Regeneration of Mammalian Skeletal Muscle: Basic Mechanisms and Clinical Implications

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Abstract: Mammalian skeletal muscles can regenerate following injury and this response is mediated by a specific type of stem cell, the satellite cell. We review here the three main phases of muscle regeneration, including i) the initial inflammatory response and the dual role of macrophages as both scavengers involved in the phagocytosis of necrotic debris and promoters of myogenic differentiation, ii) the activation and differentiation of satellite cells and iii) the growth and remodeling of the regenerated muscle tissue. Nerve activity is required to support the growth of regenerated myofibers and the specification of muscle fiber types, in particular the activation of the slow gene program. We discuss the regeneration process in two different settings. Chronic degenerative diseases, such as muscular dystrophies, are characterized by repeated cycles of segmental necrosis and regeneration involving scattered myofibers. In these conditions the regenerative capacity of satellite cells becomes exhausted with time and fibrosis prevails. Acute traumatic injuries, such as strain injuries common in sport medicine, cause the rupture of large myofiber bundles leading to muscle regeneration and formation of scar tissue and new myotendinous junctions at the level of the rupture. Mechanical loading is essential for muscle regeneration, therefore, following initial immobilization to avoid the risk of reruptures, early remobilization is required to induce correct growth and orientation of regenerated myofibers. Finally, we discuss the causes of age-dependent decline in muscle regeneration potential and the possibility of boosting regeneration in aging muscle and in muscular dystrophies.

Keywords: Skeletal muscle, muscle regeneration, satellite cells, muscular dystrophy, exercise, sport medicine, strain injury, IGF-1.

1. INTRODUCTION

Mammalian skeletal muscles consist of multinucleated myofibers which are formed during development by fusion of mononucleated muscle progenitors, some of which remain associated to adult myofibers as satellite cells, a specific type of stem cells. Mature myofibers are plastic cells that can undergo changes in fiber size (atrophy/hypertrophy) or fiber type (fast-to-slow or slow-to-fast switch). Muscle fibers can also repair local damages to specific subcellular structures. For example, focal plasma membrane disruptions induced by muscle contractions, especially eccentric (lengthening) contractions, can be repaired by membrane resealing through fusion of subsarcolemmal vesicles to the plasma membrane. A protein called dysferlin is essential in promoting this process, and defects in dysferlin cause limb-girdle muscular dystrophy 2B in humans and a slowly progressing skeletal muscle degenerative disease in mice. Myofibrillar alterations with Z-disk streaming can also be caused by eccentric contractions and lead to subsequent myofibrillar remodeling, including formation of new sarcomeres. Myonuclei can disappear due to focal apoptosis, occasionally seen in atrophic muscle, or increase in number by incorporation of satellite cell nuclei. All these forms of focal myofiber damage and repair, involving plasma membrane, myofibrils or myonuclei, are reversible processes not causing cell death, take place without obvious histological changes in myofibers and surrounding tissues and do not involve inflammatory responses. However, more severe injuries due either to traumatic lesions, such a muscle strains common in sport medicine, or to various diseases including genetic defects, such as muscular dystrophies, cause necrosis of whole myofibers or myofiber segments. Necrosis stimulates an inflammatory response with invasion of macrophages, followed by activation of satellite cells, which undergo proliferation, differentiation and fusion to one another or to undamaged portions of the fiber. The formation of new myofibers or myofiber segments following necrosis is called muscle regeneration, a process that in many but not all respects (see below, section 2.2) recapitulates the sequence of events observed during embryonic myogenesis.

Comprehensive reviews of muscle regeneration have been recently published, including reviews on the cellular and molecular mechanisms of muscle regeneration [1, 2] and a book dealing with different aspects of skeletal muscle regeneration [3]. In this brief review, which is not meant to be exhaustive, we present an update of the main events in muscle regeneration, discuss the signaling pathways involved in this process and point to open issues in this field.

2. THREE STAGES DURING MUSCLE REGENERATION

The time course of the main events during muscle regeneration following necrosis is summarized in Fig. (**1**). Three sequential but overlapping stages can be identified: i) the inflammatory reaction, dominated by the invasion of macrophages, ii) the activation, differentiation and fusion of satellite cells and iii) the maturation of newly formed myofibers and remodeling of regenerated muscle.

2.1. Inflammatory Phase and Role of Macrophages

Muscle fiber necrosis leads to dissolution of the plasma membrane with calcium influx and activation of calcium-dependent proteases, such as calpains, that produce rapid disintegration of myofibrils and other cell constituents. Entry of plasma proteins and activation of the complement cascade induce chemotactic recruitment of leukocytes, initially neutrophils then macrophages, mainly derived from blood monocytes. These phagocytes have been traditionally viewed as scavengers involved in the removal of necrotic debris, however recent studies demonstrate a more active role of macrophages in promoting muscle regeneration [4]. Two distinct subpopulations of macrophages sequentially invade injured muscle tissue [5]. The early invading macrophages, characterized by the expression of the CD68 surface marker and lacking the CD163 marker, reach a highest concentration in damaged muscle at about 24 hours after the onset of injury and then rapidly decline. These "inflammatory" macrophages secrete pro-inflammatory cytokines, such as tumor necrosis factor α (TNFα) and interleukin-

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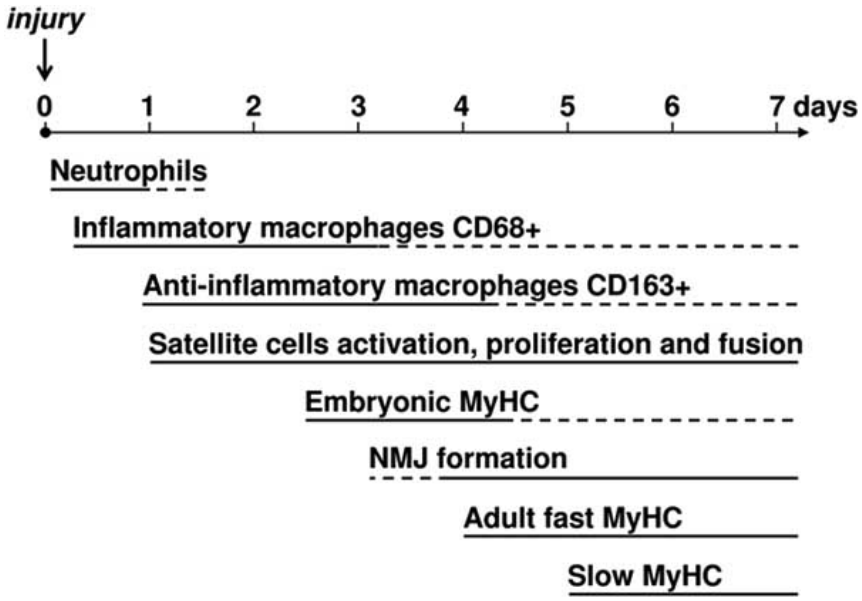

Fig. (1). Major events in muscle regeneration. Schematic representation of the temporal sequence of inflammatory and regenerative events following muscle injury.

1β (IL-1β), and are responsible for the phagocytosis of necrotic tissue. A second population of CD68-/CD163+ "anti-inflammatory" macrophages, apparently derived from inflammatory macrophages by phenotype switch, reach their peak at 2 to 4 days after injury: they secrete anti-inflammatory cytokines, such as interleukin-10 (IL-10), that contribute to the termination of inflammation. Earlier studies showed that macrophages release factors that enhance myogenic precursors proliferation, growth and differentiation [6-8]; it is now clear that this is a property of CD163+ "anti-inflammatory" macrophages [4]. Thus macrophages have a central regulatory role in the muscle response to injury, not only by removing necrotic tissue but also by promoting muscle regeneration.

2.2. Satellite Cell Activation and Differentiation

Satellite cells are muscle-specific stem cells located under the basal lamina of muscle fibers [9], which are responsible for muscle regeneration (see [10, 11]). They also contribute to the increase in the number of myonuclei during postnatal muscle growth [12] and compensatory muscle hypertrophy [13, 14] by proliferating and fusing with the myofibers. Non muscle stem cells, either cells associated to blood vessels or circulating stem cells have been hypothesized to participate in muscle regeneration, but their role appears to be minimal under normal conditions. A crucial experiment supporting the central role of satellite cells was the demonstration that grafting a single myofiber with as few as seven associated satellite cells into an irradiated host muscle can generate over 100 new myofibers with thousands of myonuclei and repopulate the host muscle with new satellite cells [15]. Thus satellite cells are able to self-renew and to give rise to differentiated cells, the two distinctive properties of stem cells.

Satellite cells can be identified by the presence of specific markers, such as Pax7, M-cadherin and CD34 (Fig. (**2**)). Pax3, the paralogue of Pax7, is also expressed in satellite cells of some adult muscles. Pax3 and Pax7 (paired box proteins 3 and 7) are transcription factors playing a crucial role in embryonic myogenesis [16]. Satellite cells undergo apoptotic cell death early after birth in Pax7 knockout mice and are absent in adult Pax7 null muscles [17]. However, a recent study using an inducible knockout technique, demonstrated that Pax7 is not necessary for the survival of satellite cells in adult animals [18]. Even in the absence of Pax7, regeneration occurs in a normal way, and muscle can recover from severe injuries. Moreover, satellite cells become activated, proliferate, and colonize again the niche between the sarcolemma and the basal lamina of the regenerated fibers. Normal muscle regeneration takes place also after dual inactivation of Pax3 and Pax7, thus excluding the possibility of a rescue of Pax7 due Pax3 activation [18]. These findings demonstrate that regeneration does not recapitulate development in this respect.

Following injury, satellite cells undergo rapid proliferation, starting during the second day after injury. Multiple signals appear to trigger satellite cells activation. Generation of sphingosine-1-phosphate in the inner side of the plasma membrane of the satellite cell is required for satellite cell activation: when its synthesis is abrogated, satellite cells do not enter the cell cycle and the regeneration process is defective [19]. Nitric oxide (NO) production by increased NO synthase (NOS) activity is also important for satellite cell activation, possibly through activation of matrix metalloproteinases, which induce the release of hepatocyte growth factor (HGF) from the extra-cellular matrix [20]. By binding to its receptor, c-Met, which is expressed by satellite cells, HGF is able to stimulate satellite cell activation [21] but in the same time inhibits muscle differentiation [22]. The switch from satellite cell proliferation to differentiation appears to be controlled by Wnt and Notch signaling, Notch signaling being prevalent during the proliferation phase [23] and Wnt signaling during the differentiation phase [24]. In the first days after injury, sustained Notch signaling is required for the expansion of satellite cell progeny, then canonical Wnt signaling drives muscle differentiation [23-25]. Defective Notch signaling leads to inhibition of satellite cell proliferation and self-renewal, while enhancement of Notch signaling promotes muscle regeneration in aged muscle (see below, section 6, [25, 26]). Increased Wnt signaling, acting through the canonical Wnt pathway, induces the differentiation of proliferating myoblasts [24]. Correct timing is crucial, since premature Wnt signaling activation leads to premature differentiation and fusion of myoblasts, when the number of proliferating myoblasts is still not sufficient to completely repair the lesion [24]. A recent study point to a more complex role of Wnt signaling during muscle regeneration. Wnt7a, which is upregulated during regeneration and acts through a non-canonical Wnt pathway, is required for the expansion of satellite cell population during regeneration [27], but has no effect on growth or differentiation of myoblasts. Overexpression of Wnt7a enhances regeneration, while silencing of Frz7 (the candidate receptor for Wnt7a) abrogates Wnt7a stimulation of

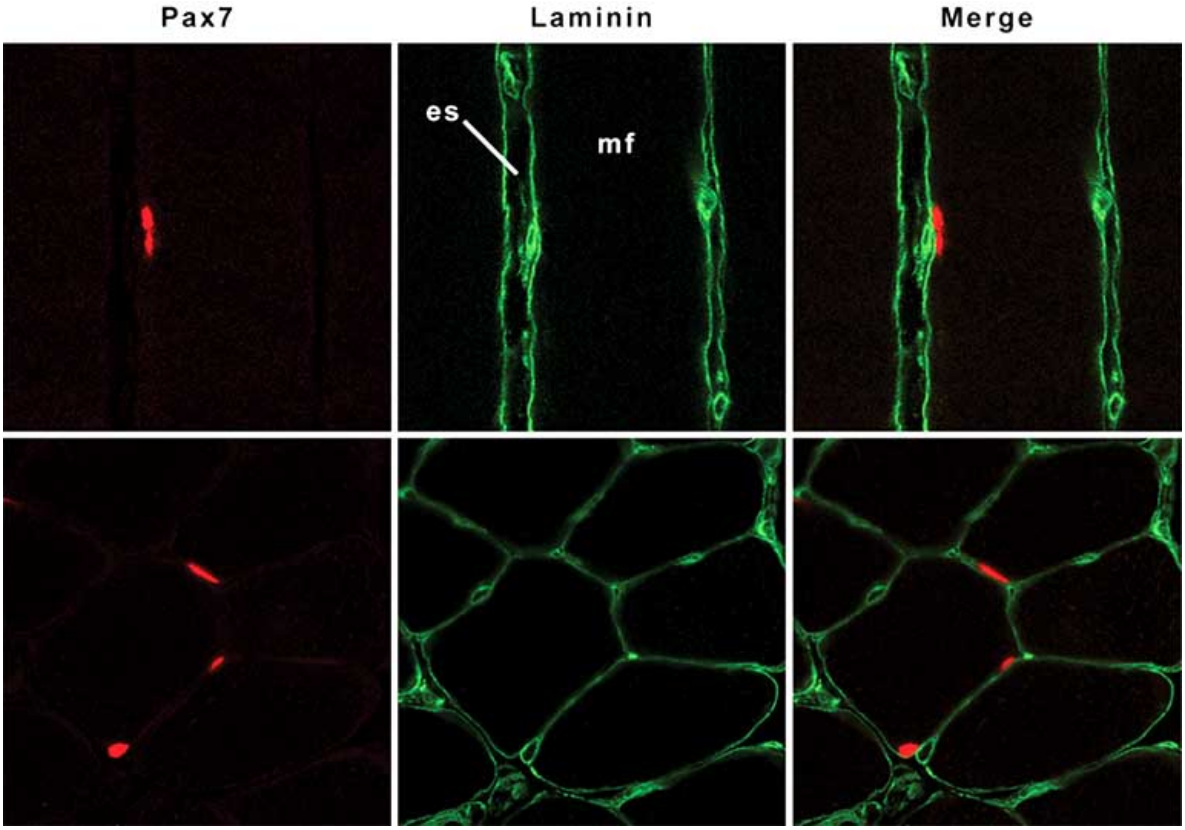

Fig. (2). Satellite cells in rat skeletal muscle. Quiescent satellite cells in adult rat soleus muscle can be recognized by the presence of Pax7 in nuclei (red) and by their localization under the basal lamina of the myofibers (laminin staining, green). Upper panels, longitudinal section. Lower panels, transverse section. *mf*, myofiber; *es*, extracellular space.

stem cell expansion. Muscles lacking Wnt7a have reduced number of myofiber-associated satellite cells after regeneration [27].

Satellite cell proliferation leads to the formation of both new stem cells, which maintain an undifferentiated state, and of committed myogenic precursor which express the MyoD family regulators of muscle determination and differentiation (MRFs), including MyoD, Myf5, myogenin and Mrf4. The transition from the quiescent to the activated state is rapidly followed by muscle differentiation, with expression of MyHCs and fusion of myoblasts to each other forming myotubes. MRF expression was examined in satellite cells using a multiplex single-cell RT-PCR assay to simultaneously monitor expression of all four MRFs in single myofiber cultures [28]. Activated satellite cells began to express either MyoD or myf5 but most cells then expressed both myf-5 and MyoD simultaneously; myogenin came on later in cells expressing both MyoD and myf5, and many cells ultimately expressed all four MRFs simultaneously. MyoD has a crucial role in this process, as MyoD null satellite cells showed reduced myogenin and completely absent Mrf4 expression, and displayed a dramatic differentiation deficit, as determined by fusion and myosin heavy chain (MyHC) expression [29]. Accordingly, muscle regeneration *in vivo* is markedly impaired in MyoD null mice [30].

During muscle regeneration, newly formed multinucleated myotubes express developmental markers, such as embryonic MyHC [31] (Fig. (**3**)), which is a useful marker of regenerating fibers also in dystrophic human muscle [32]. Cardiac-specific markers are also expressed in regenerating myofibers. This is true for troponin T which is expressed in embryonic skeletal muscle and is re-expressed during muscle regeneration [33]. In contrast, other cardiac-specific proteins, such as cardiac troponin I and cardiac myosin binding protein C, are not detected in developing and regenerating skeletal muscle [34, 35]. However, the cardiac troponin I promoter was found to be strongly upregulated in regenerating skeletal muscle by mutation of GATA elements present in this promoter, suggesting that this gene is actively repressed by GATA-like factors present in regenerating skeletal muscle [36].

2.3. Maturation of Regenerating Myofibers and Remodeling of Regenerated Muscle

The subsequent growth of regenerated muscle may vary according to various factors, including the type of muscle injury, the involvement of blood vessels and the re-establishment of neuromuscular and myotendinous connections. A crucial factor for successful muscle regeneration is the maintenance of the basal lamina of muscle fibers. Within the intact basal lamina satellite cells and myotube can proliferate and fuse to form almost normal muscle fibers in a short time period. Regenerated fibers can be recognized by the presence of centrally located nuclei which in rodents, but not in human muscle, are a hallmark of previous muscle regeneration (Fig. (**3**)). However, muscle regeneration can often lead to significant remodeling of the muscle tissue with a variety of patterns (Fig. (**4**)) [37]. Regenerating myotubes within the same basal lamina may not fuse, leading to the formation of clusters of smaller fibers, or may fuse only at one extremity, leading to the formation of forked fibers, once erroneously interpreted as "fiber splitting". After segmental necrosis, regenerative processes are concentrated at the level of the damaged stump, giving rise to appearances once erroneously interpreted as "budding", and the reconstitution of myofiber integrity may be prevented by scar tissue that separates the two stumps, leading to the formation of new myotendinous junctions (see below, section 5) [38]. Finally, regenerating myofibers may occasionally form outside the basal lamina due to migration of satellite cells or possible contribution of non muscle stem cells, and remain as small fibers embedded in the interstitial tissue.

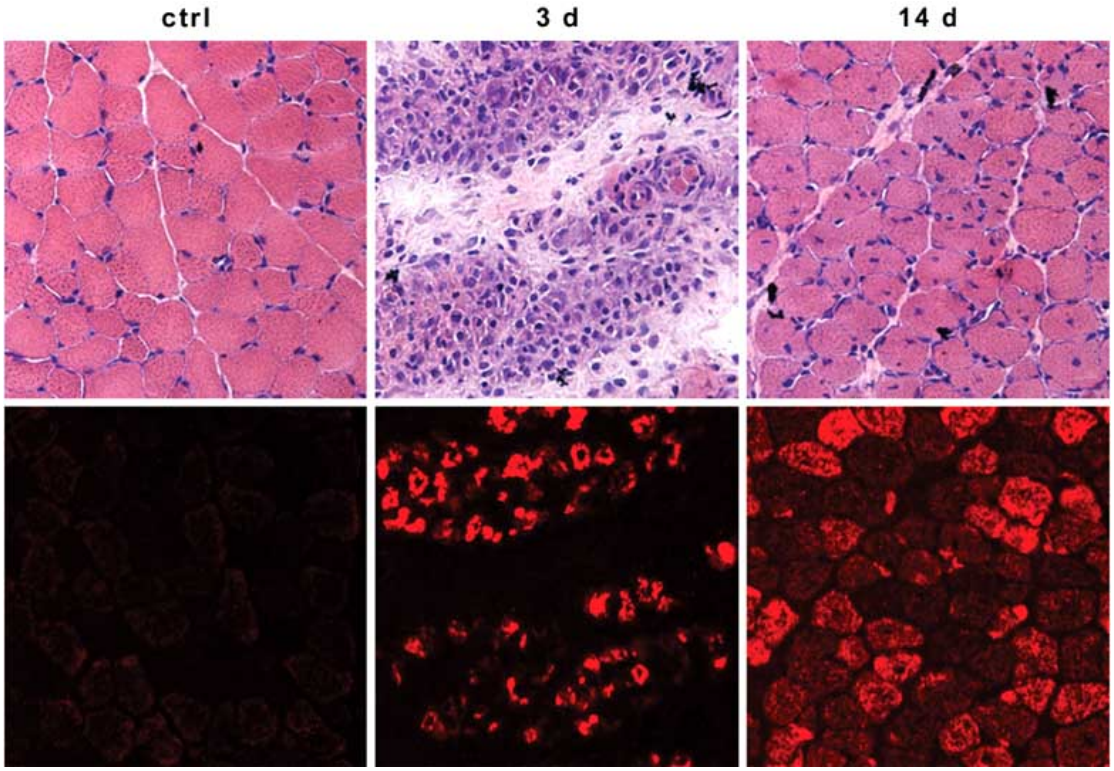

Fig. (3). Myofiber growth and embryonic myosin expression in regenerating skeletal muscle. Muscle regeneration in rat extensor digitorum longus muscle after injury induced by bupivacaine injection. Upper panels, hematoxylin and eosin staining; lower panels, immunostaining for embryonic MyHC. Control muscle fibers (left panels) do not express embryonic MyHC, which becomes detectable in regenerating small myotubes at about day 3 after injury and is still detectable in many regenerated fibers at day 14. Note the presence of centrally located nuclei in most regenerated myofibers at day 14.

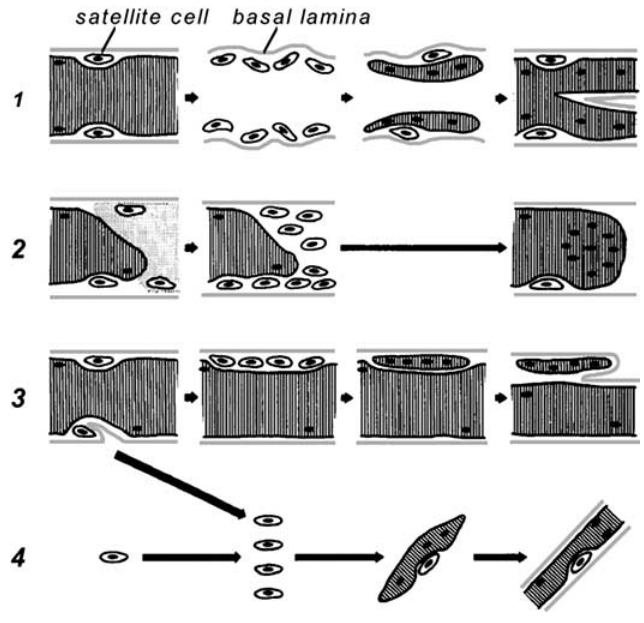

Fig. (4). Patterns of muscle regeneration. The scheme illustrates the variety of patterns of muscle regeneration, including: (**1**) incomplete fusion of newly formed myotubes with formation of forked fibers; (**2**) segmental necrosis followed by satellite cell fusion with the viable myofiber stump; (**3**) satellite cell differentiation and fusion under the basal lamina of surviving fibers with formation of satellite myofibers; (**4**) formation of new myofibers outside the basal lamina from migrated satellite cells or stem cells from other sources (a rare event). Inflammatory changes, including invasion by macrophages, are not shown in this scheme. Modified from [37].

3. ROLE OF NERVE ACTIVITY IN MUSCLE REGENERATION

The first stages of muscle regeneration after injury, including activation, proliferation, differentiation and fusion of satellite cells, can take place in the absence of the nerve. However, subsequent growth and maturation of newly formed myofibers requires the presence of the nerve [39]. If neuromuscular connections are not re-established, regenerating myofibers remain atrophic (see also below, section 5).

The role of nerve activity in muscle regeneration is clearly illustrated in the rat soleus muscle after muscle injury induced by bupivacaine injection. Bupivacaine (marcaine) causes the necrosis of all soleus muscle fibers, leaving satellite cells and nerve terminals, as well as blood vessels, intact. In this experimental model, until about day 3 after injury the regeneration is almost indistinguishable between innervated and denervated muscles, which are both composed of thin myotubes expressing embryonic MyHC. Nerve terminals start to contact newly formed myotubes at about day 3 after injury [40] and in the next 1-2 days innervation appears to be fully functional in many fibers [41]. Whereas innervated muscles undergo a rapid growth after day 3, denervated regenerating myofibers do not display further growth. Nerve activity is the crucial factor, as electrical stimulation is able to promote muscle growth in denervated regenerating muscle [42].

Regenerating muscles initially express MyHCs that are typical of developing muscle, such as embryonic and neonatal MyHCs, but soon start to express adult fast MyHCs [43]. This MyHC switch is a default program which occurs also in the absence of the nerve [44], however a second switch to slow MyHC, which can be detected by day 5 after injury in regenerating soleus, is clearly dependent on slow motor neuron activity because it does not occur in denervated muscle but can be induced by electrical stimulation of denervated

regenerating muscle with a slow-type impulse pattern (Fig. (**5**)) [42].

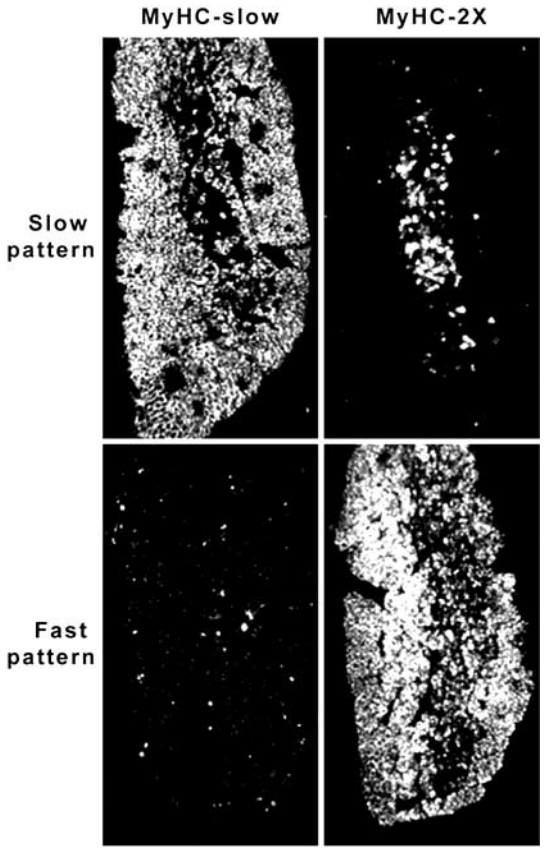

Fig. (5). Effect of electrical stimulation on myosin gene expression in regenerating rat soleus muscles. Denervated soleus muscles were stimulated *via* electrodes implanted on the muscles from 3 to 30 days after bupivacaine injection, receiving either a slow 20 Hz stimulus pattern, resembling the firing pattern of slow motor neurons, or a fast 100 Hz stimulus pattern, resembling the firing pattern of fast motor neurons. In situ hybridization using probes specific for MyHC-slow and fast MyHC-2X. Note that MyHC-slow is induced and fast MyHC-2X is repressed by the slow pattern, except in a central core of atrophic non stimulated fibers that maintain the default fast profile of MyHC gene expression. In contrast, MyHC-slow is not induced in muscles stimulated with the fast pattern, which show widespread expression of MyHC-2X transcripts.

What are the signaling pathways that mediate the effect of nerve activity on muscle growth and fiber type specification? We have previously shown that different pathways are involved in these effects. Muscle growth but not MyHC slow expression can be rescued by transfecting regenerating denervated soleus muscle with a constitutively active Ras double mutant (RasV12C40), which is know to activate selectively the Akt-mTOR pathway [45]. Constitutively active Akt is also able to prevent muscle fiber atrophy when transfected in regenerating denervated muscles [46]. Conversely, rapamycin, a specific mTOR inhibitor, blocks the growth of regenerating innervated soleus but does not interfere with MyHC slow upregulation.

However, Akt activation is not able to drive the expression of MyHC-slow in regenerating muscle. Two other signaling pathways have been implicated in this process: the Ras-ERK and the calcineurin/NFAT pathway. A constitutively active Ras double mutant (RasV12S35), that selectively activates the ERK pathway, is able to induce the expression of MyHC-slow in denervated regenerating soleus [45]. The calcineurin-NFAT pathway is also involved in the fiber-type regulation, as shown by the finding that calcineurin inhibitors, cyclosporin A, FK506 and cain/cabin-1, are able to block the up-regulation of MyHC-slow in regenerating innervated soleus muscles [47]. Calcineurin is a phosphatase which dephopshorylates the transcription factors of the NFAT family (NFATc1, -c2, -c3 and -c4), inducing their nuclear translocation. NFATs mediate the effect of innervation on fiber type specification in regenerating muscle, as shown by the finding that i) constitutively active NFATc1 is sufficient to drive MyHC-slow expression when transfected in regenerating denervated rat soleus muscle [48] and ii) the expression of MyHC-slow in regenerating innervated soleus is blocked by VIVIT, a specific peptide that interferes with calcineurin-mediated activation of NFATs [48], or by inactivation of NFAT transcripts by specific siRNAs [49].

4. MUSCLE REGENERATION IN MUSCULAR DYSTROPHIES

Different human muscular dystrophies, including Duchenne muscular dystrophy (DMD) and most limb girdle dystrophies, and inflammatory myopathies, such as dermotomyositis or polymyositis, are characterized by repeated cycles of muscle fiber necrosis and regeneration. Similar changes are observed in animal models of muscular dystrophy, including the dystrophin-deficient *mdx* mouse, a model of DMD with a milder phenotype compared to DMD. These degeneration/regeneration cycles usually involve scattered myofibers or small fiber bundles, and lead with time to alterations in muscle structure with variation in myofiber size, due to incomplete fusion of regenerating myotubes leading to forked fibers (Fig. (**4**)), progressive exhaustion of the regenerative capacity of satellite cells and substitution of muscle tissue with fibrous and adipose tissue [50]. The defective regeneration of dystrophic muscle is especially evident at advanced stages of the dystrophic process. We have compared the regenerative response to freezing injury in skeletal muscles from young and old normal and dystrophic mice, using the dy^{2J}/dy^{2J} mouse, an animal model of laminin alpha2 deficient congenital muscular dystrophy [51]. As shown in Fig. (**6**), muscle regeneration following freezing injury, evaluated by the presence of regenerating myofibers reactive for embryonic myosin, was strikingly impaired in old dystrophic muscle. Several hypotheses have been proposed to account for the progressive inability of muscle precursor cells to proliferate and/or differentiate [52]. Myoblasts isolated from DMD patients demonstrate decreased proliferation potential *in vitro* [53, 54], possibly due to replicative aging. Delayed fusion and differentiation of satellite cells from dystrophic muscle has also been described [55, 56]. An alternative possibility is that the microenvironment of dystrophic muscle, including the presence of inflammatory and immune cells, may interfere with muscle regeneration [57]. Cytokines like TGF-β, a known fibrosis mediator, which is increased in dystrophic muscle, could inhibit muscle regeneration, a notion supported by the amelioration of the dystrophic phenotype induced *in vivo* by anti-TGF-β antibodies in the *mdx* mouse model [58]. Another cytokine, osteopontin, has been implicated in muscular dystrophy, as genetic ablation of osteopontin from *mdx* mice caused a considerable reduction in intramuscular inflammatory cells and in TGF-β levels, correlated with a marked decrease in fibrosis of both diaphragm and cardiac muscles [59]. The fact that the regeneration defect of dystrophic muscle is especially obvious with age may be due to an age-related impairment of the Delta-Notch pathway [25, 60] (see below, section 6).

5. MUSCLE REGENERATION IN SPORT MEDICINE

Muscle injuries resulting from contusions and strains are the most common traumas occurring in many sports [38]. In both contusions, due to extrinsic blows, and strains, due to excessive intrinsic tensile forces resulting from muscle contractions, the injury involves not only the myofibers but also the basal lamina and the endomysial/perimysial sheaths. The mildest form of strain injury is represented by the so called "delayed onset muscle soreness"

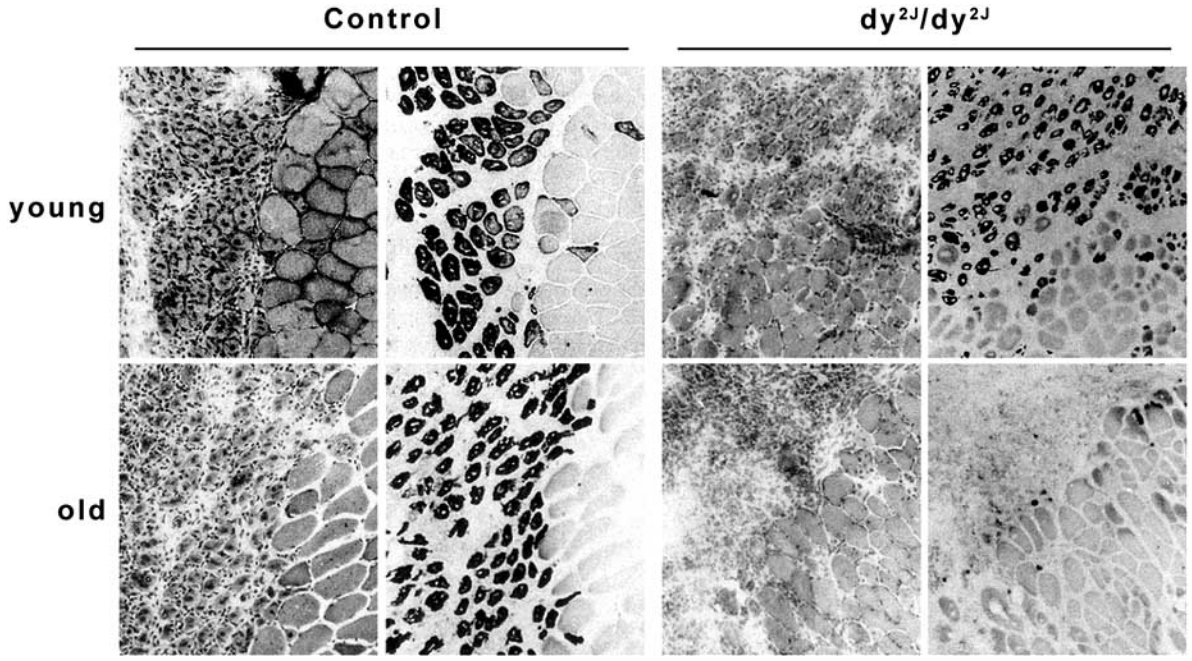

Fig. (6). Muscle regeneration is impaired in dystrophic old mice. Immunostaining for embryonic myosin at day 5 after freeze injury in cryosections of tibialis anterior muscle from young (2-month old) and old (12-month old) control mice and young and old dystrophic mice. The dy^{2J}/dy^{2J} mouse is an animal model of laminin alpha2 deficient congenital muscular dystrophy. No significant difference in the number of labeled regenerating fibers is detectable between muscles from young and old control animals, although the size of regenerating myofibers is smaller in old muscles. In contrast, muscle regeneration is severely impaired in old dystrophic animals, regenerating fibers being almost absent. First and third columns, hematoxylin and eosin; second and fourth columns, immunoperoxidase staining with antibody to embryonic MyHC. Modified from [51].

(DOMS), characterized by stiffness and soreness in the first few days after physical exercise that usually disappears within a week. DOMS is a consequence of excessive exercise of untrained muscle, which is commonly elicited by eccentric work, i.e. lengthening of contracting muscles like in running downhill. DOMS may cause increased levels of creatine kinase levels in serum and satellite cell activation and proliferation, however myofiber necrosis and muscle regeneration are not present in this condition [61]. In contrast, real strains cause rupture of myofibers, basal lamina and blood vessels, ruptures being most frequently located close to the myotendinous junctions. Following the strain, ruptured myofibers contract and the gap is filled by a hematoma, which is invaded first by macrophages then by fibroblasts which produce collagen and form scar tissue. Satellite cell activation, proliferation and fusion take place at the level of the stumps of the ruptured myofibers, where new myotendinous junctions between the regenerating myofiber stumps and the intervening scar tissue are formed (Fig. (**7A**)). These specialized junctions consist of fingerlike projections of myofibers that penetrate into the connective tissue and are enriched in dystrophin and integrin complexes involved in the transmission of the mechanical force to the extracellular matrix. Early immobilization is required after a strain to allow the formation of scar tissue that must withstand contraction-induced forces, thus avoiding the risk of reruptures. However, mechanical loading is essential for the subsequent maturation of myotendinous junctions and muscle remodeling, therefore cautious and gradual remobilization should be started some days after muscle injury. If immobilization is prolonged, regenerated myofibers remain atrophic and their orientation is more disordered. Rehabilitation and functional recovery after muscle strains thus requires difficult decisions about when starting remobilization. Restoration of muscle function after injury may require the innervation of myofiber stumps separated from the neuromuscular junction by scar tissue (Fig. (**7B**)). If nerve sprouts do not succeed in establishing new neuromuscular junctions, denervated myofibers undergo atrophy and cannot contribute to muscle function.

6. AGE-DEPENDENT DEFICIT IN MUSCLE REGENERATION

Loss of muscle mass, also called sarcopenia, and corresponding decrease in muscle force and endurance occur with aging and contribute to a state of frailty, a major cause of morbidity and mortality in the elderly. Muscle atrophy is due to decrease in size of single myofibers and in their total number, and a parallel decrease in the number of motor units [62, 63]. These changes are accompanied by changes in fiber type profile, for example in rat skeletal muscles there is a switch from fast type 2B to 2X fibers in fast muscles and from type 2A to type 1/slow fibers in slow muscles [64, 65].

A loss of myonuclei which precedes the decrease in fiber size has been reported in aged skeletal muscle [66]. There are conflicting reports about changes in the number of satellite cells with aging, but most studies point to an age-dependent reduction of muscle regenerative capacity due to reduced satellite cell proliferation and differentiation (see [67]). The balance between Notch and Wnt signaling, which control satellite cell proliferation and differentiation, respectively, appears to be altered in aged muscle. Notch signaling is impaired, as the Notch ligand, Delta, is not upregulated following injury in aged muscle, and forced activation of this pathway with a Notch-activating antibody can restore the regenerative potential [25]. On the other hand, Wnt signaling is activated in aged myogenic precursors and this can affect cell lineage: satellite cells from aged mice show in fact a conversion from a myogenic to a fibrogenic lineage phenotype, that may be responsible for the increased tissue fibrosis seen with aging [68].

Cell-extrinsic influences on satellite cell function may also change with age [67]. Heterochronic transplantation experiments show that muscles from old rats grafted into young hosts regenerate greater muscle mass and force compared to similar grafts into old hosts; conversely, regeneration of old muscle into an old host is impaired compared to that into a young host [69]. This results was confirmed by parabiosis experiments in mice, showing that satellite

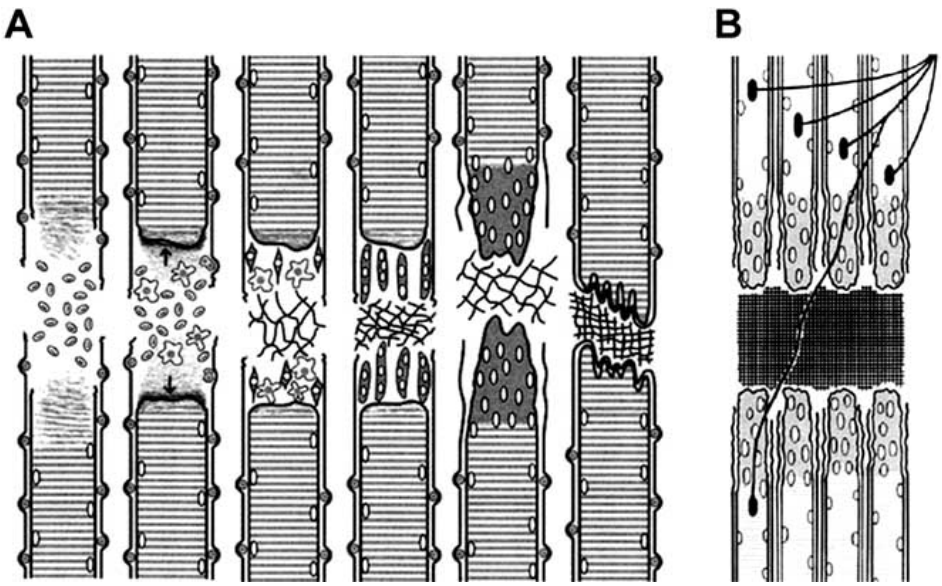

Fig. (7). Muscle regeneration following strain injury. A. Myofiber ruptures after strain injury cause the formation of scar tissue that prevents the reunion of myofiber stumps. Newly formed myotendinous junctions are formed between regenerated myofiber stumps and the intervening scar. **B.** As a consequence of myofiber ruptures and separation of the stumps by scar tissue, distal stumps not containing neuromuscular junctions become denervated but can be reinnervated by axon sprouts (one sprout depicted). Modified from [38].

cell proliferation and muscle regeneration is improved and the Notch ligand, Delta, is upregulated in muscles from an aged mouse parabiotically joined to a young mouse [60]. This effect is not due to the engraftment of circulating progenitor cells from the young partner but must result from an increase of positive factors in young mouse serum and/or a decrease or dilution of inhibitory factors present in old mouse serum. *In vitro* experiments further showed that young serum promotes the activation and proliferation of aged satellite cells, suggesting that muscle-specific stem cells retain their intrinsic proliferative potential but age-related changes in the systemic environment preclude full activation of these cells and muscle regeneration. Heterochronic parabiosis also prevented the increase in fibrogenesis during muscle regeneration in aged mice and reversed the age-related myogenic-to-fibrogenic conver-sion, and these changes were accompanied by decreased Wnt signaling observed in cultured satellite cells exposed to serum from aged mice [68]. The systemic factors that affect the response of satellite cell to injury and thus the success of muscle regeneration have not yet been identified.

7. BOOSTING MUSCLE REGENERATION

Muscle wasting and corresponding decrease in muscle force and endurance occur not only in muscle diseases, such as muscular dystrophy, but also as a result of disuse, such as prolonged bed rest, and during aging. Loss of muscle mass in these conditions is in part due to impaired muscle regeneration and altered satellite cell function, which has been clearly demonstrated in dystrophic and aged muscle (see above, section 4 and 6). The finding that systemic factors present in serum may affect the response of satellite cell to injury and muscle regeneration opens the way to the development of therapeutic programs to boost muscle regeneration and thus rescue muscle loss.

Both positive and negative regulators of muscle growth have been identified. Insulin-like growth factor 1 (IGF-1) secreted by the liver under the control of growth hormone (GH) or produced locally in various tissues, including skeletal muscle, is the best characterized positive growth regulator. The *IGF-1* gene can generate multiple isoforms by use of variable transcriptional start site and alternative splicing, specific IGF-1 isoforms produced by muscle cells being especially potent in promoting muscle growth and regeneration (see [70]). IGF-1 overexpression in transgenic mice was found to enhance the regenerative capacity of aged skeletal muscle [71]. In addition, IGF-1 affects the balance between protein synthesis and protein degradation thus inducing muscle hypertrophy and preventing muscle atrophy, promotes the recruitment of circulating non muscle stem cells to the injured muscle and mediates the resolution of the inflammatory response [70]. We have recently examined the effect of a major downstream effector of IGF-1, the kinase Akt/PKB, using a muscle-specific inducible Akt transgene. Muscle hypertrophy was rapidly induced upon induction of Akt and this was found to rescue the functional impairment produced by eccentric contractions in the dystrophin-deficient *mdx* mouse [72]. However, Akt-dependent muscle hypertrophy was not accompanied by satellite cell activation and proliferation [73].

Myostatin (*alias* GDF8), belongs to the TGFβ superfamily of growth factors and is produced predominantly by skeletal muscle. Acting via the activin receptor IIB (ActRIIB) and Smads transcription factors, myostatin negatively controls muscle growth, as shown by the finding that myostatin knockout animals show marked increase in muscle mass due to both increase in fiber number and size [74]. However, myostatin has no significant effect on satellite cell proliferation *in vitro* and muscle hypertrophy arising from lack myostatin is not associated with increase in either the number or recruitment of satellite cells [75]. Accordingly, muscle hypertophy induced by transfecting adult skeletal muscles with a dominant negative ActRIIB is not accompanied by prolife-ration of satellite cells, as determined by BrdU incorporation experiments [76].

An alternative approach to compensate for the muscle regene-ration deficit found in muscular dystrophy is cell therapy using either muscle or non muscle stem cells. A discussion of this aspect is outside the scope of this review, this topic has been recently reviewed elsewhere [77].

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