6
Peptide Growth Factors in Myogenesis and Regeneration

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Introduction

It is now known that many growth factors (GF) affect the behaviour of muscle precursor cells (mpc). Much of this information is derived from tissue culture studies using myogenic cell lines or mpc from embryonic or mature muscle and is reviewed in detail by Grounds (1). There is general correlation between the response to GF of mpc derived from embryonic or adult muscle in tissue culture (Table 1), although the response may be modified by interaction between these different factors.

Table 1 Factors affecting the behaviour of muscle precursor cells.

	Proliferation	Differentiation and Fusion
Fibroblast GFI	↑	↓
Platelet derived GF	↑	↓
Bischoff muscle GF	↑	–
Leukaemia Inhibitory Factor (LIF)	↑	–
Interleukin-6	↑	–
Adrenocorticotropin	↑	–
Insulin GF-1	↑	↑
Prostaglandin E1	–	↑
Transforming GF-b	↑	↓
Interferon	–	↓

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The precise relationship between mpc derived from embryonic and adult muscle is unclear; while they share many features their behaviour is not always completely equivalent (1). This paper is confined to myogenesis in mature muscle which is regenerating in response to injury, and summarizes the role of GF in this process. It must be emphasized that many other factors such as the availability of GF receptors, changes in composition of the extracellular matrix, and the complex role of other cells particularly macrophages, are also of central importance and are reviewed elsewhere (1).

Activation of Quiescent Satellite Cells

Muscle precursor cells of mature muscle are considered to be the satellite cells which lie between the sarcolemma and external lamina of muscle fibres. Whether other cells can also contribute to myogenesis of mature muscle *in vivo* is unclear. The satellite cells are normally quiescent and are considered to be in the Go stage of the cell cycle. It is not known what maintains this quiescent state: it may be the absence of available GF mitogens (such as fibroblast growth factor [FGF]) or of receptors for these mitogens, or it might be due to the presence of negative growth factors such as transforming growth factor-beta (TGF-β).

Experiments in our laboratory with probes to the recently described skeletal muscle specific regulatory genes MyoD1 and myogenin (2) show almost no *in situ* hybridization to tissue sections of uninjured tibialis anterior muscles of mature mice. However, when muscles are crush injured, mRNA for MyoD1 and myogenin is detected after 6 h and is conspicuous by 24 h after injury. Mononuclear cells co-expressing these genes are concentrated in a zone 1.6–2.6 mm distal to the site of injury (3). Although the expression of these genes enables the early identification of many skeletal mpc, it was not possible to confirm whether these were actually satellite cells. The rapid activation of mpc distant from the site of injury suggests a response to a change in the surface or internal environment of the muscle fibre as a result of the injury which is rapidly translated throughout the length of the fibre (e.g. altered electrical properties, changes in Ca++ levels or other ion fluxes, release of proteases or possibly muscle specific GF) rather than a sudden availability of mitogenic GF in the external environment of the satellite cells.

Whether these activated mpc are subsequently stimulated to move through G1 and synthesise DNA may well depend on changes in GF availability in their immediate environment. Our results suggest that some of the activated mpc migrate towards the injured site, where they will certainly encounter changes in GF levels. Large numbers of inflammatory cells accumulated near the site of injury will result in the enzymatic release of FGF bound to the extracellular matrix, plus the production of FGF and other GF by macrophages and other inflammatory cells, and the production of platelet-derived growth factor (PDGF) particularly by platelets. Both FGF and PDGF are potent mitogens for mpc and other mesenchymal cells. In addition, FGF is highly angiogenic and both factors are closely implicated in many aspects of general tissue repair. Leukaemia

Inhibitory Factor (LIF) and interleukin-6 which are also produced by macrophages, have recently been shown in tissue culture to be mitogenic for myoblasts, but not fibroblasts (4). LIF is a particularly potent myoblast mitogen and may well play a central role *in vivo*. The precise relationship of the muscle specific mitogen described by Bischoff (5) to FGF is unclear, and there is little information about the time course of this GF production by traumatized muscle fibres.

The association between the presence of inflammatory cells and the availability of mitogenic GF described above, is supported by our autoradiographic studies which show that the onset of DNA synthesis in mpc is delayed in transplanted as compared with injured muscles (6). These results indicate that the stimulus for replication is closely connected with diffusible substances produced by macrophages and revascularization. Similarly, other studies such as those by Bischoff (7) show that the onset of DNA synthesis in satellite cells is specifically dependent upon GF such as FGF.

Cossu and his colleagues (8) have reported that adrenocorticotrophic hormone (ACTH) is a specific mitogen for mpc. Although circulating levels of ACTH increase very rapidly and transiently in response to stress, it does not seem physiologically sensible that fluctuations which would occur in response to any general stress in mature animals would activate mpc. It seems more appropriate that local changes in ACTH or related neuropeptides might be important as appears to be the case during embryonic myogenesis. Similarly it is unlikely that changes in circulating glucocorticoid levels after stress are important for myogenesis of mature muscle *in vivo*, and the normal levels *in vivo* are probably not limiting. Tissue culture studies show that glucocorticoids such as dexamethasone potentiate the effects of GF mitogens by upregulating cellular receptors for these GF.

Replication of mpc

Once mpc are activated other growth factors are required for the cells to move through G1 to the DNA synthesis phase; these include the insulin growth factors (IGFs) and epidermal growth factor (EGF). IGF-1 and IGF-2 are produced by mpc and IGF-1 stimulates mpc replication in an autocrine and/or paracrine manner. IGF-2 appears to act later during myogenesis and may be involved in synaptogenesis. Although IGFs mediate the effects of growth hormone, studies with growth hormone deficient (*lit/lit*) mice (Grounds and McGeachie, unpublished) and rats indicate that the circulating levels of growth hormones and IGF are unimportant during muscle regeneration; it appears that only the local production of IGF is critical.

EGF is produced by endothelial cells (and other cell types) and is associated with revascularization. Like thrombin it acts in conjunction with FGF to increase the proliferation and inhibit the differentiation of mpc in tissue culture.

Proliferation will also be enhanced by high levels of GF which inhibit mpc differentiation and thus prevent mpc leaving the cell cycle. In tissue culture mpc differentiation is inhibited by high levels of FGF and PDGF (which

complements their mitogenic role) and by TGF-β and possibly interferon. The potent inhibition of mpc differentiation by TGF-β is not readily overcome by combinations of FGF and IGF-1 (9, 10). TGF-β also inhibits proliferation but this can be overcome by FGF (9). TGF-β has an important role in enhancing the formation of the extracellular matrix which is important in new muscle formation: it is produced in a biologically inactive form by many cell types including platelets.

In summary, it seems likely that in mature muscle *in vivo*, mpc replication is enhanced by high levels of FGF, PDFG, Bischoff GF, TGF-β, IGF-1, EGF and thrombin.

Differentiation and Fusion of Myogenic Cells

At some point during the early part of the G1 phase of the cell cycle mpc start to differentiate and become committed to fusion. Differentiation results in the expression of a range of muscle specific genes including those for the contractile proteins (actins, myosins, tropomyosins): some of these genes are expressed before fusion and others after fusion.

Detailed ultrastructural studies of regenerating murine muscle carried out in our laboratories indicate that the process of fusion *in vivo* between immature myogenic cells with closely apposed plasmalemmae is slightly different to that where one or both myogenic cells are well differentiated and surrounded by some form of glycocalyx (10). Fusion of mpc to form myotubes *in vivo* is normally not seen before 2.5 to 3 days after injury. Fusion is a highly complex process and it is difficult to assess the role that growth factors play *in vivo*.

In tissue culture, fusion between mpc is partially dependent upon their density: this affects the balance of GF and leads to a relative increase in those produced by the mpc themselves, in particular IGF-1 and prostaglandin E1 (PGE1). The prostaglandins are a family of compounds derived from essential fatty acids such as linoleic acid and are closely associated with changes in many factors which are important for cell fusion (e.g activation of protein kinases, turnover of phospholipids and intracellular Ca++ levels). It seems likely that the autocrine and/or paracrine effect of prostaglandins may be central to the extensive membrane reorganization associated with myogenic fusion.

The receptors for IGF (unlike other GF) are not down-regulated during differentiation, and IGF-I may play a central role in stimulating mpc differentiation. The potent inhibition by TGF-β of mpc differentiation (and subsequent fusion) suggests that levels of this GF should decrease for differentiation and fusion to occur: however, the combination of high levels of IGF-1 and PGE1 might be able to overcome this inhibition in tissue culture. In tissue culture FGF, PDGF and interferon also inhibit mpc differentiation. There may be a decrease *in vivo* in the availability of FGF from extracellular matrix or a decreased production of these GF by infiltrating cells which facilitates differentiation. Regardless of changes in these GF levels, it seems likely that the local

increases in IGF-1 and PGE1 produced by myogenic cells, are of critical importance for differentiation and fusion respectively.

In summary, the balance of GF which probably favour differentiation and fusion of myogenic cells *in vivo* are increased IGF-1 and PGE1, and decreased TGF-β, FGF, and PDGF.

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