Regulation of Satellite Cells during Skeletal Muscle Growth and Development (43060)

RONALD E. ALLEN AND LUCINDA L. RANKIN
Muscle Biology Group, Departments of Animal Sciences and Physiology, University of Arizona, Tucson, Arizona 85721

Abstract. **Satellite cells are myogenic cells attributed with the role of postnatal growth and regeneration in skeletal muscle. Following proliferation and subsequent differentiation, these cells will fuse with one another or with the adjacent muscle fiber, thereby increasing myonuclei numbers for fiber growth and repair. The potential factors which could regulate this process are many, including exercise, trauma, passive stretch, innervation, and soluble growth factors. Three classes of growth factors in particular (fibroblast growth factor, insulin-like growth factor, and transforming growth factor-β) have been studied extensively with respect to their effects on satellite cell proliferation and differentiation in culture. Fibroblast growth factor has been shown to stimulate proliferation but depress differentiation. Insulin-like growth factor stimulates both proliferation and differentiation, although the latter to a much greater degree. Transforming growth factor-β slightly depresses proliferation but inhibits differentiation. When administered in combination, these factors can induce satellite cell activities in culture which mimic those typical of satellite cells found *in vivo* in growing, regenerating, or healthy mature muscle. Alterations in the concentrations of these growth factors in the muscle environment as well as alterations in the cell's sensitivity or responsiveness to these factors represent potential mechanisms for regulating satellite cell activity *in situ*.**

[P.S.E.B.M. 1990, Vol 194]

Mammalian skeletal muscle exhibits a robust capacity for growth and repair. In the embryo, the presumptive myoblast is responsible for this potential. During myogenesis, these cells proliferate, differentiate, and fuse to form myotubes and eventual muscle fibers. In postnatal muscle, the satellite cell is attributed with this role. As first described by Mauro (1), the satellite cell lies wedged between the basal lamina and the plasma membrane of the muscle fiber and is characterized by a heterochromatic nucleus, sparse cytoplasm with few organelles, and an absence of myofilaments (2). Although considered mitotically quiescent in healthy mature muscle (3), given the appropriate stimulus, satellite cells will proliferate and fuse with the adjacent fiber (4), thereby increasing myonuclei numbers for fiber growth and repair.

Both *in vivo* and *in vitro*, satellite cells and embryonic myoblasts appear to be functionally equivalent. However, recent evidence suggests that the satellite cell represents a unique subclass of myogenic cells (5). Morphologically, satellite cells and embryonic myoblasts from mouse exhibited distinct cell shapes when grown in culture (6). Biochemically, several differences have been noted. The tumor-promoting agent, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), reversibly inhibits differentiation in mouse embryonic myoblasts; whereas satellite cells from adult muscle appear to be resistant to TPA, continuing to differentiate in its presence (7). Similarly, a difference in sensitivity to transforming growth factor-β (TGF-β) was demonstrated by Allen and Boxhorn (8); cultured neonatal rat myogenic cells were much more sensitive to TGF-β than were satellite cells from young adult rats. With regard to the expression of muscle specific proteins, the accumulation of α-actin per myonucleus was considerably lower in myotubes derived from juvenile, adult, or aged rat satellite cells as compared with myotubes derived from neonatal myogenic cells (9). Data from Yablonka-Reuveni and Nameroff (10) suggest a difference in desmin expression; desmin appeared in a percentage of replicating myogenic cells from late stage embryos (probable satellite cells) but not in cells from early embryos (predominantly myoblasts). And finally, Cossu *et al.* (11) found that undifferentiated mouse satellite cells expressed functional acetylcholine receptors *in vitro*,

Received October 12, 1989. [P.S.E.B.M. 1990, Vol 194]
Accepted January 30, 1990.

0037-9727/90/1942-0081$2.00/0

whereas embryonic myoblasts did not. More recently, however, this finding has been challenged by Bader *et al.* (12). Satellite cells which remained attached to their resident muscle fiber did not exhibit electrophysiologic characteristics associated with functional acetylcholine receptors, while dissociated cells cultured for more than 24 hr were indeed responsive to acetylcholine. These results suggest that perhaps the properties of the satellite cell are altered by the process of dissociation from the muscle fiber. Taken together, this latter suggestion notwithstanding, the data reported above support the idea that satellite cells do represent a distinct subset of myogenic cells. The question of when and from what source they are derived, however, remains unresolved.

The satellite cell plays a critical role in both normal muscle growth and regeneration following injury. The significance of satellite cell activity to the process of postnatal growth and hypertrophy resides in the ability of these cells to contribute nuclei to normal muscle fibers. Numerous studies have demonstrated that (i) the nuclei of the myotube do not divide and (ii) that the majority of nuclei in mature postnatal muscle are added to fibers during postnatal life, after the formation of new myofibers has been completed (13). During rapid growth, satellite cell numbers are relatively high to provide increased DNA content for fiber growth. Then as growth slows, these numbers decrease to reach a small stable population in mature muscle, ranging from 4 to 15% of total nuclei in the fiber. Schulz *et al.* (3) showed a virtual absence of thymidine incorporation in muscle-associated nuclei in normal adult mice, supporting the hypothesis that satellite cells are not mitotically active in healthy mature muscle. However, recent experiments have shown that when normal muscle is stressed, for example, by a significantly increased workload (14, 15) or by prolonged muscle stretch (16), satellite cells become activated and reenter the replicative phase of the cell cycle to add further to the myonuclei population.

The second major function of satellite cells is the regeneration of muscle fibers. Following an injury that results in death of fibers, satellite cells begin to proliferate rapidly in the injured muscle, and a large population of mononucleated myogenic cells is generated (17). During later phases of regeneration, these satellite cells differentiate and fuse to form either fibers within the persisting basal lamina of the necrosed fiber or new fibers in a manner analogous to myogenesis (18). In response to a focal injury, satellite cells from remote, undamaged portions of the fiber will migrate to contribute to the repair of the injured area (19). The stimulus responsible for such rapid activation is unknown at present. However, Bischoff (20) has hypothesized that muscle-cell-specific mitogens are released from the fibers as a direct result of the injury. Murray and Robbins (21) proposed the release of a similar proliferation-enhancing signal from denervated muscle.

As can be seen from the above discussion, the activation of satellite cells may be regulated by a variety of factors, including innervation, injury-related metabolites, exercise, heredity, and blood-borne growth factors. The remainder of this review will focus on the latter, soluble growth factors, and the mechanisms by which they could potentially regulate satellite cell activity.

Regulation by Protein Growth Factors

Identification of factors that may stimulate or inhibit satellite cell proliferation and differentiation has resulted almost exclusively from *in vitro* experiments with cultured satellite cells. Using culture procedures originally developed by Bischoff (22), mass cultures of rat satellite cells have been employed to demonstrate the mitogenic influence of fibroblast growth factor (FGF) and insulin-like growth factors (IGF-I and II) on this myogenic cell type (23, 24). Furthermore, Bischoff (25) used a single fiber culture system to demonstrate the mitogenic effect of FGF on satellite cells in regenerating fibers. It is important to note that the observations on satellite cells were preceded by similar observations on growth factor and hormone stimulation of myogenic cells from cell lines or embryonic muscle tissue (26–29).

The most exciting new addition to the list of growth factors that regulate myogenesis is TGF-β. Evinger-Hodges *et al.* (30) first made the observation that a factor in conditioned medium from BRL cells was a potent inhibitor of myoblast differentiation. Subsequently, Florini *et al.* (31) showed that this differentiation inhibitor was TGF-β. The differentiation inhibitory activity of TGF-β has been demonstrated with other muscle cell lines and embryonic muscle cells by Massague *et al.* (32) and Olson *et al.* (33). Experiments conducted in our laboratory extended these observations to satellite cells (8).

Unfortunately, many of the muscle cell lines used in the growth factor work are not responsive to all three factors (i.e., FGF, IGF, and TGF-β). Primary cultures of rat satellite cells, however, are responsive to all three factors, and the interactions of these three factors have been documented (34). Using optimum concentrations of each factor, all possible combinations have been examined (Fig. 1).

In the presence of serum-free, defined medium, cultured satellite cells exhibited enhanced proliferation but depressed differentiation when exposed to FGF (Fig. 1). IGF-I stimulated proliferation only to a small degree but stimulated differentiation profoundly. TGF-β depressed proliferation and inhibited differentiation. In evaluating combinations of these factors, the differentiation-inhibiting effect of TGF-β could not be coun-

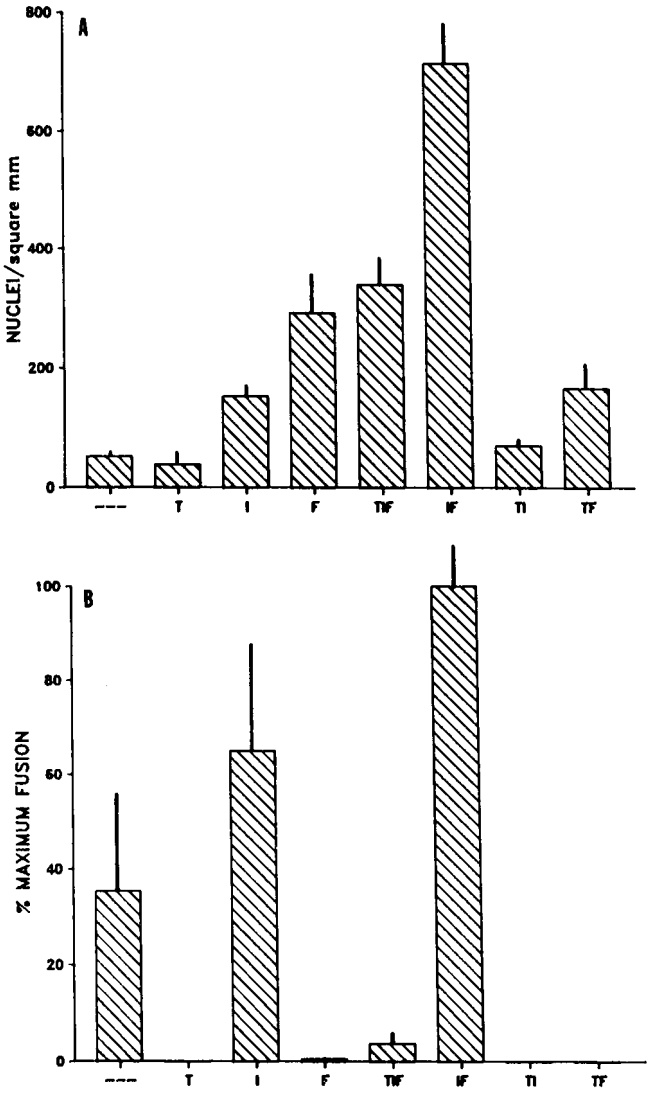

Figure 1. Interactions of TGF-β, IGF-I, and basic FGF in serum-free defined medium. Cultures were initially established in 10% Dulbecco's minimal essential medium for a 48-hr period prior to replacing the medium with serum-free defined treatment medium. Treatment medium contained 10^{-9} *M* insulin and various combinations of TGF-β (1 ng/ml), basic FGF (50 ng R&D basic FGF/ml), and IGF-I (50 ng/ml). Medium was replaced daily. At the end of a 72-hr treatment period, cultures were stained and evaluated. Total cell density (A) and percentage maximum fusion (B) are presented as the means and standard deviations of four cultures per treatment for one of three separate experiments (maximum fusion in this experiment was 45%). Growth factor combinations are indicated on the x-axis: T, TGF-β; F, basic FGF; and I, IGF-I. Reprinted with permission from Allen and Boxhorn (34).

teracted by any combination of FGF or IGF. The proliferation-depressing activity of TGF-β, however, could not inhibit the mitogenic activity of FGF. The greatest stimulation of proliferation occurred in the presence of FGF and IGF-I, and maximum differentiation was found in cultures exposed to IGF-I, in the absence of TGF-β.

Based on work in the rat satellite cell culture system, the primary action of IGF may be to stimulate differentiation when in the absence of FGF; however, when in the presence of FGF, the primary action may be to stimulate proliferation (the exception being demonstrated in Fig. 1 where significant differentiation also occurred in the presence of FGF as a probable result of the extremely high cell density). Clearly, significant proliferation does not occur in the absence of FGF and, therefore, the primary function of FGF or related mitogens may be to stimulate proliferation. The most important role for TGF-β may be inhibiting differentiation, with its effect on proliferation being secondary.

Based on the experiments described above, a model for growth factor regulation of satellite cell activity was generated (Table I). Satellite cells, as discussed, exist in a variety of states. During growth and muscle hypertrophy, they proliferate and differentiate to add nuclei to existing fibers; in culture, active proliferation and differentiation are stimulated by the combination of FGF and IGF-I. During early phases of regeneration, the primary activity of satellite cells is proliferation, without significant differentiation or myotube formation. This generates a large population of mononucleated satellite cells in preparation for fusion and myotube formation, which is the predominant activity during later stages of regeneration. *In vitro*, proliferation without differentiation, as in early regeneration, is stimulated by FGF and IGF-I in the presence of TGF-β, the latter serving to block differentiation. Subsequent differentiation without further proliferation, as occurs in late phases of regeneration, can be induced in culture by IGF-I once FGF and TGF-β have been removed. By lifting the differentiation block mediated by TGF-β and eliminating the mitogenic signals of FGF, IGF-I is able to stimulate differentiation. Finally, the quiescent state, typical of satellite cells in mature healthy muscle, can be mimicked *in vitro* by the addition of TGF-β in the absence of FGF and IGF-I.

In vitro experiments in our laboratory have established the ability of satellite cells to respond to TGF-β, IGF-I, and FGF, and indicate that the *in vivo* state of satellite cell activity can be reproduced in culture with combinations of these factors. But, our experiments do not provide evidence that IGF-I, FGF, and TGF-β are indeed the physiologic regulators of these activities in living organisms.

Of the three factors discussed, IGF-I and FGF have been localized at the cellular level in skeletal muscle. Jennische and Hansson (35) used IGF-I-specific antibodies to localize this factor in satellite cells of regenerating muscle. Based on *in situ* hybridization experiments, IGF-I mRNA appears to be synthesized in satellite cells. Therefore, at least some of the IGF-I visualized by immunocytochemical techniques was actually synthesized in these cells (36). In nonregenerating tissue IGF-I would be expected to circulate in the bloodstream in relatively high concentrations most of

Table I. Satellite Cell Functions

Growth	Early regeneration	Late regeneration	Quiescence
Activities			
Proliferate	Proliferate	Proliferate	No division or differentiation
Differentiate	No differentiation	Differentiate	
Mediators			
FGF	FGF	IGF-I	TGF-β
IGF-I	TGF-β		
	IGF-I		

the time, although it is complexed with binding proteins that may modulate its activity (37–39).

The physiologic functions of basic and acidic FGF have not been firmly established, but several cell types have been identified that have the capacity to synthesize and secrete FGF (40). Macrophages (41) and vascular endothelial cells (42) have both been shown to be a source of this factor. Recently, FGF has been specifically localized in skeletal muscle (43); by immunofluorescence techniques, basic FGF was localized to the periphery of muscle fibers, presumably sequestered by elements of the basement membrane. The site of FGF synthesis, however, was not revealed by these experiments. One possibility is that FGF was derived from vascular endothelial cells and subsequently dispersed in the tissue. In other studies, the heparin-binding ability of FGF has been exploited in its purification and may be an important factor in its physiology.

The ubiquitous nature of TGF-β has become apparent (44), and its importance in development and wound healing are active areas of current research. Although platelets are an important source of TGF-β for purification, it has been isolated from many tissues. Specific antibodies have been used to localize TGF-β in specific cells in the developing embryo (45–47), and *in situ* hybridization experiments (48) indicate that TGF-β is an autocrine or paracrine factor, in that it is synthesized in specific cells in developing tissues. TGF-β has yet to be localized in postnatal skeletal muscle, even though the effects of this factor on myogenic cells and satellite cells are well documented (8, 33, 34, 49).

The mere presence of these growth factors may not ensure activity, however. All three can be found complexed to other macromolecules. IGF are most frequently found in association with binding proteins, FGF may be bound to heparin in the basement membrane, and TGF-β can be found in a latent form in association with a larger protein. Nonetheless, if these factors are active in skeletal muscle growth regulation via their actions on satellite cells, it should be possible to localize them in association with satellite cells during growth.

Regulation by Altered Sensitivity and Responsiveness

The discussion thus far has centered around the direct effects of growth factors on satellite cells. The implication is that growth factors in the satellite cell's environment play an important role in stimulating or inhibiting cellular activities. Varying the concentrations of these factors in the muscle environment may indeed represent one mechanism for regulating satellite cell activity. Alterations in the cell's sensitivity or responsiveness to these growth factors represent a second possible mechanism.

Table II. Altered Responsiveness in Activated Satellite Cells

Pulse label period (hr)	Labeling index (%)	
	Marcaine[a]	Control[b]
0–8	19% (±4)	0%
24–32	32% (±3)	19% (±1)

[a] Cells were harvested from the tibialis anterior and EDL muscles 60 hr after marcaine injection.
[b] Cells were harvested from noninjected tibialis anterior and EDL muscles from the contralateral side.

Age-related alterations in satellite cell growth provide an example of altered sensitivity or responsiveness. Schultz and Lipton (50) provided the first evidence that satellite cells from old rats initiate DNA replication more slowly when placed in culture at clonal density than myogenic cells from very young or fetal rats. Not only did the older cells initiate growth more slowly but they formed smaller colonies than cells from younger animals, even though medium conditions were identical. More recently, Dodson and Allen (51) monitored the growth of satellite cells in mass cultures and compared satellite cells from young, rapidly growing rats, 12-month-old adult rats, and 24-month-old rats. When compared with the two adult groups, the cells from young rats displayed a shorter lag period after being placed in culture before they began dividing. Apparently, cells from older animals are not able to immediately respond to regulatory factors in culture medium that readily stimulate cells from young animals.

In a closely related example from our laboratory, the initiation of DNA replication by satellite cells cultured from adult rat tibialis anterior and extensor digitorum longus (EDL) muscle was monitored by pulse

labeling cultures with [^{3}H]thymidine from 0 to 8 hr in culture or from 24 to 32 hr. These cells were taken from muscles that had been injected 60 hr earlier with marcaine (a local anesthetic with known myotoxic effects (52)) or from the noninjected contralateral muscles. As indicated in Table II, satellite cells from the marcaine-treated leg began synthesizing DNA almost immediately after being cultured, whereas cells from control muscle did not initiate DNA synthesis until 24 hr later. This provides another example of altering the activity of satellite cells by altering the state of the muscle *in vivo*.

A final example of altered satellite cell response capabilities has been reported by Thompson *et al.* (53). These experiments were designed to document the mechanisms responsible for trenbolone-induced muscle hypertrophy. Trenbolone (TBOH), a synthetic anabolic steroid, has been studied for several years and has been demonstrated to increase animal growth rate and feed efficiency (54, 55). The mode of action has generally been attributed to the effect of TBOH on muscle protein synthesis and degradation.

In our experiments, female rats were injected with TBOH daily for 2 weeks. Then several growth parameters were measured, and cells were prepared for culture. One of the most striking findings was the consistent elevation in muscle DNA content in muscles from the TBOH-treated rats. These observations suggested that increased satellite cell activity may be involved in TBOH action. When the effect of TBOH was examined in culture with satellite cells from control rats, there was no evidence for a direct stimulatory interaction of TBOH on satellite cells. When cultures of satellite cells were prepared from control rats or TBOH-treated rats and examined for their ability to respond to IGF-I and FGF, satellite cells from TBOH-treated rats consistently responded with greater IGF-I-induced differentiation and greater FGF-induced proliferation. Therefore, it appears that the ability of satellite cells to respond to specific growth factors known to regulate proliferation and differentiation was altered by treating the rats with TBOH.

Satellite cells play an important role in muscle growth and regeneration and, depending on the circumstance, they may be called upon to proliferate, differentiate, or remain quiescent. Three growth factors in particular (IGF-I, FGF, and TGF-β) have been shown to stimulate or inhibit satellite cell growth in culture; these factors may have similar effects in the living animal. In addition to regulating satellite cell activity by exposure to different combinations of growth factors or hormones, satellite cells may also be regulated by altering their ability to respond to these growth factors or hormones. Regulation of satellite cell activity *in vivo* is undoubtedly complex, involving the interactions of various tissues and cells and being mediated by a variety of factors.

This work was supported by United States Public Health Serve Grant AG 03393, CRGO/USDA Grant, and the Arizona Agriculture Experiment Station. This communication is Arizona Agriculture Experiment Station journal article no. 7152.

1. Mauro A. Satellite cell of skeletal muscle fibers. J Biophys Biochem Cytol **9**:493–495, 1961.
2. Campion DR. The muscle satellite cell: A review. Int Rev Cytol **87**:225–251, 1984.
3. Schultz E, Gibson MC, Champion T. Satellite cells are mitotically quiescent in mature mouse muscle: An EM and radioautographic study. J Exp Zool **206**:451–456, 1978.
4. Moss FP, Leblond CP. Satellite cells as the source of nuclei in muscles of growing rats. Anat Rec **170**:421–436, 1971.
5. Cossu G, Molinaro M. Cell heterogeneity in the myogenic lineage. Curr Top Dev Biol **23**:185–208, 1987.
6. Cossu G, Zani B, Colette M, Bouche M, Pacifici M, Molinaro M. In vitro differentiation of satellite cells isolated from normal and dystrophic mammalian muscles. A comparison with embryonic myogenic cells. Cell Differ **9**:357–368, 1980.
7. Cossu G, Molinaro M, Pacific M. Differential response of satellite cells and embryonic myoblasts to a tumor promoter. Dev Biol **98**:520–524, 1983.
8. Allen RE, Boxhorn LK. Inhibition of skeletal muscle satellite cell differentiation by transforming growth factor-beta. J Cell Physiol **133**:567–562, 1987.
9. Allen RE, McAllister P, Masak M, Anderson GR. Influence of age on accumulation of alpha-actin in satellite cell-derived myotubes in vitro. Mech Ageing Dev **18**:89–95, 1982.
10. Yablonka-Reuveni Z, Nameroff M. Desmin is expressed in dividing cells in skeletal muscle mass cultures and clones. J Cell Biochem [Suppl] **9B**:43, 1985.
11. Cossu G, Eusebi F, Grassi F, Wanke E. Acetylcholine receptor channels are present in undifferentiated satellite cells but not in embryonic myoblasts in culture. Dev Biol **123**:43–50, 1987.
12. Bader CR, Bertrand D, Cooper E, Mauro A. Membrane currents of rat satellite cells attached to intact skeletal muscle fibers. Neuron **1**:237–240, 1988.
13. Allen RE, Merkel RA, Young RB. Cellular aspects of muscle growth: Myogenic cell proliferation. J Anim Sci **49**:115–127, 1979.
14. Snow MH. Satellite cell activity during hypertrophy of the rat soleus muscle following surgical ablation of the soleus synergists. Anat Rec **214**:125A, 1986.
15. Darr KC, Schultz E. Exercise-induced satellite cell activation in growing and mature skeletal muscle. J Appl Physiol **63**:1816–1821, 1987.
16. Summers PJ, Ashmore CR, Lee YB, Ellis S. Stretch-induced growth in chicken wing muscles: Role of soluble growth-promoting factors. J Cell Physiol **125**:288–294, 1985.
17. Snow MH. Origin of regenerating myoblasts in mammalian skeletal muscle. In: Mauro A, Ed. Muscle Regeneration. New York: Raven Press, pp 91–100, 1979.
18. Carlson BM, Faulkner JA. The regeneration of skeletal muscle fibers following injury: a review. Med Sci Sports Exerc **15**:187–198, 1983.
19. Schultz E, Jaryszak DL, Valliere CR. Response of satellite cells to focal skeletal muscle injury. Muscle Nerve **8**:217–222, 1985.
20. Bischoff R. A satellite cell mitogen from crushed adult muscle. Dev Biol **115**:140–147, 1986.
21. Murray MA, Robbins N. Cell proliferation in denervated muscle:

Time course, distribution and relation to disuse. Neuroscience **7**:1817–1822, 1982.
22. Bischoff R. Enzymatic liberation of myogenic cells from adult rat muscle. Anat Rec **180**:645–662, 1974.
23. Allen RE, Dodson MV, Luiten LS. Regulation of skeletal muscle satellite cell proliferation by bovine pituitary fibroblast growth factor. Exp Cell Res **152**:154–160, 1984.
24. Dodson MV, Allen RE, Hossner KL. Ovine somatomedin, multiplication-stimulating activity and insulin promote skeletal muscle satellite cell proliferation in vitro. Endocrinology **117**:2357–2363, 1985.
25. Bischoff R. Proliferation of muscle satellite cells on intact myofibers in culture. Dev Biol **115**:129–135, 1986.
26. Gospodarowicz D, Weseman J, Moran JS, Lindstrom J. Effect of fibroblast growth factor on the division and fusion of bovine myoblasts. J Cell Biol. **70**:395–405, 1976.
27. Florini JR, Nicolson ML, Dulak NC. Effects of peptide anabolic hormones on growth myoblast in culture. Endocrinology **101**:32–41, 1977.
28. Ewton DZ, Florini JR. Relative effects of the somatomedins, multiplication-stimulating activity and growth hormone on myoblasts and myotubes in culture. Endocrinology **106**:577–583, 1980.
29. Linkhart TA, Clegg CH, Hauschka SD. Myogenic differentiation in permanent clonal mouse myoblast cell lines: Regulation by macromolecular growth factors in the culture medium. Dev Biol **86**:19–30, 1981.
30. Evinger-Hodges MJ, Ewton DZ, Seifert SC, Florini JR. Inhibition of myoblast differentiation in vitro by a protein isolated from liver cell medium. J Cell Biol **93**:395–401, 1982.
31. Florini JR, Roberts AB, Ewton DZ, Falen SL, Flanders KC, Sporn MB. Transforming growth factor-β. A very potent inhibitor of myoblast differentiation, identical to the differentiation inhibitor secreted by buffalo rat liver cells. J Cell Biol **261**:16509–16513, 1986.
32. Massague J, Cheifetz S, Endo T, Nadal-Ginard B. Type beta transforming growth factor is an inhibitor of myogenic differentiation. Proc Natl Acad Sci USA **83**:8206–8210, 1986.
33. Olson EN, Sternberg E, Hu JS, Spizz G, Wilcox C. Regulation of myogenic differentiation by type beta transforming growth factor. J Cell Biol **103**:1799–1806, 1986.
34. Allen RE, Boxhorn LK. Regulation of skeletal muscle satellite cell proliferation and differentiation by transforming growth factor-beta, insulin-like growth factor I, and fibroblast growth factor. J Cell Physiol **138**:311–315, 1989.
35. Jennische E, Hansson H-A. Regenerating skeletal muscle cells express insulin-like growth factor I. Acta Physiol Scand **130**:327–332, 1987.
36. Jennische E, Andersson G, Edwall D, Norsteadt G. Expression of insulin-like growth factor I after tissue injury in the rat. Abstracts, Annual Meeting, Endocrine Society **70**:9, 1988.
37. Busby WH, Klapper DG, Clemmons DR. Purification of a 31,000 Dalton insulin-like growth factor binding protein from human amniotic fluid. J Biol Chem **263**:14203–14210, 1988.
38. Nissley SP, Rechler MM. Insulin-like growth factors: Biosynthesis, receptors and carrier proteins. In: Li CH, Ed. Hormonal Proteins and Peptides. New York: Academic Press, pp127–203, 1984.
39. Clemmons DR, Elgin RG, Han VKM, Casella SJ, D'Ercole AJ, Van Wyk JJ. Cultured fibroblast monolayer secretion that alters the cellular binding of somatomedin C/insulin-like growth factor I. J Clin Invest **77**:1548–1556, 1986.
40. Gospodarowicz D, Ferrara N, Schweigerer L, Neufield G. Structural characterization and biological functions of fibroblast growth factor. Endocr Rev **8**:95–114, 1987.
41. Baird A, Mormede P, Bohlen P. Immunoreactive fibroblast growth factor in cells of peritoneal exudate suggests its identity with macrophage-derived growth factor. Biochem Biophys Res Commun **126**:358–364, 1985.
42. Schweigerer L, Neufeld G, Friedman J, Abraham JA, Fiddes JC, Gospodarowicz D. Capillary endothelial cells express basic fibroblast growth factor, a mitogen that stimulates their own growth. Nature **325**:257–259, 1987.
43. Dimario J, Buffinger N, Yamada S, Strohman RC. Fibroblast growth factor in the extracellular matrix of dystrophic (dys) mouse muscle. Science **244**:688–690, 1989.
44. Sporn MB, Roberts AB, Wakefield LM, de Crombrugghe B. Some recent advances in the chemistry and biology of transforming growth factor-beta. J Cell Biol **105**:1039–1045, 1987.
45. Ellingsworth LR, Brennen JE, Fok K, Rosen DM, Bentz M, Piez KA, Seyedin SM. Antibodies to N-terminal portion of cartilage-inducing factor A and transforming factor-B. J Biol Chem **261**:12362–12367, 1986.
46. Flanders KC, Thompson NL, Cissel DS, VanObberghen-Schilling E, Baker CC, Kass ME, Ellingsworth LR, Roberts AB, Sporn MB. Transforming growth factor-B1: Histochemical localization with antibodies to different epitopes. J Cell Biol **108**:653–660, 1989.
47. Thompson NL, Flanders KC, Smith JM, Ellingsworth LR, Roberts AB, Sporn MB. Expression of transforming growth factor-B1 in specific cells and tissues of adult and neonatal mice. J Cell Biol **108**:661–669, 1989.
48. Wilcox JN, Derynck R. Developmental expression of transforming growth factors alpha and beta in mouse fetus. Mol Cell Biol **8**:3415–3422, 1988.
49. Florini JR, Ewton DZ. Actions of transforming growth factor-β on muscle cells. J Cell Physiol **135**:301–308, 1988.
50. Schultz E, Lipton BH. Skeletal muscle satellite cells: changes in proliferation potential as a function of age. Mech Ageing Dev **20**:377–383, 1982.
51. Dodson MV, Allen RE. Interaction of multiplication stimulating activity/rat insulin-like growth factor II with skeletal muscle satellite cells during aging. Mech Ageing Dev **39**:121–128, 1987.
52. Benoit PW, Belt WD. Destruction and regeneration of skeletal muscle after treatment with a local anesthetic, bupivicaine (Marcaine). J Anat **107**:547–556, 1970.
53. Thompson SH, Boxhorn LK, Kong W, Allen RE. Trenbolone alters the responsiveness of skeletal muscle satellite cells to fibroblast growth factor and insulin-like growth factor I. Endocrinology **124**:2110–2117, 1989.
54. Best JMJ. The use of trenbolone acetate implants in heifer beef production at pasture. Vet Rec **91**:624–626, 1972.
55. Vernon BG, Buttery PJ. Protein turnover in rats treated with trenbolone acetate. Br J Nutr **36**:575–579, 1976.