Proc. Indian natn Sci Acad. **B69** No.5 pp 719-740 (2003)

Skeletal Muscle Progenitor Cells in Development and Regeneration

CHETANA SACHIDANANDAN* and JYOTSNA DHAWAN#
Center for Cellular and Molecular Biology, Hyderabad, 500007

(Received on 24 September 2002; Accepted after revision on 24 October 2002)

In mammals, the repair of skeletal muscle damage in the adult shares many features with embryonic muscle formation. The aim of this review is to outline the cellular and molecular mechanisms that govern muscle development and regeneration. Skeletal muscle tissue is comprised of multinucleated myofibres that arise from the fusion of mononucleated myoblasts during embryonic development. In muscle precursor cells, elaborate mechanisms co-ordinate regulation of the cell cycle with the onset of tissue-specific gene expression and may protect these progenitors from precocious differentiation. Differentiated myofibres are incapable of resuming active proliferation, but damaged adult muscle can regenerate. This regenerative capacity resides in a population of undifferentiated myogenic precursor cells known as satellite cells (SC) that are associated with the myofibres. SC contribute to postnatal muscle fibre growth as well as regeneration and are believed to function as the stem cells of adult skeletal muscle. Recently, progenitors from sources extrinsic to muscle have also been shown to participate in muscle regeneration. The implications of recent findings in developmental biology are discussed both with respect to the fundamental mechanisms of growth and differentiation as well as in the light of potential therapies for muscle diseases.

Key Words: Muscle progenitors, Stem cells, Myogenesis, Regeneration, Tissue repair, Muscle regulatory factors, Cell cycle

Introduction

The formation of embryonic tissues and the repair of tissue damage in adult organisms share many features despite the distinct constraints of development vs. regeneration. Here we outline the cellular and molecular mechanisms that govern the genesis and repair of skeletal muscle, a tissue that comprises ~40% of the human body. Mammalian skeletal muscle is comprised of multinucleated myofibres that are formed by the fusion of mononucleated myoblasts during embryonic development (figure 1). Fusion is accompanied by withdrawal from the cell cycle and the activation of a battery of muscle specific differentiation-dependent genes, many of which encode components of the specialized cytoskeleton involved in contraction (reviewed in Rosenthal 1989). Damage to muscle is a frequent occurrence due to the wear and tear that this superficially located highly contractile tissue experiences. Yet, skeletal muscle is one of several adult tissues that can undergo regeneration. Once differentiated,

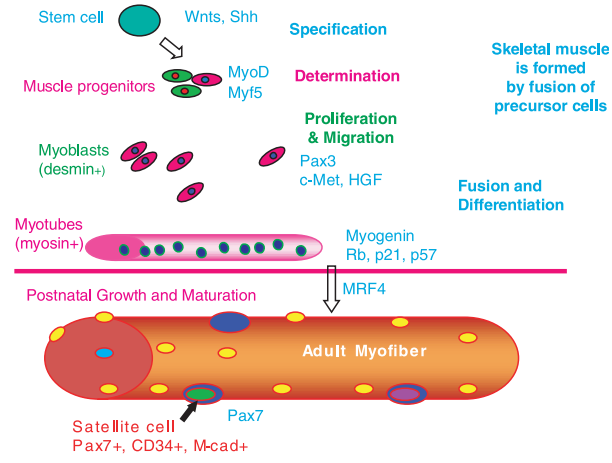

Figure 1 Formation of skeletal muscle tissue involves progressive commitment to the myogenic fate and fusion of myoblasts to form multinucleated myofibers. Specification of myogenic cells from early embryonic stem cells occurs under environmental influences (Wnts, Shh). Subsequently, the expression of MyoD/Myf5 in proliferating muscle progenitors commits them to a muscle fate and expression of muscle-specific proteins such as desmin. Cell cycle arrest, fusion of determined myoblasts into myotubes and activation of differentiation-specific genes such as myosin are regulated by the MRFs in conjunction with cell cycle regulators. Maturation of myotubes into large myofibers progresses postnatally. Satellite cells (mononucleated muscle precursors) are associated with myofibers and marked by Pax7 expression.

*Present Address: Cancer Research UK (previously Imperial Cancer Research Fund), PO Box 123, Lincoln's Inn Fields, London WC2A 3PX
#Corresponding Author: *Email:* jdhawan@ccmb.res.in; *Tel:* 91-40-2719-2747; *Fax:* 91-40-2716-0591/0311

myofibres are incapable of resuming active proliferation. Thus, the burden of repair falls on a population of undifferentiated myogenic precursor cells (MPC) known as satellite cells (SC) that are associated with the syncytial myofibres. SC contribute to postnatal growth as well as regeneration and are believed to function as the stem cells of adult skeletal muscle (reviewed in Seale & Rudnicki 2000). However, recent evidence suggests that sources extrinsic to muscle may also provide muscle precursor cells.

The aim of this review is to provide an understanding of the cells, molecules and mechanisms that govern development and repair of mammalian skeletal muscle. Since cellular interactions underlie tissue organization and homeostasis, the implications for muscle diseases and potential cell-based therapies are also discussed.

Embryonic Muscle Progenitors and The Developmental Myogenic Program

Origin of muscle in the embryo: Development of skeletal muscle is a relatively late event during embryogenesis, and is initiated at around embryonic day 8 in the mouse (reviewed in Tajbakhsh & Buckingham 2000). Skeletal muscle tissue originates during the patterning of the mesoderm into somites, segmental structures that form on either side of the notochord (see figure 2 for a schematic representation). Somites differentiate into two classes of cells depending upon their position with respect to the notochord. In response to signals from the notochord different regions of the somite differentiate into the dermomyotome (skin and muscle) and sclerotome (ribs & cartilage) (reviewed in Arnold & Braun 2000). The region of the dermomyotome that gives rise to muscles of the body wall and limbs is distinct from that which differentiates into the muscles of the ribcage and back. This regional patterning is influenced by diffusible signals such as *sonic hedgehog* (shh) and Wnts that emanate from the adjacent neural tube and notochord. Localized action of these signaling molecules results in the localized expression of a family of transcription factors known as the myogenic regulatory factors or MRFs.

Myogenic Regulatory Factors: The myotome is demarcated first by the expression of Myf5, one of four MRFs. Comprising MyoD, Myf5, Myogenin

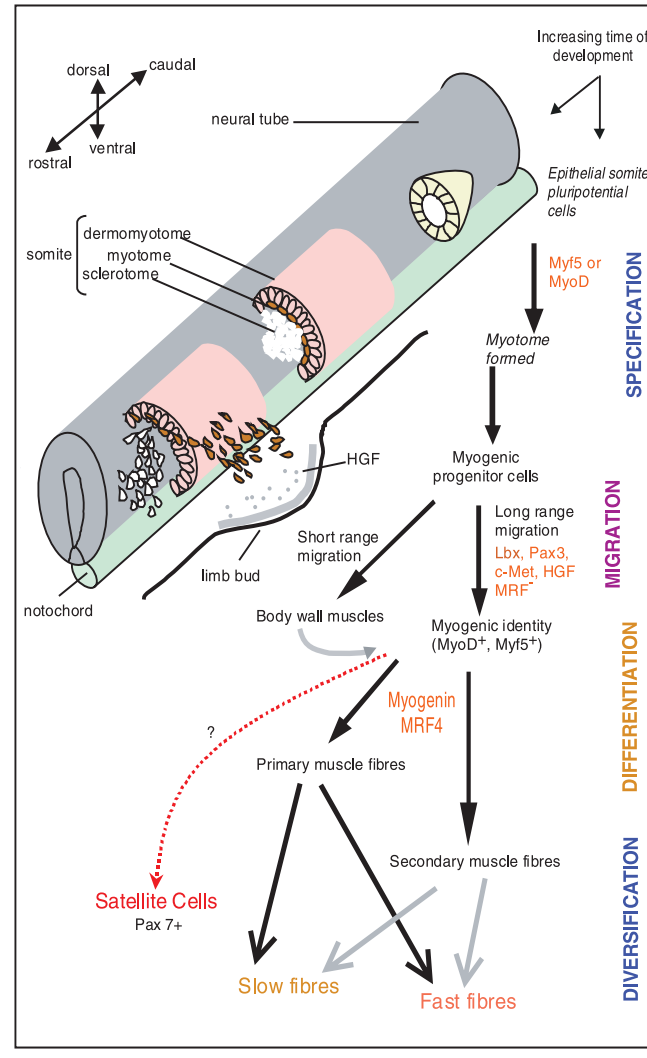

Figure 2 Overview of embryonic myogenesis (adapted from Buckingham 2001)

Schematic representation of somite development indicates the relative location of somites with respect to the axial neural tube and notochord. Although the somite is the source of many cell types (cartilage, muscle, skin), this schematic emphasizes the genes involved in the determination and differentiation of myogenic precursors. Interestingly, muscle progenitors from somites at different axial levels are influenced by different environmental cues. For example, muscle progenitors from somites positioned at the level of the developing limb bud, are induced by HGF to migrate long distances and form the limb muscles. These cells are marked by a distinct set of genes that are not expressed by the precursors of body wall muscles that migrate only short distances. Fusion and differentiation occur in two temporal waves to form primary and secondary myofibers that subsequently mature and diversify into fast and slow fiber types with distinct patterns of myosin expression. Neural signals influence fiber diversification.

and MRF4, this group of basic helix-loop-helix (bHLH) transcription factors plays an important role in the determination and differentiation of muscle (reviewed Molkentin & Olson 1996). Although all 4 molecules can play similar roles in cultured myoblasts, they have distinct functions *in vivo*. MyoD, the first of this family to be discovered, was cloned on the basis of its ability to convert non-muscle cells into multinucleated differentiated myotubes (Davis et al. 1987). Indeed, MyoD was the first "master regulator" found to possess such potent cell fate determining activity and established a new paradigm in developmental control. Subsequent findings revealed that MyoD is situated at a nodal control point in an intricate web of transcription factors and signal transduction pathways (Weintraub 1993). MRFs are transcriptional activators that act by binding to specific DNA elements known as E-boxes, in the enhancers of muscle specific genes. Efficient binding to DNA is achieved by hetero-dimerization with ubiquitously expressed bHLH proteins called the E-proteins (E12, E47). A network of cross-regulatory and auto-regulatory interactions exists between the MRFs and other activators/repressors of the myogenic pathway (Olson 1992). For example, on many muscle specific enhancer elements MRFs also cooperate with the muscle specific MADS-box proteins of the MEF2 family to initiate and maintain myogenic differentiation (reviewed in Puri & Sartorelli 2000).

Specification of myoblasts: Although initially identified from cultured myoblasts, the strict restriction of MRF expression patterns to muscle-forming cells *in vivo* provided support for their involvement in embryonic myogenesis. Subsequently, targeted inactivation of these genes in mice furnished proof of their central role in determination and differentiation of muscle cells albeit with some redundancy in their function (reviewed in Arnold & Winter 1998). The finding that the MyoD knockout mouse was largely normal was a surprise at the time (Rudnicki et al. 1992) but subsequent studies showed subtle defects in some muscle groups (Kablar et al. 1997) and a requirement for MyoD in the adult organism (Megeney et al. 1996, see below). In Myf5 knockout mice, formation of the earliest skeletal muscle, the myotome, is delayed and ribcage defects lead to perinatal lethality. An elegant demonstration of Myf5 function was made possible using a "knock-in" where the Myf5 locus was mutated by insertion of a lacZ gene. Cells that activated the Myf5 promoter expressed β-galactosidase, but lacking Myf5, showed improper migration into dermis and sclerotome where they acquire non-muscle fates (Tajbakhsh et al. 1996). However, muscle forms apparently normally later because of compensation by MyoD. Similarly, in MyoD null mice myogenesis is grossly normal but Myf5 levels are upregulated, confirming the regulation of Myf5 by MyoD (Rudnicki et al. 1992) and revealing a compensatory or fail-safe mechanism for the formation of this important tissue. Further, although muscle is largely normal in mice that lack either MyoD or Myf5, each factor seems to play a non-redundant role in specific muscle groups (Kablar et al. 1997). Mice deficient for both MyoD and Myf5 lack differentiated myofibres due to a complete loss of MPCs and die immediately after birth (Rudnicki et al. 1993) demonstrating the requirement for at least one of these factors in the specification of embryonic myoblasts.

Migration of embryonic myoblasts: Muscles of the body wall are formed by short-range migration of muscle progenitors from somites; those somites adjacent to the limb buds contribute muscle progenitors that migrate long distances to found new muscle masses in the developing limbs (reviewed in Buckingham 2001). Craniofacial muscles have a separate origin: they are derived from the prechordal and presomitic mesoderm (reviewed in Buckingham 2001). A number of genes have been implicated in the survival and migration of muscle progenitors to the limbs and other parts of body (Birchmeier & Brohmann 2000). Mice null for Lbx1, a homeobox gene, lack most hind limb muscle and show abnormal forelimb musculature, as muscle cells fail to migrate correctly (Gross et al. 2000). The c-Met receptor tyrosine kinase and its ligand hepatocyte growth factor/scatter factor (HGF/SF) are essential for the separation of individual muscle progenitors from the dermomyotome and for guiding migration to the limbs (Bladt et al. 1995, Dietrich et al. 1999). Migrating muscle progenitors are MRF negative, continue to proliferate and only initiate the differentiation program when they

reach their destination. Protection against premature differentiation is ensured by multiple mechanisms that modulate MRF expression and function (Fuchtbauer 2002).

Differentiation: Differentiation of muscle progenitor cells requires the MRF Myogenin. Mice lacking Myogenin show normal numbers of determined myoblasts (MyoD and Myf5 positive cells) but have almost no differentiated muscle fibres (Hasty et al. 1993, Nabeshima et al. 1993). Although very similar in structure, Myogenin only partially compensates for Myf5 loss of function. A knock-in of Myogenin into the Myf5 locus that results in the early expression of Myogenin and simultaneous ablation of Myf5 expression, overcomes the loss of Myf5 expression but with reduced efficiency (Wang & Jaenisch 1997). MRF4 deficiency causes only mild phenotypes (Patapoutian et al. 1995, Braun & Arnold 1995), and is thought to play a role in the maturation of muscle fibres. Despite the moderate effects of individual ablation of MyoD and MRF4, mice lacking both MyoD and MRF4 have almost no differentiated muscle fibres, although expression of Myogenin was normal. Thus, a critical level of MRFs may be required to activate muscle structural genes, a threshold that may be achieved by different combinations of MRFs. Based on the phenotypes of single and double knockout mice, as well as a large body of evidence from cultured myoblasts, the MRFs can be sub-divided as follows. Either MyoD or Myf5 is required for determination of myoblasts, Myogenin is critical for differentiation, while MRF4 plays a role in myofibre maturation.

Fusion: Muscle fibre formation occurs in two waves of differentiation in the embryo. Studies in which myonuclei were birth-dated by injection of ^{3}H-thymidine or BrdU into developing muscles concluded that primary myotubes form by a nearly synchronous fusion of myoblasts (Harris et al. 1989b). Subsequently, formation of secondary myotubes occurs in a progressive fashion. The formation of multinucleated myofibres is promoted by many cell surface adhesion molecules of the cadherin, CAM and integrin families, but the mechanism of membrane fusion is still obscure. In Drosophila, there is compelling evidence that embryonic myoblasts can be classified as founder cells and feeder cells based on early expression of transcription factors and cell adhesion molecules that act as selective attractants (Ruiz-Gomez et al. 2000). Groups of feeder cells fuse to founder cells and are entrained by founder cell nuclei in the resulting syncytium (reviewed in Dworak & Sink 2002). In mammalian muscle, partitioning of progenitors into selectively fusing subsets has not been demonstrated but could well be revealed by newer sensitive methods such as comprehensive expression profiling.

Diversification: The final stage of differentiation is a diversification of fibres into slow and fast types, a late process occurring only after primary myogenesis (Harris et al. 1989a). Whereas fast fibres exhibit rapid rates of contraction that cannot be sustained due to the primarily glycolytic mode of energy generation, slow fibres are capable of sustained tonic contraction because of their capacity for oxidative metabolism. The diversification of muscle fibres into slow and fast types is controlled by a combination of extrinsic signals provided by nerve type and pattern and intrinsic differences in the expression of isoforms of the contractile protein myosin as well as metabolic enzymes (Hughes & Salinas 1999).

Integration of Myogenic Differentiation with the Cell Cycle

Individual cells within developing tissues do not normally initiate cell type-specific functions until proliferation ceases. The coupling of growth arrest with the onset of differentiation has been a major focus of research, and studies on skeletal muscle have provided many insights that are applicable to other tissues (Lassar 1994). In early postnatal skeletal muscle, proliferating mononucleated myoblasts arrest prior to fusion with fully functional multinucleated muscle fibres. In later postnatal life, mononucleated precursors enter a quiescent state where neither proliferation nor differentiation is evident. Reactivation of these precursors by damage to the tissue results in a return to both the cell cycle and the capacity to differentiate. The following section reviews what is known about the interaction of these two programs.

Growth control in myoblasts: A large body of evidence from studies of cultured myoblasts suggests that the relationship between cell division and tissue specific function is mutually exclusive. In

culture, proliferating myoblasts can be induced to exit the cell cycle and differentiate simply by withdrawal of mitogens from the medium. Cells that exit the cell cycle in this manner are normally incapable of re-entering the proliferative phase. Growth arrest is thought to be a prerequisite for differentiation and the two processes are tightly coupled (Walsh & Perlman 1997). However, even in clonal cultures of myoblasts, not all cells respond to the differentiation signals. A population of cells that exhibits stem-like self-renewal properties has been described (Baroffio et al. 1996) and are resistant to differentiation signals. These resistant cells will exit the cell cycle but do not differentiate, and have been termed "reserve cells" (Yoshida et al. 1998). An analysis of gene expression reveals that like SC *in vivo*, reserve cells in culture do not express the MRFs, and can be induced to return to the cell cycle with a concomitant re-expression of MyoD and Myf5 (Yoshida et al. 1998, Kitzman et al. 1998).

In vivo, Myogenin is essential for differentiation since in its absence normal numbers of myoblasts are specified but no differentiated myotubes are seen (Hasty et al. 1993). In culture, Myogenin expression precedes phenotypic differentiation, but does not commit myoblasts to irreversible arrest and differentiation (Andrés & Walsh 1996). Until the expression of the cyclin-dependent kinase inhibitor (CKI), $p21^{cip1}$ follows that of Myogenin, myoblasts are capable of returning to the proliferating phase. However, once p21 protein is induced in myogenin-expressing cells, myoblasts are committed to become post-mitotic. The induction of p21 also accounts, in part, for the decrease in CDK activity in differentiating myocytes. Thus, the participation of Myogenin as well as p21 (or the related CKI p57, Zhang et al. 1999c) is required to bring about cell cycle arrest coupled to differentiation. Inactivation of both p21 and the related CKI p57 has a strong muscle phenotype that resembles loss of Myogenin function: no differentiated myotubes are formed. Since Myogenin is not required for expression of p21 or p57, these growth-inhibitory molecules are thought to function in a pathway parallel to the myogenic regulator (Zhang et al. 1999c).

The retinoblastoma protein Rb is also thought to work in conjunction with Myogenin or MEF2C to activate a network of muscle specific genes that executes differentiation (Zhang et al. 1999c). Cell cycle progression is facilitated by the phosphorylation of Rb by the cyclinD1/CDK4 complex during the G_1 phase, thus enabling the S-phase regulator E2F (that is sequestered by hypo-phosphorylated Rb) to activate transcription of genes required for DNA synthesis (Sherr 1995). Rb null myocytes fuse into myotubes but the expression of muscle specific genes is defective and arrest can be reversed (Schneider et al. 1994, Novitch et al. 1999). Hence, for cell cycle arrest to occur, active (hypophosphorylated) Rb is necessary. In growth-arrested cells, active Rb, through its interaction with E2F, recruits the chromatin-remodeling enzyme histone deacetylase (HDAC1) to E2F target genes and causes their repression (Brehm et al. 1998, Luo et al. 1998, Magnaghi-Jaulin et al. 1998).

Antagonistic interactions between the MRFs and the cell cycle regulators appear to govern the balance between proliferation and differentiation. MyoD itself is known to play an anti-proliferative role, and inhibits the cell cycle independent of its ability to induce myogenic differentiation (Crescenzi et. al. 1990, Sorrentino et al. 1990). During differentiation of myoblasts, MyoD inhibits the CDK4-dependent phosphorylation of Rb, which is sufficient to promote cell cycle arrest and differentiation (Zhang et al. 1999b). Conversely, in growing myoblasts over-expression of cyclin D1 induces nuclear localization of CDK4 which binds MyoD directly, blocking both DNA binding and trans-activating function (Zhang et al.1999a). According to this model, the reduction of cyclin D1 levels in myoblasts undergoing growth arrest relieves the CDK4-induced repression of MyoD activity and thus promotes differentiation. A schematic representation of the network of interactions taking place in myoblasts during growth and differentiation is presented in figure 3. Further, studies in synchronized myoblasts have shown that MyoD and Myf5 themselves exhibit cell-cycle dependent oscillations in expression, supporting the notion that the MRFs are functional at particular times during proliferation (Milasincic et al. 1996, Yoshida et al. 1998, Lindon et al. 1998, Kitzmann et al. 1998, Sachidanandan et al. 2002).

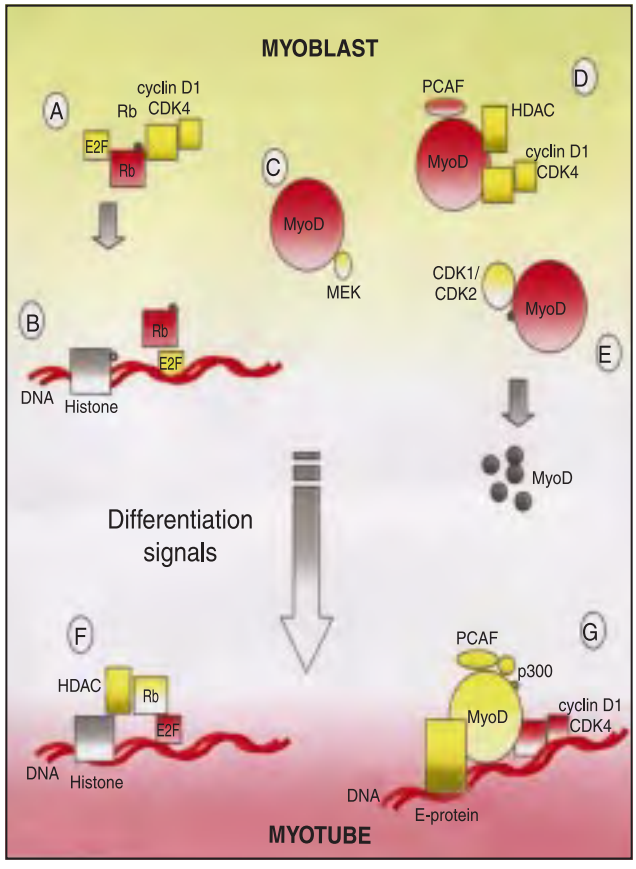

Figure 3 Reciprocal regulation of proliferation and differentiation occurs at multiple levels.

A schematic representation of interactions between cell cycle regulators and the myogenic regulator MyoD emphasizes the role played by post-translational modifications in controlling activity of the different proteins. In Myoblasts **A,** Phosphorylation by cyclin-CDK complexes leads to Rb inactivation; **B,** Phosphorylated Rb releases E2F from repression allowing transactivation of S phase genes. Acetylated (*yellow* star) histones remodel chromatin and aids transcription; **C,** DNA-binding activity of MyoD is repressed by MEK which is activated by mitogen induced signals; **D,** HDAC prevents acetylation of MyoD and CDK4/cyclinD1 complex inactivates MyoD. (E) CDK1&CDK2 phosphorylate (*green* star) MyoD which targets it for degradation. In Myotubes; **F,** Hypophosphorylated (active) Rb recruits HDAC on to E2F targets causing repression of transcription by histone deacetylation. MyoD inhibits the phophorylation of Rb by cyclin-CDK complex (not shown). In the absence of HDAC (sequestered by Rb), PCAF activates MyoD, by acetylation. Active MyoD cooperates with E-proteins to enhance transcription of myogenic genes when bound to E boxes. Proteins shown in shades of *yellow* are in their active form and in shades of *red* are in their inactive form.

Inhibitors of differentiation: MyoD activity is incompatible with cell cycle progression. Intriguingly however, proliferating somitic myoblasts in the embryo and asynchronously growing myoblasts in culture express MyoD. What prevents their differentiation? Mitogen induced pathways have long been known to inhibit MyoD function by their positive effects on cell cycle proteins such as cyclins and CDKs (reviewed in Kitzmann & Fernandez 2001, Wei & Paterson 2001). In addition, recent reports suggest that MEK, a MAP kinase kinase, interacts directly with MyoD leading to its inactivation (Perry et al. 2001). A number of negative regulatory factors are also known which inhibit the activity of MRFs. The Id (originally *inhibitor of DNA binding* but often expanded as *inhibitor of differentiation*) family is comprised of proteins with an HLH dimerization domain but no basic DNA binding domain. In undifferentiated myoblasts, Id family members sequester E-proteins (dimerization partners of MRFs) thus inhibiting MRF activity. Twist, another inhibitor of muscle differentiation in vertebrates, is a bHLH protein that is expressed in the whole somite in early development and is down-regulated in the prospective myotome compartment prior to myogenesis. Twist inhibits muscle differentiation in many ways: through direct interactions with MEF2 and MyoD, by sequestering E-proteins into inactive complexes and by inactivating acetyl transferase activity of PCAF that is required for MyoD function (reviewed in Puri & Sartorelli 2000). The histone deacetylase HDAC1 has also been suggested to have inhibitory effects on MyoD and upon growth arrest, which are negated by active Rb (Puri et al. 2001). Multiple mechanisms to inhibit differentiation may exist to safeguard against the precocious differentiation of progenitors.

Post-translational modifications play a significant role in modulating the activity of MRFs. Phosphorylation of MyoD targets its rapid degradation via the ubiquitin pathway (Song et al. 1998). CDK1 and CDK2 have been implicated in this pathway to maintain low levels of MyoD in undifferentiated myoblasts (Kitzmann et al. 1999) and this mechanism might also be responsible for the cell-cycle dependant fluctuations in the levels of MyoD (Kitzmann et al.

1998). Acetylation of MyoD increases during differentiation of myoblasts (reviewed in Puri & Sartorelli 2000) and is also implicated in regulating its function. PCAF and p300 are proteins that regulate transcription dually: by inducing chromatin remodeling through acetylation of histones and by acetylation of transcription factors such as MyoD. p300 appears to stabilize PCAF-MyoD interactions while PCAF itself is capable of acetylating MyoD protein leading to augmentation of MyoD-DNA interactions. MyoD-p300-PCAF complexes have been detected on E-boxes and this interaction appears to be important for the myogenic and growth inhibitory functions of MyoD (Sartorelli et al. 1999).

Thus, a general conclusion that can be drawn is that to ensure that differentiation is tightly coordinated with cell cycle exit, multi-pronged mechanisms of control are exerted on MyoD as well as cell cycle regulators. Given that multiple means are used to suppress MyoD activity, it was thought that this MRF had no transcriptional targets in proliferating myoblasts per se, but only became active when conditions were conducive to differentiation. The recent identification of Id3 and Np1 as direct targets of MyoD in growing cells challenges this notion, and adds new complexity to the role of this determination factor in muscle progenitor biology (Wyzykowski et al. 2002).

Reversal of differentiation: Although nuclei in differentiated muscle cells normally arrest irreversibly, there are unusual situations where myotubes retain the ability to reenter the cell cycle. For example, Rb null myocytes can fuse into myotubes without irreversible arrest (Schneider et al. 1994, Novitch et al. 1996). Similarly, ectopic expression of viral oncogenes such as E1A and SV40 T antigen that sequester Rb causes myotube nuclei to reenter cell cycle (Endo & Nadal-Ginard 1998, Iujvidin et al. 1990), mimicking the Rb null phenotype. In lower vertebrates such as newts, dedifferentiation of myofibres occurs during limb regeneration and extracts of regenerating newt muscle cause de differentiation of C2C12 myotubes. The active component is yet to be defined (McGann et al. 2001), but its existence may explain the different modes of regeneration in urodeles and mammals. Cleavage of differentiated myotubes has been artificially induced using myoseverin, a synthetic microtubule-binding purine identified by combinatorial chemistry (Rosania et al. 2000). Over-expression of Msx1 (a homeodomain protein) in differentiated myotubes not only induces re-entry into the cell cycle followed by fission, but also imparts a pluri-potentiality to the resulting mono-nucleated progeny, enabling them to differentiate along osteogenic, chondrogenic and adipogenic pathways (Odelberg et al. 2000). Thus, both arrest and differentiation are controlled by active mechanisms and do not represent static "end states". However, in mammalian myofibres *in vivo*, there are no clear reports of de-differentiation, suggesting that even if cleavage does occur it is rare and not likely to account for the bulk regeneration of the tissue.

Adult Muscle Progenitors: Growth and Repair

Postnatal Growth of Muscle: Muscle fibre number, type, patterning and innervation are determined prior to birth, but during postnatal development, muscle fibres increase in cross sectional caliber as well as length (Grounds 1991). Myoblasts that participate in this phase of proliferation and differentiation to increase the mass of pre-existing muscle fibres are derived from satellite cells (SC; Schultz 1986, 1996). SC were first described in amphibian muscle by their anatomical location as mono-nucleated cells that reside between the plasma membrane and the basal lamina sheath that surrounds individual myofibres (Mauro 1961), and have since been identified in all vertebrates studied (Campion 1984). As they contribute to increased myofibre girth by fusion, the population of SC decreases during the period of postnatal growth, from about 32% of muscle nuclei at birth to less than 5% in the adult (2 months in mice) (Bischoff 1994). Subsequently, SC number is fairly constant at between 1-4% of nuclei in different muscles. In undisturbed adult muscle, most SC are quiescent, cycling very slowly or not at all.

Regeneration of Damaged Muscle: In addition to their role in normal postnatal growth, SC are involved in regeneration of damaged muscle tissue (Bischoff 1990). When muscles experience acute injury induced by increased workload, or by mechanical, toxic, or genetic causes, damaged myofibres die.

Satellite cells associated with dying myofibres and myofibres in their vicinity, are activated to return to the cell cycle and proliferate and differentiate resulting in restoration of tissue form and function. Even mild injury that causes no more than myofibre ECM/membrane damage is sufficient to induce SC proliferation.

The regenerative response of skeletal muscle to diverse types of injury follows a definite pattern. Damage to myofibres releases proteases and activation factors that may serve as chemo-attractants for leukocytes as well as MPC (Grounds 1991, Tidball 1995). As a result, damage is followed by infiltration of the injured region by phagocytic cells: initially neutrophils followed by macrophages and finally, lymphocytes (Orimo et al. 1991). While a primary role of phagocytic cells is to remove the debris of dead fibres, they also secrete cytokines that affect SC behavior. A schematic representation of the process of regeneration is presented in figure 4.

The proximal activating signal for SC activation is not known, but hepatocyte growth factor (HGF), the ligand for the c-met receptor tyrosine kinase is a leading candidate as it is sequestered in the myofibre ECM and released upon injury (Tatsumi et al. 1998, described in detail below). Activated SC enter the cell cycle and proliferate for a period of 2-3 days. The newly formed progeny MPC subsequently arrest and differentiate to form new fibres, or repair damaged ones by fusion. Some MPC must also repopulate the SC compartment, as is suggested by the observation that regenerated myofibres can mount a continued response to repeated rounds of damage (Gross et al. 1999). Further, studies using injection of marked myoblasts into regenerating muscle show that although the majority of surviving donor cells contribute to the formation of differentiated muscle soon after transplantation, marked mono-nucleated cells can be recovered even a year after transplantation (Blaveri et al. 1999, Heslop et al. 2001). These cells are thought to represent the small fraction of donor cells that enter the SC compartment, but direct evidence for a replenishment pathway is still lacking. A number of other factors are thought to augment the proliferation and differentiation of MPC (reviewed in Tidball 1995). Systemic hormones are known to affect MPC proliferation and differentiation

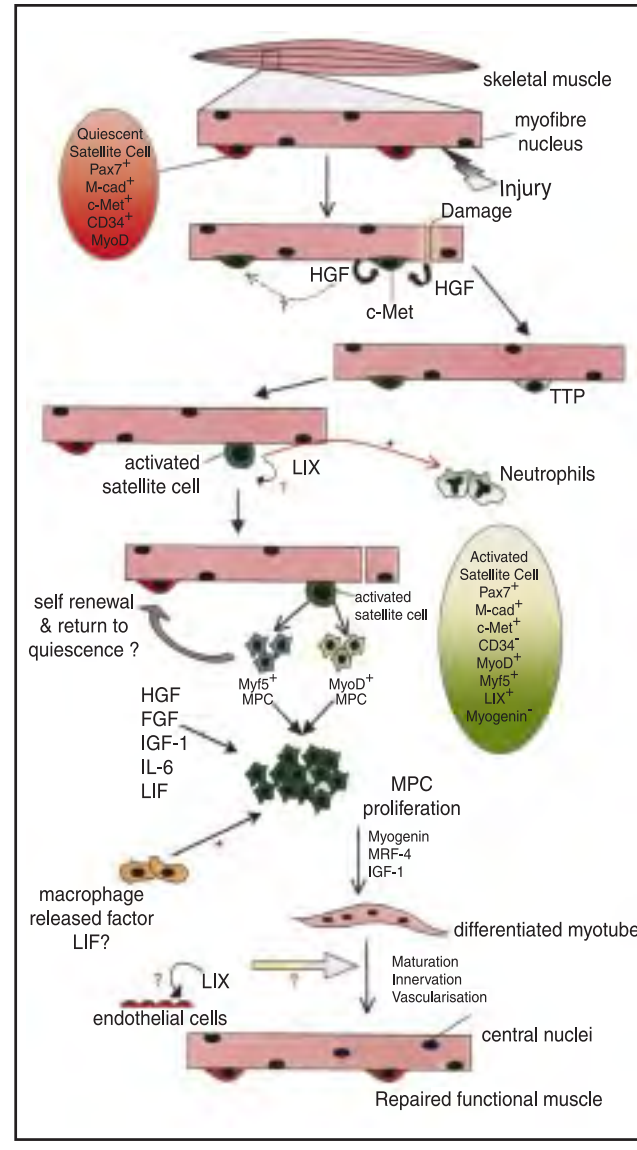

Figure 4 Overview of cells and molecules involved in muscle regeneration (adapted from Seale & Rudnicki 2000)

Quiescent satellite cells associated with mature myofibers do not express MyoD but do express several specific markers such Pax7, a transcription factor that may be involved in the commitment to myogenesis and c-met, the receptor for HGF. Myofiber injury releases HGF from extra-cellular sources and results in SC activation. Early response genes TTP and LIX are induced in activated SC prior to MyoD expression and may amplify the damage signal . MRF expression is followed by a series of events that resemble the stages of embryonic myogenesis and result in the formation of new myofibers, although some SC also fuse with pre-existing myofibers. Regenerated myofibers grow and mature over time and acquire a blood supply and innervation, but are marked by persistent central nuclei.

(reviewed in Grounds 1991). Resident non-muscle cells such as endothelial cells and fibroblasts as well as infiltrating cells recruited during injury may release factors that act locally (Lescaudron et. al. 1999).

The Origin and Identity of Satellite Cells

Although the involvement of SC in repair and regeneration is well documented, the mechanism by which they retain their myogenic identity is still uncertain, since quiescent SC do not express any of the known MRFs. Thus, the developmental origin of SC is unclear: since SC are defined on the basis of their position relative to the myofibre extracellular matrix, they are first detected in muscle only after the basal lamina has formed [at embryonic day 17.5 in the mouse] (Seale & Rudnicki 2000). The discovery that SC are absent in the Pax7 null mouse, suggests that this upstream regulator of MyoD may play this role. Yet, in light of recent evidence of multi-potentiality of the MPC population in muscle, whether SC are committed to form only MPC and the molecular mechanisms that allow cell fate choices are unknown. Further, SC may not be the only progenitors that can participate in regeneration (see below).

Origins of satellite cells: Although skeletal myofibres have been established to be primarily somitic in origin, evidence for the derivation of SC in adult muscle from somites is not conclusive (Armand et al. 1983, reviewed in Bailey et al. 2001). The notion that adult SC are embryonic MPC, trapped underneath the basal lamina during development seems not to be correct, as many physiological differences between these temporally distinct groups have been noted (Cossu et al. 1983, 1987, Grounds & Yablonka-Reuveni 1993). However, there is no evidence to suggest that SC do not derive from embryonic MPC by the acquisition of novel characteristics, possibly under the influence of as yet unknown extrinsic factors. The possibility that myogenic progenitors present in muscle could be derived from aortal or endothelial precursors (De Angelis et al. 1999) is supported by the observation that MPC express a number of endothelial markers such as VE-cadherin, VEGF-R2, P-selectin, and PECAM. Other studies have also demonstrated expression of hematopoietic lineage markers such as CD34, CD43, Sca-1, CD11, Gr-1 and CD5, in MPC isolated from muscle (Gussoni et al. 1999). Thus, it is possible that cells of different lineages contribute to the MPC population in adult muscle, an issue that is discussed further when we consider the developmental fate of stem cells derived from adult muscle.

Specification of satellite cells: Until recently, few molecules were known to specifically mark adult satellite cells. Pax7, a close relative of Pax3 (a known upstream regulator of MyoD) has now been implicated in the specification of SC (Seale et al. 2000). A member of the paired box family of transcription factors, Pax7 is also expressed in the brain and is important for the development of the central nervous system (Jostes et al. 1990, Mansouri et al. 1996). In adult muscle, Pax7 is expressed specifically in SC and not in myonuclei or resident non-muscle cells. More importantly, EM studies showed that muscles of Pax7 null mice contain almost no SC (Seale et al. 2000), despite otherwise normal structure, suggesting that embryonic myogenesis is unaffected. Taken together with a dramatic reduction in body size (Mansouri et al. 1996), these findings support the role assigned to SC in the postnatal growth of muscle (Schultz 1986, 1996). Further, the frequency of $Pax7^{+}$ cells in dystrophic mice is greatly increased over wild type, consistent with the increase in SC progeny during the cycles of degeneration/regeneration that occur in these animals (Seale et al. 2000). Moreover, mononucleated muscle-derived cells from the Pax7 null mice exhibit no myogenic properties, but surprisingly, enhanced hematopoietic potential, implicating Pax7 in the specification of SC as a myogenic stem cell reserve (Seale et al. 2000). This finding may suggest that the progenitors of SC prior to Pax7 expression are bi-potential at least with respect to muscle and blood formation. The status of regeneration in Pax7 knockout mice has not yet been reported, probably due to their poor survival as adults.

The identity and heterogeneity of satellite cells: In order to study the biology of SC during their quiescence or activation following injury, unambiguous markers are required. Prior to the discovery of Pax7, M-cadherin (M-cad) (Irintchev et al. 1994) emerged as a widely used marker of SC (Cooper et al. 1999, Beauchamp et al. 2000).

Cadherins are transmembrane intercellular adhesion proteins with important functions in muscle differentiation (Zeschnigk et al. 1995), and in development and maintenance of tissue architecture (reviewed in Kaufmann et al. 1999). M-cadherin (M-cad) a muscle-specific isoform is found in quiescent undifferentiated SC, where it localizes to the surface that is in contact with the myofibre (Irintchev et al. 1994). It is possible that interactions of M-cad in SC, with unknown ligands on the myofibre surface play a role in the quiescence of these cells in uninjured muscle. However, a recent study that used M-cad as a marker also enumerated SC by independent means and concluded that not all SC express M-cad, indicating an inherent heterogeneity in the reserve population (Beauchamp et al. 2000). Our studies have showed that M-cad is cell cycle regulated in cultured myoblasts suggesting a possible origin for this heterogeneity *in vivo* (Sachidanandan et al. 2002).

Studies that followed DNA synthesis in SC during postnatal growth of muscle have also found that there are at least two populations of SC (Schultz 1996). In growing muscle, 80% of SC were found to traverse the cell cycle slowly while the remaining 20% are arrested in G0. This minor population is thought to represent the true stem cells, which go through self-renewal at a slow rate in order to provide reserve cells for muscle. In adult muscle, this heterogeneity was reiterated when it was found that ~20% of cells located in the SC position are negative for M-cad and CD34, two known markers for SC (Beauchamp et al. 2000). Although it is not clear whether the cells identified by marker analysis and tracer studies are the same, it is evident that SC are not a homogeneous population.

Quiescence of satellite cells: SC are mitotically quiescent in adult muscle but the mechanism for entry into and maintenance of this quiescence is largely unknown. Recently, CD34, a marker of hematopoietic stem cells (HSC) that is expressed on quiescent SC, has been implicated in this role (Beauchamp et al. 2000). CD34 is the standard clinically applied marker used for isolating HSC from blood and bone marrow (Krause et al. 1996). Although the function of this trans-membrane protein has not yet been elucidated it has been implicated in differentiation (Fackler et al. 1995), adhesive interactions (Healy et al. 1995, Puri et al. 1995) and liver regeneration (Omori et al. 1997). CD34 mRNA expression is lost within a few hours of SC activation, leading to the speculation that CD34 may be involved in the maintenance of quiescence of SC (Beauchamp et al. 2000). Consistent with this notion, we have found that CD34 expression is induced in during G0 arrest and down-regulated as synchronized myoblasts return to the cell cycle (Sachidanandan et al. 2002).

Inhibitory signaling molecules may also play a role in SC quiescence. Myostatin/GDF8, a member of the TGFβ family, localizes to quiescent SC in adult muscle (McCroskery et al. 2001), is down-regulated in activated SC and inhibits myoblast proliferation in culture (Thomas et al. 2000). Myostatin knockout mice are approximately 200% larger in size than their wild type littermates, further implicating this molecule in a growth suppressive role (McPherron et al. 1997).

Activation of satellite cells: When muscle is damaged, the information of the injury spreads from the focus of injury to undamaged tissue. Thus, cells nearer to the injury are likely to respond before cells situated farther away, and the activation of SC probably occurs asynchronously following the initial damage. The factors activating SC during injury have been elusive although NO has been implicated (Anderson 2000). Using isolated fibres with their associated quiescent SC, it was demonstrated that an extract from lightly crushed muscle tissue contained a factor that could stimulate quiescent SC to proliferate in culture (Bischoff 1986b) and enhance muscle regeneration *in vivo* (Bischoff & Heintz 1994). Subsequently, the active component was found to be HGF/SF (Tatsumi et al. 1998).

HGF/SF is a heparin binding growth factor present in uninjured muscle, associated with the ECM, and is thought to be released as a result of proteolytic activity during injury (Tatsumi et al. 1998, Miyazawa et al. 1994). The HGF receptor, c-Met, is expressed on the surface of SC in uninjured muscle (Tatsumi et al. 1998, Cornelison & Wold 1997), consistent with a role in signal reception from HGF during muscle injury. HGF is the only factor shown to be capable of the activation of the SC out of quiescence. HGF knockout mice die before birth due

to pleiotropic requirements for this growth factor (Uehara et. al. 1995). However, the embryonic lethality of HGF knockout mice has recently been rescued by injection of the factor into the amniotic cavity of the embryo (Uehara et al. 2000) and it would be interesting to see whether muscle regeneration is impaired in the mutant.

Activation of SC leads to alterations in gene expression, notably the return of MRF expression (Grounds et al. 1992, Cornelison & Wold 1997) and a down-regulation of CD34 expression (Beauchamp et al. 2000). Using synchronized myoblasts in culture as a means of isolating genes induced at different stages of the cell cycle, we have found that early response genes precede the induction of MyoD, and include molecules such as LIX and TTP that are involved in inflammation (Sachidanandan et al. 2002). LIX, a potent chemoattractant for neutrophils and TTP, a regulator of TNFα expression are both rapidly and transiently induced in SC in response to muscle injury. Our studies suggest that in addition to providing new myoblasts for muscle repair, activated SC may be a source of signaling molecules during tissue remodeling.

Self-renewal or differentiation: the choices for a stem cell: Little is known about the mechanisms by which MPCs in regenerating muscle decide when to cease proliferating and initiate differentiation (reviewed in Seale & Rudnicki 2000, Zammit & Beauchamp 2001). Progeny MPC derived from injury-activated SC continue to proliferate for a period of about 3 days. Subsequently, they exit the cell cycle and enter the differentiation program, as evidenced by the decrease in $MyoD^+$ $BrdU^+$ cells and the appearance of embryonic myosin heavy chain in regenerating muscle (Pavlath et. al. 1998). Whether this switch is a cell-intrinsic feature or whether signals from the milieu trigger this transition is unclear but almost certainly both aspects are likely to be involved. Mutations in key genes which steer the MPC towards a particular choice i.e. self-renewal or differentiation have provided some clues.

Quiescent SC do not express MyoD, but when activated following muscle injury, SC induce MyoD expression rapidly (Grounds et al. 1992). Subsequently, all SC express both MyoD and Myf5 (Cooper et al. 1999, Cornelison et al. 2000) suggesting either that these MRFs are involved in the activation of SC or that SC represent uncommitted precursors whose fate is then specified by the induction of MRF expression. The Myf5 locus is known to be active in the majority of SC in undamaged muscle (Beauchamp et al. 2000) as evidenced by β-gal reporter activity in the Myf5nlacZ heterozygous mouse which harbors lacZ targeted to one Myf5 allele (Tajbakhsh et al. 1996). However, since neither the endogenous Myf5 transcript nor protein has been detected in these cells, Myf5's activity in quiescent SC is still unclear. Since the Myf5 null mutation leads to perinatal lethality, it has not been possible to assess the role of Myf5 in adult muscle regeneration.

Not surprisingly, regeneration in $MyoD^{-/-}$ mice is severely impaired (Megeney et al. 1996). However, activation of SC per se does not appear to be defective. Rather, it is the transition from proliferation to differentiation that is impaired, as adult MyoD null mice possess higher numbers of proliferating MPCs than wild type mice (Megeney et al. 1996). Consistent with the anti-proliferative role ascribed to MyoD, these cells show enhanced proliferation and delayed differentiation *in vitro.* In addition, $MyoD^{-/-}$ MPC express higher than normal levels of PEA3 (Sabourin et al. 1999), a transcription factor expressed by activated SC (Taylor et al. 1997). Interestingly, after 96 hr in culture the $MyoD^{-/-}$ 'activated MPC' now re-express Myostatin and Msx1, both genes implicated in SC quiescence (Cornelison et al. 2000), and are unable to induce the late myogenic factor MRF4. Taken together, these data have been interpreted to suggest that MyoD deficiency 'stalls' the activated SC at a point prior to differentiation and diverts SC down a pathway of self-renewal (Megeney et al. 1996, Cornelison et al. 2000). It should be noted that there is as yet no direct evidence for self-renewal of SC, although asymmetric distribution of Numb (an inhibitor of the Notch signaling pathway), has been recently reported in activated SC associated with isolated fibres in culture (Conboy & Rando 2002). Expression of Pax7 and Myf5 appears to differ between the

Numb-expressing and Numb-negative progeny, which may lead to divergent cell fates. As the Notch pathway has been previously implicated in asymmetric cell divisions during embryogenesis (Artavanis-Tsakonas et al. 1999) asymmetric distribution of Numb may influence the choice between self-renewal and differentiation in adult SC.

Mice deficient for myocyte nuclear factor (MNF/Foxk1) also exhibit severely impaired muscle regeneration (Garry et al. 2000). MNF, a transcription factor of the fork-head family is represented by two isoforms: MNFα is expressed by growing and differentiated muscle cells in culture, while MNFβ is transiently expressed when differentiation is initiated (Yang et al. 1997). *In vivo*, SC express one or both isoforms of this protein (Garry et al. 1997). Cultured MPC isolated from MNF mutant mice are incapable of self-renewal and arrest in G_0/ G_1 phase, but appear to be capable of differentiation in response to stimuli (Hawke et al. 2001). A consensus MNF-binding site has been found in the upstream regions of key cell cycle regulatory genes cdc2, c-myc and $p21^{cip1}$ (Yang et al. 1997) and in particular, MNF is thought to act as a transcriptional repressor of the growth inhibitor p21 (Hawke et al. 2001). Consistent with this notion, MNF mutant MPC show higher levels of p21 transcripts and reduced proliferation rates.

Stem Cells and Muscle Precursors in Muscle and Beyond

The long-standing assumption that SC were the only source of MPC has been challenged by recent findings (reviewed in Seale & Rudnicki 2000, Zammit & Beauchamp 2001). Additional MPC may migrate to sites of injury from neighboring uninjured areas, in response to HGF (a potent chemo-attractant for SC *in vitro*, Bischoff 1997) released from injured tissue. In addition to local sources, several lines of evidence now suggest that MPC can be recruited from distant sites during regeneration. When injected intravenously, marked clones of donor MPC home to muscle and form new myofibres (Lee et al. 2000).

More remarkably, recent studies show that not only muscles other than the site of injury, but also cells of other lineages, are capable of contributing to regenerating muscle. Citing the discrepancy between the low frequency of resident SC in normal adult muscle and the hundreds of activated MPC (MyoD+) detected hours after injury, it was suggested that myogenic cells could be recruited from systemic sources (Ferrari et al. 1998). These studies showed that aortic and endothelial derivatives could contribute to new muscle formation, albeit at very low frequencies (Ferrari et al. 1998, Bianco & Cossu 1999), but provided the impetus for further investigation of non-muscle sources for muscle progenitors.

Highly purified hematopoietic stem cells (HSC) can contribute to limb muscle regeneration when injected into the tail vein of mice (Gussoni et al. 1999). Notably, the injected HSC were not purified on the basis of stem cell marker (CD34) expression, but on the basis of their weak staining with the DNA-binding dye Hoechst 33342. Stem cell function was found to reside in this faintly fluorescent population, which results from the expression of a stem-cell specific membrane transporter whose activity leads to dye expulsion (Zhou et al. 2002). This minor population revealed by flow cytometric dye exclusion analysis is also called the SP or side population. SP cells have now been isolated from many adult tissues and appear to possess potent stem cell activity in all cases (Jackson et al. 2002).

Cells isolated from several sources appear to be capable of contributing to muscle formation. For example, cells of the embryonic dorsal aorta (De Angelis et al. 1999), clonally isolated neural stem cells from mice and humans (Galli et al. 2000) form muscle upon transplantation into adult animals, although these cells required a 'muscle-environment' for the conversion. Conversions of bone marrow derived cells (BMDC) to neuronal (Brazelton et al. 2000) and hepatic phenotypes (Theise et al. 2000) have also been reported, although other studies failed to detect such events (Castro et al. 2002, Wagers et al. 2002). However, these observations do suggest a previously unforeseen versatility, and challenge earlier dogma that in the adult, stem cells are committed to contribute only to the tissue in which they reside. Given that the frequency with which these newly identified stem cells contribute to other tissues is generally very low, such rare conversions are not likely to account for the repair observed in tissues such as muscle, except perhaps

in extreme stress-induced circumstances. A higher frequency of donor stem cell incorporation into myofibres was recently reported (LaBarge & Blau 2002), when BMDC were implanted into lethally irradiated mice. Marked GFP+ BMDC were able to colonize not only the bone marrow, leading to host survival, but also the stem cell niche vacated by endogenous SC that were killed by irradiation. That these donor BMDC cells converted to bona fide SC was determined both by their peripheral location on isolated myofibres and in intact muscle and by detecting the co-expression of SC markers with GFP. More importantly, subsequent exercise-induced damage of the implanted muscle led to substantial numbers of GFP+ regenerated myofibres, demonstrating that the BDMC differentiated into functional SC that could contribute to new muscle formation.

Plasticity of muscle precursor cells: In addition to the phenomenon of cells of other lineages contributing to muscle tissue, recent observations show that MPC are capable of differentiation into other cell types (reviewed in Zammit & Beauchamp 2001). MPC derived from adult mouse muscle can repopulate all blood lineages when injected into lethally irradiated mice leading to their survival, the classical test for HSC activity (Jackson et al. 1999). Interestingly, MPC isolated from adult skeletal muscle also contain a population with similar properties as the hematopoietic SP cells with respect to dye exclusion (Jackson et al. 1999), and are capable of functionally reconstituting the hematopoietic compartment of lethally irradiated mice albeit less effectively (Gussoni et al. 1999). These muscle SP cells were however negative for a number of hematopoietic lineage markers that were present in the HSC as well as in the main population (non-SP muscle precursor cells) (Gussoni et al. 1999). The muscle SP cells also showed delayed differentiation into muscle and fibroblast cell types when compared to the main population isolated from adult muscle. Muscle SP cells do not appear to express Pax7 and it would seem that these cells are less committed towards either the muscle or the hematopoietic lineage. Interestingly, in the Pax7 knockout mouse which has drastically reduced numbers of SC, muscle-derived SP cells give rise to hematopoietic colonies with 10-fold greater efficiency than wild type muscle SP cells (Seale & Rudnicki 2000). Thus, Pax7 expression appears to commit SP cells to the myogenic lineage. A recent investigation of the relationship between muscle derived SP cells and SC suggests that they are in fact distinct populations based on the expression of markers such as Sca-1 (Asakura et al. 2002). Muscle SP cells are thought to have a hematopoeitic origin (McKinney-Freeman et al. 2002), but can undergo myogenic specification following inductive interactions with differentiated muscle cells both in vitro and *in vivo* (Asakura et al. 2002).

The potential plasticity of adult stem cells has profound implications for cell-based therapies of degenerative diseases including muscular dystrophies. However, proof of this versatility awaits clonal analysis, in which single cells are unambiguously demonstrated to give rise to progeny of different types. Indeed, when single marked adult HSC were implanted into lethally irradiated mice, no evidence was found for differentiation of the donor cells into cells other than those of the hematopoietic lineage (Wagers et al. 2002). Thus, the extent to which contribution of cells of other lineages is effective in regeneration of muscle, and conversely that of MPC in forming cells of hematopoietic and other lineages, will need to be addressed before therapeutic applications can be envisaged.

Degenerative Diseases of Muscle

Cycles of degeneration and regeneration are an ongoing process in muscle diseases such as muscular dystrophies, a large and heterogeneous group of inherited disorders characterized by progressive muscle wasting (reviewed in Toniolo & Minetti 1999). A number of genes have been implicated in this family of diseases, but most muscular dystrophies are the result of mutations that affect proteins of a large transmembrane complex that links the ECM and the muscle cytoskeleton. The most common muscular dystrophy, Duchenne muscular dystrophy (DMD) results from deficiency of dystrophin, a sub-membrane cytoskeletal protein (Hoffman et al. 1987). The *mdx* mouse, model for the human DMD, shares many of the pathogenetic features of the human disease (Bulfield et al. 1984). The absence of the dystrophin-dystroglycan complex at the surface of

the fibres is thought to compromise membrane strength, rendering it susceptible to damage during muscle contraction (Petrof et al. 1993). Loss of dystrophin leads to depletion of nitric oxide synthase (NOS) from the membrane (Brenman et al. 1995). The resulting reduction of the anti-inflammatory radical NO leads to accumulation of macrophages, massive inflammation and membrane damage common to DMD muscle (Wehling et al. 2001). Although there is no evidence that NO, itself a free radical, causes any oxidative damage to the dystrophic muscle (Zhuang et al. 2001), it is known that *mdx* muscle experiences more oxidative stress than wildtype (Disatnik et al. 1998, Disatnik et al. 2000). Thus, the primary defects in muscular dystrophies are associated with myofibre damage, and not with defective stem cell activity. However, there is also some evidence that secondary effects are seen in the MPC of dystrophic muscle (Webster & Blau 1990 Melone et al. 1999, Heslop et al. 2000, Luz et al. 2002).

Therapeutic interventions for dystrophies: Pharmacological: The preferred and most non-invasive therapeutic approach would involve pharmacological treatments (reviewed in Biggar et al. 2002, Escolar & Scacheri 2001). Steroids such as prednisone appear to have a limited ameliorative effect, but at present, the process of muscle repair is not well enough understood to design molecules that can enhance regeneration or attenuate degeneration. A simple compound that may be effective for DMD is the aminoglycoside antibiotic, gentamicin. Gentamicin suppresses premature stop codons in coding regions of mRNAs by inducing read-through translation. Interestingly, administration of this antibiotic to *mdx* mice (which harbor a point mutation in the dystrophin gene) led to the production and correct localization of full-length dystrophin protein (Barton-Davis et al. 1999). About 15% of the cases of DMD result from spontaneous mutations that introduce stop codons in the dystrophin gene and might be corrected by treatment with gentamicin (Senior 1999). Compounds that are not targeted at dystrophin reversal per se may also be effective. Curcumin, one of the active components of turmeric, has recently emerged as a possible candidate. In mice, intraperitoneal administration of this inhibitor of the transcription factor NF-kB stimulates MPC proliferation as well as differentiation, thus accelerating regeneration in damaged muscle (Thaloor et al. 1999). Recently, functional improvement of dystrophic muscle was achieved in mdx mice by inhibition of myostatin activity, either by injections of neutralizing antibodies (Bogdanovich et al. 2002) or by genetic ablation (Wagner et al. 2002). Taken together, these reports suggest that new therapies may emerge from unexpected sources.

Gene Delivery: Several strategies to deliver normal copies of the dystrophin gene into the diseased muscle have been tried with varying success (reviewed in Burton & Davies 2002, Clemens et al. 2001, Stedman 2001). Direct injection of naked DNA leads to engineered muscle fibres that express dystrophin gene but the efficiency is not high enough for therapeutic use. Chimeraplasts (hybrid RNA-DNA molecules) have been shown to result in reversion *in vivo* of the most common frame shift mutation in mdx mice, both in myofibres (Rando et al. 2000) and in the MPC (Bertoni & Rando 2002) but as with DNA injection would require higher efficiency for therapeutic effects. Adenoviral vectors are the most efficient for dystrophin minigene delivery in animal models but immunological considerations have precluded their use clinically (reviewed in Moisset & Tremblay 2001).

Cell Based Therapies: In DMD, muscle degeneration outstrips the replenishing effects of regeneration. Thus, therapies may be broadly classified into those that mitigate the degenerative process vs. those that potentiate the regenerative process. Injection of MPC into muscle to ameliorate the disease condition is a strategy of the latter class (Watt et al. 1982, Partridge et al. 1989, reviewed in Moisset & Tremblay 2001). Normal myoblasts showed great promise in animal models but the efficiency of myoblast transfer into human dystrophic muscle has been disappointing (Partidge 2002). With recent suggestions that a number of stem cell populations may exhibit multi-potentiality (Pelinkovic et al. 2001, Jankowski et al. 2002) hematopoietic stem cells (HSC) or aortal derivatives may emerge as alternative sources of cells. The intravenous route of injection may serve as a better option rather than direct muscle injection if means of

muscle homing can be devised so that dissemination to all affected muscles occurs. However, it is clear that in addition to an understanding of the pathogenesis of the degenerative process, the basic mechanisms governing the fate of endogenous or transplanted MPC will need to be uncovered before therapies for dystrophies can become a reality. In this context, understanding the regulation of quiescence and activation of MPC in animal models becomes imperative. Thus, a critical step in understanding muscle disease is an understanding of the biology of progenitors of new muscle fibres.

Development vs Repair

The process of muscle regeneration in the adult recapitulates many facets of embryonic muscle development. These events include proliferation of MPC, their withdrawal from the cell cycle, activation of differentiation-dependent genes, fusion to form syncytial myofibres and finally maturation to form a fully functional muscle tissue with its resident non-muscle cells, associated blood supply and neural control (reviewed in Grounds 1991, Seale & Rudnicki 2000). However, there are both molecular and histological differences between the two processes. Muscle precursors in the embryo are not known to exit the cell cycle without differentiation, yet their adult counterparts must enter a quiescent undifferentiated state so as to play their stem cell role. Further, by contrast to myofibres formed during early postnatal growth and development, regenerated fibres in adult muscle are marked by centrally located nuclei in addition to the peripherally located ones. This histological marker allows distinction between muscle fibres that have undergone regeneration and those that have not, but the physiological implications of this phenomenon are unknown.

Molecular differences between development and regeneration: Although molecules such as MyoD, HGF and c-met that are important for embryonic myogenesis also play a role in regeneration, there are some notable differences. In particular, the finding that absence of Pax7 leaves embryonic myogenesis unscathed but obliterates adult MPC suggests functional differences between development and regeneration.

An intriguing notion that emerges from a review of the developmental and regenerative aspects of myogenesis is that during the irreversible linear progression of development, embryonic and neonatal myoblasts are committed to differentiation since the function of these cells is to provide building blocks for growing new tissues. Yet adult myogenic stem cells functioning to maintain tissue homeostasis, essentially a cyclical process, must retain the capacity for regulated proliferation to provide new cells for repair. We suggest that this notion can be articulated in molecular terms as the ability of adult progenitors to reversibly couple and uncouple cell cycle arrest from the differentiation pathway. The mechanisms by which such reversible coupling occurs is unknown, but must play a key role in the function of adult myoblasts and regulation of their fate. Further, we propose that this context makes a search for potential mechanisms possible since reversible arrest must in these terms differ from irreversible arrest. Such events that occur in a stage-specific fashion, for example cell cycle dependent events, are difficult to study in an asynchronous population. To address these questions, we have employed a system of synchronized myoblasts in culture to model the cell cycle dependent events during arrest and activation, especially those that differ from the irreversible events that occur during differentiation.

In normal cells, cell cycle progression depends on both soluble growth factors as well as attachment to a substratum. Growth factor deprivation of adherent cells or abrogation of adhesion in mitogen-rich medium can individually trigger arrest (Benecke et al. 1978, Milasincic et al. 1996). In myogenic cells, the major difference between these two situations is that while cell cycle exit induced by serum withdrawal is coupled to differentiation and therefore irreversible, quiescence induced by suspension culture is reversible and uncoupled from the differentiation program. Suspension culture of myoblasts causes growth arrest as well as down-regulation of MyoD (Milasincic et al. 1996, Sachidanandan et al. 2002). We found that synchronized myoblasts in culture resemble SC *in vivo* in the expression of several genes known to play a role during muscle regeneration (Sachidanandan et al. 2002). Further, the isolation

from synchronized myoblasts of cDNAs that were not expressed in either asynchronously growing or differentiated muscle cells gave credence to the notion that reversible arrest differs from irreversible arrest in molecular terms. Interestingly, two genes isolated in this screen, LIX and TTP encode molecules involved in the inflammatory process and more importantly, both are rapidly and transiently induced in SC *in vivo* in response to muscle damage. LIX is a chemokine with potent neutrophil attracting activity and TTP is an RNA binding protein that regulates cytokine expression. Thus, in addition to the response of SC to injury-induced signals, these muscle precursors might themselves be a source of signaling molecules that play a role in regeneration (Sachidanandan et al. 2002). An exploration of other molecular differences of reversible and irreversible arrest using gene trapping has also yielded several candidate genes (S Ramkumar & J Dhawan, unpublished).

Conclusion

Skeletal muscle tissue has served as an informative model for understanding the molecular mechanisms of determination, development and differentiation in part because of the existence of readily cultured cell lines (Yaffe & Saxel 1977, Blau et al. 1983) that allowed these processes to be studied *in vitro*. This review highlights the findings that demonstrate that despite its unique multinucleated character, skeletal muscle also provides an excellent model for the study of regenerative processes and the involvement of stem cells *in vivo*. The recent explosion of information regarding the versatility and/or multiple origins of stem cells in muscle is likely to enhance our understanding of how cell fate is determined and maintained in adult progenitors. In the future, this information could lead to new therapeutic applications. Developmental mechanisms may differ from regenerative events because the constraints of linear progression in growing systems are different from those operating when cyclical events are required to maintain a system in homeostasis.

References

Anderson J E 2000 A role for nitric oxide in muscle repair: nitric oxide-mediated activation of muscle satellite cells; *Mol. Biol. Cell* **11** 859-1874

Andres V and Walsh K 1996 Myogenin expression, cell cycle withdrawal, and phenotypic differentiation are temporally separable events that precede cell fusion upon myogenesis; *J. Cell Biol.* **132** 657-666

Armand O, Boutineau A M, Mauger A, Pautou M P and Kieny M 1983 Origin of satellite cells in avian skeletal muscles; *Arch. Anat. Microsc. Morphol. Exp.* **72** 163-181

Arnold H H and Winter B 1998 Muscle differentiation: more complexity to the network of myogenic regulators; *Curr. Opin. Genet. Dev.* **8** 539-544

_____ and Braun T 2000 Genetics of muscle determination and development; *Curr. Top Dev. Biol.* **48** 129-164

Artavanis-Tsakonas S, Rand M D and Lake R J 1999 Notch signaling: cell fate control and signal integration in development; *Science* **284** 770-776

Asakura A, Seale P, Girgis-Gabardo A, Rudnicki M A 2002 Myogenic specification of side population cells in skeletal muscle; *J. Cell Biol.* **159** 123-134

Bailey P, Holowacz T and Lassar A B 2001 The origin of skeletal muscle stem cells in the embryo and the adult; *Curr. Opin. Cell Biol.* **13** 679-689

Baroffio A, Hamann M, Bernheim L, Bochaton-Piallat M L, Gabbiani G and Bader C R 1996 Identification of self-renewing myoblasts in the progeny of single human muscle satellite cells; *Differentiation* **60** 47-57

Barton-Davis ER, Cordier L, Shoturma D I, Leland S E and Sweeney H L 1999 Aminoglycoside antibiotics restore dystrophin function to skeletal muscles of mdx mice; *J. Clin. Invest.* **104** 375-381

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** 1221-1234

Benecke B J, Ben-Ze'ev A and Penman S 1978 The control of mRNA production, translation and turnover in suspended and reattached anchorage-dependent fibroblasts; *Cell* **14** 931-939

Bertoni C and Rando T A 2002 Dystrophin gene repair in mdx muscle precursor cells *in vitro* and *in vivo* mediated by RNA-DNA chimeric oligonucleotides; *Hum. Gene. Ther.* **13** 707-718

Bianco P and Cossu G 1999 Uno, nessuno e centomila: searching for the identity of mesodermal progenitors; *Exp. Cell Res.* **251** 257-263

Biggar W D, Klamut H J, Demacio P C, Stevens D J and Ray P N 2002 Duchenne muscular dystrophy: current knowledge, treatment, and future prospects; *Clin. Orthop.* **401** 88-106

Birchmeier C and Brohmann H 2000 Genes that control the development of migrating muscle precursor cells; *Curr Opin Cell Biol.* **12** 725-730

Bischoff R 1986a Proliferation of muscle satellite cells on intact myofibres in culture; *Dev. Biol.* **115** 129-139

_____1986b A satellite cell mitogen from crushed adult muscle; *Dev. Biol.* **115** 140-147

_____1990 Interaction between satellite cells and skeletal muscle fibres; *Development* **109** 943-952

_____ 1994 The satellite cell and muscle regeneration; in *Myology* ed. A G Engel and C Franzini-Armstrong, pp 97-133. (New York: McGraw Hill Inc.)

_____ and Heintz C 1994 Enhancement of skeletal muscle regeneration; *Dev. Dyn.* **201** 41-54

_____ 1997 Chemotaxis of skeletal muscle satellite cells; *Dev. Dyn.* **208** 505-515

Bladt F, Riethmacher D, Isenmann S, Aguzzi A and Birchmeier C 1995 Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud; *Nature* **376** 768-771

Blaveri K, Heslop L, Yu DS, Rosenblatt J D, Gross J G, Partridge T A, Morgan J E 1999 Patterns of repair of dystrophic mouse muscle: studies on isolated fibres; *Dev. Dyn.* **216** 244-256

Blau H M, Chiu C P and Webster C 1983 Cytoplasmic activation of human nuclear genes in stable heterocaryons; *Cell* **32** 1171-1180

Bogdanovich S, Krag T O, Barton E R, Morris L D, Whittemore L A, Ahima R S and Khurana T S 2002 Functional improvement of dystrophic muscle by myostatin blockade; *Nature* **420** 418-421

Borycki A G and Emerson C P 2000 Multiple tissue interactions and signal transduction pathways control somite myogenesis; *Curr. Top. Dev. Biol.* **48** 165-224

Braun T, Buschhausen-Denker G, Bober E, Tannich E and Arnold H H 1989 A novel human muscle factor related to but distinct from MyoD1 induces myogenic conversion in 10T1/2 fibroblasts; *EMBO J.* **8** 701-709

_____ and Arnold H H 1995 Inactivation of Myf-6 and Myf-5 genes in mice leads to alterations in skeletal muscle development; *EMBO J.* **14** 1176-1186

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

Brehm A, Miska E A, McCance D J, Reid J L, Bannister A J and Kouzarides T 1998 Retinoblastoma protein recruits histone deacetylase to repress transcription; *Nature* **391** 597-601

Brenman J E, Chao D S, Xia H, Aldape K and Bredt D S 1995 Nitric oxide synthase complexed with dystrophin and absent from skeletal muscle sarcolemma in Duchenne muscular dystrophy; *Cell* **82** 743-752

Buckingham M 2001 Skeletal muscle formation in vertebrates; *Curr. Opin. Genet. Dev.* **11** 440-448

Bulfield G, Siller W G, Wight P A and Moore K J 1984 X chromosome-linked muscular dystrophy (mdx) in the mouse; *Proc. Natl. Acad. Sci. USA* **81** 1189-1192

Burton E A and Davies K E 2002 Muscular dystrophy—reason for optimism? *Cell* **108** 5-8

Campion D R 1984 The muscle satellite cell: a review; *Int. Rev. Cytol.* **87** 225-251

Castro R F, Jackson K A, Goodell M A, Robertson C S, Liu H and Shine H D 2002 Failure of bone marrow cells to transdifferentiate into neural cells *in vivo; Science* **297** 1299

Clemens P R and Duncan F J 2001 Progress in gene therapy for Duchenne muscular dystrophy; *Curr. Neurol. Neurosci. Rep.* **1** 89-96

Conboy I M and Rando T A 2002 The regulation of Notch signaling controls satellite cell activation and cell fate determination in postnatal myogenesis; *Dev. Cell* **3** 397-409

Cooper R N, Tajbakhsh S, Mouly V, Cossu G, Buckingham M and Butler-Browne G S 1999 *In vivo* satellite cell activation via Myf5 and MyoD in regenerating mouse skeletal muscle; *J. Cell Sci.* **112** 2895-2901

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

_____, Olwin B B, Rudnicki M A and Wold B J 2000 MyoD(-/-) satellite cells in single-fibre culture are differentiation defective and MRF4 deficient; *Dev. Biol.* **224** 122-137

Cossu G, Molinaro M and Pacifici M 1983 Differential response of satellite cells and embryonic myoblasts to a tumor promoter; *Dev. Biol.* **98** 520-524

_____, Eusebi F, Grassi F and Wanke E 1987 Acetylcholine receptor channels are present in undifferentiated satellite cells but not in embryonic myoblasts in culture; *Dev. Biol.* **123** 43-50

_____, Kelly R, Tajbakhsh S, DiDonna S, Vivarelli E and Buckingham M 1996 Activation of different myogenic pathways: Myf-5 is induced by the neural tube and MyoD by the dorsal ectoderm in mouse paraxial mesoderm; *Development* **122** 429–437

Crescenzi M, Fleming T P, Lassar A B, Weintraub H and Aaronson S A 1990 MyoD induces growth arrest independent of differentiation in normal and transformed cells; *Proc. Natl. Acad. Sci. USA* **87** 8442-8446

Davis R L, Weintraub H and Lassar A B 1987 Expression of a single transfected cDNA converts fibroblasts to myoblasts; *Cell* **51** 987-1000

De Angelis L, Berghella L, Coletta M, Lattanzi L, Zanchi M, Cusella-De Angelis M G, Ponzetto C and Cossu G 1999 Skeletal myogenic progenitors originating from embryonic dorsal aorta coexpress endothelial and myogenic markers and contribute to postnatal muscle growth and regeneration; *J. Cell Biol.* **147** 869-878

Dietrich S, Abou-Rebyeh F, Brohmann H, Bladt F, Sonnenberg-Riethmacher E, Yamaai T, Lumsden A, Brand-Saberi B and Birchmeier C 1999 The role of SF/HGF and c-Met in the development of skeletal muscle; *Development* **126** 1621-1629

Disatnik M H, Dhawan J, Yu Y, Beal M F, Whirl M M, Franco A A and Rando T A 1998 Evidence of oxidative stress in mdx mouse muscle: studies of the pre-necrotic state; *J. Neurol. Sci.* **161** 77-84

_____, Chamberlain J S and Rando T A 2000 Dystrophin mutations predict cellular susceptibility to oxidative stress; *Muscle Nerve* **23** 784-792

Dworak H A and Sink H 2002 Myoblast fusion in Drosophila; *Bioessays* **24** 591-601

Endo T and Nadal-Ginard B 1998 Reversal of myogenic terminal differentiation by SV40 large T antigen results in mitosis and apoptosis; *J Cell Sci.* **111** 1081-93

Escolar D M and Scacheri C G 2001 Pharmacologic and genetic therapy for childhood muscular dystrophies; *Curr. Neurol. Neurosci. Rep.* **1** 168-174

Fackler M J, Krause D S, Smith O M, Civin C I and May W S 1995 Full-length but not truncated CD34 inhibits hematopoietic cell differentiation of M1 cells; *Blood* **85** 3040-3047

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

Fuchtbauer E M 2002 Inhibition of skeletal muscle development: less differentiation gives more muscle; *Results Probl. Cell Differ.* **38** 143-161

Galli R, Borello U, Gritti A, Minasi M G, Bjornson C, Coletta M, Mora M, De Angelis M G, Fiocco R, Cossu G and Vescovi A L 2000 Skeletal myogenic potential of human and mouse neural stem cells; *Nat. Neurosci.* **3** 986-991

Garry D J, Yang Q, Bassel-Duby R and Williams R S 1997 Persistent expression of MNF identifies myogenic stem cells in postnatal muscles; *Dev. Biol.* **188** 280-294

Garry D J, Meeson A, Elterman J, Zhao Y, Yang P, Bassel-Duby R and Williams R S 2000 Myogenic stem cell function is impaired in mice lacking the forkhead/winged helix protein MNF; *Proc. Natl. Acad. Sci. U S A* **97** 5416-5421

Gross J G, Bou-Gharios G and Morgan J E 1999 Potentiation of myoblast transplantation by host muscle irradiation is dependent on the rate of radiation delivery; *Cell Tissue Res.* **298** 371-375

Gross M K, Moran-Rivard L, Velasquez T, Nakatsu M N, Jagla K and Goulding M 2000 Lbx1 is required for muscle precursor migration along a lateral pathway into the limb; *Development* **127** 413-424

Grounds M D 1991 Towards understanding skeletal muscle regeneration; *Pathol. Res. Pract.* **187** 1-22

_____, Garrett K L, Lai M C, Wright W E and Beilharz M W 1992 Identification of skeletal muscle precursor cells *in vivo* by use of MyoD1 and myogenin probes; *Cell Tissue Res.* **267** 99-104

_____ and Yablonka-Reuveni Z 1993 Molecular and cell biology of skeletal muscle regeneration; in *Molecular and Cell Biology of Muscular Distrophy* ed. T Partridge, pp.210-256 (London: Chapman & Hall)

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

Harris A J, Fitzsimons R B and McEwan J C 1989a Neural control of the sequence of expression of myosin heavy chain isoforms in foetal mammalian muscles; *Development* **10** 751-769

_____, Duxson M J, Fitzsimons R B and Rieger F 1989b Myonuclear birthdates distinguish the origins of primary and secondary myotubes in embryonic mammalian skeletal muscles; *Development* **107** 771-784

Hasty P, Bradley A, Morris J H, Edmondson D G, Venuti J M, Olson E N and Klein W H 1993 Muscle deficiency and neonatal death in mice with a targeted mutation in the myogenin gene; *Nature* **364** 501-506

Hawke T J, Yan Z, Bassel-Duby R and Garry D J 2001 Cell cycle progression is impaired in myogenic stem cells lacking the winged helix transcription factor Foxk1; *Proc. 2nd Internat. Meeting on Muscle Satellite and Stem Cells.*

Healy L, May G, Gale K, Grosveld F, Greaves M and Enver T 1995 The stem cell antigen CD34 functions as a regulator of hemopoietic cell adhesion; *Proc. Natl. Acad. Sci. USA* **92** 12240-12244

Heslop L, Morgan J E and Partridge T A 2000 Evidence for a myogenic stem cell that is exhausted in dystrophic muscle; *J. Cell Sci.* **113** 2299-2308

_____, Beauchamp J R, Tajbakhsh S, Buckingham M E, Partridge T A, Zammit P S 2001 Transplanted primary neonatal myoblasts can give rise to functional satellite cells as identified using the Myf5nlacZl+ mouse; *Gene. Ther.* **8** 778-783

Hoffman E P, Brown R H Jr and Kunkel L M 1987 Dystrophin: the protein product of the Duchenne muscular dystrophy locus; *Cell* **51** 919-928

Hughes S M and Salinas P C 1999 Control of muscle fibre and motoneuron diversification; *Curr. Opin Neurobiol.* **9** 54-64

Irintchev A, Zeschnigk M, Starzinski-Powitz A and Wernig A 1994 Expression pattern of M-cadherin in normal, denervated, and regenerating mouse muscles; *Dev. Dyn.* **199** 326-337

Iujvidin S, Fuchs O, Nudel U and Yaffe D 1990 SV40 immortalizes myogenic cells: DNA synthesis and mitosis in differentiating myotubes; *Differentiation* **43** 192-203

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

_____, Majka S M, Wulf G G and Goodell M A 2002 Stem Cells: A Minireview; *J. Cell. Biochem. Suppl.* **38** 1–6

Jankowski R J, Deasy B M and Huard J 2002 Muscle-derived stem cells; *Gene. Ther.* **9** 642-647

Jostes B, Walther C and Gruss P 1990 The murine paired box gene, Pax7, is expressed specifically during the development of the nervous and muscular system; *Mech. Dev.* **33** 27-37

Kablar B, Krastel K, Ying C, Asakura A, Tapscott S J and Rudnicki M A 1997 MyoD and Myf-5 differentially regulate the development of limb versus trunk skeletal muscle; *Development* **124** 4729-4738

Kaufmann U, Martin B, Link D, Witt K, Zeitler R, Reinhard S and Starzinski-Powitz A 1999 M-cadherin and its sisters in development of striated muscle; *Cell Tissue Res.* **296** 191-198

Kitzmann M, Carnac G, Vandromme M, Primig M, Lamb N J and Fernandez A 1998 The muscle regulatory factors MyoD and myf-5 undergo distinct cell cycle-specific expression in muscle cells; *J. Cell Biol.* **142** 1447-1459

_____, Vandromme M, Schaeffer V, Carnac G, Labbe JC, Lamb N and Fernandez A. 1999 cdk1- and cdk2-mediated phosphorylation of MyoD Ser200 in growing C2 myoblasts: role in modulating MyoD half-life and myogenic activity; *Mol. Cell Biol.* **19** 3167-3176

_____ and Fernandez A 2001 Crosstalk between cell cycle regulators and the myogenic factor MyoD in skeletal myoblasts; *Cell Mol. Life Sci.* **58** 571-579

Krause D S, Fackler M J, Civin C I and May W S 1996 CD34: structure, biology, and clinical utility; *Blood* **87** 1-13

LaBarge M A and Blau H M 2002 Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fibre in response to injury; *Cell* **111** 589-601

Lassar A B, Skapek S X and Novitch B 1994 Regulatory mechanisms that coordinate skeletal muscle differentiation and cell cycle withdrawal; *Curr. Opin. Cell Biol.* **6** 788-794

Lee J Y, Qu-Petersen Z, Cao B, Kimura S, Jankowski R, Cummins J Usas A, Gates C, Robbins P, Wernig A and Huard J 2000 Clonal isolation of muscle-derived cells capable of enhancing muscle regeneration and bone healing; *J. Cell Biol.* **150** 1085-1100

Lescaudron L, Peltekian E, Fontaine-Perus J, Paulin D, Zampieri M, Garcia L and Parrish E 1999 Macrophages enhance muscle satellite cell proliferation and delay their differentiation; *Muscle Nerve* **22** 724-732

Lindon C, Montarras D, Pinset C. 1998 Cell cycle-regulated expression of the muscle determination factor Myf5 in proliferating myoblasts; *J. Cell. Biol.* **140** 111-118

Luo R X, Postigo A A and Dean D C 1998 Rb interacts with histone deacetylase to repress transcription; *Cell* **92** 463-73

Luz M A, Marques M J and Santo Neto H 2002 Impaired regeneration of dystrophin-deficient muscle fibres is caused by exhaustion of myogenic cells; *Braz. J. Med. Biol. Res.* **35** 691-695

Magnaghi-Jaulin L, Groisman R, Naguibneva I, Robin P, Lorain S, Le Villain J P, Troalen F, Trouche D and Harel-Bellan A 1998 Retinoblastoma protein represses transcription by recruiting a histone deacetylase; *Nature* **391** 601-605

Mansouri A, Stoykova A, Torres M and Gruss P 1996 Dysgenesis of cephalic neural crest derivatives in Pax7-/- mutant mice; *Development* **122** 831-838

Marcelle C, Stark M R and Bronner-Fraser M 1997 Coordinate actions of BMPs, Wnts, Shh and noggin mediate patterning of the dorsal somite; *Development* **124** 3955-3963

Mauro A 1961 Satellite cells of skeletal muscle fibres; *J. Biophys. Biochem. Cytol.* **9** 493-494

McCroskery S, Thomas M, Maxwell L, Sharma M and Kambadur R 2001 Myostatin in satellite cell quiescence and activation. Proceedings of 2nd International Meeting on Muscle Satellite and Stem Cells

McGann C J, Odelberg S J and Keating M T 2001 Mammalian myotube dedifferentiation induced by newt regeneration extract; *Proc. Natl. Acad. Sci. USA* **98** 13699-13704

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

McPherron A C, Lawler A M and Lee S J 1997 Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member; *Nature* **387** 83-90

Megeney L A, Kablar B, Garrett K, Anderson J E and Rudnicki M A 1996 MyoD is required for myogenic stem cell function in adult skeletal muscle; *Genes. Dev.* **10** 1173-1183

Melone M A, Peluso G, Petillo O, Galderisi U and Cotrufo R 1999 Defective growth *in vitro* of Duchenne Muscular Dystrophy myoblasts: the molecular and biochemical basis; *J. Cell Biochem.* **76** 118-132

Milasincic D J, Dhawan J and Farmer S R 1996 Anchorage-dependent control of muscle-specific gene expression in C2C12 mouse myoblasts; *In vitro Cell Dev. Biol. Anim.* **32** 90-99

Miyazawa K, Shimomura T, Naka D and Kitamura N 1994 Proteolytic activation of hepatocyte growth factor in response to tissue injury; *J. Biol. Chem.* **269** 8966-8970

Moisset P A and Tremblay J P 2001 Gene therapy: a strategy for the treatment of inherited muscle diseases? *Curr. Opin. Pharmacol.* **1** 294-299

Molkentin J D and Olson E N 1996 Combinatorial control of muscle development by basic helix-loop-helix and MADS-box transcription factors; *Proc. Natl. Acad. Sci. USA*. **93** 9366-9373

Nabeshima Y, Hanaoka K, Hayasaka M, Esumi E, Li S, Nonaka I and Nabeshima Y 1993 Myogenin gene disruption results in perinatal lethality because of severe muscle defect; *Nature* **364** 532-535

Novitch B G, Spicer D B, Kim P S, Cheung W L and Lassar A B 1999 pRb is required for MEF2-dependent gene expression as well as cell-cycle arrest during skeletal muscle differentiation; *Curr. Biol.* **9** 449-459

Odelberg S J, Kollhoff A and Keating M T 2000 Dedifferentiation of mammalian myotubes induced by msx1; *Cell* **103** 1099-1109

Olson E N 1992 Interplay between proliferation and differentiation within the myogenic lineage; *Dev. Biol.* **154** 261-72

Omori N, Omori M, Evarts R P, Teramoto T, Miller M J, Hoang T N and Thorgeirsson S S 1997 Partial cloning of rat CD34 cDNA and expression during stem cell-dependent liver regeneration in the adult rat; *Hepatology* **26** 720-727

Orimo S, Hiyamuta E, Arahata K and Sugita H 1991 Analysis of inflammatory cells and complement C3 in bupivacaine-induced myonecrosis; *Muscle Nerve* **14** 515-520

Partridge T 2002 Myoblast transplantation; *Neuromuscular Disorders* **12** S3-S6

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

Patapoutian A, Yoon J K, Miner J H, Wang S, Stark K and Wold B. 1995 Disruption of the mouse MRF4 gene identifies multiple waves of myogenesis in the myotome; *Development* **121** 3347-3358

Pavlath G K, Thaloor D, Rando T A, Cheong M, English A W and Zheng B 1998 Heterogeneity among muscle precursor cells in adult skeletal muscles with differing regenerative capacities; *Dev. Dyn.* **212** 495-508

Perry R L, Parker M H and Rudnicki M A 2001 Activated MEK1 binds the nuclear MyoD transcriptional complex to repress transactivation; *Mol. Cell* **8** 291-301

Pelinkovic D, Lee J Y, Adachi N, Fu F H and Huard J 2001 Muscle-based gene therapy and tissue engineering; Crit; *Rev. Eukaryot. Gene. Expr.* **11** 121-129

Petrof B J, Shrager J B, Stedman H H, Kelly A M and Sweeney H L 1993 Dystrophin protects the sarcolemma from stresses developed during muscle contraction; *Proc. Natl. Acad. Sci. USA* **90** 3710-3714

Puri K D, Finger E B, Gaudernack G and Springer T A 1995 Sialomucin CD34 is the major L-selectin ligand in human tonsil high endothelial venules; *J. Cell Biol.* **131** 261-270

Puri P L and Sartorelli V 2000 Regulation of muscle regulatory factors by DNA-binding, interacting proteins, and post-transcriptional modifications; *J. Cell Physiol.* **185** 155-173

_____, Iezzi S, Stiegler P, Chen T T, Schiltz R L, Muscat G E, Giordano A, Kedes L, Wang J Y and Sartorelli V 2001 Class I Histone Deacetylases Sequentially Interact with MyoD and pRb during Skeletal Myogenesis; *Mol. Cell* **8** 885-897

Rando T A, Pavlath G K and Blau H M 1995 The fate of myoblasts following transplantation into mature muscle; *Exp. Cell Res.* **220** 383-389

_____, Disatnik M H and Zhou L Z 2000 Rescue of dystrophin expression in mdx mouse muscle by RNA/DNA oligonucleotides, *Proc. Natl. Acad. Sci. USA* **97** 5363-5368

Rawls A, Valdez M R, Zhang W, Richardson J, Klein W H and Olson E N 1998 Overlapping functions of the myogenic bHLH genes MRF4 and MyoD revealed in double mutant mice; *Development* **125** 2349-2358

Rosania G R, Chang Y T, Perez O, Sutherlin D, Dong H, Lockhart D J and Schultz P G 2000 Myoseverin, a microtubule-binding molecule with novel cellular effects; *Nat. Biotechnol.* **18** 304-308

Rosenthal N 1989 Muscle cell differentiation; *Curr. Opin. Cell Biol.* **1** 1094-1101

Rudnicki M A, Braun T, Hinuma S and Jaenisch R 1992 Inactivation of MyoD in mice leads to up-regulation of the myogenic HLH gene Myf-5 and results in apparently normal muscle development; *Cell* **71** 383-390

_____, Schnegelsberg P N, Stead R H, Braun T, Arnold H H and Jaenisch R. 1993 MyoD or Myf-5 is required for the formation of skeletal muscle; *Cell* **75** 1351-1359

Ruiz-Gomez M, Coutts N, Price A, Taylor M V and Bate M 2000 *Drosophila* dumbfounded: a myoblast attractant essential for fusion; *Cell* **102** 189-98

Sabourin LA, Girgis-Gabardo A, Seale P, Asakura A and Rudnicki MA. 1999 Reduced differentiation potential of primary MyoD-/- myogenic cells derived from adult skeletal muscle; *J. Cell Biol.* **144** 631-643

Sachidanandan C, Sambasivan R and Dhawan J 2002 Tristetraprolin and LPS-inducible CXC chemokine are rapidly induced in presumptive satellite cells in response to skeletal muscle injury; *J. Cell Sci.* **115** 2701-2712

Sartorelli V, Puri PL, Hamamori Y, Ogryzko V, Chung G, Nakatani Y, Wang JY and Kedes 1999 Acetylation of MyoD directed by PCAF is necessary for the execution of the muscle program; *Mol. Cell* **4** 725-734

Schneider J W, Gu W, Zhu L, Mahdavi V and Nadal-Ginard B 1994 Reversal of terminal differentiation mediated by p107 in Rb-/- muscle cells; *Science* **264** 1467-1471

Schultz E, Jaryszak D L, Gibson M C and Albright D J 1986 Absence of exogenous satellite cell contribution to regeneration of frozen skeletal muscle; *J. Muscle Res. Cell Motil.* **7** 361-367

_____1996 Satellite cell proliferative compartments in growing skeletal muscles; *Dev. Biol.* **175** 84-94

Seale P and Rudnicki M A 2000 A new look at the origin, function, and "stem-cell" status of muscle satellite cells; *Dev. Biol.* **218** 115-124

_____, Sabourin L A, 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

Senior K 1999 Duchenne muscular dystrophy improved by gentamicin; *Mol. Med. Today* **5** 461

Sherr C J 1995 D-type cyclins; *Trends Biochem Sci.* **2**187-190

Song A, Wang Q, Goebl M G and Harrington M A 1998 Phosphorylation of nuclear MyoD is required for its rapid degradation; *Mol. Cell Biol.* **18** 4994-4999

Sorrentino V, Pepperkok R, Davis R L, Ansorge W and Philipson L 1990 Cell proliferation inhibited by MyoD1 independently of myogenic differentiation; *Nature* **345** 813-815

Stedman H H 2001 Molecular approaches to therapy for Duchenne and limb-girdle muscular dystrophy; *Curr. Opin. Mol. Ther.* **3** 350-356

Tajbakhsh, S and Buckingham M 2000 The birth of muscle progenitor cells in the mouse: spatiotemporal considerations; *Curr. Top. Dev. Biol.* **48** 225-268

_____, Rocancourt D and Buckingham M 1996 Muscle progenitor cells failing to respond to positional cues adopt non-myogenic fates in myf-5 null mice; *Nature* **384** 266–270

Tatsumi R, Anderson JE, Nevoret CJ, Halevy O and Allen R E 1998 HGF/SF is present in normal adult skeletal muscle and is capable of activating satellite cells; *Dev. Biol.* **194** 114-128

Taylor J M, Dupont-Versteegden E E, Davies J D, Hassell J A, Houle J D, Gurley C M and Peterson C A 1997 A role for the ETS domain transcription factor PEA3 in myogenic differentiation; *Mol. Cell Biol.* **17** 5550-5558

Thaloor D, Miller K J, Gephart J, Mitchell P O and Pavlath G K 1999 Systemic administration of the NF-kappaB inhibitor curcumin stimulates muscle regeneration after traumatic injury; *Am. J. Physiol.* **2** C320-329

Theise N D, Badve S, Saxena R, Henegariu O, Sell S, Crawford J M and Krause D S 2000 Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation; *Hepatology* **31** 235-240

Thomas M, Langley B, Berry C, Sharma M, Kirk S, Bass J and Kambadur R 2000 Myostatin, a negative regulator of muscle growth, functions by inhibiting myoblast proliferation; *J. Biol. Chem.* **275** 40235-40243

Tidball J G 1995 Inflammatory cell response to acute muscle injury; *Med. Sci. Sports Exerc.* **27** 1022-1032

Toniolo D and Minetti C 1999 Muscular dystrophies: alterations in a limited number of cellular pathways? *Curr. Opin. Genet. Dev.* **9** 275-282

Uehara Y, Minowa O, Mori C, Shiota K, Kuno J, Noda T and Kitamura N 1995 Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor; *Nature* **373** 702-705

_____, Mori C, Noda T, Shiota K and Kitamura N 2000 Rescue of embryonic lethality in hepatocyte growth factor/scatter factor knockout mice; *Genesis* **27** 99-103

Wagers A J, Sherwood R I, Christensen J L and Weissman I L 2002 Little evidence for developmental plasticity of adult hematopoietic stem cells; *Science* **297** 2256-2259

Wagner K R, McPherron A C, Winik N and Lee S J 2002 Loss of myostatin attenuates severity of muscular dystrophy in mdx mice; *Ann. Neurol.* **52** 832-836

Walsh K and Perlman H 1997 Cell cycle exit upon myogenic differentiation; *Curr. Opin. Genet. Dev.* **7(5)** 597-602

Wang Y and Jaenisch R 1997 Myogenin can substitute for Myf5 in promoting myogenesis but less efficiently; *Development* **124** 2507-2513

Watt D J, Lambert K, Morgan J E, Partridge T A and Sloper J C 1982 Incorporation of donor muscle precursor cells into an area of muscle regeneration in the host mouse; *J. Neurol. Sci.* **57** 319-331

Webster C and Blau H M 1990 Accelerated age-related decline in replicative life-span of Duchenne muscular dystrophy myoblasts: implications for cell and gene therapy; *Somat. Cell Mol. Genet.* **16** 557-565

Wehling M, Spencer M J and Tidball J G 2001 A nitric oxide synthase transgene ameliorates muscular dystrophy in mdx mice; *J. Cell Biol.* **155** 123-131

Wei Q and Paterson B M 2001 Regulation of MyoD function in the dividing myoblast; *FEBS Lett.* **490** 171-178

Weintraub H 1993 The MyoD family and myogenesis: redundancy, networks, and thresholds; *Cell* **75** 1241-1244

Wyzykowski J C, Winata T I, Mitin N, Taparowsky E J and Konieczny S F 2002 Identification of novel MyoD gene targets in proliferating myogenic stem cells; *Mol. Cell Biol.* **22** 6199-6208

Yaffe D and Saxel O 1977 Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle; *Nature* **270(5639)** 725-727

Yang Q, Bassel-Duby R and Williams R S 1997 Transient expression of a winged-helix protein, MNF-beta, during myogenesis; *Mol. Cell Biol.* **17** 5236-5243

Yoshida N, Yoshida S, Koishi K, Masuda K and Nabeshima Y 1998 Cell heterogeneity upon myogenic differentiation: down-regulation of MyoD and Myf-5 generates 'reserve cells'; *J Cell Sci.* **111** 769-779

Zammit P S and Beauchamp J R 2001 The skeletal muscle satellite cell: stem cell or son of stem cell? *Differentiation* **68** 193-204

Zeschnigk M, Kozian D, Kuch C, Schmoll M and Starzinski-Powitz A 1995 Involvement of M-cadherin in terminal differentiation of skeletal muscle cells; *J. Cell Sci.* **108** 2973-2981

Zhang J M, Wei Q, Zhao X and Paterson B M 1999a Coupling of the cell cycle and myogenesis through the cyclin D1-dependent interaction of MyoD with cdk4; *EMBO J.* **18** 926-933

_____, Zhao X, Wei Q and Paterson B M 1999b Direct inhibition of G(1) cdk kinase activity by MyoD promotes myoblast cell cycle withdrawal and terminal differentiation; *EMBO J.* **18** 6983-6993

Zhang P, Wong C, Liu D, Finegold M, Harper J W and Elledge S J 1999c p21(CIP1) and p57(KIP2) control muscle differentiation at the myogenin step; *Genes Dev.* **13** 213-224

Zhou S, Schuetz J D, Bunting K D, Colapietro A M, Sampath J, Morris J J, Lagutina I, Grosveld G C, Osawa M, Nakauchi H and Sorrentino B P 2002 The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype; *Nat Med.* **7** 1028-1034

Zhuang W, Eby J C, Cheong M, Mohapatra P K, Bredt D S, Disatnik M H and Rando T A 2001 The susceptibility of muscle cells to oxidative stress is independent of nitric oxide synthase expression; *Muscle Nerve.* **24** 502-511