansson C B, Momma S, Clarke D L, Risling M, Lendahl U and Frisen J 1999 Identification of a neural stem cell in the adult mammalian central nervous system; *Cell* 96 25-34

Johannsson G, Svenson J and Bengtsson B A 2000 Growth hormone and ageing; *Growth Horm. IGF Res.* 10 25-30

Johnstone B, Ju Y and Barry F 1996 *in vitro* chondrogenesis of bone marrow derived mesenchymal cells; *Trans. Ortho. Res. Soc.* **21** 65

Kenyon C 2001 A conserved regulatory system for aging; *Cell* **105** 165-168

Kocher A A, Schuster M D, Szabolcs M J, Takuma S, Burkhoff D, Wang J, Homma S, Edwards N M and Itescu S 2001 Neovascularization of ischemic Myocardium by human bone-marrow-derived angioblast prevents cardiomyocytes apoptosis, reduces remodeling and improves cardiac function; *Nature Medicine* 7 430-436

Krause D S, Theise N D, Collector M I, Henegariu O, Hwang S, Gardner R, Neutzel S and Sharkis S J 2001 Multi-organ, Multi-lineage Engraftment by a Single Bone Marrow-Derived Stem Cell; *Cell* **105** 369-377

Kuhn H G, Dickinson-Anson H and Gage F H 1996 Neurogenesis in the Dentate Gyrus of the Adult Rat: Age-Related Decrease of Neuronal Progenitor Proliferation; *J. Neurosci.* 16 2027-2033

Lagasse E, Connors H, Al-Dhalimy, Reitsma M, Dohse M, Osborne L, Wang X, Fiengold M, Weissman I L and Grampe M 2000 Purified hematopoietic stem cells can differentiate into hepatocytes *in vitro; Nature Medicine* 6 1229-1234

Lansdorp P M, Poon S, Chavez E, Dragowska V, Zijlmans M, Bryan T, Reddel R, Egholm M, Bacchetti S and Martens U 1998 Telomeres in the haematopoietic system; *Ciba Foundation Symposia* **211** 209-218

Lexel J, Henriksson-Larsen K, Winblad B and Sjostrom M 1983 Distribution of different fibre types in human skeletal muscles: effects of ageing studied in whole muscle cross sections; *Muscle & Nerve* 6 588-595

Majumdar M K, Thiede M A, Mosca J D, Moorman M and Gerson S L 1998 Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (MSCs) and stromal cells; *J Cell. Physiol.* **176** 57-66

Marshman E, Booth C and Potten C S 2002 The intestinal epithelial stem cell; *Bioassays* 24 91-98

Martens U M, Chavez E A, Poon S S S, Schmoor C and Landsdorp P M 2000 Accumulation of short telomeres in human fibroblasts prior to replicative senescence; *Exp. Cell Res.* 256 291-299

Martin G R 1978 in *Genetic effects on Aging, Birth defects: Original articles,* Eds D Bergsma and D E Harrison (Liss, New York), Series 14, No 1, pp 177-221

Martin K, Potten C S, Roberts S A and Kirkwood T B L 1998a Age changes in stem cells of murine small intestinal crypts; *Exp. Cell Res.* **241** 316-23

_____, _____, _____ and _____ 1998b Altered stem cell regeneration in irradiated intestinal crypts of senescent mice; *J. Cell Sci.* **111** 2297-2303

McKinney-Freeman S L, Jackson K A, Camargo F D, Ferrari G, Mavilio F and Goodell M A 2002 Muscle-derived hematopoietic stem cells are hematopoietic in origin; *Proc. Natl. Acad. Sci. (USA)* 99 1341-1346

Merrick D, Badr I, Watkins M and Smith J 2002 IGF2 function in skeletal muscle differentiation; *Genet. Res. Camb.* **80** 63-75

Merrill B J, Gat U, DasGupta R and Fuchs E 2001 Tcf3 and Lef1 regulates lineage differentiation of multipotent stem cells in skin; *Genes & Development* 15 1688-1705

Mezey E, Chandross K J, Harta G, Maki R A and McKercher S R 2000 Turning blood into brain: cells bearing antigens generated *in vivo* from bone marrow; *Science* **290** 1779-1782

Michel M, Torok N, Godbout M-J, Lussier M, Gaudreau P, Royal A and Germain L 1996 Keratin 19 as a biochemical marker of skin stem cells *in vivo* and in vitro: keratin 19 expressing cells are differentially localized in function of anatomic sites, and their number varies with donar age and culture stage; *J. Cell Science* **109** 1017-1028

Miller R A 2000 Telomere diminution as a cause of immune failure in old age: an unfashionable demurral; *Biochem. Soc. Trans.* **28** 241-245

Mills J C and Gordon J I 2001 The intestinal stem cell niche: There grows the neighborhood; *Proc. Natl. Acad. Sci. (USA)* **98** 12334-12336

Minasi M G, Riminucci M, De Angelis L, Borello L, Beraducci B, Innocenzi A, Caprioli A, Sirabella D, Baiocci M, De Maria R, Boratto R, Jaffredo T, Broccoli V, Bianco P and Cossu G 2002 The meso-angioblast: a multipotent, self-renewing cell that originates from the dorsal aorta and differentiates into most mesodermal tissues; *Development* 129 2772-2783

Miura T, Katakura Y, Yamamoto K, Uehara N, Tsuchiya T, Kim E H and Shirahata S 2001 Neural stem cells loose telomerase activity upon differentiating into astrocytes; *Cytotechnol.* 36 137-144

Monemi M, Eriksson P O, Kadi F and Burtler-Browne G S 1999 Opposite changes in myosin heavy chain composition of human masseter and biceps brachii muscles during aging; *J. Mus.*

Morshead C M, Benveniste P, Iscove N N and van der Kooy 2002 Hematopoietic competence is a rare property of neural stem cells that may depend on genetic and epigenetic alterations; *Nature Medicine* **8** 268-273

_____, Reynolds B A, Craig C G, McBurney M W, Staines W A, Morassutti D, Weiss S and van der Kooy D 1994 Neural Stem Cells in the Adult Mammalian Forebrain: A Relatively Quiescent Subpopulation of Subependymal Cells; *Neuron.* 13 1071-1082

Muraglia A, Cancedda R and Quarto R 2000 Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model; *J. Cell Sci.* **113** 1161-1166

Nishimura E K, Jordans S A, Oshima H, Yoshida H, Osawa M, Moriyama M, jackson I J, Barrandon Y, Miyachi Y and Nishikawa S-I 2002 Dominant role of the niche in melanocyte stem-cell fate determination; *Nature* **416** 854-860

Novak K, MacFarlane J and Aamodt S 2000 Stem cells find their niche; *Nature Med.* **6** 1220

Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson S M, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine D M, Leri A and Anversa P 2001 Bone marrow cells regenerate infarcted myocardium. *Nature* 410 701-705

O'Shea LC, Johnson C, Rooney M, Gleeson R, Woods K & Smith J (2001). Adipogenesis and skeletal muscle ageing *Mech.; Ageing Dev.* **122** 1354-1355

Oshima H, Rochat A, Kedzia C, Kobayashi K and Barrandon Y 2001 Morphogenesis and renewal of hair follicles from adult multipotent stem cells; *Cell* **104** 233-245

Ouellette M M, Liao M, Herbert B S, Johnson M, Holt S E, Liss H S, Shay J W and Wright W E 2000 Subsenescent telomere lengths in fibroblasts immortalised by limiting amounts of telomerase; *J. Biol. Chem.* **275** 10072-10076

Partridge T A, Morgan J E, Coulton G R, Hoffman E P and Kunkel L M 1989 Conversion of mdx myofibers from dystrophin-negative to dystrophin-positive by injection of normal myoblasts; *Nature* **337** 176-179

Potten C S and Loeffler M 1990 Stem-cells - attributes, cycles, spirals, pitfalls and uncertainties - lessons for and from the crypt; *Development* **110** 1001-1120

_____, Booth C and Pritchard M 1997 The intestinal epithelial stem cell: the mucosal governor; *Int. J. Exp. Pathol.* **78** 219-243

Pescatello L S, Murphy D and Costanzo D 2000 Low-intensity physical activity benefits blood lipids and lipoproteins in older adults living at home; *Age Ageing* 29 433-439

Pittenger M F, Mackay A M, Beck S C, Jaiswal R K, Douglas R, Mosca J D, Moorman M A, Simonetti D W, Craig S and Marshak D R 1999 Multilineage Potential of Adult Mesenchymal Stem Cells; *Science* **284** 143-147

Reynolds B A and Weiss S 1992 Generation of Neurons and Astrocytes from Isolated Cells of the Adult Mammalian Central Nervous System; *Science* **255** 1707-1710

Rochart A, Kobayashi K and Barrandon Y 1994 Location of stem cells of human follicles by clonal analysis; *Cell* **76** 1063-1073

Savine R and Sonksen P 2000 Growth hormone - Hormone replacement for the somatopause? *Hormone Res.* **51** 37-41

Seale P, Sabourin LA, Girgis-Gabardo A, Mansouri A, Gruss P and Rudnicki M A 2000 Pax7 is required for the specification of myogenic satellite cells; *Cell* **102** 777-786

Schultz E and Lipton B H 1982 A quantitative study of satellite cells in soleus and extensor digitorum longus muscles following cross transplantation; *Anat. Rec.* **202** 171

Serra V and von Zglinicki T 2002 Human fibroblasts in vitro senesce with a donor-specific telomere length; *FEBS Letters* **516** 71-74

Shay J W and Wright W E 2002 Telomerase: a target for cancer therapeutics; *Cancer Cell* 2 257-265

Shinohara T, Orwig K E, Avarbock M R and Brinster R L 2001 Remodeling of the postnatal mouse testis is accompanied by dramatic changes in stem cell number and Niche accessibility; *Proc. Natl. Acad. Sci. (USA)* **98** 6186-6191

Smith D E, Roberts J, Gage F H and Tusznski M H 1999 Age-associated neuronal atrophy occurs in the primate brain and is reversible by growth factor gene therapy; *Proc. Natl. Acad. Sci. (USA)* **96** 10893-10898

Smith J and Schofield 1994 The effects of fibroblast growth factors in long term primary culture of dystrophic (mdx) mouse muscle myoblasts; *Exp. Cell Res.* .210 86-93

_____, Fowkes G and Schofield P N 1995 Programmed cell death in dystrophic (mdx) muscle is inhibited by IGF-II; *cell Death & Differentiation* 2 243-251

_____ and Schofield P N 1997 Stable integration of an mdx skeletal muscle cell line into dystrophic (*mdx*) skeletal muscle: evidence for stem cell status; *Cell Growth & Diff.* 8 927-934

_____, Goldsmith C, Ward A and LeDieu R 2000 IGF-II ameliorates the dystrophic phenotype and coordinately down-regulates programmed cell death; *Cell Death and Diff.* 7 1109-1118

Song H, Stevens C F and Gage F 2002 Astroglia induce neurogenesis from adult neural stem cells; *Nature* 417 39-44

Sonntag W E, Lynch C, Thornton P, Khan A, Bennett S and Ingram R 2000 The effects of growth hormone and IGF-1 deficiency on cerebrovascular and brain ageing; *J. Anat.* **197** 575-585

Soares M J, Piers L S, O'Dea K and Collier G R 2000 Plasma leptin concentrations, basal metabolic rates and respiratory quotients in young and older adults; *Int. J. Obesity* 24 1592-1599

Sozou P D and Kirkwood T B L 2001 A stochastic model of cell replicative senescence based on telomere shortening, oxidative stress and somatic mutations in nuclear and mitochondrial DNA; *J. Theor. Biol.* 213 573-586

Sprangrude G J, Heimfeld S and Weissman I L 1988 Purification and Characterization of Mouse Hematopoietic Stem Cells; *Science* **241** 58-62

Stewart C E and Rotwein P 1996 Insulin-like growth factor-II is an autocrine survival factor for differentiating myoblasts; *J. Biol. Chem.* **271** 11330-11338

Svendsen C N et al. 1998 A new method for the rapid and long term growth of human neural precursor cells; *J. Neursci. Methods* **85** 141-152

Tang D G, Tokumoto Y M, Apperly J A, Lloyd A C and Raff M C 2001 Lack of replicative senescence in cultured rat-Ologodendrocyte precursor cells; *Science* **291** 868-871

Taylor G, Lehrer M S, Jensen P J, Sun T-T and Lavker R M 2000 Involvement of Follicular Stem Cells in Forming Not Only the Follicle but Also the Epidermis; *Cell* **102** 451-461

Taylor-Jones J M, McGhee R E, Rando T A, Lecka-Czernik B, Lipschitz D A and Peterson C A 2002 *Mech. Ageing Dev.* **123** 649-661

Terada N, Hamazaki T, Oka M, Hoki M, Mastalerz D M, Nakano Y, Meyer E M, Morel L, Petersen B E and Scott E W 2002 Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion; *Nature* **416** 542-545

Till J E and McCulloch E A 1961 A direct measurement of the radiation sensitivity of normal mouse bone marrow cells; *Radiation Res.* **14** 213-222

Thomson J A, Itskovitz-Eldor J, Shapiro S S, Waknitz M A, Swiergiel J J, Marshall V S and Jones J M 1998 Embryonic stem cell lines derived from human blastocysts; *Science* **282** 1145-1147

Toma J G, Akhavan M, Fernandes K J L, Barbabé-Heider F, Sadikot A, Kaplan D R and Miller F D 2001 Isolation of multipotent adult stem cells from the dermis of mammalian skin; *Nature Cell Biol.* **3** 778-784

Tordjman R, Ortega N, Coulombel L, Plouet J, Romeo P-H and Lemarchandel V 1999 *Blood* **94** 2301-2309

Tropepe V, Craig C G, Morshead C M and van der Kooy D 1997 Transforming Growth Factor-± Null and senescent Mice Show Decreased Neural Progenitor Cell Proliferation in the Forebrain Subependyma; *J. Neurosci.* **17** 7850-7859

Villareal D T, Binder E F, Williams D B, Schechtman K B, Yarasheski K E, Kohrt W M 2001 Bone mineral density response to estrogen replacement in frail elderly women: a randomized controlled trial; *JAMA* **286** 815-820

Watt F M and Hogan 2000 Out of Eden: Stem cells and Their Niches; *Science* **287** 1427-1430

Wesbury J, Watkins M, Ferguson-Smith A C and Smith J 2001 Dynamic and temporal regulation of the cdk inhibitor $P57^{kip2}$ during embryo morphogenesis; *Mech. Development* **109** 83-89

Withers H R and Elkind M M 1970 Mictocolony survival assay for cells of mouse intestinal mucosa exposed to radiation; *Int. J. Radiat. Biol.* **17** 261-267

Woods K, Marrone A and Smith J 2000 Programmed cell death and senescence in skeletal muscle stem cells; *Ann. Proc. Natl. Acad. Sci* **908** 331-335

Wright D L, Jones E L, Mayer J F, Oehninger S, Gibbons W E and Lanzerdorf S E 2001 Characterisation of telomerase activity in the human oocyte and preimplanataion embryo; *Molec. Human Reprod.* **7** 947-955

Wright E G 1999 Inherited and inducible chromosomal instability: a fragile bridge between genome integrity mechanisms and tumourigenesis; *J. Pathol.* **187** 19-27

Wyllie F S, Jones C J, Skinner J W, Haughton M F, Wallis C, Wynford-Thomas D, Faragher R G A and Kipling D 2000 Telomerase prevents the accelerated cell aging of Werner syndrome fibroblasts; *Nature Genet.* **24** 16-17

Ying Q-L, Nichols J, Evans E P and Smith A G 2002 Changing potency by spontaneous fusion; *Nature* **416** 545-548

Young H E, Steele T A, Bray R A, Hudson J, Floyd J A, Haukins K, Thomas K, Austin T, Edwards C, Cuzzourt J, Duenzl M, Lucas P A and Black A C Jr 2001 Human reserve pluripotent mesenchymal stem cells are present in the connective tissue of skeletal muscle dermis derived from Fetal, Adult and Geriatic Donors; *Anat. Record* **264** 51-62

Zacksenhaus E, Jiang Z, Chung D, Marth J D, Phillips R A and Gallie B L 1996 pRb controls proliferation differentiation, and death of skeletal muscle cells and other lineages during embryogenesis; *Genes & Devel.* **10** 3051-3064

Zorzano A, Kaliman P, Guma A and Palacin M 2003 Intracellular signals involved in the effects of insulin-like growth factors and neuregulins on myofibre formation; *Cell Signal* **15** 141-149