Cell Cycle Commitment of Rat Muscle Satellite Cells

Richard Bischoff

Department of Anatomy and Neurobiology, Washington University School of Medicine, Saint Louis, Missouri 63110

Abstract. Satellite cells of adult muscle are quiescent myogenic stem cells that can be induced to enter the cell cycle by an extract of crushed muscle (Bischoff, R. 1986. *Dev. Biol.* 115:140–147). Here, evidence is presented that the extract acts transiently to commit cells to enter the cell cycle. Satellite cells associated with both live and killed rat myofibers in culture were briefly exposed to muscle extract and the increase in cell number was determined at 48 h in vitro, before the onset of fusion. An 8–12-h exposure to extract with killed, but not live, myofibers was sufficient to produce maximum proliferation of satellite cells. Continuous exposure for over 40 h was needed to sustain proliferation of satellite cells on live myofibers.

The role of serum factors was also studied. Neither serum nor muscle extract alone was able to induce proliferation of satellite cells. In the presence of muscle extract, however, satellite cell proliferation was directly proportional to the concentration of serum in the medium. These results suggest that mitogens released from crushed muscle produce long-lasting effects that commit quiescent satellite cells to divide, whereas serum factors are needed to maintain progression through the cell cycle. Contact with a viable myofiber modulates the response of satellite cells to growth factors.

SATELLITE cells are the source of new muscle fibers during skeletal muscle regeneration. During the postnatal growth of muscle, most satellite cells fuse with the developing myofibers, but a few withdraw from the cell cycle, heterochromatize their DNA, and become quiescent mononucleated cells beneath the basal lamina of the maturing myofiber (Armand et al., 1983; Armand and Kieny, 1984). The cells remain in this condition as reserve myogenic stem cells until triggered by muscle injury to reenter the cell cycle and generate a new population of myoblasts during regeneration (Mauro, 1979). Signals that control the passage of satellite cells to and from the cell cycle are poorly understood.

Surviving satellite cells in mature muscle can be stimulated to enter the cell cycle by overt muscle trauma such as that produced by temperature extremes (Shafiq and Gorycki, 1965), toxins (Kouyoumdjian et al., 1986; Maltin et al., 1983), anesthetics (Foster and Carlson, 1980), and mechanical damage (Carlson, 1968; McGeachie and Grounds, 1987). In these cases the associated myofibers degenerate, but there are also situations in which dormant satellite cells are stimulated to divide without death of the myofibers. These include muscle denervation (Murray and Robbins, 1982; Ontell, 1974), mild compression (Teravainen, 1970), and various types of muscle overwork (Appell et al., 1988; Darr and Schultz, 1987; Schiaffino et al., 1976; Taylor and Wilkinson, 1986). The variety of conditions leading to satellite cell proliferation suggests that multiple signals or pathways are involved.

In vitro studies using monolayer cultures of cell lines and primary explants have identified several hormones and mitogens that stimulate the growth of myogenic cells (reviewed in Florini, 1987). These include fibroblast growth factor (FGF),[1] insulin-like growth factors (IGF), thyroxine, and testosterone. Because cells in monolayers are removed from their normal relationship with the myofibers, and continuous cell lines may have deficient or altered growth control pathways, results from these experiments may not be applicable in vivo. To provide a more comprehensive model for study of satellite cell growth control, I have used cultures of isolated mature myofibers bearing attached satellite cells beneath the basal lamina (Bischoff, 1986*a*,*b*). Each myofiber contains ~125 myonuclei and 3 satellite cells. The cells on these myofibers are in the null phase of the cell cycle (Go) when placed in culture (Bischoff, 1986*a*; 1989*a*). The cells can be recruited into the cell cycle, however, by exposure to a saline extract of crushed adult muscle (Bischoff, 1986*b*). There is a 16-h delay between exposure to extract and entry into the DNA synthetic phase of the cell cycle. The long lag raises the possibility that the mitogen(s) released from crushed muscle acts as a trigger to initiate a chain of growth-related events and may not be required continuously. This recalls the model for growth control that has emerged from studies of quiescent 3T3 fibroblasts (Pledger et al., 1977; Stiles et al., 1979). The model postulates the existence of two classes of serum factors that control growth; competence factors which, after brief exposure, induce persistent changes that commit cells to divide, and progression factors that are needed continuously throughout the cell cycle. Platelet-derived growth factor and FGF act as cell cycle

1. *Abbreviations used in this paper*: FGF, fibroblast growth factor; IGF, insulin-like growth factor.

competence factors for cultured cells (Shipley and Ham, 1983; Stiles et al., 1979), while IGF and epidermal growth factor are thought to be cell cycle progression factors (Doi et al., 1989; Leof et al., 1982).

To investigate growth control in muscle, satellite cells associated with both live and killed myofibers were exposed to muscle extract for various times and the resulting proliferation of satellite cells was monitored at 48 h in vitro. Results show that a 10-h exposure of satellite cells on killed, but not live, myofibers is sufficient to produce the maximum growth response. Satellite cells on live myofibers require continuous exposure.

The role of serum was also studied. Serum alone is ineffective in initiating satellite cell proliferation, but in the presence of extract, serum factors are needed for progression through the cell cycle.

Materials and Methods

Single myofibers were prepared from the *flexor digitorum brevis* muscle of 200–300-g male Sprague-Dawley rats (SASCO, Omaha, NE) as described (Bischoff, 1986*a*). Briefly, the epimysium was removed and the muscles were incubated for 1 h at 37°C in Earle's balanced salt solution containing 0.4 mg/ml each of the collagenase and neutral protease peaks of crude collagenase, type I (Sigma Chemical Co., St. Louis, MO) purified by chromatography on SP-Sephadex (Pharmacia Fine Chemicals, Piscataway, NJ) as described (Hefley et al., 1983). The myofibers were dissociated by pipetting, and separated from free mononucleated cells by repeated 1-*g* sedimentation. A drop of balanced salt solution containing several hundred myofibers was mixed with a drop of collagen (3 mg protein/ml), spread on a coverslip in a 35-mm culture dish, and gelled at 37°C for 10 min. The collagen was prepared from rat tail tendons by extraction with acetic acid, purified by salt precipitation, and brought to physiological pH and salt concentration before mixing with the myofibers (Elsdale, 1972). The basal culture medium was MEM (Sigma Chemical Co.) with 10% selected horse serum (Gibco Laboratories, Grand Island, NY) and antibiotics. Medium was changed daily unless otherwise indicated. A saline extract of the crushed leg and thigh muscles of adult rats prepared as described (Bischoff, 1986*b*) was added to stimulate satellite cell proliferation.

Satellite cell growth was measured by counting labeled nuclei in radioautographs after incubating cultures in medium containing 0.1 or 0.5 μCi/ml [*methyl*-^{3}H]thymidine ([^{3}H]-TdR, 6.7 Ci/mM, New England Nuclear, Boston, MA). The coverslips bearing myofibers were fixed in ethanol, formalin, acetic acid (20:2:1), attached to slides, and coated with bulk emulsion (NTB-2, Eastman Kodak, Rochester, NY) diluted 1:1 with water. The cells were stained with hematoxylin after photographic processing according to the manufacturer's directions. At least 50 myofibers from each treatment were scored.

In some experiments the myofibers were killed by exposure to the myotoxic anesthetic Marcaine (preservative free, Winthrop-Breon, New York, NY). Cultures were incubated at 37°C for 10 min in balanced salt solution containing 0.05% Marcaine, washed twice with balanced salt solution, and returned to normal culture medium. The myofibers rupture and undergo hypercontraction during this treatment leaving the satellite cells attached to the inner surface of the basal lamina (Bischoff, 1980; Bischoff, 1986*a*; Bischoff, 1990). In some cases the myofibers were killed at 0 time to test the effect of a viable myofiber on satellite cell proliferation. In other cases, the myofibers were killed at the end of the experiment at 48 h, simply to make it easier to visualize and count satellite cells.

Results

Growth Fraction and Fusion

Satellite cells on single myofibers in culture proliferate after stimulation by mitogen, then some begin to withdraw from the cell cycle and fuse to form new multinucleated myofibers (Bischoff, 1980; 1986*a*). Before undertaking quantitative studies of satellite cell growth, it was necessary to determine, (*a*) whether all satellite cells have the capacity to proliferate, and (*b*) when fusion begins. The inability of some cells to proliferate, or the withdrawal of cells from the cell cycle before fusion could result in noncycling cells and thus complicate growth measurements. Although it has been determined previously that the increase in satellite cells is exponential for the first 3 d (Bischoff, 1989*b*), exponential growth may occur in populations that contain noncycling cells (Cleaver, 1967). To answer the first question, isolated myofibers in culture were briefly treated with Marcaine to kill the myofibers while leaving satellite cells within the basal lamina tubes. Eliminating the myofibers makes it easier to visualize all satellite cells. The cultures were grown in muscle extract to stimulate proliferation, and exposed continuously to a low level of [^{3}H]-TdR to label all cells that synthesized DNA. Counts of labeled satellite cells in radioautographs showed that >97% of the cells were labeled at 24 (199/205) and 48 h (362/370) in vitro (Fig. 1). Thus, under these conditions of culture virtually all the satellite cells proliferate and the growth fraction is 1. To determine the onset of fusion, cultures grown in muscle extract were killed at intervals and examined microscopically for myotube formation. The earliest evidence of satellite cell fusion was found at 84 h in vitro (Fig. 2). Taken together, these observations suggest that all satellite cells on single myofibers proliferate during exposure to a saturating concentration muscle extract. The cells remain in the cell cycle for at least 2–3 d, after which they begin to fuse.

Cell Cycle Commitment

To determine whether muscle extract acts transiently to commit satellite cells to proliferate or whether continuous exposure is needed, single myofiber cultures were exposed to muscle extract for various intervals, washed three times with balanced salt solution, and continued in medium containing serum but no extract. The medium contained [^{3}H]-TdR throughout the experiment to label all satellite cells as they synthesized DNA. All cultures were killed at 48 h, well before the onset of fusion, and labeled satellite cells were counted. The experiments were carried out with satellite cells on both living and Marcaine-killed myofibers since it was previously found that contact with the surface of a viable myofiber can partially suppress the mitogenic effect of muscle extract on satellite cells (Bischoff, 1990).

Results of these experiments are shown in Fig. 3 along with a chart giving the approximate timing of cell cycle events of satellite cells on single myofibers grown continuously in muscle extract. These times are based upon a 16-h lag period before DNA synthesis and a cell cycle time of 12 h (Bischoff, 1986*b*; 1989*a*). With living myofibers (Fig. 3 *A*), the total satellite cell number per myofiber measured at 48 h begins to increase after 15 h of exposure to muscle extract. The cell yield as a function of duration of exposure increases linearly to 42 h. The number of satellite cells at each time point is approximately the same as the value predicted from continuous exposure. All the labeled nuclei were in satellite cells. In contrast, satellite cells on dead myofibers are committed to proliferate after 5 h exposure to muscle extract and the maximum cell yield is reached after only 12 h exposure (Fig. 3 *B*). Removal of muscle extract and thorough washing of the cultures after 12 h does not decrease the total cell yield at 48 h as produced by continuous exposure. The half-time

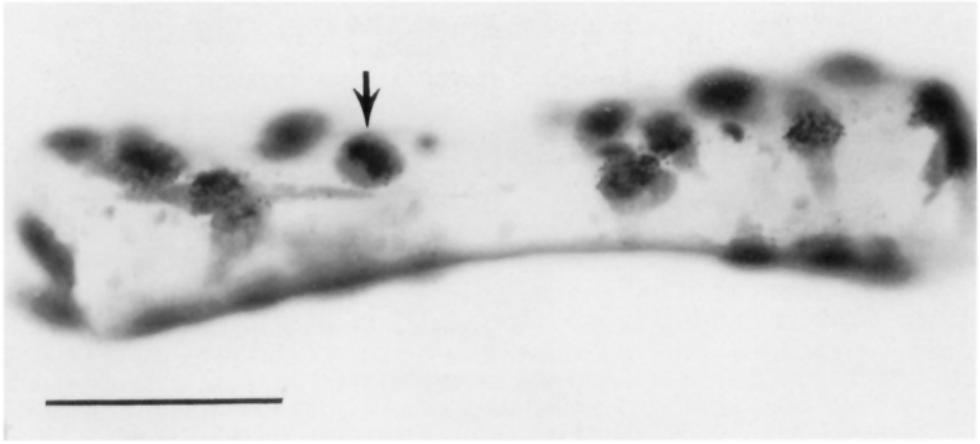

Figure 1. Radioautograph of myofiber and satellite cells incubated continuously for 48 h in basal medium containing 0.5 mg/ml muscle extract and 0.1 μCi/ml [^{3}H]-TdR. The cultures were treated with Marcaine at 0 time to kill myofibers, leaving the satellite cells attached to the inner surface of the basal lamina tube where they have proliferated. Most of the myofibrillar debris and myonuclei have dissolved by 48 h. All satellite cells associated with this myofiber are labeled with [^{3}H]-TdR; only those on the upper surface of the basal lamina tube are in focus. One of the labeled satellite cells is in metaphase (*arrow*). Hematoxylin stain. Bar, 25 μm.

of exposure needed for maximum satellite cell proliferation over the 2-d period is 30 h for cells on living myofibers and 8 h for cells on killed myofibers.

As a check on nonspecific binding of growth factor to the culture dish in these experiments, fresh myofibers at 0 time were incubated in the used medium removed from the dishes at 20 h. There was little or no loss of growth factor activity in the used medium when compared with unused medium containing 0.5 mg/ml muscle extract.

Although the experiments in Fig. 3 show that continuous exposure to muscle extract is not required for satellite cell proliferation, serum was present in the medium during the transient exposure to muscle extract. To determine whether muscle extract alone, in the absence of serum, is able to commit satellite cells to proliferate, Marcaine-killed myofibers were cultured for 11 h in muscle extract alone, then thoroughly washed and grown in serum alone for a total of 48 h in culture. Control cultures were grown in muscle extract alone, serum alone, or a combination of both for the entire 48-h period. Results show that an 11-h exposure to muscle extract alone followed by serum alone supports almost as much satellite cell proliferation as a combination of the two (Fig. 4). There was little satellite cell proliferation in cultures grown in serum alone or muscle extract alone.

Previous dose-response studies showed that the satellite cell yield after 48 h growth in muscle extract is directly proportional to the concentration of extract in the medium up to about 0.5 mg/ml (Bischoff, 1986*b*; 1989*a*). Muscle extract could control the number of cells that commit to the cell cycle, the cell cycle time of the proliferating cells, or both factors. To address this problem, single live myofibers were cultured in basal medium containing various concentrations of muscle extract plus [^{3}H]-TdR and killed at 20 h in vitro. At this time the committed satellite cells have begun DNA

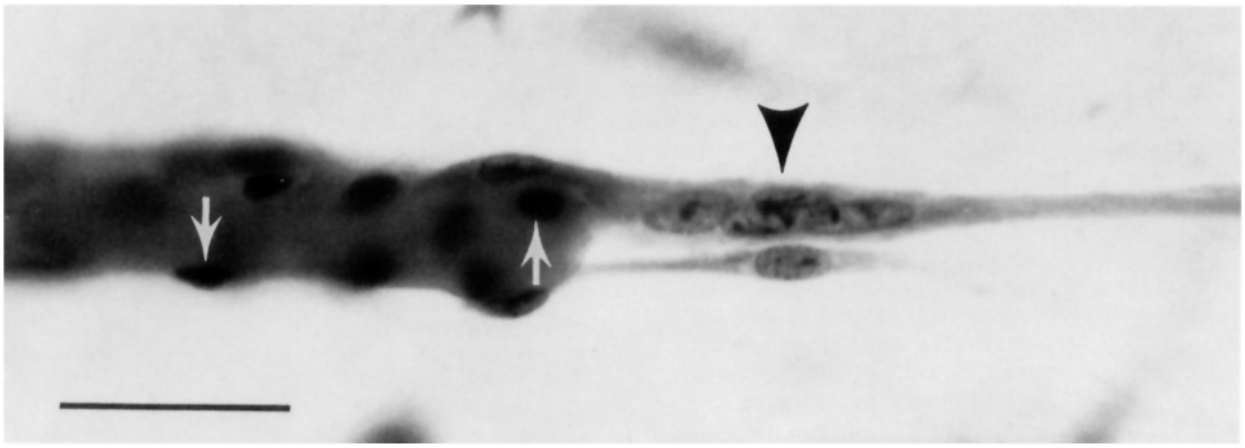

Figure 2. Photomicrograph of myofiber and satellite cells incubated in 0.5 mg/ml muscle extract in basal medium for 84 h. The cultures were not treated with Marcaine and the myofibers were alive at the time of fixation. The satellite cells have proliferated between the basal lamina and the plasmalemma of the myofiber and some have fused to form a multinucleated myotube (*arrowhead*) that extends beyond the end of the myofiber. This is the earliest time in culture at which fusion of satellite cells was observed. Some of the myonuclei of the mature myofiber are also in focus (*arrows*). Hematoxylin stain. Bar, 25 μm.

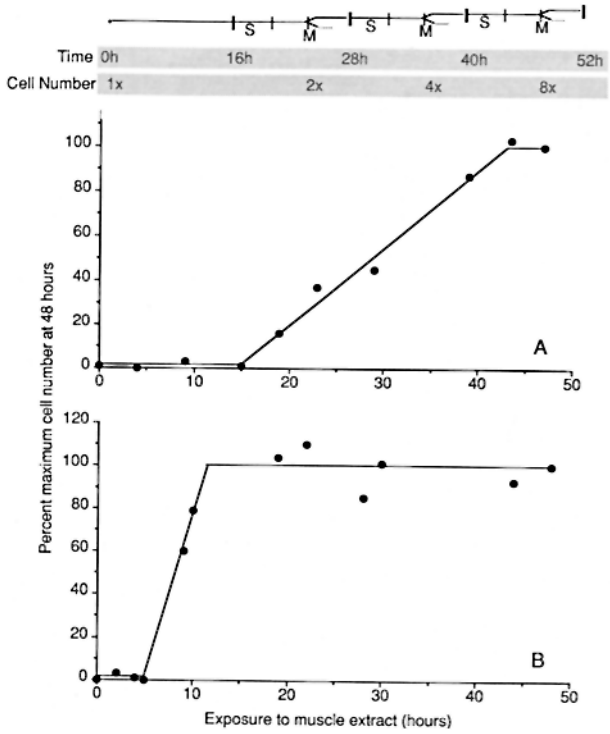

Figure 3. Effect of the viable myofiber on muscle extract-induced commitment to DNA synthesis. Isolated myofibers were left intact (*A*) or killed with Marcaine (*B*) and incubated in basal medium containing 0.5 mg/ml muscle extract plus 0.1 μCi [^{3}H]-TdR for various periods of time. The medium was then removed and the cultures were washed three times over a 10-min interval and incubated in basal medium with [^{3}H]-TdR. All cultures were killed at 48 h and the number of labeled satellite cells per myofiber was counted in radioautographs. The chart at the top shows the approximate cell number and cell cycle stage estimated from parameters determined previously (see text).

synthesis and incorporated the label, but not yet undergone mitosis, so the number of labeled cells should reflect the number recruited into the cell cycle. The results show that the number of cells entering the DNA synthetic phase of the cell cycle is directly proportional to the concentration of muscle extract up to the maximum number of cells per myofiber, about three (Fig. 5).

Role of Serum

Brief exposure to muscle extract is able to trigger proliferation of satellite cells, but other factors may be needed to sustain their proliferation. To examine the role of serum, single myofibers were cultured in a range of horse serum from 1–15%, with and without muscle extract. After 48 h, the myofibers were killed with Marcaine to facilitate visualization of satellite cells, and the total satellite cells per myofiber was determined in fixed and stained cultures. In the absence of muscle extract, satellite cells underwent little proliferation regardless of the serum concentration (Fig. 6). As a check for satellite cell viability in serum alone, one group of cultures that had been incubated in 10% serum for 48 h was switched to serum plus muscle extract and grown for another 48 h. The cells remained viable for at least 48 h under these

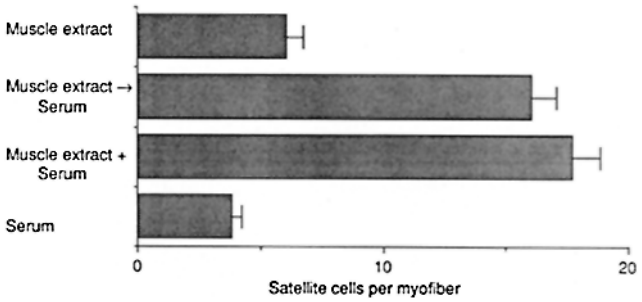

Figure 4. Effect of muscle extract and serum alone and in combination. Isolated myofibers were killed with Marcaine at 0 time and incubated in either, (*a*) MEM containing muscle extract, (*b*) MEM containing muscle extract for 11 h, then washed and incubated in MEM with serum (*c*) MEM with muscle extract and serum, and (*d*) MEM with serum. All cultures were killed at 48 h and the total number of satellite cells per myofiber was counted after fixing and staining. Muscle extract was used at 0.5 mg/ml medium and serum at 10%. Statistical evaluation using *t* test revealed no difference between treatments 1 and 4 or 2 and 3 (probability = 0.05).

conditions and were able to proliferate when given muscle extract. When muscle extract was present continuously, satellite cells increased in direct proportion to serum concentration over the range of 1 to 15%.

Discussion

Satellite cells on killed myofibers that are exposed briefly to muscle extract then washed and incubated in basal medium are stimulated to proliferate during the following 2 d to the same extent as cells exposed to muscle extract continuously. The half-time of cell cycle commitment is 8 h; however, the cells do not begin DNA synthesis until 16 h after exposure to muscle extract (Bischoff, 1986*b*). Thus, brief exposure induces a stable change which persists throughout the prereplicative lag period and during the subsequent 2 d. Based upon the number of satellite cells per myofiber at 48 h (e.g., Fig. 6) and measurements of cell cycle parameters (Bischoff, 1986*b*; 1989*a*), it can be calculated that committed satellite

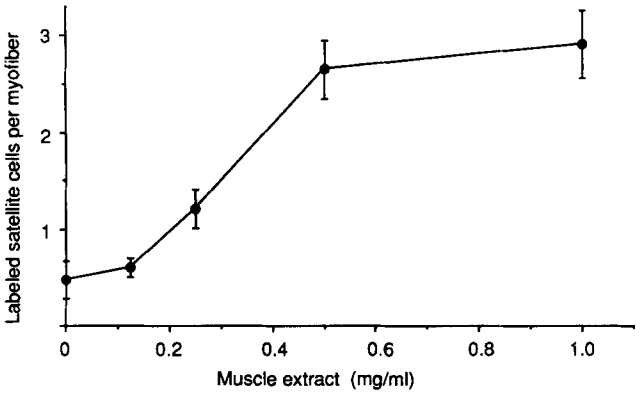

Figure 5. Effect of the concentration of muscle extract on the number of satellite cells entering the DNA synthetic phase of the cell cycle. Single live myofibers and attached satellite cells were incubated in basal medium containing 0 to 1 mg/ml muscle extract and 0.5 μCi [^{3}H]-TdR and killed at 20 h. The number of labeled satellite cells was counted in radioautographs.

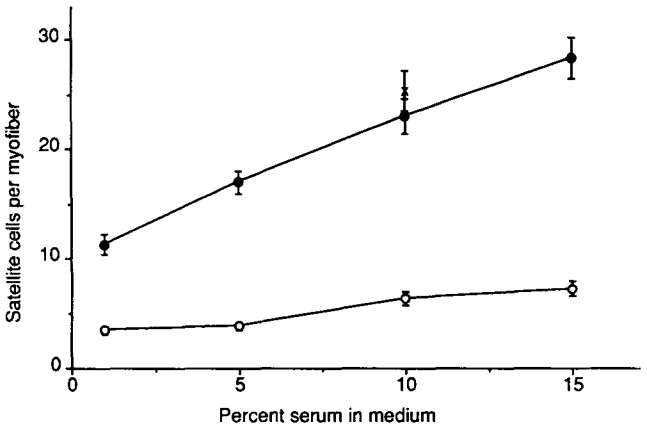

Figure 6. Effect of serum concentration on muscle extract-induced proliferation of satellite cells. Single myofibers with satellite cells were incubated in MEM containing from 1 to 15% horse serum alone (-o-) or with 0.5 mg/ml muscle extract (-•-). After 48 h the cultures were treated for 10 min with Marcaine to kill myofibers and the total number of satellite cells per myofiber was counted after fixing and staining. Some cultures (-x-) were incubated in MEM plus 10% serum for 48 h then incubated in the same medium containing 0.5 mg/ml muscle extract for another 48 h.

cells are able to complete at least three rounds of the cell cycle in the absence of further stimulation with muscle extract.

Although these results could also be explained by incomplete removal of mitogen, i.e., binding to the cultures followed by slow release after washing, this seems unlikely. First, prolonged exposure for up to 40 h does not have a lasting effect with satellite cells on live myofibers; when the cultures are washed, the growth stimulation vanishes. If there were nonspecific binding to the cultures, it should be equivalent for both types of cultures. Second, a 5-h exposure has no effect, while only 3 more hours produces half maximum response; the nonspecific binding would have to be exquisitely time dependent. Third, cultures of live myofibers exposed to muscle extract for 15 h, then washed and grown in basal medium, do not exhibit satellite cell proliferation. Finally, the dose of muscle extract used is close to the threshold needed for maximum response (Bischoff, 1986*b*). If residual mitogen resistant to washing were responsible for the sustained effect, almost all the mitogen would have to be in this form, yet full mitogenic activity was still present in the used medium after removal from the test cultures.

There is a striking difference between the response of satellite cells on live and dead myofibers. Previous studies have shown that continuous exposure to a wide range of muscle extract concentrations produces more satellite cell growth on dead myofibers than on live myofibers (Bischoff, 1990). This study further demonstrates that contact with a live myofiber is able to reverse or suspend the cell cycle commitment produced by exposure to muscle extract. With live myofibers the increase in satellite cell number measured at 48 h first begins after 15 h exposure to muscle extract, about the time the cells are initiating DNA synthesis (Bischoff, 1986*b*). If the muscle extract is subsequently removed, the cell number does not proceed much beyond that predicted from the generation time (see Fig. 3). Because many studies have shown that withdrawal of growth factors promotes differentiation of myogenic cells in monolayer culture (reviewed in Florini and Magri, 1989), perhaps the satellite cells were induced to fuse with their associated myofiber when extract was removed. Although fusion of a small number of nuclei cannot be ruled out, the great majority of labeled nuclei were in satellite cells at 48 h. Also, experiments in which fusion competent myoblasts were added to these preparations have shown that the mature myofibers are resistant to fusion (Bischoff, 1990). Thus, it seems likely that the satellite cells stopped proliferating but did not fuse after the extract was removed. Fusion of satellite cells at longer intervals after mitogen withdrawal was not examined. It is impossible to determine from these data whether the cells are arrested in the cell cycle phase they were in at the time of mitogen withdrawal, or whether they are able to progress to the next G1. Results from other studies, however, show that cells are usually able to complete a cell cycle and return to G1 after removal of mitogen (Prescott, 1987).

There are many examples of contact-mediated regulation of the cell cycle, but this is the first instance in which a differentiated end cell has been shown to influence the proliferation of its precursor stem cell by direct contact. In both live and dead myofibers, the satellite cells are in contact with the basal lamina and appear to be the same shape in both situations (Bischoff, 1989*a*; 1990), so these factors are probably not involved. Instead, growth suppression appears to be mediated by contact with the plasma membrane of the myofiber (Bischoff, 1990). The isolated myofibers twitch spontaneously in culture and preliminary experiments have shown that electrical activity may be responsible for influencing satellite cells, since the growth suppression is prevented by incubation in the Na^+ channel blocker, tetrodotoxin (Bischoff, 1989*b*).

The mitogenic factor in muscle extract has not yet been completely purified and, although it has been regarded as a single component for the purpose of these experiments, it is likely that multiple active factors are involved (Bischoff, 1989*a*, *b*). FGF is mitogenic for myogenic cells (Allen et al., 1984; Clegg et al., 1987; DiMario and Strohman, 1988; Lathrop et al., 1985) and has been isolated from adult muscle (DiMario et al., 1989; Kardami et al., 1985). Immunocytochemistry has been used to localize FGF in the extracellular matrix of adult rat muscle where it appears to be bound to the myofiber basal lamina (Yamada et al., 1989). In chick embryo muscle however, FGF was found intracellularly in the myotubes, and not in the extracellular matrix (Joseph-Silverstein et al., 1989). Because satellite cells are in direct contact with the basal lamina, it is difficult to envision a mechanism regulating the availability of FGF stored in this location. Release of FGF from intracellular stores after injury is more consistent with other examples of growth factor mediators such as platelet-derived growth factor release from platelets and cytokine release from macrophages. Although FGF may be a component of the crushed muscle extract, there are sufficient differences in purification and activity to suggest that other factors from the extract are also involved in triggering cell cycle commitment in quiescent satellite cells. For example, saturating doses of FGF and muscle extract have additive effects (Bischoff, 1989*b*), and partially purified satellite cell mitogen from muscle extract is not active with cultured endothelial cells that respond strongly to authentic FGF (Bischoff, 1989*a*). Furthermore,

while FGF is relatively unspecific, the active factor from crushed muscle exhibits both source and target specificity (Bischoff, 1986*b*; 1989*a*).

The crude extract may contain components also found in serum, but the extract is deficient in factors needed for DNA synthesis, while serum is deficient in factors needed for cell cycle commitment. The role of serum in this regard differs between satellite cells on myofibers and those in monolayer culture. Allen et al. (1984) have shown that the growth of rat satellite cells in monolayer is directly proportional to serum concentration between 2 and 15%, whereas in the present study serum had little effect on satellite cell proliferation in the absence of muscle extract. Purified IGF alone is also ineffective in stimulating proliferation of myofiber-attached satellite cells (Bischoff, 1986*a*). At 0 time, satellite cells on single myofibers are in Go, whereas the cell cycle status of satellite cells at the onset of monolayer culture has not been determined.

These studies provide a broad outline of how satellite cell reproduction may be controlled in the animal. Satellite cell proliferation occurs in a wide range of settings such as development, regeneration, hypertrophy, and denervation (reviewed in White and Esser, 1989). The existence of several regulatory elements affords a finer control that can be adapted to a variety of situations. For example, during late development, satellite cells would be encouraged to withdraw from the cell cycle and become quiescent as the concentration of nonspecific growth factors declines and the satellite cells become increasingly subject to cell cycle inhibition by contact with the maturing myofibers. Both the levels of IGF in serum (Moses et al., 1980) and the expression of IGF in muscle tissue (Beck et al., 1988) declines during the first month in rats. This period corresponds to the time of proliferation restriction in muscle (Kelly, 1978; Schultz, 1979). The myofibers may influence both proliferation and differentiation of attached satellite cells. Myofibers become refractory to the fusion of competent myoblasts at some point in their maturation (Bischoff, 1990; Bischoff and Holtzer, 1969), and this event may prevent attached satellite cells from differentiating. Muscle-specific growth factors such as those present in crushed muscle extract may not play a role in embryonic myogenesis since the satellite cells are already engaged in the cell cycle at this time and may not require commitment factors. During the first day of muscle injury, high local concentration of muscle-specific growth factor released from necrotic myofibers, combined with loss of contact inhibition by death of the myofibers, would commit satellite cells to enter the cell cycle. There is a rapid induction of IGF in regenerating muscle (Jennische and Hansson, 1987; Jennische et al., 1987), suggesting a local action of this factor also during the period of satellite cell growth. The recent finding that satellite cell proliferation in free muscle grafts occurs before revascularization (Roberts et al., 1989) is consistent with the local origin of growth factors. Later, as vascularization increases, serum growth factors could maintain cell cycle progression as muscle-specific growth factor disappears. When the new myofibers mature, unfused satellite cells would again become quiescent owing to contact inhibition. Variations are also possible. During muscle overwork, leakage of mitogen from the myofibers, perhaps along with the well documented leakage of creatine kinase during exercise (Noakes, 1987; Hortobagyi and Denahan, 1989), may trigger cell cycle commitment in some satellite cells, but continued contact with a viable myofiber would limit their proliferation. These notions are speculative, but the model suggests future experiments on muscle growth, especially in vivo.

I thank Kym Hallsworth for expert technical assistance with these experiments.

This research was supported by National Institutes of Health grant AM-36853.

Received for publication 20 November 1989 and in revised form 23 March 1990.

References

Allen, R. E., M. V. Dodson, and L. S. Luiten. 1984. Regulation of skeletal muscle satellite cell proliferation by bovine pituitary fibroblast growth factor. *Exp. Cell Res.* 152:154–160.

Appell, H. J., S. Forsberg, and W. Hollmann. 1988. Satellite cell activation in human skeletal-muscle after training: evidence for muscle-fiber neoformation. *Intl. J. Sports Med.* 9:297–299.

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

Armand, O. and M. Kieny. 1984. Ontogeny of the myosatellite cell. *Arch. Anat. Microsc. Morphol. Exp.* 73:75–90.

Beck, F., N. J. Samani, S. Byrne, K. Morgan, R. Gebhard, and W. J. Brammar. 1988. Histochemical localization of IGF-I and IGF-II mRNA in the rat between birth and adulthood. *Development.* 104:29–39.

Bischoff, R. 1980. Plasticity of the myofiber–satellite cell complex in culture. *In* Plasticity of Muscle. D. Pette, editor. de Gruyter & Co., Berlin. 119–129.

Bischoff, R. 1986*a*. Proliferation of muscle satellite cells on intact myofibers in culture. *Dev. Biol.* 115:129–139.

Bischoff, R. 1986*b*. A satellite cell mitogen from crushed adult muscle. *Dev. Biol.* 115:140–147.

Bischoff, R. 1989*a*. Analysis of muscle regeneration using single myofibers in culture. *Med. Sci. Sports Exer.* 21:S164–S172.

Bischoff, R. 1989*b*. Control of satellite cell proliferation. *In* Myoblast Transfer Therapy. A. B. Eastwood, G. Karpati, and R. Griggs, editors. Plenum Publishing Corp., New York. In press.

Bischoff, R. 1990. Interaction between satellite cells and skeletal muscle fibers. *Development.* In press.

Bischoff, R., and H. Holtzer. 1969. Mitosis and the processes of differentiation of myogenic cells in vitro. *J. Cell Biol.* 41:188–200.

Carlson, B. M. 1968. Regeneration of the completely excised gastrocnemius muscle in the frog and rat from minced muscle fragments. *J. Morphol.* 125:447–472.

Cleaver, J. E. 1967. Thymidine Metabolism and Cell Kinetics. Elsevier North-Holland, Amsterdam. 259 pp.

Clegg, C. H., T. A. Linkhart, B. B. Olwin, and S. D. Hauschka. 1987. Growth factor control of skeletal muscle differentiation: commitment to terminal differntiation occurs in G1 phase and is repressed by fibroblast growth factor. *J. Cell Biol.* 105:949–956.

Darr, K. C., and E. Schultz. 1987. Exercise-induced satellite cell activation in growing and mature skeletal muscle. *J. Appl. Physiol.* 63:1816–1821.

DiMario, J., N. Buffinger, S. Yamada, and R. C. Strohman. 1989. Fibroblast growth factor in the extracellular matrix of dystrophic (MDX) mouse muscle. *Science (Wash. DC).* 244:688–690.

DiMario, J., and R. C. Strohman. 1988. Satellite cells from dystrophic (mdx) mouse muscle are stimulated by fibroblast growth-factor in vitro. *Differentiation.* 39:42–49.

Doi, T., L. J. Striker, S. J. Elliot, F. G. Conti, and G. E. Striker. 1989. Insulin-like growth factor-1 is a progression factor for human mesangial cells. *Am. J. Pathol.* 134:395–404.

Elsdale, T., and J. Bard. 1972. Collagen substrate for the study of cell behavior. *J. Cell Biol.* 41:298–311.

Florini, J. R. 1987. Hormonal control of muscle growth. *Muscle & Nerve.* 10:577–598.

Florini, J. R., and K. A. Magri. 1989. Effects of growth factors on myogenic differentiation. *Am. J. Physiol.* 256:C701–C711.

Foster, A. H., and B. M. Carlson. 1980. Myotoxicity of local anesthetics and regeneration of the damaged muscle fibers. *Anesth. Analg.* 59:727–736.

Hefley, T. J., P. Stern, and J. S. Brand. 1983. Enzymatic isolation of cells from neonatal calvaria using two purified enzymes from clostridium histolyticum. *Exp. Cell Res.* 149:227–236.

Hortobagyi, T., and T. Denahan. 1989. Variability in creatine kinase: methodological, exercise, and clinically related factors. *Int. J. Sports Med.* 10:69–80.

Jennische, E., and H. A. Hansson. 1987. Regenerating skeletal muscle cells express insulin-like growth factor I. *Acta Physiol. Scand.* 130:327–332.

Jennische, E., A. Skottner, and H. A. Hansson. 1987. Satellite cells express

the trophic factor IGF-I in regenerating skeletal muscle. *Acta. Physiol. Scand.* 129:9–15.

Joseph-Silverstein, J., S. A. Consigli, K. M. Lyser, and C. Ver Pault. 1989. Basic fibroblast growth factor in the chick embryo: immunolocalization to striated muscle cells and their precursors. *J. Cell Biol.* 108:2459–2466.

Kardami, E., D. Spector, and R. C. Strohman. 1985. Myogenic growth factor present in skeletal muscle is purified by heparin-affinity chromatography. *Proc. Natl. Acad. Sci. USA.* 82:8044–8047.

Kelly, A. M. 1978. Satellite cells and myofiber growth in the rat soleus and extensor digitorum longus muscles. *Dev. Biol.* 65:1–10.

Kouyoumdjian, J., J. B. Harris, and M. A. Johnson. 1986. Muscle necrosis caused by the sub-units of crotoxin. *Toxicon.* 24:575–583.

Lathrop, B., E. Olson, and L. Glaser. 1985. Control by fibroblast growth factor of differentiation in the BC_3H1 muscle cell line. *J. Cell Biol.* 100:1540–1547.

Leof, E. B., W. Wharton, J. J. Van Wyk, and W. J. Pledger. 1982. Epidermal growth factor (EGF) and somatomedin C regulate G1 progression in competent BALB/c-3T3 cells. *Exp. Cell Res.* 141:107–115.

Maltin, C. A., J. B. Harris, and M. J. Cullen. 1983. Regeneration of mammalian skeletal muscle following the injection of the snake-venom toxin taipoxin. *Cell Tissue Res.* 232:565–577.

Mauro, A., editor. 1979. Muscle Regeneration. Raven Press, New York. 560 pp.

McGeachie, J. K., and M. D. Grounds. 1987. Initiation and duration of muscle precursor replication after mild and severe injury to skeletal muscle of mice. An autoradiographic study. *Cell Tissue Res.* 248:125–130.

Moses, A. C., S. P. Nissley, P. A. Short, M. M. Rechler, R. M. White, A. B. Knight, and O. Z. Higa. 1980. Increased levels of multiplication-stimulating activity, an insulin-like growth factor, in fetal rat serum. *Proc. Natl. Acad. Sci. USA.* 77:3649–3653.

Murray, M. A., and N. Robbins. 1982. Cell proliferation in denervated muscle: identity and origin of dividing cells. *Neuroscience.* 7:1823–1833.

Noakes, T. D. 1987. Effect of exercise on serum enzyme activities in humans. *Sports Med.* 4:245–267.

Ontell, M. 1974. Muscle satellite cells: a validated technique for light microscopic identification and a quantitative study of changes in their population following denervation. *Anat. Rec.* 178:211–228.

Pledger, W. J., C. D. Stiles, H. N. Antoniades, and C. D. Scher. 1977. Induction of DNA synthesis in BALB/c 3T3 cells by serum components: reevaluation of the commitment process. *Proc. Natl. Acad. Sci. USA.* 74:4481–4485.

Prescott, D. M. 1987. Cell reproduction. *Int. Rev. Cytol.* 100:93–128.

Roberts, P., J. K. McGeachie, M. D. Grounds, and E. R. Smith. 1989. Initiation and duration of myogenic precursor cell replication in transplants of intact skeletal-muscles: an autoradiographic study in mice. *Anat. Rec.* 224:1–6.

Schiaffino, S., S. Pierobon Bormioli, and M. Aloisi. 1976. The fate of newly formed satellite cells during compensatory muscle hypertrophy. *Virchows Arch. B Cell Pathol.* 21:113–118.

Schultz, E. 1979. Quantification of satellite cells in growing muscle using electron microscopy and fiber whole mounts. *In* Muscle Regeneration. A. Mauro, editor. Raven Press, New York. 131–136.

Shafiq, S. A., and M. A. Gorycki. 1965. Regeneration in skeletal muscle of mouse: some electron-microscope observations. *J. Path. Bacteriol.* 90:123–127.

Shipley, G. D., and R. G. Ham. 1983. Control of entry of Swiss 3T3 cells into S phase by fibroblast growth factor under serum-free conditions. *Exp. Cell Res.* 46:261–270.

Stiles, C. D., G. T. Capone, H. N. Antoniades, J. J. Van Wyk, and W. J. Pledger. 1979. Dual control of cell growth by somatomedins and platelet-derived growth factor. *Proc. Natl. Acad. Sci. USA.* 76:1279–1283.

Taylor, N. A., and J. G. Wilkinson. 1986. Exercise-induced skeletal muscle growth. Hypertrophy or hyperplasia? *Sports Med.* 3:190–200.

Teravainen, H. 1970. Satellite cells of striated muscle after compression injury so slight as not to cause degeneration of the muscle fibers. *Z. Zellforsch. Mikros. Anat.* 103:320–327.

White, T. P., and K. A. Esser. 1989. Satellite cell and growth factor involvement in skeletal muscle growth. *Med. Sci. Sports Exer.* 21:S158–S163.

Yamada, S., N. Buffinger, J. Dimario, and R. C. Strohman. 1989. Fibroblast growth factor is stored in fiber extracellular matrix and plays a role in regulating muscle hypertrophy. *Med. Sci. Sports Exer.* 21:S173–S180.