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## Review

# Changes in muscle mass and phenotype and the expression of autocrine and systemic growth factors by muscle in response to stretch and overload

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ABSTRACT

The study of the underlying mechanisms by which cells respond to mechanical stimuli, i.e. the link between the mechanical stimulus and gene expression, represents a new and important area in the morphological sciences. Several cell types ('mechanocytes'), e.g. osteoblasts and fibroblasts as well as smooth, cardiac and skeletal muscle cells are activated by mechanical strain and there is now mounting evidence that this involves the cytoskeleton. Muscle offers one of the best opportunities for studying this type of mechanotransduction as the mechanical activity generated by and imposed upon muscle tissue can be accurately controlled and measured in both in vitro and in vivo systems. Muscle is highly responsive to changes in functional demands. Overload leads to hypertrophy, whilst decreased load force generation and immobilisation with the muscle in the shortened position leads to atrophy. For instance it has been shown that stretch is an important mechanical signal for the production of more actin and myosin filaments and the addition of new sarcomeres in series and in parallel. This is preceded by upregulation of transcription of the appropriate genes some of which such as the myosin isoforms markedly change the muscle phenotype. Indeed, the switch in the expression induced by mechanical activity of myosin heavy chain genes which encode different molecular motors is a means via which the tissue adapts to a given type of physical activity. As far as increase in mass is concerned, our group have cloned the cDNA of a splice variant of IGF-1 that is produced by active muscle that appears to be the factor that controls local tissue repair, maintenance and remodelling. From its sequence it can be seen that it is derived from the IGF-1 gene by alternative splicing but it has different exons to the liver isoforms. It has a 52 base insert in the E domain which alters the reading frame of the 3′ end. Therefore, this splice variant of IGF-1 is likely to bind to a different binding protein which exists in the interstitial tissue spaces of muscle, neuronal tissue and bone. This would be expected to localise its action as it would be unstable in the unbound form which is important as its production would not disturb the glucose homeostasis unduly. This new growth factor has been called mechano growth factor (MGF) to distinguish it from the liver IGFs which have a systemic mode of action. Although the liver is usually thought of as the source of circulating IGF-l, it has recently been shown that during exercise skeletal muscle not only produces much of the circulating IGF-l but active musculature also utilises most of the IGF-l produced. We have cloned both an autocrine and endocrine IGF-1, both of which are upregulated in cardiac as well as skeletal muscle when subjected to overload. It has been shown that, in contrast to normal muscle, MGF is not detectable in dystrophic mdx muscles even when subjected to stretch and stretch combined with electrical stimulation. This is true for muscular dystrophies that are due to the lack of dystrophin (X-linked) and due to a laminin deficiency (autosomal), thus indicating that the dystrophin cytoskeletal complex may be involved in the mechanotransduction mechanism. When this complex is defective the necessary systemic as well as autocrine IGF-1 growth factors required for local repair are not produced and the ensuing cell death results in progressive loss of muscle mass. The discovery of the locally produced IGF-1 appears to provide the link between the mechanical stimulus and the activation of gene expression.

*Key words*: Mechanotransduction; myosin gene switching; muscle fibre types; muscle mass; autocrine growth factors; IGF-1; muscular dystrophy.

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## INTRODUCTION

It has been appreciated for some time that many cell types respond to mechanical signals. However, we know very little about how these signals are transduced into chemical signals and how these in turn, regulate gene expression. Indeed, in these days of molecular genetics, people tend to think of cell development as being strictly programmed in the genome. Although early differentiation may occur according to set programmes it is known that gene expression in cells such as osteocytes, fibroblasts as well as skeletal, cardiac and smooth muscle cells is very much influenced by mechanical factors. These cell types have been termed mechanocytes (Goldspink & Booth, 1992). However, most cells respond to mechanical stimuli, e.g. if part of the gastrointestinal system, one kidney or part of the liver is removed, the tissue mass of that organ system is rapidly increased to compensate. Indeed, the eventual size an organ attains during normal development appears to be governed by mechanical factors as well as metabolic activity. Thus even in these noncontractile tissues the cells apparently respond to gravity, hydrodynamic pressure and movement. Mechanical strain has also been found to induce cardiac myocyte hypertrophy (Sadoshima et al. 1993) and to increase cell number and DNA synthesis in cultured bone (Hasegawa et al. 1985), epithelial cells (Brunette, 1984), endothelial cells (Sumpio et al. 1987) and mesangial cells (Harris et al. 1992). Cyclic strain induces reorganisation of the cytoskeleton in endothelial cells (Sumpio et al. 1988) and increases formation of collagen and other extracellular matrix proteins in bone (Jones et al. 1991) and early gene expression in endothelium (see review by Burnstock, 1999) which apparently involves autocrine/paracrine regulation of vascular smooth muscle via ATP purinoceptors (Hamada et al. 1998). The way these mechanical signals are transduced must involve moving parts, yet this aspect of the morphological sciences has thus far received little attention. Using new molecular and cellular biology methods we can now study how mechanical factors 'regulate the regulators' of gene expression as well as the resulting structural changes.

Muscle gene expression has been quite extensively studied during differentiation and early development and has led to the discovery of a range of myogenic factors which include myogenin and MyoD (Buskin & Hauseka, 1989; Weintraub et al. 1989). These induce terminal differentiation of pluripotent cells into muscle cells which then divide and differentiate into muscle fibres under the influence of growth factors including myostatin which is a member of the TGF$\beta$ family and which represses muscle development. Transgenic mice in which this gene has been knocked out showed more than double the muscle weight of normal mice (McPherron et al. 1997). Thus there is both negative and positive regulation. However, it is unlikely that none of the factors that operate during early differentiation and perinatal development are regulated by mechanical signals of the type which operate after birth. The production of differentiation and growth factors during early stages of tissue differentiation has to be preprogrammed in the genome. Tissue adaptability after birth which is very much a feature of skeletal muscle is also conceivably regulated by growth factors but these include ones that are induced by mechanical signals.

For the purpose of studying the responses to physical factors, striated muscle is a useful tissue as it generates the mechanical strain as well as responding to it and one can exercise, electrically stimulate or stretch muscle for the purpose of elucidating mechanotransduction mechanisms. In our work we have been particularly interested in the way mechanical signals influence the expression of subsets of genes which determine muscle mass and muscle phenotype. This has led to the search for systemic as well as the local growth factors that may be involved. With regard to the latter it has been appreciated for some time that if a muscle is exercised, it is mainly that muscle that undergoes hypertrophy and not all the muscles of the limb or the whole body. Throughout life, all tissues sustain microdamage and this is a particular problem in a mechanical tissue and in a tissue in which there is no cell replacement, e.g. neuronal tissue as well as skeletal and cardiac muscle. Therefore the search for the local mechanisms of gene activation goes beyond the remodelling of the tissue growth and adaptation to increased activity but includes the local repair and protection mechanisms involved in tissue maintenance throughout life.

There have been some developments in understanding mechanotransduction mechanisms which result in electrical signals. The patch clamp technique has revealed that mechanosensitive channels exist, for example stretch activated $Ca^{2+}$ channels in muscle. When looking for factors that activate gene expression, the influx of anions or cations, particularly calcium and nitric oxide, have to be considered. However, as far as muscle is concerned extensive studies on cardiac myocytes by Izumo and coworkers indicate that stretch induced hypertrophy of cardiac myocytes involves the production of autocrine growth factors (Sadoshima & Izumo, 1997). There is evidence

that other cell systems also produce autocrine growth factors and the morphological basis of the mechanism involves the cytoskeleton and the extracellular matrix, the deformation of which leads to the activation of certain enzymes which result in the rapid production of signalling molecules (Ingebar, 1997). These in turn presumably induce a cascade of events and the expression of transcriptional factors that activate the structural genes required for the repair and adaption of the individual cells.

Some years ago my group commenced the study of the cellular adaptation of muscle to both force development and stretch. During the last decade or so this has been extended to studying the molecular mechanisms involved in determining muscle phenotype and muscle mass. The intention here is not to review all the factors that induce changes in muscle gene expression but to describe the way these are influenced by mechanical factors and the underlying mechanisms involved, i.e. mechanochemical transduction.

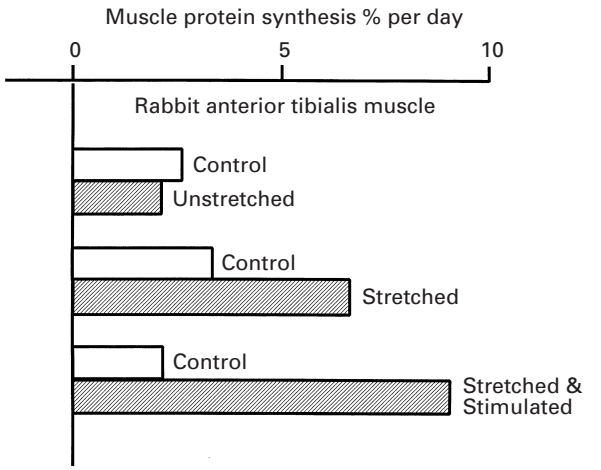


Fig. 1. Effect of stretch and stretch combined with electrical stimulation on the rate of protein synthesis in the anterior tibialis (cranial tibialis) muscle of the adult rabbit. Stretch was achieved by immobilisation of the lower limb in the plantar flexed (extended) position. Stimulation was through implanted electrodes connected to a minature stimulation circuit that generated biphasic pulses at a frequency of 5 Hz. Data from Goldspink & Goldspink (1986).

## STRUCTURAL CHANGES OF MUSCLE CELLS IN RESPONSE TO STRETCH AND OVERLOAD

Stretch has been shown to be a powerful stimulant of muscle protein synthesis and muscle growth. During postnatal growth, skeletal muscles fibres elongate by adding new sarcomeres (Goldspink, 1964) serially to the ends of existing myofibrils (Griffin et al. 1971). Even mature muscles have been shown to be capable of adapting to a new functional length by adding or removing sarcomeres in series (Tabary et al. 1972; Williams et al. 1973; Goldspink, 1984). In this way sarcomere length is adjusted back to the optimum for force generation, velocity and hence power output. The stretch effect and the adaptation to an increased functional length is known to be associated with increased protein synthesis (Goldspink & Goldspink, 1986; Loughna et al. 1986). More recently we have studied the way gene expression in muscle is influenced by stretch by casting the limb with the muscle either in the shortened or lengthened position (Loughna et al. 1990). Several interesting findings emerged from this study including the fact that the slow soleus muscle which does not normally express fast type IIb myosin hc genes, begins to transcribe the fast myosin hc gene after only a day if its muscle fibres are not subjected to stretch or are not producing force. Stretch combined with electrical stimulation was also found to induce very rapid hypertrophy of tibialis anterior in the adult animal (Goldspink et al. 1992). Both force generation and stretch are major factors in activating protein synthesis and the combination of these stimuli apparently has a pronounced additive effect (Fig. 1). Indeed, if mammalian muscles are stimulated and not held in a stretched position the fibres undergo a decrease in size (Tabary et al. 1981). Thus it seems that of the 2 mechanical factors stretch is the more important. If, as suspected, the sensor is located in the membrane this will be subjected to greater mechanical strain if it is stretched and pulled in the opposite direction by the electrical stimulation inducing the muscle to contract. Nevertheless, the rapidity and magnitude of the hypertrophy induced by stimulation combined with stretch was surprising.

Associated with this very significant increase in muscle size (35% in 4 d) there was a marked increase (up to 250%) in RNA content of the muscles which was found to peak after 2 d of the commencement of stretch and stretch combined with stimulation. This rapid increase in total RNA, which is mainly ribosomal RNA, indicates that muscle fibre hypertrophy may be controlled mainly at the level of translation and that the rapid increase in the number of ribosomes means that more message can be translated into protein. There are situations when an abundant message is present but the fibres are still undergoing atrophy, e.g. lack of stretch (with and without stimulation). This again indicates that muscle size, unlike muscle phenotype, may be regulated mainly at the level of gene translation rather than transcription.

## MECHANICAL FACTORS AND THE REGULATION OF MUSCLE PHENOTYPE

Skeletal muscles in the mammals are composed of mixtures of different types of muscle fibres that express myosin isoform genes (for review see Pette & Staron, 1990) which encode different molecular motors. The slow oxidative (type 1) fibres have a molecular motor that has a long cycle time and uses ATP at a slow rate. Thus they are adapted for maintaining isometric force very economically or carrying out repetitive slow isotonic contractions efficiently. They also contain many mitochondria so that ATP can be replenished almost as quickly as it is used. Hence type 1 fibres are fatigue resistant. In addition there are fast muscle fibre types which express molecular motors that produce more rapid myosin cross bridge cycling rates. These are recruited when more powerful movements are required or the isometric force produced by the slow fibres is insufficient. There are several subtypes of fast fibres including 2A which is reasonably fast and adapted for producing sustained power. Then there are 2X fibres which are faster and non-oxidative so they are fatigued rapidly. These and the 2A fibres are the fast types in human muscle (Ennion et al. 1995) not the very fast 2B which are the main fast type in small mammals.

The interconvertibility of fibre types was demonstrated by cross-innervation and by chronic stimulation and has been reviewed (Pette & Vrbova, 1986). It became generally accepted that the frequency of stimulation was the important factor in determining fibre type transition. However, it was shown that higher stimulation frequencies were just as effective in producing the fast to slow switch (Streter et al. 1982). Using plaster cast limb immobilisation, stretch alone (Fig 3.) was found to induce fast fibres to lay down slow type sarcomeres (Williams et al. 1986) and under these conditions virtually no EMG signal can be detected (Hnik et al. 1985). Therefore it is not possible that stimulation frequency per se is the primary cause of muscle phenotype determination. As mentioned above, more complete reprogramming of the muscle was obtained when stretch was combined with electrical stimulation irrespective of the frequency. This indicates that the signal for the fast to slow change is mechanical strain rather than stimulation frequency per se. This makes physiological sense as it can be argued that the muscle cells by responding to isometric overload are adapting to an increased postural role.

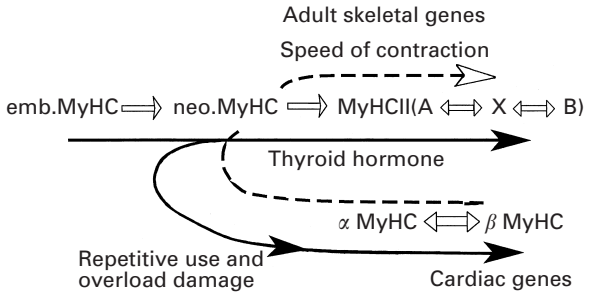


Fig. 2. Diagram illustrating the way muscle can adapt to mechanical signals and hormones by switching expression of the individual myosin hc genes and how this changes contractile properties.

When a muscle is subjected to stretch and/or electrical stimulation not only is the slow type 1 myosin expressed but the fast myosin genes are turned off, thus demonstrating complete reprogramming (Goldspink et al. 1992). Indeed, it seems that all muscle fibres stay phenotypically fast unless they are subjected to stretch and isometric force development. As shown by the soleus muscle, when immobilised in the shortened position (Loughna et al. 1990), subjected to surgical overload (Gregory et al. 1986) or subjected to hypogravity (Oganov & Potapov, 1976) it reverts to expressing fast myosin genes. More recent work strongly indicates that the determination of muscle phenotype is controlled at the level of gene transcription and is strongly influenced (McKoy et al. 1998). However, muscle fibre hypertrophy which is also related to mechanical signals, apparently involves a different mechanism with regulation at the level of translation rather than transcription (although the latter is important for the production of more ribosomal RNA and protein). The induction of hypertrophy seems to have a shorter time window than the induction of the slow genes. Therefore for adaptation for sprinting short intensive bouts of exercise are required as these result in increase in mass without the upregulation of slow myosin.

Other subsets of genes are also involved in the interconversion of fibre types including mitochondrial and cytoplasmic enzyme genes and those that induce changes in the vascularity of the tissue. These are apparently not coordinated. Indeed the signals involved in switching of myosin isoform gene expression are probably different from those that induce mitochondrial or sarcoplasmic enzyme expression but under most training conditions the directions happen to coincide. Williams et al. (1986) reported that 10 Hz chronic stimulation of the rabbit tibialis anterior muscle for 21 d which is known to increase type 1 myosin expression, resulted in a 5-fold increase in cytochrome b mRNA but a 4-fold reduction in the levels of aldolase mRNA. These 3 subgroups of genes

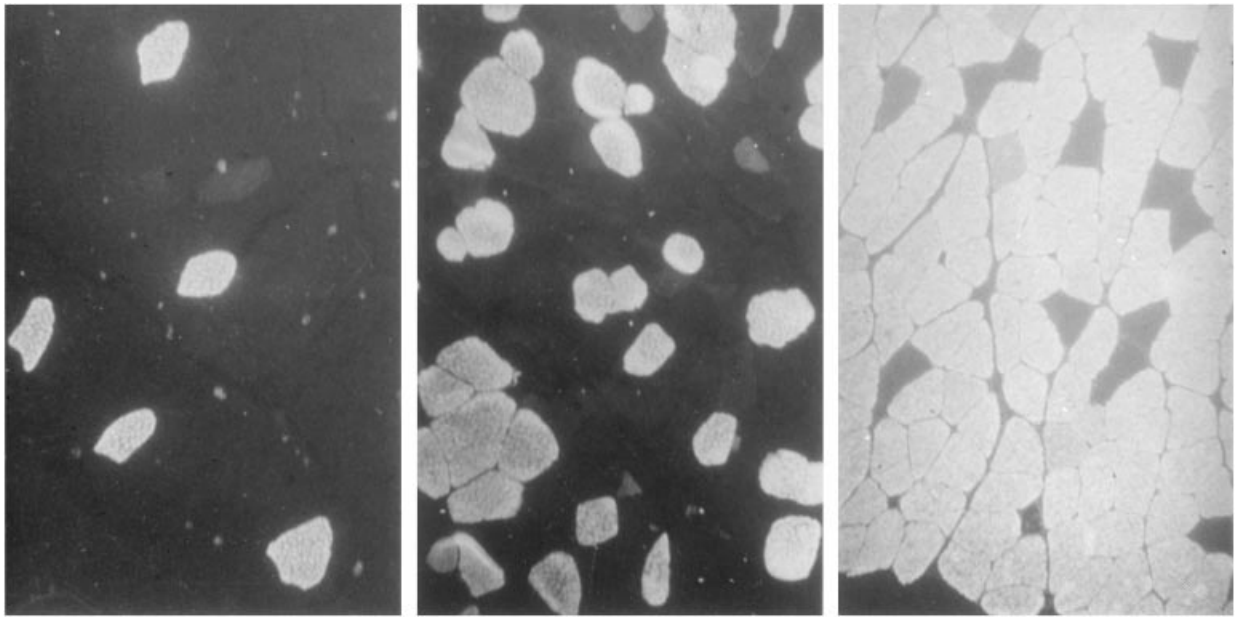

Fig. 3. Switching of myosin gene expression in the rabbit tibialis anterior (cranial tibialis) muscle of the adult rabbit in response to stretch. *Left*: normal muscle stained for slow ($\beta$) type I myosin antibody. *Middle*: the same muscle in a limb that has been surgically lengthened for 1 mo showing the marked increase in slow myosin gene expression. *Right*: immunostaining of the stretched muscle using an antibody to embryonic/neonatal myosin which indicates that the muscle fibres revert back to the embryonic form when converting from the fast to the slow phenotype in response to overload. Collaborative work with Drs P Williams and A. H. R. W. Simpson and Professor J Kenwright (unpublished work) .

have a different time course for expression which illustrates that duration as well as the intensity of the mechanical signals is important. It is known from EMG studies that postural muscle fibres, such as those of the soleus, are activated about 90% of the time during standing or walking whilst the fibres in other skeletal muscles are activated only 5% of the time (Hnik et al. 1985). Therefore slow postural muscles are subjected to stretch and stimulation long enough for the full transition to the slow type I phenotype to be achieved. Only some details of the molecular mechanism(s) involved in isoform gene switching are known.

## MECHANICAL ACTIVITY AND THE EXPRESSION OF GROWTH FACTORS BY MUSCLE

It was previously known that muscle expresses growth factors such as FGF (Clarke & Feeback, 1996) and IGF-1 and that they are upregulated by physical activity (Czerwinski et al. 1994; DF Goldspink et al. 1995; Perrone et al. 1995). However, as far as IGF-1 is concerned it was not appreciated until recently, that there are 2 main muscle forms, one of which is similar to the main IGF-1 isoform produced in liver and another which is apparently designed for an autocrine/paracrine mode of action (Yang et al. 1996). This latter form of IGF-1 is produced by overloaded muscle and appears to be one of the main growth factors that controls local tissue repair and remodelling in cardiac (Skarli et al. 1998) as well as skeletal muscle. The difference between the liver types of IGF-1 and that produced by muscle was implied by the in vitro studies by Vandenburg's group who showed release of IGF-1 from stretched chicken skeletal myocytes and also added recombinant (mature) IGF-1 into the cell culture medium. They reached the conclusions that the IGF-1 released from stretched cells is more effective in increasing protein synthesis than the added (basic) rIGF-1 although stretched muscle is more responsive than nonstretched muscle cells to both (Perrone et al. 1995).

The effects the different liver IGF-l splice variants elicit include muscle differentiation (Florini et al. 1994; Enger et al. 1996), nerve sprouting (D'Ercole et al. 1996), mitosis (Pietrzkowski et al. 1992; Coolican et al. 1997), prevention of apoptosis and stimulation of protein synthesis and muscle fibre hypertrophy (Coleman et al. 1995) with or without any of the other effects. Although there is a growing appreciation that these diverse effects depend on the particular IGF-1 isoform expressed and the distribution of its binding protein(s), in these respects the muscle IGF-1s were likely to differ from those expressed in muscle tissue.

## MECHANOTRANSDUCTION MECHANISM RESULTING IN MYOSIN GENE EXPRESSION SWITCHING

The expression of the individual myosin genes is to a large extent regulated by mechanical signals. In human skeletal muscle there are at least 7 separate skeletal myosin hc genes including the embryonic, neonatal and adult fast and slow myosin hc genes (Fig. 4). These are arranged in series on chromosome 17 in man (Leinwand et al. 1983*a*). As well as being expressed in different tissues and in different cell types, different myosin hc genes including embryonic and neonatal isoforms are expressed at various stages during development (Butler-Brown & Whalen, 1984; Swynghedauw, 1986). There are also 2 cardiac myosin hcs ($\alpha$ and $\beta$) which are located in tandem on chromosome 14 in man (Leinwand, 1983*b*) and 2 smooth muscle myosin hc genes on chromosome 16 (Deng et al. 1993). The cardiac genes are also expressed in skeletal muscles. Indeed, the slow $\beta$ cardiac myosin hc is the predominant gene expressed in the type I fibres of the soleus muscle of mammals and can be regarded as the very slow skeletal muscle myosin that is suitable for postural activity as the slow myosin ATPase activity means that isometric and semi-isometric forces are maintained very economically.

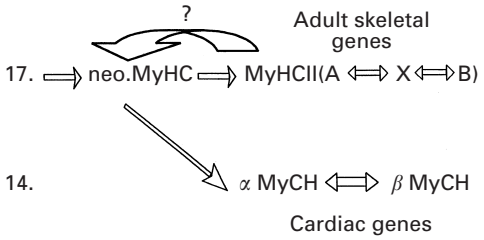


Fig. 4. The myosin heavy chain family of genes in mammals and the position of individual genes on chromosomes 17 and 14 in man.

We have studied the expression of the $\beta$ cardiac (type 1) myosin heavy chain gene as it is upregulated by stretch in cardiac as well as skeletal muscle. All the muscle myosin genes (Fig. 5) have regulatory or promoter sequences in their 5′ flanking sequence that are not too far upstream. This sequence is not transcribed into mRNA but it is needed to 'drive' the gene. The 3′ flanking sequence appears to have a regulatory function and is important in determining the stability of the message. Experiments have shown that without this 5′ sequence expression levels are very low. When spliced to reporter gene sequences and introduced into transgenic animals, the 5′ regulatory sequence is important for the stretch response (Tsika et al. 1996). Therefore one must assume that there is a stretch response element in the promoter region but what transactivating factor or factors are involved needs to be elucidated. As it is also known that myosin gene expression is influenced by hormones, e.g.

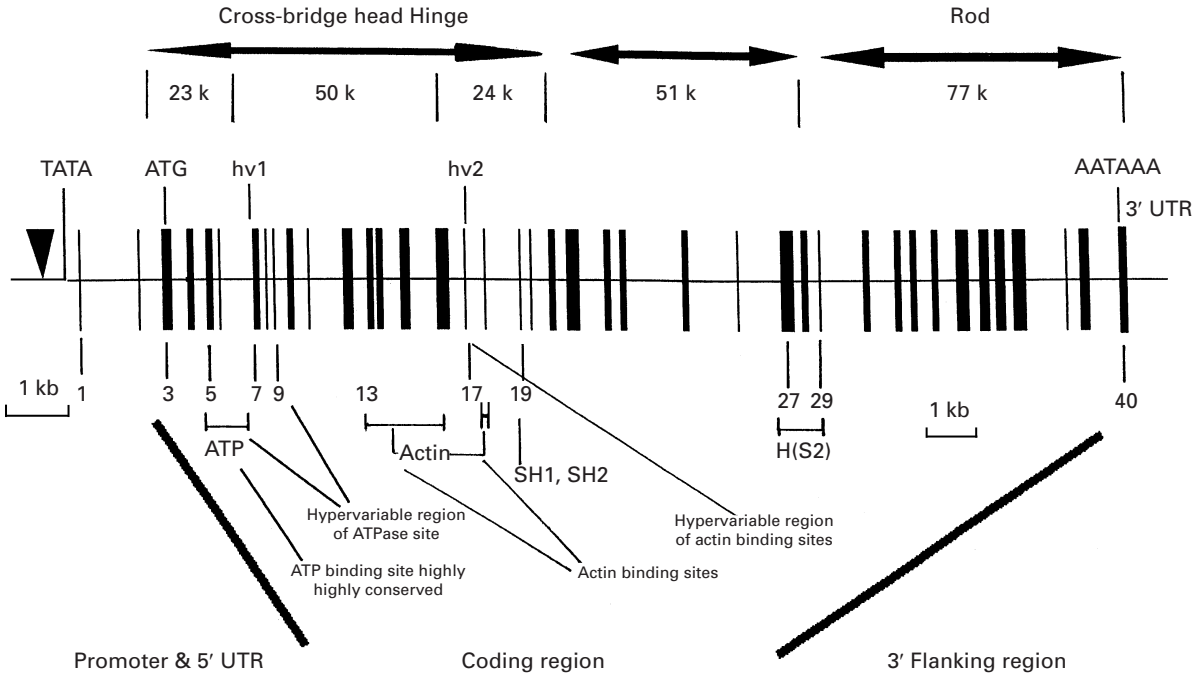


Fig. 5. Generalised structure of the myosin heavy chain genes in skeletal and cardiac muscle. Note the region encoding the ATPase site of the cross-bridges coding region and the 5′ flanking region where the regulatory response elements are located. These are activated by transcriptional factors that are presumably induced by mechanical signals as well as hormones.

insulin, thyroid hormone as well as IGF-1, there may be a common pathway involving the same second messengers and transcriptional factors.

From studies on other genes it is known that the binding of certain proteins or transcriptional factors at or near the promoter region results in activation or repression of the gene. The usual feature of a promoter sequence is the presence of a 'TATA' box that is believed to be the binding site for TFII transcriptional complex of proteins and which is usually a few bases 'upstream' from the transcription start site. The complex has been described as the 'signpost' for the RNA polymerase II. This enzyme binds to TFIID and depending on which other trans-activator or trans-repressor proteins, will or will not, transcribe the gene in question into RNA (primary transcript). Gene activation or repression is more complex than first thought as it does not involve just one activating protein but a complex of quite large protein molecules (Roberts & Green, 1995). Some of these contain a zinc finger motif and others such as myogenin, are known as helix loop helix transcriptional factors. In addition to the TATA box there is often a CCAAT box which is thought to be the binding site for transcriptional factors which 'upregulate' or 'downregulate' transcription. In the myosin hc genes 5′ nontranscribed region there are positive and negative elements which activate or repress transcription respectively. Other regulatory sequences, such as enhancers, may also be involved in facilitating the binding of the transcriptional factor proteins to DNA. These may be at the 5′ or 3′ end of the gene and many bases from the start sequence. Enhancer sequences are also sometimes found in untranslated introns as well as exons. In the myosin hc genes these are found in introns 1 and 2 which are untranslated (Gauvry et al. 1996; Chang et al. 1993, 1995). In addition, in muscle genes there are also regulatory elements known as E boxes (CAANTG). These represent binding regions for myogenic factors which determine whether a cell is going to be a muscle cell or not by specifically turning on the muscle genes (Buskin & Hauschka, 1989; Weinbtraub et al. 1989).

## LOCAL CONTROL OF MUSCLE REPAIR, MAINTENANCE AND ADAPTATION

It has been known for some time that there are local factors as well as systemic factors that regulate tissue growth. The growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis is the main regulator of tissue mass during early life. Our group have cloned the cDNA of a splice variant of IGF-1 that is produced by active muscle that appears to be the factor that controls local tissue repair, maintenance and remodelling. From its sequence it can be seen that it is derived from the IGF-1 gene by alternative splicing but it has different exons to the liver isoforms (Fig. 6). Unlike the liver isoforms it is not glycosylated, is therefore smaller and probably has a shorter half life and is thus suited for an autocrine/paracrine rather than a systemic mode of action. It has a 52 base insert in the E domain which alters the reading frame of the 3′ end. Therefore this splice variant of IGF-1 is likely to bind to a different binding protein, e.g. BP5, which exists only in the extracellular matrix of muscle, neuronal tissue and bone. This would be expected to localise its action as it would be unstable in the unbound form which is important as its production would not disturb unduly the glucose homeostasis mechanism. This new growth factor which is designed for an autocrine function has been called mechano growth factor (MGF) to distinguish it from the liver IGFs which have a systemic mode of action. In addition to the autocrine form we also find that muscle expresses an isoform that is similar to the main liver type with a similar exon sequence. Because of the similarity in exon structure we have designated this muscle L.IGF-1 as like the liver IGF-1s it probably has a systemic or endocrine mode of action.

Using specific gene probes, derived from the sequences of the liver and muscle type IGF-1 isoforms we have shown that MGF is expressed in human (Yang et al. 1996), mouse (Goldspink et al. 1996) and rabbit (Yang et al. 1997; McKoy et al. 1999) muscle when subjected to stretch by plaster cast immobilisation with the muscle in its lengthened position. We have also shown that in contrast to normal muscle, the mRNA for MGF is not detectable in dystrophic mdx muscles even when subjected to stretch and stretch combined with electrical stimulation (Goldspink et al. 1996). The systemic levels of IGF-1s are mainly controlled by circulating growth hormone at least those produced by the liver. Interestingly, it has recently been shown that during intensive exercise most of the circulating IGF-1 is actually derived from the active muscles and that most of the IGF-1 circulating IGF-1 is actually utilised by the musculature (Brahm et al. 1997). In recent work in which we have used RNase protection to distinguish and quantify the expression of different muscle IGF-1 isoforms we have found that both the autocrine (MGF) and the systemic type (muscle L.IGF-1) that is also produced by the muscle are both markedly upregulated by stretch and stretch combined with stimulation (McKoy et al. 1999).

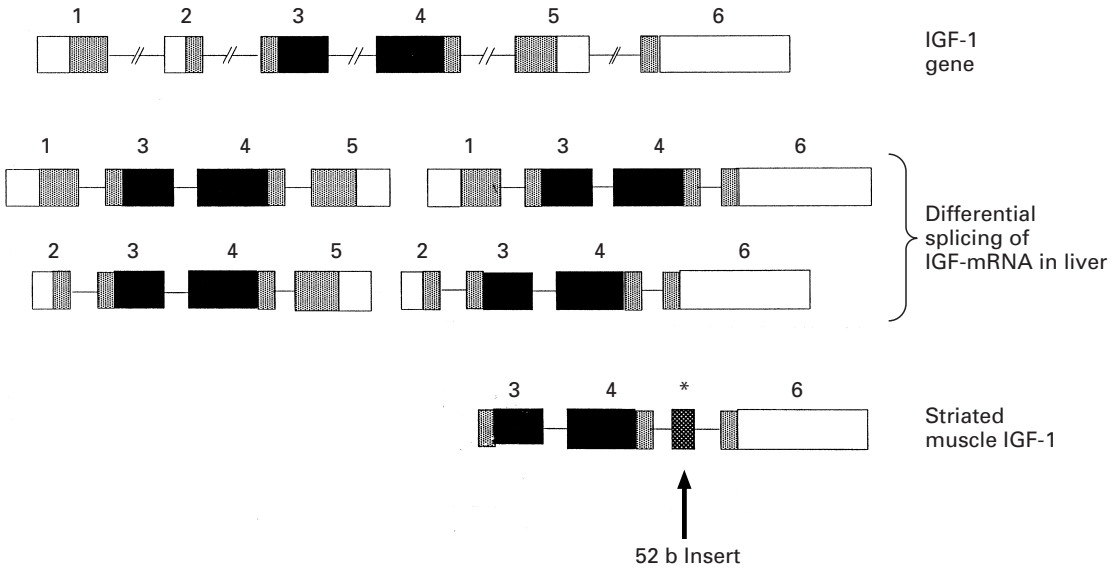


Fig. 6. Diagram showing the way the insulin-like growth factor (IGF-1) gene can be alternatively spliced to yield the liver-type forms and the splice variant that is expressed in overloaded muscle. The latter has been called mechano growth factor (MGF) as it is apparently designed for an autocrine/paracrine mode of action. The 52 base insert results in a reading frame shift so that the carboxyl terminal end of the peptide is different and this probably results in different binding characteristics for receptor and binding proteins. Details of sequences are published in Yang et al. (1996).

During ageing, however, the circulating growth hormone and IGF-1 are known to decrease markedly particularly after the initial growth spurt (Rudman et al. 1981). Although IGF-1s produced via growth hormone stimulation are important during early postnatal muscle development, it appears that IGF-1 produced by muscle during exercise becomes more important for the maintenance of muscle mass. The decline in the production by the liver and the inability to supplement it by locally produced IGF-1 is most probably one of the main reasons for the progressive nature of the dystrophies.

As we have cloned the human MGF cDNA we have been able to design specific probes which allowed us to use a syringe needle aspiration RT-PCR technique on the forearm flexor muscles of a human subject who had engaged in eccentric exercise (Yang et al. 1996). At 2 h after the exercise MGF was shown to be strongly expressed which is too short a time interval to explain the response in terms of satellite cell proliferation. Indeed, using in situ hybridisation with cRNA probes on sections of stretched rabbit muscle we have shown that the IGF-1 is expressed in the muscle fibres themselves as well as in smaller cells which appear to be satellite cells or fibroblasts (Yang et al. 1996). The fact that we can monitor MGF expression in human muscle using a minimally invasive technique means the work could be easily extrapolated to patients with muscular dystrophy once the fundamental studies have been carried out using animal models.

## CYTOSKELETAL DEFECTS AND FAILURE TO MAINTAIN MUSCLE MASS IN MUSCULAR DYSTROPHY

Using specific probes for RT-PCR and RNase protection, derived from the sequences of the liver and muscle type IGF-1 isoforms we have shown that MGF is expressed in mouse, rabbit and human muscle when subjected to stretch and overloaded. We have also shown that in contrast to normal muscle, the mRNA for MGF is not detectable in dystrophic murine muscles when subjected to stretch even when using the sensitive PCR method (Goldspink et al. 1995). The systemic muscle IGF-1 (muscle L.IGF-1) is however detectable in the dystrophic muscle. The inability to express MGF in response to stretch and overload was shown both for the X-linked Duchenne type dystrophy in which the protein dystrophin is lacking and for the autosomal recessive type in which one of the extracellular proteins, merosin, a muscle form of laminin, is missing. The latter anchors the dystrophin complex to the extracellular matrix and the absence of this attachment appears to result in lack of mechanotransduction. The dystrophin complex at the N end terminal is attached to actin filaments. At the C terminal end dystrophin is attached to an elaborate array of sarcoglycans, dystroglycans as well as the extracellular matrix via merosin. Tyrosine kinase and nNOS moieties are also associated with the dystrophin complex. It seems inconceivable therefore that this elaborate structure is

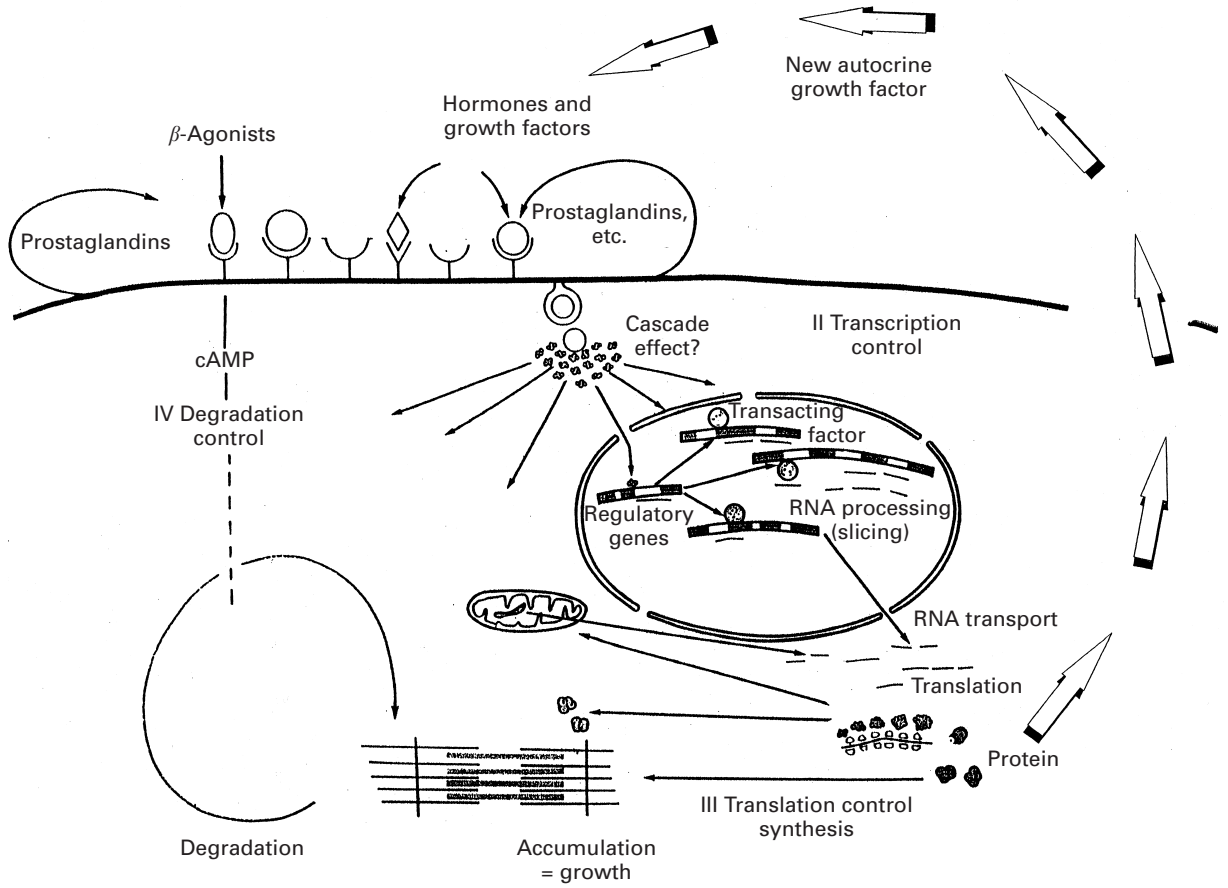


Fig. 7. Diagram showing the types of membrane signals involved in regulating muscle mass and phenotype with special reference to the autocrine action of the new growth factor (MGF) that is expressed in response to stretch and overload.

present merely to stiffen the membrane. As with other cytoskeletal systems we believe it is involved in mechanosignalling and gene regulation Even if dystrophin is not directly involved in the mechanotransduction mechanism the evidence indicates that it is indirectly involved as membrane stiffness is critical for the response of muscle fibres to stretch.

Other evidence that IGF-1 is important in determining muscle mass and preventing dystrophy has been obtained from transgenic mouse experiments. Transgenic mice produced by introduction of the human IGF-1 cDNA under the control of a chicken actin promoter showed elevated muscle but not systemic levels of IGF-1 and muscle fibre hypertrophy but no significant increase in body weight (Coleman et al. 1995). As far as dystrophy is concerned the gene experiments give an indication regarding its aetiology. These include those in which the IGF-1 gene (Baker et al. 1993; Powell-Baxton et al. 1993) was truncated or the IGF-1 receptor(s) were knocked out. In both situations the result was early, severe muscular dystrophy and as a consequence these mice died at or just after birth. Administration of recombinant IGF-1 has been shown to have a markedly beneficial affect on murine muscular dystrophy (Zdanowitz et al. 1995). This same group has recently shown that the influence of IGF-1 involves the upregulation of MyoD (Hsu et al. 1997) which is one of the myogenic factors known to be involved in the induction of muscle specific genes.

We have now produced a specific antibody to MGF which works well with tissue sections as well as Western blots. We have also put the cDNA into an expression system and we are using the antibody to purify the MGF peptide so that protein therapy can now be carried out. The expressed peptide contains a marker sequence so that the localisation of the peptide can be determined and used in competition assays. The progressive nature of the musclular dystrophies appears to be linked to the inability to respond to mechanical signals by producing MGF and thus the inability to induce local tissue repair and to prevent apoptosis. This may not prevent the initial fibre hypertrophy which does occur in some dystrophic muscles as IGF-1 is not completely absent because of the circulating levels of that derived from the skeletal

muscle as well as the liver. However, associated with the deficiency is the local growth factor production in response to mechanical stimuli.

## MORPHOLOGICAL ASPECTS OF LOCAL SIGNALLING AND GENE REGULATION

When cells sustain damage cytokines are released which initiate blood clotting, scar formation and cell proliferation, etc. Some of these may simply be released into the extracellular compartment on rupture of the membrane. This may be the way the purinoceptors are activated by ATP which seems to be an immediate signalling mechanism, as is nitric oxide production. However, in nonmitotic tissue such as neuronal and muscle cells it appears that a more sophisticated mechanism is required to initiate cell repair and adaptation.

At the present time all we can say is that when muscle and probably other types of cells respond to mechanical overload, IGF-1 appears to be the main longer duration signalling molecule, and that it exists both in autocrine and systemic forms. In muscle, both are upregulated by stretch which requires an intact cytoskeletal system that is attached to the extracellular system. In muscle this involves the dystrophin complex which is a rather sophisticated cytoskeletal system/mechanotransducer. If this is defective it seems that local repair is not initiated and this results in cell death and loss of muscle fibres. Our group is now actively investigating the autocrine mechanism that result in IGF-1 signalling (Fig. 7). Genetic abnormalities of the cytoskeletal system do exist in animals and humans and this enables us to stretch cells in vitro that are derived from patients and animal models. As well as specific mutations there is the possibility to see if the ability to generate signal molecules can be restored by gene therapy. For example we have a collaborative project with groups who have transferred parts or the whole of the dystrophin or utrophin cDNA into mdx dystrophic mice using transgenic methods. With this approach we can determine which parts of these cytoskeletal complexes are required for the mechanotransduction that results in signal molecules with particular interest in the autocrine form of IGF-1 in muscle. It is of interest that a nuclear receptor for IGF-1 had been demonstrated (Chen & Roy, 1996). In addition to activating the normal membrane IGF-1 receptor the autocrine IGF-1 may also have an intracellular route. Understanding the mode of action and the mechanotransduction of these autocrine signalling molecules therefore represents a very interesting challenge as it appears to be the mechanism via which local tissue repair is initiated.

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