Review

THE SATELLITE CELL OF SKELETAL MUSCLE FIBRES

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ABSTRACT

Satellite cells of adult skeletal muscle fibres are myogenic monoculeated cells that are closely attached to muscle fibres. These cells provide new myonuclei during growth and regeneration; myonuclei are postmitotic. Three to 11 of myonuclei seen by light microscopy in reality are satellite cell nuclei. Developing muscles contain up to 35% satellite cells. Their incidence decreases after denervation and possibly also with age. They are more numerous in slow-twitch than in fast-twitch muscles. The turnover rate of myonuclei in normal muscles is at most 1% per week, and satellite cells are quiescent. Injury, excessive muscle activity, mechanical stretching and also androgens induce proliferation and eventually fusion into myotubes. Myostatin keeps the satellite cells quiescent, and hepatocyte growth factor (HGF) induces activation. Myoblasts during muscle development express Pax3 while satellite cells express Pax7. Pax3 is upregulated in activated satellite cells. Satellite cells form a self-sustaining population, and when labelled satellite cells are grafted into a necrotic muscle, the label occurs not only in the new myonuclei but also in the satellite cells of the new fibres. Satellite cells are stem cells that may form haematopoetic colonies, and bone-marrow- and endothelium-derived cells may become myogenic. Nevertheless, the capability of these cells to replace each other is apparently limited. Satellite cells from aged individuals are activated with delay, possibly also their number declines. The delay is due to impaired Notch signaling and becomes normal in parabiotic old-young animals. The length of the telomeric DNA decreases with the number of mitotic cycles and therefore is shorter in aged individuals, in overworked muscles and in children with muscular dystrophy. The number of mitotic cycles is 50 to 60 when human satellite cells are harvested at birth, but it is only 15 to 20 beyond the age of 20 years. Satellite cells have gained much interest during recent years, because they might provide an entry for therapeutic genes into a stable tissue like muscle and thereby guarantee long-term gene expression. Furthermore, grafting satellite cells from healthy donors into muscles from patients with Duchenne and other types of muscular dystrophy may once become a therapy for these disorders.

Key words: Activation, myoblasts, denervation, old age, regeneration

INTRODUCTION

Satellite cells are mononucleated myogenic cells attached to skeletal muscles fibres and in impressions of the myofibre enclosed by its basal lamina tube (Fig. 1). The gap between the plasmamembranes is only 20 to 40 nm wide and hence invisible by light microscopy. Although satellite cell nuclei are slightly more heterochromatic than myonuclei, this criterion is not distinct enough to distinguish nuclei of satellite cells and of muscle fibre by means of routine light microscopy. The discovery of satellite cells by electron microscopy by Mauro [66] in 1961 marks a turning point in our understanding of muscle development, growth and regeneration. More than 100 years of misconceptions about these matters still influence the interpretation of morphology and histogenesis of diseased muscle. It may therefore be justified to give a brief historical overview. The last few years have seen a surge of interest in satellite cells, because skeletal muscle is a postmitotic tissue with the ability to regenerate by means of stem cells.

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History

In 1839, Schwann [97], who first discovered that cells are constituents of all animal tissues,

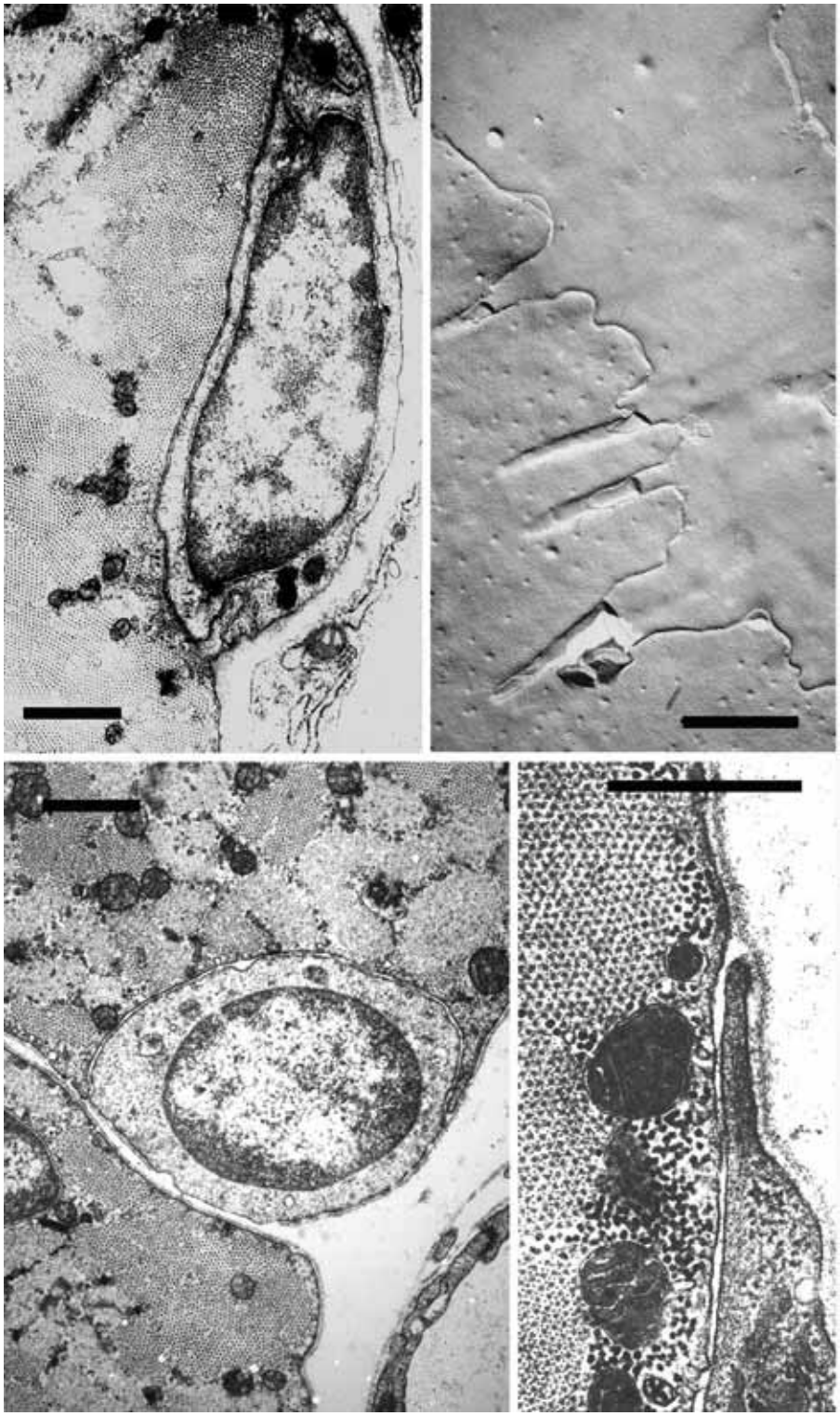

Figure 1. Electronmicrographs of satellite cells**. Top left**: Quiescent satellite cells of normal rat soleus muscles. **Top right**: Freeze-fracture of a satellite cell from a rat soleus muscle. The P-faces of the satellite cell and the underlying muscle fibre is seen. The satellite cell has several lateral extensions some of which are broken away. Note that the plasmamembrane of the muscle fibre but not that of the satellite cell carries numerous caveolae. **Below left**: Activated satellite cell of a regenerating rat soleus muscle. Note the increased cytoplasmic volume of the activated cell. **Below right**: Human brachial biceps muscle. High magnification to show the position of the satellite cell within the basal lamina tube of the muscle fibre. The gap between the two plasmamembranes is too narrow to be detected by light microscopy. Bars: 1 μm

correctly observed that multinucleated muscle fibres arise from the coalescence of mononucleated cells, which means that muscle fibres are syncytia. This finding was contested by Kölliker [55], an influential anatomist of his time, and for 100 years the textbook teaching was that that muscle fibres arise from a single cell that becomes multinucleated by repeated nuclear divisions. According to this hypothesis, myofibres were plasmodia. The striking lack of mitotic figures in muscle fibres was explained by amitosis, i.e., a mechanism of nuclear division without anteceding duplication of chromosomes.

Muscle fibres were believed to grow by gaining more and more nuclei by more and more amitotic divisions, and to multiply by longitudinal fission inside muscle spindles [114]. It was not before the fifties and sixties of the last century that amitosis, which would increasingly reduce the DNA content of myonuclei, was disproved and that the fusion of myoblasts into myofibres was accepted. In 1970, the probably last paper claiming amitosis in skeletal muscle fibres was published [43]. Interestingly, the 2004 edition of Encyclopaedia Britannica does not even list the term amitosis any more. Incompletely fused and therefore branching fibres that may form during regeneration are misinterpreted as evidence of splitting [89,90]. Although the true nature of muscle spindles has long been established, longitudinal fission ("splitting") of muscle fibres is still widely accepted among pathologists.

Regeneration of skeletal muscle was explained along the plasmodium theory, or it was even claimed that skeletal muscles do not regenerate [55]. The second point contradicted obvious regeneration meticulously described and illustrated already in 1864 by Zenker [119] in patients who had died from typhoid fever but had survived long enough to allow partial recovery from muscle necrosis. This author even depicted mononucleated cells that fused into small myotubes, an observation widely neglected, probably because of Kölliker's preconception. Volkmann [110] experimentally transected muscles and described that the fibre fragments formed buds and sprouts with nuclear clusters. These observations were the basis of the regeneration *per continuum* theory [18] that prevailed into the seventies of the last century. Even after Mauro's [66] observation, the existence of mononucleated myoblasts in normal muscle was contested, and it was claimed that they only occur after muscle injury. It was assumed that myonuclei released from damaged fibres "gather" cytoplasm and thereby become myoblasts. Still in 1976, a referee recommended rejection of a manuscript on satellite cells in normal human muscle by the present author [93], because "it is known, that normal human muscle do not have satellite cells." Today, it is firmly established that myonuclei are postmitotic and diploid, that muscle fibres are formed by fusion of myoblasts, and that mononuclear cells present in all skeletal muscle provide new myonuclei if needed during growth or regeneration (for refs. [91]).

The number of satellite cells

As stated above, it is not possible reliably to identify satellite cells by means of routine light microscopy. Even counts obtained after membrane staining with anti-dystrophin or anti-laminin - procedures that facilitate identifying of satellite cells - must be considered questionable. Originally, satellite cells had to be counted on cross sections in the electron microscope, and their number was given relative to myonuclei. Electron microscopy is still the gold standard for counting satellite cells. It is obvious, that the raw counts have to be corrected for differences in nuclear lengths. Usually nuclei of satellite cells are shorter than myonuclei and the incidence of satellite cells is therefore underestimated without correction. An increased length of satellite cell nuclei that may occur under experimental conditions will then mimic an increased incidence.

For practical reasons, satellite cells are now mostly identified by immunohistochemistry. The two commonly used markers are N-CAM [15] or M-cadherin [13,48] (Fig. 2), less often Pax7 (see below). There exists a number of other markers that are specific for satellite cells (for refs. [16]), but they are either less easily used than N-CAM or M-cadherin, or they identify only a fraction of the satellite cell population. While immunological markers are important to label myogenic cells and to determine the stage of activation and differentiation, counts should always be related to electron microscopic counts in order to ensure that the label marks really all satellite cells.

In various muscles of different animals, 3% to 11% of all nuclei within the contour of the muscle fibres belong to satellite cells (refs in [91]). Higher numbers are found in newborn rodents (30 to 35%). In mice and rats, a steep decrease occurs during the

first weeks of life; the decrease with age becomes less pronounced in older animals [1]. The levator ani of rats 4 and 32 months of age, respectively, contains the same percentage of satellite cells [79]. There is possibly a tendency for the percentage of satellite cells to decrease in humans, but the results are controversial. Kadi *et al.* [51] stained the cells against N-CAM and found fewer satellite cells as related to myonuclei in humans aged 70-83 years than in humans aged 20-33 years; the number of myonuclei per fibre, however, had increased in the elderly subjects while that of satellite cells was found to have decreased. Sajko *et al.* [88] observed a decrease of the number of anti-M-Cadherin stained satellite cells between the age of 30 and 70 years. Roth *et al.* [87], however, by means of electron microscopy were unable to find differences between 14 subjects aged 20 to 30 years and 15 subjects aged 65 to 75 years, also when data from males and females were separately analyzed.

It is possible to determine the total number of myonuclei per unit volume or weight muscle, either morphometrically or by measuring the DNA content and correcting for the contribution of connective tissue nuclei. Absolute numbers for satellite cells can then be computed from their incidence relative to myonuclei. In rats of 200-250 g weight, 5,000 satellite cells per mm^3 (mg) are in the diaphragm and the soleus muscle and 1,000 in the superficial white part of the anterior tibial muscle [94]. The number in the biceps muscle of adult humans is about 800/mm^3 (Schmalbruch, unpublished). Gibson and Schultz [39] found in the extensor digitorum longus muscle of rats aged 1, 12 and 24 months 7,700, 1,150 and 700 satellite cells/mg and in the soleus muscle at the same ages 12,600, 3,300 and 2,400 satellite cells/mg muscle. This dramatic decrease is mainly due to the growth of the muscles, at the same time the number of myonuclei in a muscle increases by 60 to 100% between one and 24 months. The total number of satellite cells per muscle remains constant in the soleus muscle but decreases from 3.1 $x10^5$ to 1.3 x 10^5 in the extensor digitorum longus muscle [39]. This demonstrates that the percentage of satellite cells in relation to muscle nuclei may be deceiving when different muscles are compared.

Nuclei of satellite cells in contrast to myonuclei synthesize DNA and are able to divide. Moss and Leblond [75,76] observed that in growing rat muscles after each mitotic division one of the daughter cells fuses with the underlying muscle fibre; the other daughter cell retains its position as satellite cell

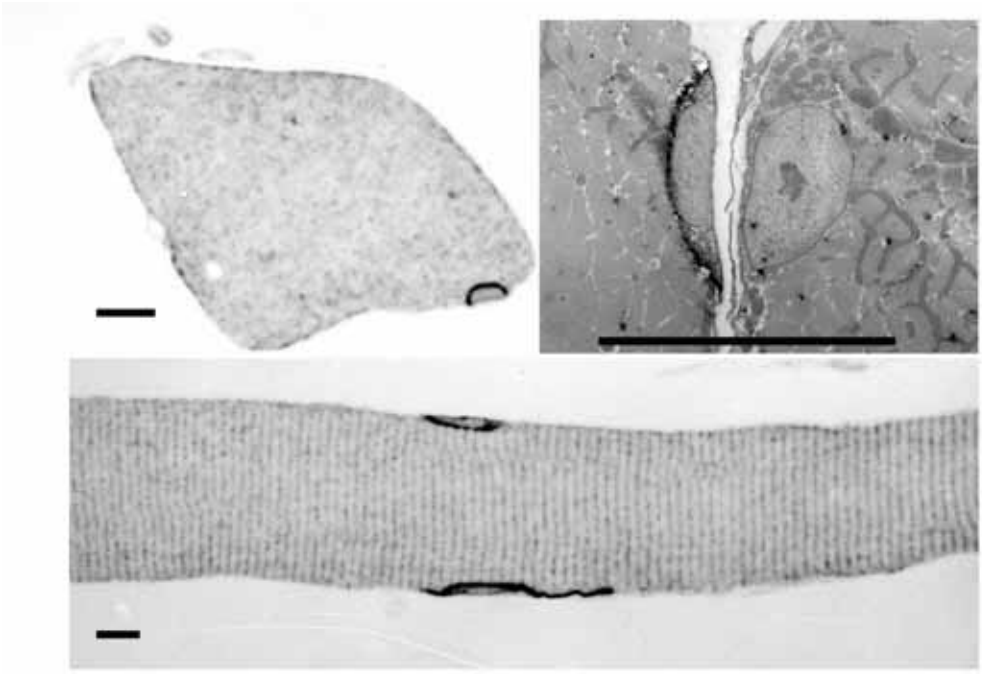

Figure 2. Rat soleus muscle, immunostained for M-Cadherin and embedded in epoxy resin, ultrathin section for electron microscopy (top right) and semithin sections for light microscopy (top left and below). The reaction product is bound to the plasmamembrane of the satellite cell facing the underlying muscle fibre. Opposite to the satellite cell nucleus (top right) is a myonucleus. Bars: 10 μm (modified from [13]).

close to the new myonucleus. This mechanism would ensure that the number of satellite cells remains constant while that of myonuclei increases. Nevertheless, this cannot be true for all muscles given the distinct decrease of the total number of satellite cells reported by Gibson and Schultz [39] for the extensor digitorum longus muscle of adult rats. Either, some satellite cells die in this muscle or both daughter cells fuse.

When isolated and transferred to a culture medium, a satellite cell is deprived of the possible controlling influence of the muscle fibre it adheres to and may become activated. In order to circumvent this problem, intact muscle fibres may be kept in culture. This allows evaluating the reaction of quiescent satellite cells to external influences [115]. A useful marker in this situation is Pax7 that is expressed in quiescent satellite cells only but not in activated myoblasts. There might, however, be transitory stages with overlapping expression of Pax7 and Pax3, a marker for activated satellite cells [84]. Single fibres of the extensor digitorum longus muscle of mice have an average of 7 quiescent satellite cells attached to it, and fibres of the tibialis anterior muscle have 9 cells, while there are 22 satellite cells on fibres of the soleus muscle [19]. Similar figures are reported by Shefer *et al.* [102] for young adult mice. These authors furthermore found a decrease to only 3 cells/fibre in both soleus and extensor digitorum longus muscles in old age (28-33 months).

The origin of satellite cells

Myoblasts are derived from mesodermal somatic cells localized in the dermatomyotome, the dorsal part of the somite. Cranial muscles have a different origin that is not to be discussed here. Mesodermal cells become committed myoblasts and migrate laterally to form the myotome and eventually the skeletal musculature. Pax3 expression promotes myogenesis and proliferation of these myoblasts. Early myoblasts express the myoregulatory factors Myf5 or MyoD or both. Eventually, the cells withdraw from the cell cycle and become terminally differentiated myoblasts that express the myoregulatory factors Myogenin and MRF4. Terminally differentiated myoblasts fuse and express other muscle specific proteins such as myosin heavy chains. Satellite cells appear rather late during fetal development (for refs. [16]).

While some authors assume that satellite cells have the same origin as myoblasts forming muscle fibres during myogenesis, i.e., the dorsal part of the somite [3,40], others maintain that they originate from endothelial cells. The reason for the latter hypothesis is that blood vessels and also other tissues can provide myogenic cells during muscle regeneration (see below). This is not necessarily a contradiction because the aortic endothelium and the somites are adjacent during early embryogenesis. Although endothelial cells and myogenic cells may have a common origin, it has not been demonstrated that endothelial-derived myogenic cells can become quiescent satellite cells [16]. Satellite cells specifically express the transcription factor Pax7 gene and thereby differ from myoblasts that express Pax3 that, however, is a closely related gene. Also cells that express both Pax3 and Pax7 do not express muscle specific markers [84,85]. Pax7 knock-out mice lack satellite cells [99] or have only very few [81]. Interestingly, although regeneration in these mice is limited, some myotubes are formed after muscle injury. These originate from myogenic cells in the interstitium of the muscle and, in contrast to typical satellite cells, express Pax3. Myofibre-attached satellite cells in wild-type mice never express Pax3 [56].

It has been found that bone marrow cells when grafted into a muscle may start to express Pax7, form myogenic cells and contribute to muscle regeneration [32,38,58]. Similar results were obtained with fetal liver [38], mesenchymal cells of the synovial membrane [26], or adipose tissue-derived stromal cells [4]. Bone-marrow derived cells grafted into irradiated mice appear in the interstitium of the muscles or beneath the basal lamina of muscle fibres, express satellite cell markers and form myotubes [35]. Sherwood *et al.* [104] labelled and separately isolated original muscle-resident satellite cells and putative myogenic cells derived from bone-marrow transplants. Bone-marrow derived cells expressed fewer satellite cell markers than original satellite cells and showed very little myogenic potential in culture. Cells isolated from the interstitial space of skeletal muscles might differentiate into endothelial cells or into myogenic cells [106]. On the other hand, satellite cells isolated from adult mouse muscles typically expressing Pax7 might stop to do so and become mature adipocytes or smooth muscle cells [103] or haematopoetic cells [49,98]. Stem cells isolated from muscles of Pax7 knock-out mice that lacked typical satellite cells had an increased ability

to form haematopoetic colonies as compared to stem cells from wild-type mice [99]. Satellite cells grafted into the heart after experimental myocardial infarction not only survive but, at least according to some [9,44,47], but not all authors [83], improve cardiac function and express cardiac-specific contractile proteins. In summary, satellite cells apparently belong to a pluripotential stem cell pool. It should, however, be kept in mind that regeneration of skeletal muscles of Pax7 knock-out mice is dramatically reduced and that the contribution of non-muscle cells to regeneration of muscle, if any, is small [82].

Activation of satellite cells

The turnover rate of myonuclei determined by BRDU incorporation in normal muscle of adult rats is 1-2 % per week [96]. While mitotic spindles in satellite cells are extremely rare in adult intact muscles, they become abundant within 2 days after injury to the muscle [92] (Fig. 3). Bischoff [7,8] isolated intact muscle fibres in culture and observed that the satellite cells remained quiescent and did not proliferate. When he added extract from crushed muscle, the cells started to divide after 16 h, but proliferation stopped when the extract was withdrawn. On the other hand, when the muscle fibre had been killed with the myotoxic local anesthetic bupivacaine, proliferation continued even when the extract was withdrawn after 8-12 h. These early experiments demonstrate that an intact muscle fibre blocks proliferation while a factor from damaged muscle initiates proliferation.

Myostatin, a TGF-beta member, inhibits satellite cell proliferation, and an increased number of proliferating satellite cells are found in myostatin knock-out mice [69]. Muscle regeneration in these mice is enhanced, probably not only because more satellite cells are activated but also because invasion and removal of inflammatory and phagocytotic cells are accelerated [70]. Another factor that has been implicated with keeping the satellite cells quiet is caveolin-1 [111]. Caveolin-1 is expressed in satellite cells but not in muscle fibres, and it is down-regulated by hepatocyte growth factor (HGF) which is produced after muscle injury and which is generally considered to be the principal initiator of satellite cell proliferation [25,73,107]. Fibroblast growth factor (FGF) is apparently equally effective as HGF in initiating proliferation. Neutralizing antibodies against FGF inhibit satellite cell proliferation, and quiescent satellite cells express receptors for FGF [50,100,117]. It has, however, been argued, that FGF (and other growth factors)

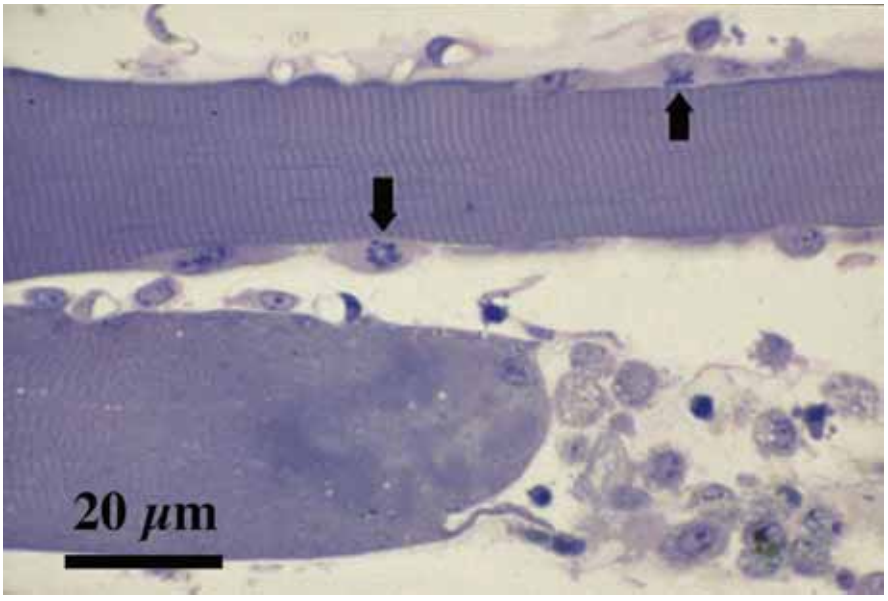

Figure 3. Rat soleus muscle, 2 days after injection of hot Ringer solution (60°C). Longitudinal resin sections stained with toluidine blue. Two muscle fibres are visible, the one below shows segmental necrosis with macrophages invading the basal lamina tube (right). The upper fibre is still intact. Attached to it are at least four activated satellite cells with distinct metachromatic staining. Mitotic figures (**arrows**) are seen in two of the satellite cells. (modified from [92]).

only promote proliferation but are not able to initiate activation [46,116]. HGF is activated by binding to its c-met receptor, that is expressed by most but possibly not all satellite cells [25]. Release of HGF is mediated by nitric oxide (NO) that is produced by a skeletal muscle specific nitric oxide synthetase [2]. Inhibiting this enzyme prevents activation of satellite cells and the colocalization of HGF and c-met. Additional pathways leading to activation have tentatively been suggested, some bypass the release of NO, others lead directly from NO to activation and bypass binding of HGF (for details, see [116]).

An early event during activation is the binding of HGF to c-met. The immunohistochemical demonstration of c-met is, however, not always reproducible across laboratories [116]. When fully activated, satellite cells start to express either Myf5 or MyoD or both, and eventually all activated cells express MyoD but only some Myf5 [24]. Some authors have, however, found that transcription of Myf5 takes place already in quiescent satellite cells, but that the protein itself is lacking. These cells, which are believed to be committed myogenic cells still express CD34, a marker of haematopoetic stem cells together with M-cadherin, the accepted marker of satellite cells. Transcription of CD34 is shut down when the cells differentiate. The process is driven by signaling to the Notch receptor. Inhibition of Notch, as mediated by the Numb protein, leads to progression to the myoblast stage [23,62].

A minority of satellite cells fails to express CD34 and no evidence for the activation of Myf5 is found. The authors speculate, that the latter cells are involved in maintaining the lineage–committed majority. In differentiating cultures, cells that do not express CD34 or MyoD never fuse [6]. Activated satellite cells initially express Pax7 and MyoD, but some downregulate MyoD and become quiescent cells with satellite cell characteristics [118].

Satellite cells are activated not only when the muscle is injured but also by muscle activity [52,53,112], mechanical stretching [108], and androgens [17,80,105]. Chronic low-frequency stimulation of a fast-twitch rat muscle changed its contractile properties towards slow-twitch and induced a 8-fold increase in satellite cell proliferation; the number of myonuclei increased 3-fold. Gamma-irradiation reduced the increase in satellite cell number and attenuated the fast-to-slow transition [65].

Markers of quiescent and activated satellite cells

Quiescent satellite cells among other proteins express Pax7, CD34, c-met, M-cadherin and myostatin. It has been proposed, that myostatin in an autocrine fashion ensures the resting state while HGF is the autocrine factor for activation [101].

When the cells move from G_0 to G_1 and enter their first mitotic cycle, Pax3 expression is upregulated [33]. Once activated, satellite cells undergo rapid proliferation along the myogenic lineage. Decreasing expression of CD34 and high levels of Pax3 expression accompany this.

Most recently, it has been claimed that a decrease of the sphingomyelin content of the plasmamembrane is the earliest signs of satellite cell activation, possibly because the lipid composition of the membrane relates to the binding of signaling molecules [78].

The effect of age on satellite cells

Satellite cells derived from old animals when activated enter the mitotic cycle with delay. This has been linked to loss of Notch signaling [21]. In parabiotic mice when an old animal is exposed to the serum of a young mouse, the regenerative capacity of the satellite cells is restored as is Notch signaling [22].

Another factor that has been discussed is the length of the telomeric DNA in satellite cells of old muscles, possibly because repeated bouts of regeneration exhaust their regenerative capacity. Decary *et al.* [28,29] found that human satellite cells divide more often and survive longer the younger the donor has been. When isolated at birth, the cells divide 60 times, but at all ages beyond 20 years only 15 to 25 cell divisions occur. Similar results had already in 1982 been published by Hauschka [42] who counted 70 cycles in fetal and 30 cycles in adult myogenic cells (for refs. [91]). Telomeres in satellite cells from young children are longer than in adults, but in adult life the length remains relatively constant. A pronounced shortening has, however, been found in children with muscular dystrophy, indicating that extensive regeneration had already occurred [27,29,77]. Athletes who suffered from the "fatigued athlete myopathic syndrome" had dramatic shortened telomere length as compared to control subjects [20], presumably because overload and damage of skeletal muscles had required repeated rounds of regeneration which thus exhausted the proliferative capacity of the satellite cells.

Intuitively, one would expect that the decreased regenerative capacity in old muscles might be due to a decreased number of satellite cells. The findings both in animals and man are controversial and may differ for fast and slow muscles. Dreyer *et al.* [34] investigated young (23-35 years) and old (60-75 years) men before and after a single bout of eccentric contractions and found by means of N-CAM staining in both groups an increased number of satellite cells after the exercise; the increase was greater in young than in old men. The incidences before the exercise did not differ. This observation points toward more rigorous activation in young than in old subjects rather than to different numbers of cells before the exposure to eccentric contractions. Collins *et al.* [19] transplanted single intact muscle fibres into radiation-ablated muscles of nude mdx-mice and found that 7 satellite cells bound to one muscle fibre can give rise to hundreds of new fibres with thousands of new myonuclei. This finding suggests that a modest decline of the number of satellite cells, if any, hardly explains the decreased regenerative capacity of aged muscle. Shefer *et al.* [102], on the other hand, isolated individual fibres of different mouse muscles and identified quiescent satellite cells by staining for Pax7 and CD34. They unequivocally found that the number decreased between 3 and 30 months of age. The authors did not find any differences between the myogenic potential of the cells when cultured and conclude that the declining size of the satellite cell pool is responsible for age-related muscle atrophy.

Inhomogeneity of the satellite cell pool

The source of new satellite cells after bouts of regeneration has been discussed. Some assume, that a fraction of the original satellite cells does not proliferate to become myoblasts but rather stay in a non-differentiating compartment in order to replace those cells that have been used. Others suggest that some of the proliferating cells fail to differentiate and then become quiescent satellite cells. A third opinion is that stem cells that are present in the interstitium or in the bone marrow become myogenic and take the typical satellite cell position on the new fibres. Experiments by Collins *et al.* [19] who grafted single muscle fibres with labelled satellite cells into irradiated (i.e., stem-cell depleted) muscles indicate, that the satellite cells after grafting expand by a factor 10 to form new myonuclei. If the regenerated muscle was injured and allowed to regenerate once more, the new population of satellite cells still carried the label of the first graft, indicating that a satellite cell population is self-sustaining. These experiments also showed that the regenerative capacity of grafted fibre-attached satellite cells from soleus and EDL was about the same whereas grafts from the anterior tibial muscle were far less effective. Montarras *et al.* [74] generated mice that express fluorescent Pax3 in their satellite cells, isolated these cells by flow cytometry, and grafted them into muscles of nude mdx mice. The labeled cells participated in muscle regeneration and contributed to the satellite cell compartment. The results are thus in accordance with those of Collins *et al.* [19]

Already in 1990, Miller [72] reported that mouse myoblast cell lines differed in the expression of myosin heavy chains. Hartley *et al.* [41] investigated chick myoblasts and concluded that the different expression of myosin reflected that developmental stages of the myoblasts differ or that distinct populations are present in the developing muscles. Hughes and Blau [45] injected labelled myoblasts into muscles of young rats. They found that a clone from one myoblast might contribute to fast and slow fibres and suggested that external influences would override any intrinsic commitment of a myoblast nucleus. Düsterhöft and Pette [36] cultured satellite cells from slow-twitch soleus and fast-twitch anterior tibial muscles of rat and found that myotubes expressed myosin corresponding to the muscle of origin. Similar results were obtained by Cantini *et al.* [14] who used regenerating fast and slow rat muscles as myoblast source. Wehrle *et al.* [113] electrically stimulated cultured myotubes derived from fast or slow muscles and found that a stimulus pattern suited to enhance slow myosin expression was far more effective in cultures from slow than from fast muscles. *In situ* stimulation of non-innervated regenerates of rat muscles again supported the view that fast and slow fibres regenerate from intrinsically different satellite cells [54]. Myoblasts derived from rabbit slow muscles differentiated faster and expressed adult slow myosin isoforms long before myoblasts from rabbit fast muscles did [5]. In contrast, Edom *et al.* [37] cultured human myoblasts and found no difference at all with respect to myosin expression. Finally, the same laboratory [10] isolated, identified and cultured single human muscle fibres; satellite cells from fast-

or slow-twitch fibres had exactly the same program of myosin expression. This suggests that, at least in man, satellite cells on fast or slow-twitch fibres do not represent distinct populations.

Effect of denervation

Denervation of muscles in chick embryos severely reduces the number of clonable myoblasts [11], and denervation in newborn rats not only rapidly leads to severe atrophy but also depletes the satellite pool almost completely [86]. Schmalbruch and Lewis [95] after denervation of adult rat soleus muscles found that the number of satellite cells initially increased. After 10 days few myotubes occurred which had become numerous after 70 days; signs of fibre breakdown were prominent. The percentage of satellite cells differed greatly between rats at day 70: in some muscles it had decreased, in others it had 3-fold increased. The mitotic activity was reduced (Fig. 4). After 20 to 30 weeks, the percentage of satellite cell nuclei had decreased to one fifth of normal, the decrease being more rapid and more distinct in the soleus than in the EDL muscle [86]. It must be noted, however, that 10 weeks denervation caused a 50% loss of myonuclei in the soleus but not in the extensor digitorum longus muscle [96]. This means that the loss of satellite cells in long-term denervated soleus muscles is underestimated if expressed as percentage of myonuclei. Viguie *et al.* [109] in the extensor digitorum longus muscle of adult rats observed an increase of the percentage of satellite cells 8 weeks after denervation, thereafter their percentage declined. Satellite cells became activated, and new myotubes were formed 10 to 20 days after denervation with a maximum after 2 and 4 months. Two patterns of myogenesis could be distinguished. First new myotubes appeared attached to atrophic but intact fibres, presumably due to loss of neurogenic control. Later, 2 to 4 months after denervation, myotubes were contained in

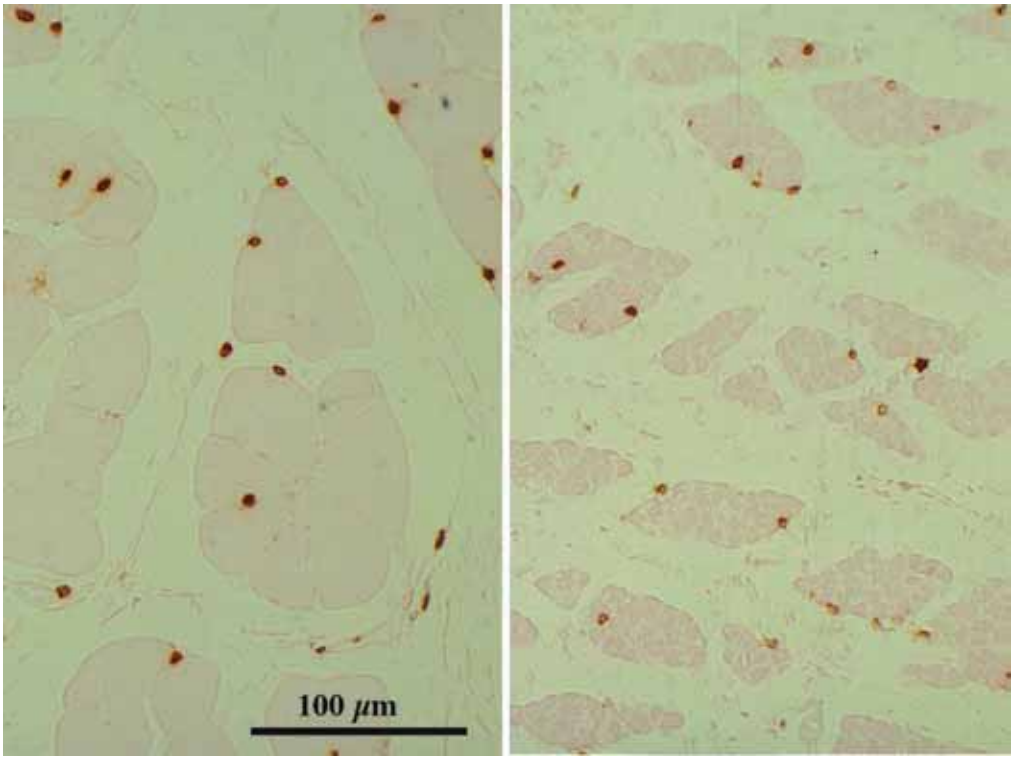

Figure 4. Rat soleus muscles, normal (**left**) and denervated for 6 weeks (**right**). Paraffin cross-sections 10-μm thick stained with eosin. The magnification is the same for both micrographs to show the pronounced atrophy of the denervated rat soleus muscle. Both muscles had been exposed to BRDU for 6 weeks, and DNA-bound BRDU is shown by immuno-histochemistry in order to label nuclei that have undergone mitosis during the experimental period. Numerous unstained nuclei are seen as staining defects in the denervated muscle. The number of fibres with reacting nuclei is much smaller in the denervated than in the innervated muscle indicating that denervation eventually halts the mitotic activity of the satellite cells. Note that also fewer connective tissue cells are labelled in the denervated as compared to the normal muscle (modified from [96]).

empty basal lamina tubes of lost muscle fibres [12]. Denervation-induced activation of the satellite cells was independent of the age of the rats (4 months vs. 24 months) [30]. It is unknown whether the eventual loss of satellite cells after denervation is due to the exhaustion of the satellite cell pool by repeated bouts of regeneration [31] or whether it is the lack of contractile activity of the muscle. The fact that satellite cells cultured from denervated adult rat muscles are less apt to become recruited into the mitotic cycle [57,63,64] favors the second point. It has been suggested that rapid loss of satellite cells and myonuclei and the exceptionally fast atrophy of the rat soleus muscle which are more pronounced than in fast rat muscle (and in the guinea pig soleus muscle [61]) are due to the fact, that the phase of post-denervation fibrillation of the rat soleus muscle is shorter than in other muscles [96]. In any case, the reaction of satellite cells to denervation differs in fast- and slow-twitch muscles of rodents, and observations in mice and rats may not always be valid for human muscles.

CONCLUDING REMARKS

Interest in satellite cells as reflected by the number of publications has followed a bimodal pattern. The sixties to eighties of the last century saw publications dealing with satellite cells in relation to development, maintenance and regeneration of skeletal muscle, obviously as a reaction of the century-old misconception regarding the nature of skeletal muscle fibres (see History). This research has been presented in several symposia sponsored by the American Muscular Dystrophy Association (MDA) [67,68,71] and is only cursorily dealt with in the present review. After a decline in publication rate in the nineties, a deluge of papers came after 2000, provoked by the widespread interest in stem cells. In this respect, satellite cells of skeletal muscle may offer three therapeutic possibilities.

i. They may lead to an adult stem cell.

ii. Muscle being a stable postmitotic tissue may be a useful tool for gene therapy if one succeeds to introduce the transgene *via* satellite cells and to ensure long-term expression by the muscle fibres.

iii. Grafting of adult satellite cells may be a therapeutic tool for genetic muscle diseases such as Duchenne muscular dystrophy. Myoblast transfer has been suggested in 1988 by Law [60] and prematurely been tested in children with muscle dystrophy [59]. Not surprisingly, the clinical results were negligible. Laboratories working in this field have returned to the *mdx* mouse or to *in vitro* models, and only future will tell whether there are practicable ways for myoblast transfer in humans.

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