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Symposium 5: Muscle hypertrophy: the signals of insulin, amino acids and exercise

Mechanotransduction and the regulation of protein synthesis in skeletal muscle

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Repeated bouts of resistance exercise produce an increase in skeletal muscle mass. The accumulation of protein associated with the growth process results from a net increase in protein synthesis relative to breakdown. While the effect of resistance exercise on muscle mass has long been recognized, the mechanisms underlying the link between high-resistance contractions and the regulation of protein synthesis and breakdown are, to date, poorly understood. In the present paper skeletal muscle will be viewed as a mechanosensitive cell type and the possible mechanisms through which mechanically-induced signalling events lead to changes in rates of protein synthesis will be examined.

Growth: Hypertrophy: Stretch

Repeated bouts of resistance exercise produce compensatory growth of skeletal muscle (Kimball *et al.* 2002). The increase in muscle mass results from an increase in protein synthesis over protein degradation with the net result being a deposition of protein (Tipton & Wolfe, 2001; Kimball *et al.* 2002). While the effect of resistance exercise on muscle mass has long been recognized, the mechanisms underlying the link between high-resistance contractions and muscle growth are, to date, poorly understood.

Currently, the proposed mechanisms in the regulation of exercise-induced compensatory growth include, but are not limited to, the involvement of growth factors (e.g. insulin-like growth factor 1) and the signalling events induced by the mechanical stimulus (Goldberg *et al.* 1975; Vandenburg, 1987; Tipton & Wolfe, 2001; Kimball *et al.* 2002). The focus of the present paper will be to view skeletal muscle as a mechanosensitive cell type and examine the possible mechanisms through which mechanically-induced signalling events lead to changes in rates of protein synthesis.

Tension regulates muscle mass

Goldberg *et al.* (1975) proposed that the development of tension is the critical event in initiating compensatory growth in mammals. This hypothesis was based on a large number of studies in which the amount of loading was manipulated in both increased and decreased directions to try and understand the effects of tension on skeletal muscle mass (Goldberg *et al.* 1975; Vandenburg, 1987). These studies indicated that chronically overloading a muscle results in an increase in mass, while chronically unloading a muscle results in a decrease in mass. In addition, placing a muscle in a chronically- or intermittently-strained state can attenuate the atrophy produced by chronically unloading the muscle (Goldberg *et al.* 1975). In combination, these observations suggest that the tension placed on the muscle plays a critical role in the regulation of its mass.

Consistent with the role that tension plays in regulating skeletal muscle mass *in vivo*, stretch models have been used to demonstrate that tension also regulates mass *in vitro* (Vandenburg, 1987). Using a cell-culture model,

Abbreviations: FA, focal adhesion; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B.
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Vandenburg (1987) has demonstrated in avian myotubes that intermittent stretch produces a large increase in protein synthesis and a smaller decrease in protein degradation, with the net result being a deposition of protein. These data suggest that in cell culture the hypertrophic response to increased tension is to a large extent the result of an increased rate of protein synthesis. An increased rate of protein synthesis also appears to be a critical mechanism for producing a hypertrophic response to increased tension *in vivo* (Goldberg, 1968; Goldberg *et al.* 1975; Vandenburg, 1987). Consistent with these observations, in models of muscle atrophy resulting from decreased tension the primary response is a rapid decrease in the rate of protein synthesis (Vandenburg *et al.* 1999; Fitts *et al.* 2000). Thus, to better understand the mechanisms through which resistance exercise induces compensatory growth, it will be critical to understand how the muscle senses mechanical signals (change in tension) and converts this stimulus into the biochemical events that regulate the rate of protein synthesis.

Mechano-transduction

Cell- and organ-culture experiments with skeletal muscle have demonstrated that mechanically-induced alterations in protein synthesis occur independently of other cell types and circulating factors such as testosterone, glucocorticoids and growth factors (Palmer *et al.* 1983; Vandenburg *et al.* 1999). These observations suggest that skeletal muscle has an intrinsic capacity for sensing mechanical information and converting it into biochemical events that regulate protein synthesis.

The process of converting mechanical energy into biological events is termed mechanotransduction. In order for mechanotransduction to occur, mechanisms must exist for coupling the mechanical information with intracellular biochemical events. This coupling will be referred to as mechanoreception. Several candidate mechanoreceptors have been proposed and the majority of these can be divided into two groups: (1) the lipid bilayer; (2) extracellular matrix–integrin–cytoskeleton.

The lipid bilayer

The lipid bilayer is composed of lipid molecules that contain hydrophilic head groups and hydrophobic acyl tails. The two monolayers are stabilized by van der Waals forces and the hydrophobic forces produced by the sequestered acyl lipid chains. Under physiological conditions the bilayer exists in a highly fluid state but is very resistant to changes in surface area, with increases of 2–4% leading to its rupture (Hamill & Martinac, 2001). However, in the presence of an intact cytoskeleton, the lipid bilayer can assume non-spherical geometries and, as a consequence, cells can maintain an excess in membrane surface area. In fact, skeletal muscle has been shown to have approximately 100% excess of membrane surface area in the form of membrane folds, microvilli and/or caveolae (Dulhunty & Franzini-Armstrong, 1975). The excess in membrane surface area serves as an immediate reservoir for the

highly-expandable cytoskeleton and relatively non-expandable lipid bilayer.

During mechanical deformation the cytoskeletal network can be expanded and the lipid bilayer smoothed out before the generation of marked tension and rupture of the lipid bilayer occurs. If the lipid bilayer is ruptured, the movement of intra- and extracellular components through the damaged membrane could serve as a mechanism of mechanoreception. This form of mechanoreception would result in rather non-specific movement of intracellular and extracellular components. A more precise form of mechanoreception could occur during the repair or resealing of the damaged membrane. Such a mechanism has been referred to as the ‘vesicle plug’, in which an intracellular vesicle fuses with the damaged membrane, in turn releasing the vesicular components into the extracellular space (Hamill & Martinac, 2001). Release of vesicular components can also be stimulated by the generation of tension within the lipid bilayer. Increased tension will promote fusion between internal vesicles and the membrane, as fusion will lower the energy (tension) in the membrane (Hamill & Martinac, 2001). Release of vesicular components, such as insulin-like growth factor 1, could induce the receptor tyrosine kinase activity, in turn enhancing the phosphorylation of the insulin receptor substrate 1 and subsequent activation of p85-dependent phosphatidylinositol 3-kinase (PI3K) activity and protein synthesis (Kimball *et al.* 2002).

The lipid membrane can also serve as a mechanoreceptor via alterations in the fluidity of the bilayer. Evidence of this mechanism has come from studies in which lipid vesicles have been reconstituted with purified G-proteins. Altering the membrane fluidity by changing the cholesterol content or applying fluid shear stress results in concomitant changes in the activity of the G-proteins (Gudi *et al.* 1998). Activated trimeric G-protein dissociates into G α and G $\beta\gamma$ subunits. The G $\beta\gamma$ subunit can then bind and activate p101 γ -dependent PI3K (Wymann & Piroola, 1998). In addition to being able to activate PI3K, inhibition of the G α_i , G α_o and G α_t classes of G-proteins with pertussis toxin has been shown to abolish numerous stretch-induced signalling events and the overall growth response to intermittent stretch *in vitro* (Vandenburg *et al.* 1995). These data suggest that the lipid membrane, via activation of G-proteins, is capable of mechanotransduction, and the possibility exists that G-proteins regulate a mechanically-induced increase in protein synthesis by signalling through a PI3K-dependent pathway.

Extracellular matrix: cytoskeleton

Recent work indicates that much of the signal transduction machinery within cells is physically immobilized to the cytoskeleton and spatially integrated within the focal adhesion (FA) and dystrophin–glycoprotein complexes (Plopper *et al.* 1995; Rando, 2001). Force-dependent alterations in the spatial organization of FA or dystrophin–glycoprotein complex signalling proteins or force-dependent changes in protein conformation could expose new binding sites and, in turn, activate signalling cascades.

Focal adhesions. FA are sites where the extracellular matrix is physically coupled to the cytoskeleton within the cell. In skeletal muscle FA can be found at the myotendinous junctions, neuromuscular junctions and in periodic structures that lie above the z-bands termed costameres (Anastasi *et al.* 1998). The FA are protein-dense regions and most of the molecules in the FA contain multiple domains that can interact with a variety of molecular partners. One of the major constituents of the FA is the family of cell surface receptors termed integrins. Integrins are composed of α and β subunit heterodimers, which have an extracellular domain that interacts with the extracellular matrix, a transmembrane region and a cytoplasmic domain that interacts with the cytoskeletal components. Thus, integrins can physically couple the extracellular matrix with the cytoskeleton (Schwartz *et al.* 1995). The direct association with extracellular matrix and cytoskeletal components suggests that integrins could act as a mechanoreceptor that transmits mechanical information between the outside and inside of the cell. In support of this hypothesis, twisting of magnetic beads coated with a ligand for the extracellular domain of integrin can transfer mechanical stress to the cell's internal cytoskeleton (Wang *et al.* 1993).

Dystrophin–glycoprotein complex. The extracellular matrix can also be physically coupled with the cytoskeleton through the dystrophin–glycoprotein complex. In this complex the extracellular matrix protein laminin is bound to α -dystroglycan, which is an extracellular protein that binds tightly with the transmembranous β -dystroglycan. β -Dystroglycan forms a tight intracellular association with dystrophin as well as the transmembranous α , β , γ and δ sarcoglycans. Dystrophin forms a strong association with F-actin, completing a bridge between the extracellular matrix and the cytoskeleton (Rando, 2001).

Both integrins and dystrophin–glycoprotein complexes lack inherent kinase or enzymic activity. However, these proteins have been shown to interact with various signalling proteins, such as the FA kinases, Grb2 and PI3K (Rando, 2001). Thus, integrins and dystrophin–glycoprotein complexes could not only serve as mechanoreceptors that transmit mechanical information between the outside and inside of the cell, but could also serve as integration sites where mechanical information is converted into biochemical signals that ultimately lead to regulation of protein synthesis.

Specificity in mechanotransduction

In addition to being able to sense mechanical stimuli, it also appears that muscle cells can differentiate between different types of mechanical forces. For example, in skeletal muscle chronic longitudinal stretch produces growth in length but not cross-section (sarcomere deposition in series to the long axes), while chronic functional overload produces cross-sectional growth with no changes in length (sarcomere deposition is parallel to the long axes). In the heart different growth phenotypes have also been well documented in response to either pressure or volume overload. Both these examples demonstrate that different types of mechanical loading produce unique phenotypic

adaptations and they also imply that specificity may exist within the cellular mechanisms sensing and subsequently responding to the mechanical forces. However, because of the complexity of the mechanical environment within muscle tissue, the concept that different types of mechanical signals can elicit unique molecular events has not been well studied.

In order to study mechanotransduction and/or mechanosensory capacities of muscle cells it is necessary to apply clearly defined mechanical stimuli. The application of such stimuli can be accomplished through the use of *in vitro* stretch devices. These devices are used within the confines of a cell culture environment and the mechanical forces delivered to the culture membrane can be easily controlled and readily defined.

In vitro stretch devices can generally be grouped into one of two classes, uniaxial or multiaxial. The major difference between uniaxial and multiaxial stretch devices is the type of mechanical forces delivered to the elastic culture membrane. Recent work (TA Hornberger, DD Armstrong, TJ Koh, TJ Burkholder and KA Esser, unpublished results) has taken advantage of these differences in an effort to determine whether muscle cells can distinguish between different types of mechanical forces. It has been determined that both uniaxial and multiaxial stretch devices promote an increase in extracellular-regulated kinase and the protein kinase B/Akt phosphorylation, but only multiaxial stretch induces ribosomal S6 kinase phosphorylation. Additionally, disrupting the actin cytoskeleton with cytochalasin D blocks the multiaxial signalling to ribosomal S6 kinase, with no effect on signalling to protein kinase B/Akt. These results demonstrate that mechanical stretch can activate distinct mechanosensory–mechanotransduction pathways, and activation to these pathways is specific to the types of mechanical forces applied. These results also suggest that mechanically-induced protein synthesis may only be activated by specific types of mechanical stimuli.

Regulation of protein synthesis

Mechanically-induced alterations in protein synthesis appear to be regulated by changes in translational efficiency (rate per ribosome) as opposed to translational capacity (concentration of ribosomes; Goldspink, 1977; Kimball *et al.* 2002). Translational efficiency can be regulated through the stages of translation initiation, elongation and/or termination. Although regulation can occur at any stage of translation, most studies to date have suggested that initiation is the primary site of regulation following mechanical stimuli (Kimball *et al.* 2002).

Translation initiation is regulated by a coordinated array of biochemical events. A simplified schematic representation of these events following insulin stimulation is shown in Fig. 1 (for a more comprehensive review, see Sonenberg *et al.* 2000; Kimball *et al.* 2002.) Most of the biochemical events involved in the regulation of translation initiation involve inputs from signalling molecules that alter the phosphorylation state of the eukaryotic initiation factors (Kimball *et al.* 2002). For example, initiation can be regulated by altering eukaryotic initiation factor 2B

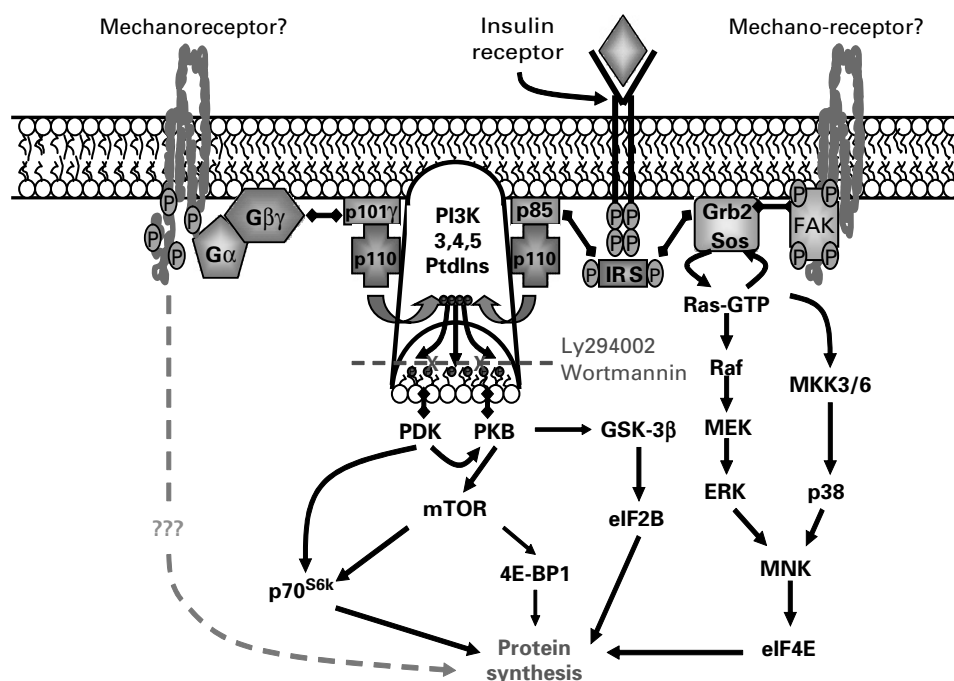


Fig. 1. Protein synthesis is regulated by a coordinated array of biochemical events. Using insulin stimulation as an example, a schematic of signalling events involved in the regulation of protein synthesis is described. Although the biochemical events that regulate protein synthesis following mitogenic and nutrient stimulation have been fairly well characterized, the molecules that sense mechanical stimuli (mechanoreceptors) and the resulting biochemical events that regulate protein synthesis in response to changes in tension are not known. P, phosphate; $G\alpha$ and $G\beta\gamma$, subunits of G-protein; PI3K, phosphatidylinositol 3-kinase; 3,4,5 PtdIns, phosphatidylinositol-3,4,5-triphosphate; IRS, insulin receptor substrate; FAK, focal adhesion kinase; PDK, phosphatidylinositol-dependent protein kinase; PKB, protein kinase B; GSK-3 β , glycogen synthase kinase 3 β ; MKK, mitogen-activated protein kinase kinase; MEK, mitogen-activated protein kinase/ERK kinase; ERK, extracellular signal-regulated kinase; mTOR, mammalian target of rapamycin; p70^{S6k}, ribosomal S6 kinase; 4E-BP1, eIF 4E-binding protein 1; eIF, eukaryotic initiation factor; p38, stress-activated protein kinase; MNK, mitogen-activated protein kinase-interacting kinase.

phosphorylation, and glycogen synthase kinase 3 appears to play a primary role in this reaction (Kimball *et al.* 2002). Formation of the eukaryotic initiation factor 4F complex, composed of initiation factors 4A, 4E and 4G, regulates cap-dependent translation, and signals from protein kinase B/Akt, extracellular-regulated kinase, eukaryotic initiation factor 4E-binding protein 1, stress-activated protein kinase and mammalian target of rapamycin have all been implicated in this process (Herbert *et al.* 2000; Kimball *et al.* 2002; Liu *et al.* 2002). The translation initiation of mRNA that contain a 5' tract of polypyrimidines (i.e. elongation factors and ribosomal proteins) is also mammalian target of rapamycin dependent and ribosomal S6 kinase appears to play a critical role in the translation of these mRNA (Jefferies *et al.* 1997). The coordinated regulation of the initiation factors by multiple signalling pathways allows for the integration of several inputs, including mitogenic signals and nutrient availability.

A large majority of the signalling pathways implicated in the regulation of translation initiation lie downstream of PI3K. In skeletal muscle inhibition of PI3K with wortmannin or LY294002 completely blocks mitogenic- and nutrient-induced increases in protein synthesis (Kimball

et al. 2002). Thus, PI3K appears to be a central integrator of the signalling pathways that regulate protein synthesis. Although a role for PI3K in regulating mitogenic and nutrient-induced protein synthesis is well established, it is not known whether this kinase plays a role in the regulation of protein synthesis induced by mechanical signals.

In many aspects the signalling events induced by mechanical signals are similar to those induced by mitogens and nutrients. For example, mechanical stimuli have been shown to induce signalling through extracellular-regulated kinase, stress-activated protein kinase, protein kinase B/Akt, glycogen synthase kinase-3 and ribosomal S6 kinase, and, as mentioned earlier, all these pathways have been implicated in the regulation of translation initiation (Ruwhof & van der Laarse, 2000; Sakamoto *et al.* 2002). Thus, although the signalling pathways that regulate protein synthesis in response to mechanical stimuli are not known, it seems probable that many of the same pathways implicated in the regulation of nutrient- and mitogenic-induced protein synthesis will be involved. Thus, it is proposed that, similar to mitogens and nutrients, signalling through PI3K is critical for mechanically-induced alterations in protein synthesis.

Summary

In closing, many questions remain about the mechanism through which mechanical stimuli are converted into the biochemical events that regulate protein synthesis. These questions are important, as the identification of the signalling events that are critical for the mechanically-induced increase in protein synthesis could guide future experiments aimed at identifying the mechanoreceptor(s) that initiate the response. With the identification of the mechanoreceptor(s), the potential exists for the development of pharmacological agents that mimic the effects of tension on skeletal muscle. These agents could be used to prevent muscle atrophy that occurs during periods of skeletal muscle unloading, such as aging, bed rest, cast immobilization and space flight.

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