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Gene expression in skeletal muscle

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Abbreviations used: hc gene, heavy chain gene; IGF-I, insulin-like growth factor-I; MGF, mechano growth factor.

Abstract

Muscle has an intrinsic ability to change its mass and phenotype in response to activity. This process involves quantitative and qualitative changes in gene expression, including that of the myosin heavy chain isogenes that encode different types of molecular motors. This, and the differential expression of metabolic genes, results in altered fatigue resistance and power output. The regulation of muscle mass involves autocrine as well as systemic factors. We have cloned the cDNAs of local and systemic isoforms of insulin-like growth factor-I (IGF-I) from exercised muscle. Although different isoforms are derived from the IGF-I gene by alternative splicing, the RNA transcript of one of them is only detectable following injury and/or mechanical activity. Thus this protein has been called mechano growth factor (MGF). Because of a reading-frame shift, MGF has a different 3´ sequence and a different mode of action compared with systemic or liver IGF-I. Although MGF has been called a growth factor, it may be regulated as a local repair factor.

Introduction

In this era of molecular biology, many people think in terms of genetic programming. However, a prerequisite for the survival of animal species is adaptability. Muscle is one of the best examples of a tissue that has an inherent ability to adapt. It provides the power not only for locomotion but also for a number of life-sustaining processes. Therefore its ability to function efficiently and economically over a range of conditions is crucial to survival. It has been shown that changes in contractile function can be brought about by switching on one subset and repressing another subset of genes (1). In this way the tissue can be optimized for power output, rapid movement or fatigue resistance. These contractile characteristics are determined by the type of myosin cross-bridge, i.e. the type of molecular motor that produces force. Different molecular motors are coded for by different myosin heavy chain (hc) isogenes that in the vertebrates comprise a family of separate genes. We have studied the regulation and switching of expression of the different myosin hc genes in response to physical signals during growth and adaptation in both mammals [1) and fish [2).

Both muscle mass and muscle phenotype determine muscle contractile performance, as maximum power output is related directly to the cross-sectional area of the fibres as well as to the speed of shortening. However, there has to be a 'trade-off' of the desirability of having a

greater cross-sectional area of muscle and the energy cost of carrying that extra mass. This is less of a problem in a buoyant aqueous environment than it is on land or in the air. Muscle has other functions as well as producing movement; in particular, it acts as a store of metabolites. As well as being a major provider of blood glucose to fuel muscle contraction, it provides amino acids, e.g. glutamine, which is required for the synthesis of a neurotransmitter and for acid–base balance. It is now appreciated that, in humans, a marked loss of muscle mass during disease or aging is a major cause of death, as the individual is unable to survive a traumatic experience which requires the increased provision of metabolites such as glutamate. In recent years we have studied the regulation of muscle mass and phenotype concomitantly, to see if these two processes involve similar mechanisms whereby physical signals result in up-regulation of the expression of specific genes.

Mechanical factors that influence gene expression and muscle phenotype

Stretch has been shown to be a very powerful stimulant of muscle growth and muscle protein synthesis. During postnatal growth, skeletal muscle fibres elongate by the serial addition of new sarcomeres to the ends of existing myofibrils (3). Even mature muscles have been shown to be capable of adapting to a new functional length by adding or removing sarcomeres in series (4,5). In this way, sarcomere length is adjusted 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 (6). Stretch combined with electrical stimulation has also been shown to induce very rapid hypertrophy of the tibialis anterior muscle in the adult animal. Both force generation and stretch are major factors in activating protein synthesis, and the combination of these stimuli apparently has a pronounced additive effect. Associated with this very significant increase in muscle size (over 30% in 4 days), there was a marked increase (up to 250%) in the RNA content of the muscles, which was found to peak 2 days after the start of stretch (6). This rapid increase in total RNA reflects mainly rRNA, indicating that muscle fibre hypertrophy may be controlled primarily at the level of translation, and that the rapid increase in the number of ribosomes means that more mRNAs can be translated into protein. There are situations when abundant mRNA 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 translation. When stretch and electrical stimulation are combined, the fast tibialis anterior of the rabbit apparently becomes completely reprogrammed for the transcription of the slow myosin hc gene, and represses the expression of the fast myosin hc gene within only 4 days (1).

Muscle phenotype changes in response to activity

Although it is generally accepted that the different fibre types in mammalian muscle are interconvertible, there is some dispute concerning the nature of the physical signal involved. At one stage it was thought 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 (7). Using plaster-cast limb immobilization, stretch alone was found to induce fast fibres to lay down slow-type sarcomeres (8), and under these conditions virtually no electromyograph signal can be detected (9). Therefore it is unlikely that stimulation frequency is the primary determinant of muscle phenotype. Instead, it appears to be the particular physical activity that this induces that is important, rather than the electrical stimulation itself. In our experiments, more complete reprogramming of the muscle was obtained when stretch was combined with high-frequency stimulation. This indicates that the signal for the fast-to-slow change is mechanical strain. 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.

We studied the way in which gene expression in muscle is influenced by stretch, by casting the limb with the muscle in either the shortened or the lengthened position (10). Several interesting findings emerged from this study, including the fact that a slow soleus muscle, which does not normally express fast-type IIb myosin hc genes, begins to transcribe the fast myosin hc gene after only 1 day if its muscle fibres are not subjected to stretch or are not producing force.

Other subsets of genes may be controlled in a similar fashion, including those encoding mitochondrial and cytoplasmic enzymes, when a muscle fibre is converted into the slow type. The changes in gene expression may not be co-ordinated; indeed, there may be different signals involved in the activation of, for example, the myosin isoform genes as compared with, for example, mitochondrial genes or sarcoplasmic enzyme genes; however, under most training conditions these will happen to coincide. For instance, during endurance training there is increased expression of slow myosin and also of mitochondrial genes. The former apparently respond to mechanical strain, while the latter may be induced by anoxia. The details of the molecular mechanism(s) involved in myosin isoform gene switching are not known. The possible cellular mechanisms that spring to mind include transient changes in internal calcium levels and metabolic signals such as the depletion of ATP. Changing the internal calcium concentration of cultured muscle cells changes the expression from fast to slow myosin (11). However, damage also induces this change (12,13).

Fibre types in human skeletal muscle

Most histochemical and immunochemical studies on muscle fibre types have been carried out using rat muscle. The laboratory rat is rather a specialized animal, and extrapolation of data derived from this species to others, particularly large animals, is sometimes misleading. Indeed, it is interesting to compare the differences between the species and to relate these to their size and mode of life. For example, in the cat the masseter muscles and other jaw muscles express an 'ultra fast' myosin (14). To illustrate further the differences between species, a new approach was developed in the author's laboratory. This involves taking human muscle biopsy samples that are freeze-dried. Fragments of single fibres are dissected out, one-third of which are used for histochemical staining and one-third for protein electrophoresis. In the remaining one-third, the mRNA of the primary myosin hc genes expressed is amplified by reverse transcriptase–PCR. This showed that the main fast hc gene expressed in human muscle was type 2x and not type 2b as in the rat (15,16). A similar conclusion was arrived at concurrently by Smerdu et al. (17), who carried out in situ hybridization studies on post-operative human muscle samples.

We believe that the expression of a different myosin hc fast gene in the human as compared with the rat is related to size scaling. It was pointed out by A. V. Hill in 1954 (18) that the intrinsic velocity of shortening of the longer muscles of larger animals with a large number of sarcomeres in series must be considerably lower than in smaller animals, otherwise the rate of development of mechanical strain would be too great. Hence the main fibre types in larger animals are 2x and 2a, which are considerably slower than 2b fibres. It is important that the fibres from different species are correctly defined, although it should also be appreciated that there is often co-expression of two or more hc genes within a given muscle fibre, as shown by our work and that of other groups (15, 19).

Local control of muscle mass

For some time it has been appreciated that there is local as well as systemic control of tissue growth. The postnatal growth spurt that occurs early in life is believed to be regulated to a large extent by growth hormone produced by the pituitary, which causes the release of insulin-like growth factor-I (IGF-I) from the liver and probably other tissues, including muscle. However, it is well known that there are a good number of cell types that respond to

mechanical signals and possess a mechanism for the local control of growth remodelling and repair. As mentioned above, tissue size and shape are not apparently strictly pre-programmed, but are regulated to a large extent by mechanical factors. Cells that have an inherent ability to respond to mechanical factors have been termed 'mechanocytes', and these include fibroblasts, keratinocytes, osteoblasts and skeletal, cardiac and smooth muscle cells. Experiments involving stretching of cardiac myocytes in culture suggested that another mechanism, involving the production of autocrine growth factors, is a further possibility (20). Skeletal muscle offers one of the best approaches to studying this type of mechanotransduction, as the mechanical activity generated by and imposed upon muscle tissue can be controlled accurately and measured in both in vitro and in vivo systems.

We have identified and cloned a growth factor that is expressed in muscle only when it is subjected to activity (21). Both the rabbit and human cDNAs for this growth factor have now been sequenced and it is apparent that this protein is derived from the IGF-I gene by alternative splicing. The structure of the cDNA of this isoform indicates that it has different exons compared with liver IGF-I and that it is not glycosylated. Therefore it is expected to be smaller and to have a shorter half-life than the liver form. This factor, called mechano growth factor (MGF), is thus designed to act in an autocrine/paracrine rather than in a systemic fashion. It is possible that MGF is the end product of mechanotransduction signalling pathways in muscle and other cell types. Questions such as whether MGF is up-regulated before membrane damage occurs or whether membrane damage initiates the production of the growth factor can be addressed. Experiments are currently being performed to determine the mechanisms via which cells respond to mechanical stimuli, as well as the link between the mechanical stimulus and gene expression, as this represents a new and important area of physiology (22). As far as skeletal muscle is concerned, it has long been appreciated that there is local control growth, because if a muscle is exercised, it is only that muscle that undergoes hypertrophy and not all the muscles of the limb. It has been shown that stretch is the major mechanical signal for the addition of new sarcomeres (3, 23), up-regulating protein synthesis (6, 24) and changing gene transcription (1, 13). The discovery of this growth factor provides a link between mechanical stimuli and gene expression.

Preliminary experiments have shown that, in mdx and dydy dystrophic mice, the mechanotransduction system is defective, as MGF is not up-regulated as it is in normal mice when the muscle is stretched (25). Dystrophin is associated with the membrane in normal muscle, but it is absent in Duchenne dystrophy and in the mdx mouse. In autosomal dystrophies and in the dydy mouse it is the one of the proteins that connect the dystrophin to the membrane and to the extracellular matrix that is missing. This suggests that the dystrophin/dystrophin-associated glycoproteins and extracellular proteins are part of a mechanotransduction system. In addition, neuronal NO synthase in muscle has been shown to be associated with dystrophin (26), which therefore seems to have a more important role than just stabilizing the membrane. Recombinant (basic) IGF-I has been shown to have a beneficial effect on the muscles of the dystrophic dydy mouse (27), but the IGF-I used was probably an inappropriate form for maximum effectiveness. In IGF-I gene knockout experiments, the transgenic offspring did not survive long after birth and examination of their muscles revealed that they were dystrophic (28, 29). Hence it appears that inadequate IGF-I and the inability of muscle to repair may be the causal mechanism in all the dystrophies.

Action of MGF in inducing muscle hypertrophy

In order to determine if the splice variant transcript that is detected in muscle following stretch or exercise has any biological activity, we introduced by intramuscular injection a plasmid gene construct containing the MGF cDNA under the control of muscle regulatory elements. We obtained up to a 20% increase in muscle mass within 2 weeks following a single injection into the mouse tibialis anterior muscle. It has been reported from transgenic

experiments that overexpression of the IGF-I gene results in increased muscle mass (30), and injection of the liver-type IGF-I cDNA in a viral vector into muscle produced a 20% increase in mass, but over a period of 4 months (31). We were therefore surprised at the potency of the MGF cDNA, which we have shown is associated with higher levels of its peptide in the muscle. In order to determine if this increase in wet tissue mass was of physiological significance, we prepared cryostat sections of the muscles and measured muscle fibre size. The mean fibre size was increased by 25%, but it was noticeable that this was due to there being more large fibres in the injected muscle rather than to an increase in size of all the fibres. With the intramuscular injection method, first described by Wollf's group (32), only some of the fibres take up and express the introduced construct. It seems, therefore, that it is these fibres that have undergone hypertrophy, the rapidity and extent of which is impressive.

In order to study the mechanisms responsible for the effects of muscle IGF-I isoforms, particularly the autocrine splice variant (MGF), we generated the peptides using a peptide synthesizer and by introducing the cDNA into a expression system. A polyclonal antibody to MGF was shown to be specific by using its peptide to block its reaction. Using a proteomics approach we used the antibody to detect a specific MGF-binding protein on two-dimensional electrophoresis gels. The binding protein was then identified by cutting out the spots and by using a mass spectrometer. The binding protein was shown to bind only MGF, and not the systemic types of IGF-I. The muscle-specific binding protein localizes and stabilizes the MGF within the muscle and also acts as a time-release mechanism. It is for this reason and others that we regard MGF as an autocrine growth/repair factor. Also, the discovery of MGF provides a link between the mechanical stimulus and gene expression, although the nature of the mechanochemical coupling process is not yet known.

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Muscle growth in response to mechanical stimuli

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Abstract:
The relative merits of the separate and combined uses of stretch and electrical stimulation at 10 Hz in influencing the rates of protein synthesis in vivo, proteolysis, and the growth of the extensor digitorum longus muscle have been investigated after 3 days in the rabbit. Continuous electrical stimulation failed to change muscle protein turnover or growth. Static stretch caused significant adaptive growth, with increases in c-fos, c-jun, and insulin-like growth factor I (IGF-I; 12-fold) mRNA levels, and protein (19%), RNA (128%), and DNA (45%) contents. Both the fractional (138%) and total (191%) rates of protein synthesis increased with stretch, correlating with increased ribosomal capacities. Combining stretch and electrical stimulation increased the mRNA concentration of IGF-I (40-fold). The adaptive growth was greater (35%), with massive increases in the nucleic acids (185 and 300%), ribosomal capacities (230%), and the rates of protein synthesis (345 and 450%). Large increases (i.e., 200-400%) in cathepsins B and L and dipeptidyl aminopeptidase I activities during stretch, with or without stimulation, suggest a role for these enzymes in tissue remodeling during muscle hypertrophy.