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The role of mechanical stress in skeletal myocytes: MAPK signal transduction pathways

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The idea of signal transduction provides the necessary link between a stimulus from the external environment of a cell, and the intricacies that occur among its intracellular components. The proteins embedded in the plasma membrane of a cell assist in this transduction in that they act as molecular antennae, capturing the initiating external stimulus and transmitting its effects to the cell cytoplasm. Albeit many molecules can freely pass through the cell membrane, those that are effectively excluded by this semipermeable barrier would have no way of communicating with the cell interior without the process of signal transduction. Consequently, many pivotal biochemical pathways would be completely hindered and the life of the organism affected would cease. The mitogen-activated protein kinase (MAPK) pathway is one of the most significant signaling systems used by an organism to elicit a variety of responses at the cellular level. This unique pathway is thought to be directly responsible for regulating cell proliferation, differentiation, and survival, all of which are vital for the existence of life. Even though the basic regulatory steps have been delineated, many fascinating features of this pathway are only beginning to emerge. The conventional study of signal amplification, which has historically encompassed the analysis of ligand interaction with cell membrane receptors, can be slightly modified to incorporate mechanical stress as the initiating component in the signaling pathway. Studying signal transduction in light of mechanical stretching may help enhance the understanding of pathway-initiating mechanisms in a way that can be applicable to mechanical manipulation of the extracellular matrix and plasma membrane domains. Such manipulation is seen *in vivo*, with normal physiological muscle contraction, as well as *in vitro*, with concentric and eccentric muscle stretching maneuvers. Furthermore, tracing the stimulus of cell stretching all the way to gene transcription demonstrates that many proteins from different families are involved in facilitating the activation of MAPKs. Theories on integrin signaling show how cell stretching induces the activation of protein kinase C (PKC), leading to the release of Ca^{2+} ions which are then sequestered by integrins. These active integrins may then activate focal adhesion kinases (FAK), which constitutes a major initiating pathway to activating MAPKs. Alternatively, integrins may immediately be activated by their associations with the extracellular matrix, which then activate FAK in a similar fashion. The dominance of ionic over mechanical activation is still unknown, and a combination of these mechanisms may contribute to the initiation of MAPKs. Observation of cell spreading and growth, two characteristics of activating MAPK pathways, can prove to verify the effects of mechanotransduction in muscle stretching. While the hypothetical models presented here provide a logical synthesis of concepts in signal transduction, the complexity surrounding the field leads to the idea that an intricate interplay of chemical processes within the world of the cell exists. The mediators of these processes include, but are certainly not limited to, integrin activation via calcium binding or dimerization of its subunits.

Key Words: Mitogen-activated protein kinase (MAPK), protein kinase C (PKC), integrin signaling, focal adhesion kinase (FAK), mechanotransduction

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A cell's decision to grow, proliferate, or terminate itself is the end product of long and complex deliberations. For instance, a quiescent (nongrowing) cell must receive and process a number of growth-stimulatory signals, notably those conveyed by growth factors, and assess whether its strength and number warrant entrance into an active proliferative phase. Decision-making such as this demands a complex signal-processing apparatus inside the cell. A helpful metaphor is an electronic circuit board constructed as a network of components that operate like resistors, transistors, and capacitors (Weinberg, 1998). Each of these components is a logical device that receives signals from other components, processes and interprets these signals, and then passes them on to other circuit elements. In the living cell, circuit components like these are proteins endowed with complex signal-processing capabilities. These proteins are capable of 'signal transduction' in that they receive signals, filter and amplify them, and then pass them on to other components. In this way, signal transduction allows the cell's external environment to govern intracellular machinery in an orderly fashion. Although there are many substances that can physically traverse the plasma membrane barrier, namely, hydrophobic substances and other relatively small molecules, there are still quite a few instrumental molecules that are effectively excluded by this barrier. Such substances would thus have no effect on cytoplasmic elements without the mechanism that is known as signal transduction. In this process, proteins function like molecular bucket brigades (Weinberg, 1998). A protein at the top of this brigade relays a signal to the next protein down the line, which in turn responds by transmitting the signal yet another step down. Such chains of command are commonly referred to as signal cascades. In actuality, the initiating signal is amplified virtually exponentially, in that each component that is activated in turn can activate several others to the extent that the effects are seen on a global scale. Of the known contributors to signaling cascades, mitogen activated protein kinases (MAPKs) are among the most versatile known. MAPKs are evolutionary conserved enzymatic complexes connecting cell membrane epitopes, or regions capable of provoking a cellular response, to regulatory intracellular endpoints. They respond to chemical and, as will be shown, mechanical stresses, in an effort to control cell survival and adaptation. MAPK activity is regulated through three-tiered cascades composed of a MAPK, a MAPK kinase (MEK), and an MEK kinase (MEKK) (English et al, 1999). These modules may be activated by small guanosine triphoshate (GTP) binding proteins, namely via G-protein linked receptors (Gutkind, 2000). In theory, all MAPK pathways activated in the forward direction, through substrate-level phosphorylation, can be inactivated by MAPK phosphatases.

Four parallel cascades of MAPKs have recently been described in mammalian cells, including extracellular signal-regulated kinase (ERK), stress-activated protein kinase p38, and cJun NH_2-terminal kinase (figure 1). An instrumental factor involved in the ERK cascade is Raf, which is a serine/threonine kinase that phosphorylates

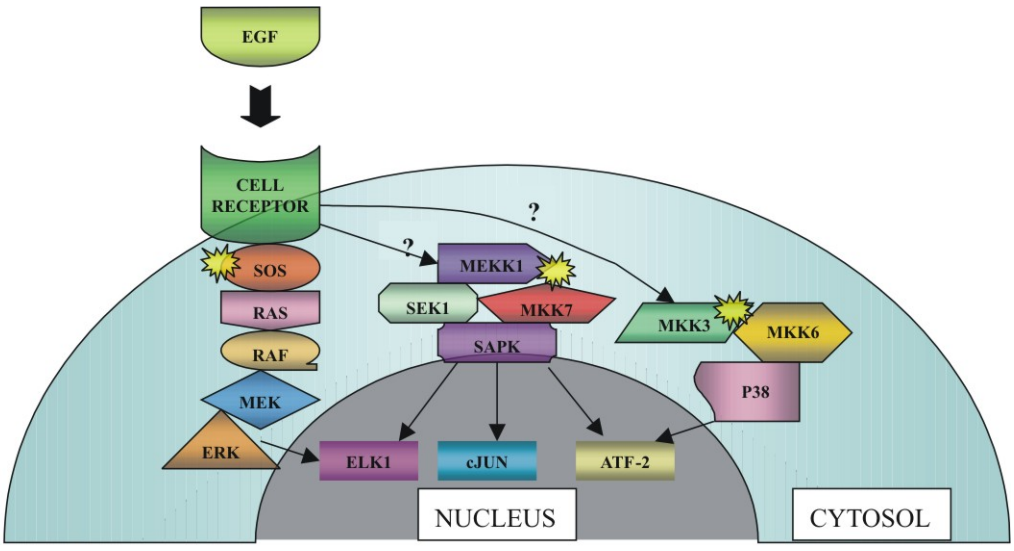

Figure 1. Hierarchy of MAPK signals

Signals from the cell surface are transduced through the cytoplasm by a cascade of protein kinases. In this case, epidermal growth factor (EGF) acts as a signaling promoter by binding to its transmembrane receptor domain with concomitant activation of Sos (the yellow sunburst symbol representing activation will be used throughout this article). The relationship between ligand binding and activation of both the SAPK and p38 pathways is still unclear. (Figure adapted from L.A. Tibbles, et. al.)

downstream targets in signaling pathways. MAPKs are activated via dual phosphorylation on threonine and tyrosine residues by MAPKs. Factors that have been shown to trigger MAPK activation include hormones, growth factors, reactive oxygen species, lowered pH, and mechanical stress (Widmann et al, 1999). Following activation, MAPKs can either phosphorylate different cytoplasmic targets or translocate to the nucleus and directly or indirectly affect transcription (Wretman et al, 2001).

The general pathway for stress activated protein kinases (SAPKs), which originates from members of the MAPK family, involves Raf, MEK, and ERK. The three main mediators of this pathway are ERK, SAPK, and p38, which each eventually have an effect on transcription factors in the nucleus (figure 1). As can be seen in figure 1, the ERK pathway is a hierarchical cascade originating at the cell membrane with receptors for either mitogens, which are substances that cause cells to undergo cell division, or growth factors. These receptors recruit, via adaptor proteins and exchange factors, the small guanosine triphosphatase (GTPase) known as Ras (Tibbles et al, 1999). Ras then subsequently activates Raf, a serine threonine kinase, which activates MEK (MAPK/ERK kinase). MEK, in turn, phosphorylates and activates ERK1 and ERK2 which translocate to the nucleus perhaps via a nuclear localization sequence (Alberts et al, 1994). These two proteins transactivate transcription factors, changing gene expression to promote cellular expansion, differentiation, or mitotic division.

It has been shown that signal transduction pathways are activated via stress and inflammatory mechanisms in mammalian somatic cells (Kyriakis et al, 2001). Environmental stresses such as physical exertion or mechanical manipulation of the muscle tissue constitute external stimuli that may employ MAPKs capable of initiating SAPKs (Tibbles et al, 2000). Both of these categories of stress will be discussed in turn, including their effects on the extracellular matrix and cytoskeletal agents.

As the regulation of mitogen activated cell signaling has been discussed in detail elsewhere (English et al, 1999), this article focuses on contemporary developments in understanding MAPK function in mammalian systems, coupled with stress-related stimuli from the external environment. The analysis of targeted mutations in mice and development of specific inhibitors have contributed to a greater understanding of the definitive role of MAPKs in mammals. It is becoming increasingly evident that MAPKs regulate almost all cellular processes, from transcription of genetic information to programmed cell death.

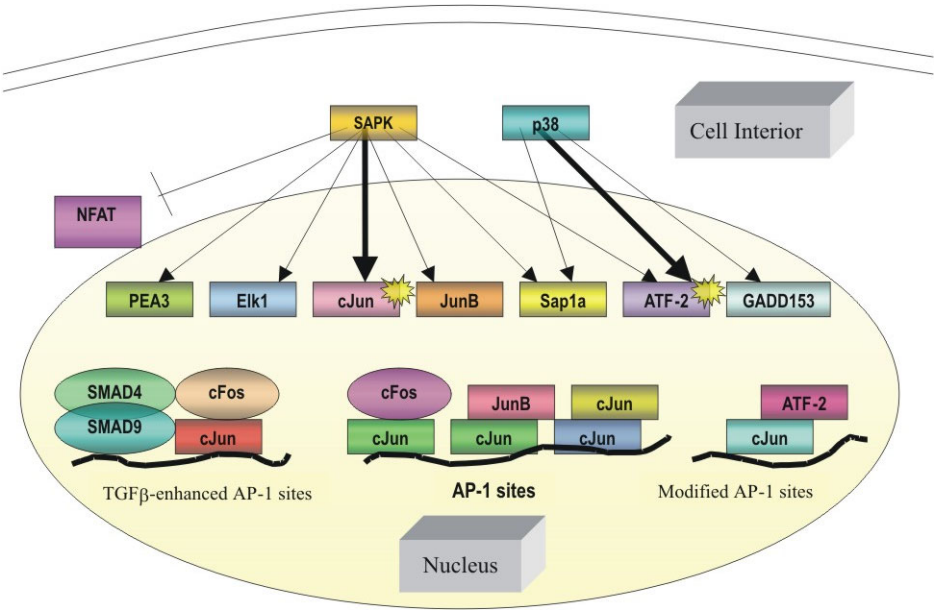

Figure 2. Transcription factor targets of the stress-response kinases

SAPK activation via an extracellular signal leads to the phosphorylation of specific downsteam transcription factors, leading to an effect at the level of the gene. Various forms of these transcription factors ultimately promote the transcription of genes with binding sites for the AP-1 complex. Arrows in bold correspond to major activation pathways outlined in Figure 1. (Figure adapted from L.A. Tibbles et al.)

Key components in MAPK activation

The stress activated protein kinases (SAPKs)

SAPKs reversibly bind and phosphorylate the transcription factor cJun (figure1). cJun is one portion of the activator protein 1 (AP-1) transcription factor complex; the remaining constituent parts include members of the cFos and cJun superfamilies. Transactivation of cJun (figure 2) by the SAPKs leads to increased expression of the genes that have AP-1 regions in their promoters (Tibbles et al, 1999). Among the primary targets of the AP-1 is the cJun gene itself, so transactivation of cJun initiates a positive feedback loop (Tibbles et al, 1999) which is presumably terminated upon transcription of the cJun gene. As can be seen in Figure 1, SAPK effectively acts as a universal pivot point, with targets to both a ternary complex transcription factor (ELK-1) and activating transcription factor 2 (ATF-2). The ternary complex factor ELK-1, once activated by SAPK, leads to positive regulation of the cFos promoter resulting in increased expression of the cFos protein with concomitant increases in AP-1 levels (Tibbles et al, 1999) (figure 2). Targeting of ATF-2, which can form heterodimers with cJun, is another suitable route to initiate increases in AP-1 expression. Given the myriad of possibilities for activating AP-1, it is quite apparent that the SAPK is a model transduction junction for amplifying a given extracellular signal. The SAPKs are encoded by at least three genes, and as with all MAPKs, each SAPK isoform contains a characteristic Thr-X-Tyr phosphoacceptor loop domain, where X indicates any amino acid structurally suitable for a loop domain (Kyriakis et al, 2001).

p38 protein kinases

p38 kinases respond to virtually the same agonists that activate the structurally similar SAPKs (i.e. members of the MAPK family), but under certain circumstances they are differentially regulated (Mendelson et al, 1996). As seen in figure 2, they activate via phosphorylating the transcription factor ATF-2 as well as growth arrest and DNA damage transcription factors (Tibbles et al, 1999). As will be discussed later, p38 activation can be mediated by protein kinase C (PKC) by an experimentally unidentified mechanism (Ryder et al, 2000). Muscle contraction has been implicated in activation of PKC in response to electrical stimulation, however (Richter et al, 1988). As a checkpoint prior to nuclear translocation, p38 appears to be instrumental in regulating a variety of cellular processes, ranging from maintenance of genetic information to preservation of the cell line.

ERK- a third class of Stress-Activated MAP Kinases

The extracellular regulated protein kinases (ERK), with a phosphoacceptor sequence of Thr-Glu-Tyr, contain an NH_2-terminal kinase domain followed by an extensive COOH-terminal tail of unknown function that has several proline-rich motifs indicative of binding sites with SH3 domains (Zhou et al, 1995). These SH3 adaptor proteins are instrumental in linking the initial activation of a kinase to the downstream components of any signal transduction pathway. Although the stimuli that recruit ERK kinases have not been well identified, environmental stresses such as osmotic shock and oxidant stress have been shown to substantially activate ERK and similar substrates (Abe et al, 1996). EGF activation of ERK has been subsequently documented in studies done on cultured cells (Chao et al, 1999) suggesting that a MAPK may be involved (figure 1).

Effects of myocyte stretching on MAPKs

As previously mentioned, there are a variety of environmental stimuli that activate MAPK pathways. Of these stimuli, manipulation of the cell plasma membrane and associated extracellular matrix appears to be especially effective in propagating signal transduction pathways. Voluntary stretching of muscle tissue, exercise induced muscle contraction, and inflammation of muscle cell (myocyte) tissue are all examples of such manipulation of the cell exterior. While many hypotheses and models for mechanotransduction exist, the role of tensile and shear forces on activating MAPKs will be the focus here.

Concentric and Eccentric contractions

Studies on isolated rat skeletal muscle have shown an increase in phosphorylation of both ERK and p38 MAPKs via mechanical alterations, whereas an increase only in ERK activity was caused by contraction-related metabolic/ ionic changes (Wretman et al, 2001). The latter effectors for ERK activation stem from acidotic cell conditions, in which the buildup of lactic acid from cellular respiration induces, either directly or indirectly, the activation of MAPKs. This idea will have some interesting implications in exercise-induced stimulation of MAPKs, as will be discussed in the following section.

Concentric muscle contractions (i.e. contractions induced in muscle fibers along a common axis situated at the geometric center) have been found to have divergent effects on MAPKs in that they induce a marked elevation in ERK phosphorylation, whereas p38 is not significantly affected (Wretman et al, 2001). In addition, the increase in phosphorylation of ERK, but not p38, can be induced by metabolic changes, such as acidification, that occur during

repeated contractions and also by mild mechanical perturbations (Wretman et al, 2001). Eccentric contractions (i.e. contractions induced in muscle fibers along an axis other than that situated at the geometric center), on the other hand, seem to markedly facilitate the phosphorylation of both ERK and p38 MAPKs (Wretman et al, 2001). Thus there seems to be an alternative mechanism at work in concentric contractions that selectively stimulates the initiation of ERK and not p38. It may be hypothesized that the concentric contractions do not generate sufficient force to exert an effect on the p38 pathway, which may give rise to the idea that the upstream components of the p38 activation pathway are more internal to the cell surface, in relation to the ERK elements. However, there still remains much to be discovered about the nature of these concentric/eccentric contractions and their relationship to the activation of these MAPK components.

The fact that p38 MAPK phosphorylation is not affected greatly by concentric contractions implies that it is little affected by metabolic alterations, and previous studies have shown no effect on phosphorylation by acidotic conditions (Wretman et al, 2001). Furthermore, since Wretman has shown that p38 phosphorylation is not induced by mild mechanical stress, by exclusion the higher mechanical stress imposed upon muscle in isometric contractions is required to induce an increase in p38 MAPK phosphorylation. This was verified in Wretman's studies by the finding that eccentric contractions markedly induced phosphorylation of p38 MAPK, an effect that also tended to occur with severe stretching maneuvers. The importance of mechanical stress in enhancing MAPK phosphorylation is now becoming increasingly evident. As far as the mechanism underlying MAPK phosphorylation with eccentric contractions is concerned, it appears that a number of internal cytoskeletal elements are involved. In eccentric contractions, the contractile units (cross bridges) and the elastic elements in series (z-lines and tendons) are involved in force generation and transmission, whereas in severe stretch, stiffness of elastic components in parallel (sarcolemma, endomysium, perimysium, and epimysium) and in series generates force (Wretman et al, 2001).

Exercise stimulation

It has been shown that exercise- and contraction-induced ERK signaling involves the same Ras/Raf/MEK pathway in the activation of ELK1 (Aronson et al, 1997) (figure 1). PKC activation is also known to lead to Ras activation and thus stimulate MAPK activity (van Biesen et al, 1996). Additionally, PKC can mediate p38 MAPK activation by an unidentified mechanism. One possible mechanism may involve a signal cascade, in which PKC phosphorylates a MAPK at its tyrosine and threonine phosphoacceptor domains (figure 3). The activation of PKC occurs via a G-protein, which is itself activated by the binding of an agonist ligand to its specific cell receptor (figure 3). The mediator, phospholipase C, cleaves phosphatidylinositol bisphosphate (PIP) into diacylglycerol (DAG) and inositol triphosphate (IP), the former of which directly activates PKC (Voet et al, 1995).

As previously mentioned, the activation of PKC has been

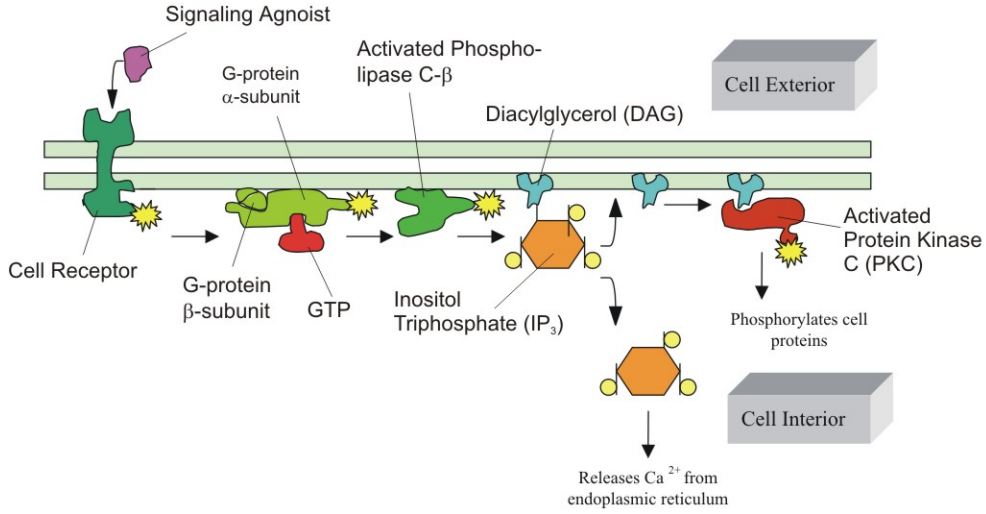

Figure 3. Activation of Protein Kinase C (PKC) via G-proteins

The activation of PKC is preceded by a number of steps, originating from the binding of an extracellular ligand that activates a G-protein on the cytosolic side of the plasma membrane. The G-protein, using GTP as an energy source, then activates PKC via the phosphatidylinositol bisphosphate (PIP2) intermediate, which is shown as the DAG/IP3 complex. (Figure adapted from Alberts, B. et al)

suggested to be caused by muscle contraction in response to electrical stimulation (Cleland et al, 1989). Coupling this idea with myocyte stretching might provide a relationship between the presence of an external force and the binding of the activating ligand. If one would imagine this ligand in the vicinity of the extracellular matrix, the direction and magnitude of force in cell stretching would become a major determining factor in the orientation and proper binding of the agonist to its transmembrane receptor. This would support the selective activation of p38 MAPKs only in eccentric contractions. Moreover, the selective responsiveness of certain upstream elements of MAPKs (i.e. PKC) to electrical stimulation may be the result of ionic changes within the cell *in vivo*.

Energy considerations within a cell and acidification of the cytosolic environment can also shed light on the regulation of MAPKs. When energy turnover in contracting muscle is high, the intracellular milieu becomes acidic due to the buildup of lactic acid discussed earlier (Aronson et al, 2000). A high energy turnover is indicative of a high rate of cellular respiration, which in turn is the result of active muscle contraction during physical exertion. *In vitro* analysis of muscle stretching indicates that acidosis, of the magnitude similar to acidosis in severe fatigue, can induce the ERK MAPK phosphorylation in skeletal muscle cells (Fitts, 1994). Thus the analogous process of *in vivo* myocyte stretching from physical exertion can be seen in myocyte stretching *in vitro*.

Ca^{2+} and positive feedback hypothesis

The aforementioned response of MAPK to electrical stimulation is noticeable in fluctuations of established electrochemical gradients within living cells. Perhaps one of the most salient ions in myocytes is Ca^{2+}. The presence of calcium ions allow for the operation of the contractile machinery within myocytes of all types. Via a well-established model, calcium ions bind to the tropomyosin protein on actin filaments, and subsequently expose various binding sites for myosin. In this way, the myosin head can bind to the actin filaments and allow for muscle contraction to occur. The presence of calcium ions may be correlated to the presence of mechanical stress on the exterior of the cell since the influx of ions through stretch sensitive mechanoreceptors has been implicated in inducing muscle contraction *in vitro* (Aronson et al, 2000). Once in the cytosol, Ca^{2+} ions exert an effect on a variety of cellular elements. Figure 4 illustrates the indirect role of PKC in releasing calcium ions. One of the major products of PKC activity, IP_3, is responsible for activating calcium voltage-gated channels in the membrane of the endoplasmic reticulum. This *in vivo* process mimics the activation of mechanoreceptors seen in many *in vitro* studies (Aronson et al, 2000). Thus, there appears to be a relationship building between the role of mechanical cell membrane stretching and calcium signaling. An intricate network of related pathways seems to be developing, since it was previously mentioned that PKC activates ERK and p38 pathways. It is possible to imagine a signaling pathway propagated by calcium ions that is directly responsible for activating these MAPK pathways. Such calcium signaling could conceivably originate in a way similar to the model in figure 5. Such a PKC-mediated pathway, however, has not yet been experimentally described.

A major consideration not yet addressed is the regulatory mechanism of this postulated signaling system. In normal physiologic systems, the effects of calcium are quickly dissipated unless there is a positive feedback mechanism to fuel the continuation of the calcium-triggered process in question. The initial release of Ca^{2+} ions may prompt an even greater release of calcium into the cytosol, as some ions can bind to allosteric sites on voltage-gated calcium channels, inducing release from reservoirs such as the sarcoplasmic reticulum in myocytes (Alberts et al, 1994). Such a feedback mechanism is supported in evidence of studies on smooth muscle. These studies illustrated that an initial calcium release induces an even greater output of systemic calcium to produce an extended

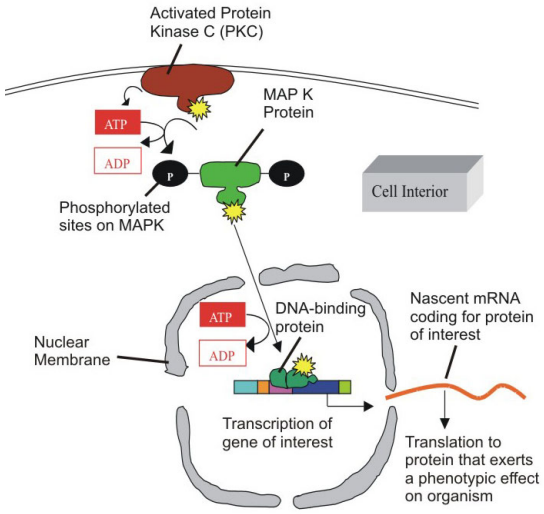

Figure 4. Intracellular pathway of MAPK activation via PKC

Activated PKC phosphorylates MAPK on its tyrosine and threonine sites, at the expense of two ATPs. MAPK then phosphorylates its downstream targets (not shown) to the level of a transcription factor that binds to a DNA element and prompts the transcription of mRNA coding for the protein of interest. Such proteins are employed in cell differentiation, proliferation, and even death.

(Figure adapted from Alberts B. et al)

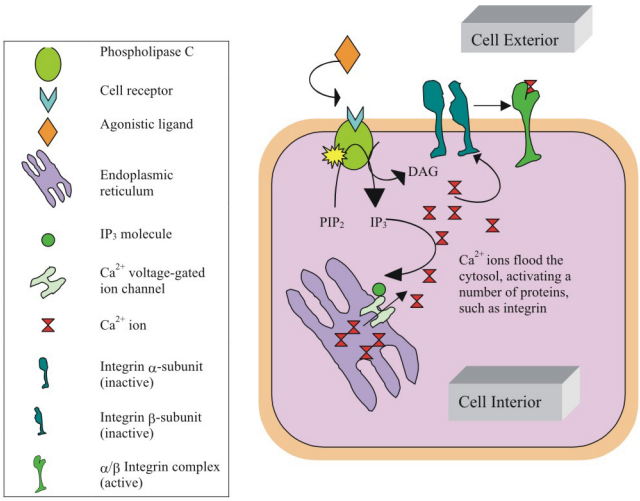

Figure 5. Role of IPs in Ca2+ release

IP3 formation during PKC activation has some interesting effects within the cytosol. A well-characterized effect of IP3 is binding to Ca2+ voltage-gated channels, inducing a conformational change that allows calcium ions to flow down their electrochemical gradient (from the lumen of the endoplasmic reticulum toward the cell interior). The presence of calcium ions within the cytosol allows them to interact with a number of calcium dependent proteins, such as integrins. Interestingly enough, their binding to such proteins effectively removes them from the cytosol, thus increasing the demand for free Ca2+ in the cell interior and stimulating the release of even more Ca2+ ions. This positive feedback loop will be important in recruiting multiple myocytes at the tissue level. (Figure adapted from Voet D., et al)

effect, such as prolonged intestinal contraction within the digestive system (Katoch et al, 1999). Extended contraction is not normally observed in skeletal muscle, except under tetanic conditions in which multiple simple muscle spasms are combined into an apparently smooth continuous effort. Even so, this positive feedback control of calcium can be involved in systems that sequester calcium for purposes of signal transduction, as will be discussed next.

Integrin signaling: a special case

Integrins comprise a major family of transmembrane proteins that allow for both cell-cell and cell-matrix associations. Although most integrins are cell-matrix, those that are involved in cell-cell contacts bind heterophilically to extracellular matrix elements on adjacent cells. Integrins are also found in hemidesmosomes (major cell surface attachment sites at contacts between the cell membrane and components of the extracellular matrix), where they connect to intermediate filaments inside the cell, as well as in focal adhesions (cell-matrix adherens junctions) where they connect to actin filaments and stress fibers (Alberts et al, 1994). The latter form of integrin interactions proves to be noteworthy in activating MAPK pathways in response to cell stretching.

There are four major characteristics of integrin that make it particularly unique as a signaling molecule (Alberts et al, 1994): (1) multiple integrins each recognize different targets (fibronectins, laminins, etc), (2) integrins can be regulated (e.g. during mitosis, phosphorylation of the cytoplasmic tail of the b-subunit of integrin impairs its ability to bind fibronectin, an extracellular matrix protein involved in cell adhesion and migration, causing the cells to round up), (3) matrix binding to integrin regulates cellular activities through focal adhesion kinase (FAK) (figure 8) signaling cascades, and (4) integrins can be either Mg^{2+} or Ca^{2+} dependent. Each of these four attributes can contribute to a greater understanding of integrin function at the level of signal transduction.

Mechanical activation

There are two main subunits for integrin proteins: the a- and the b-subunit. While there are many binding sites for a variety of proteins on each of these subunits, the focus will be on the binding site for laminin, located on the a-subunit, and the binding site for fibronectin located on the b-subunit (Disatnik et al, 1999). The intracellular signaling cascades that are activated when integrins bind to their extracellular ligands are varied. Biochemical changes in cells with integrin deficiencies indicate that integrins are true signaling molecules, transmitting information from the extracellular compartment into the cell in what constitutes “outside-in signaling” (Hynes, 1992). The current fluid-mosaic model of transmembrane proteins in the plasma membrane and the associated extracellular matrix holds that the cell membrane components are not static but rather in constant motion (Alberts et al, 1994). It is not outlandish to consider a force from mechanical stretching of this fluid membrane that is sufficient in magnitude to bring together certain transmembrane proteins of interest. This is in fact what happens with integrins during membrane stretching (Disatnik et al, 1999). One of the earliest changes initiated by integrin engagement is clustering of integrins at focal adhesions and tyrosine phosphorylation of proteins such as paxillin (a cytoskeletal component that localizes to the focal adhesions at the ends of actin stress fibers), talin (a cytoplasmic protein that links integrins to the actin cytoskeleton), and FAK (a cytosolic tyrosine kinase which is recruited at an early stage to focal adhesions and mediates many downstream cellular

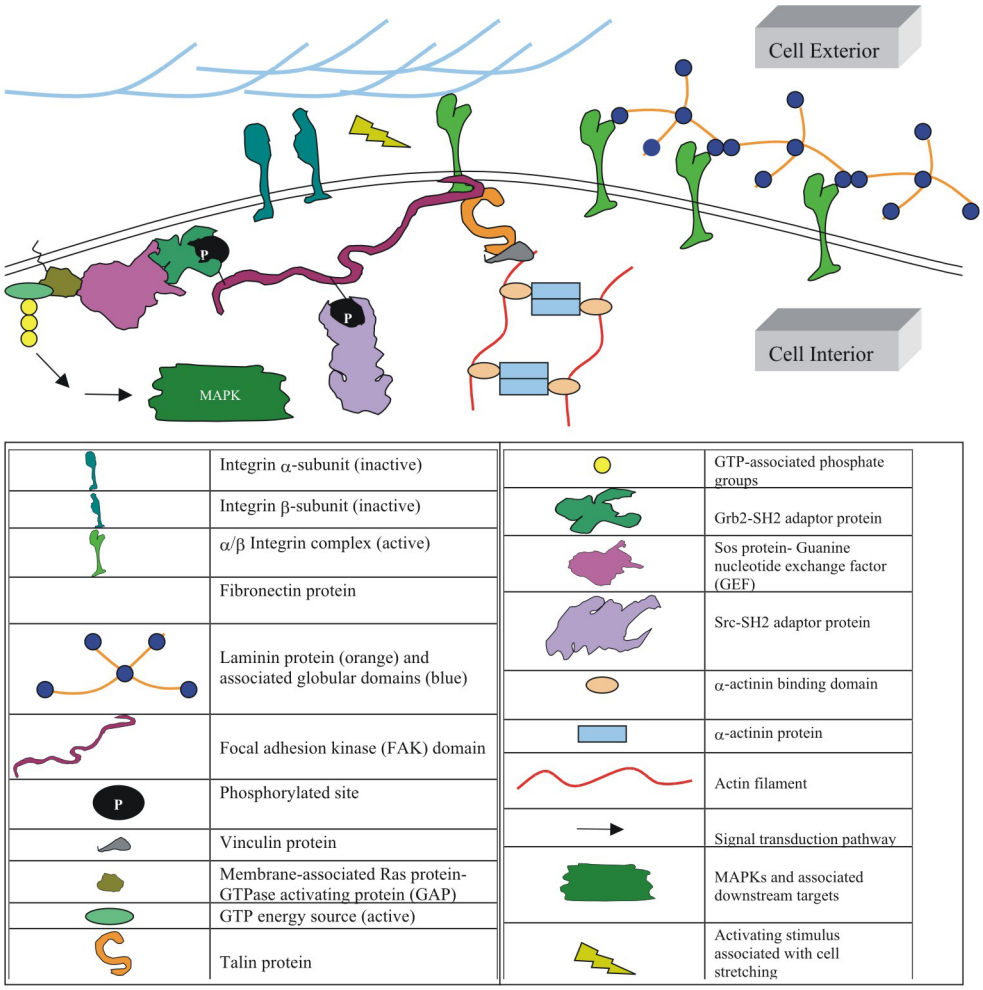

Figure 6. Model of MAPK activation via proteins of the extracellular matrix and cytoskeleton

Stretching the plasma membrane may cause a number of changes in the surrounding extracellular matrix and internal cytoskeletal architecture. These changes may, in turn, activate a MAPK signal transduction pathway, leading to a number of effects manifested in cell growth, differentiation, and proliferation. Two mechanisms may be at work here. First, tension in the cell membrane may prompt an association of a and b integrin subunits from Ca2+ binding, thus activating them. These associated units may then further activate focal adhesion kinase (FAK), with eventual activation of MAPK. Another possible mechanism may involve the association of actin filaments to the vinculin linker protein, which may activate FAK in a similar fashion. The latter mechanism may also involve the binding of Ca2+, perhaps in preparation of muscle contraction in active myocytes. In reality, a combination of these two processes may occur. (Figure adapted from Cooper, et al)

responses) (Schaller et al, 1992). FAK phosphorylation is considered to be one of the critical steps in the downstream signaling that promotes cell spreading and cell survival (Disatnik et al, 1999). Although the details of these more distal events remain to be elucidated, there is evidence that the binding of integrins to their extracellular ligands may activate pathways that prevent apoptosis in a variety of cell types, including skeletal myocytes (Zhang et al, 1995). Association of integrins with concomitant binding of fibronectin to the b-subunit is sufficient to form an activated dimer of integrin subunits. Close inspection of figure 6 illustrates that this aggregation of integrin leads to the association of various proteins, including FAK and talin, as well as their localization to focal adhesions (Kornberg et al, 1992).

It has been experimentally shown that the activation of PKC is necessary for the interaction of b integrin with fibronectin to promote FAK phosphorylation and spreading of muscle cells (Disatnik et al, 1999). Woods and Couchman found that activation of PKC leads to the localization of proteins such as talin to focal adhesions. Using specific PKC inhibitors, Haimovich *et al*. showed that PKC plays a crucial role in integrin signaling and phosphorylation of FAK in platelets. It has also been

shown that PKC isoforms translocate to nuclear structures and focal adhesions upon binding of vascular smooth muscle cells to fibronectin (Vuori et al, 1993). This adds to the growing evidence of the importance of PKC in both integrin signaling and MAPK activation, which can be mediated by activated FAK. Since it is known that MAPKs allow for growth and differentiation of cells (English et al, 1999), the observable changes in cell spreading induced by associations of integrins, which indicate growth patterns, must be the result of MAPK signal transduction.

Ionic activation

Integrin subunits also contain binding site for Ca^{2+} and Mg^{2+} on both the a- and b-subunits (Alberts et al, 1994). As noted earlier, one of the characteristics of integrins is that they are highly regulated, often by means of these divalent cation binding sites. Integrins can be activated by Ca^{2+} binding to its appropriate receptor on the transmembrane protein. Recalling the previously discussed considerations of calcium ions in cell stimulus response, it is clear that integrin has an important role in responding to mechanical stimuli. The interrelationship of PKC, Ca^{2+} ions, and integrins is beginning to be revealed: cell stretching can induce the aggregation of integrins, thus activating them. These activated integrins may then stimulate the initiation of a signal cascade through MAPK, in a postulated mechanism delineated in figure 6. The previously discussed Ca^{2+} positive feedback mechanism mediated by IP_3 binding to the endoplasmic reticulum (figure 3) may constitute the fuel needed to keep the cycle of MAPK activation running by allowing more calcium to bind to the integrin divalent cation sites. Furthermore, the activation of PKC is made possible by the presence of IP_3 (figure 3). By syllogism, this is how the action of active PKC is involved in integrin signaling. This somewhat refines the rather crude model of the positive feedback mechanism described earlier, in which Ca^{2+} binds to other voltage gated ion channels, inducing the release of more calcium ions.

Recruitment of myocytes in tissue systems

The aforementioned considerations have thus far pertained to a single cell. While it is important to understand the mechanics of cellular processes, it is equally important to investigate what occurs with multiple cells at the tissue and organismal level. Taking this into

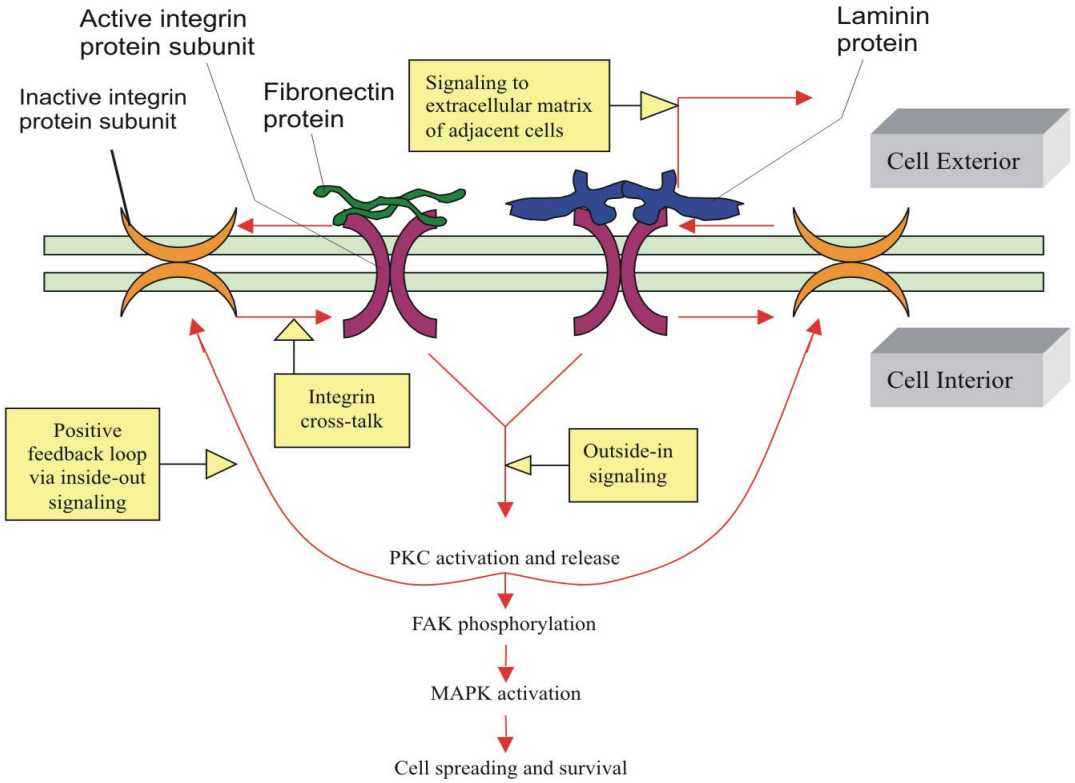

Figure 7. Activation of integins via positive feedback sources: outside-in and inside-out signaling

Clustering of integrins due to an external stimulus can induce them to become activated, with subsequent activation of PKC and downstream phosphorylation of MAPKs. These active integrins can then activate other neighboring inactive integrins in what constitutes "integrin cross-talk" via inside-out signaling. This inside-out signaling proves to be a form of positive feedback, in which more integrins are recruited to allow for cell spreading and growth, a phenomenon regulated by MAPKs. Note that since phosphorylation of FAK is the result of integrin activation, integrins are partially responsible for initiation of MAPK signal transduction pathways. (Figure adapted from Disatnik, et al.)

consideration calls to mind a mechanism of signal spreading, in which a few cells propagate an activated signal pathway to adjacent, or nearby cells. Understanding such a mechanism proves to be essential, since many biochemical processes hardly involve only a single, isolated cell. Moreover, physiology and pathology are meaningless outside the context of cell aggregates.

Although integrin engagement leads to signal cascade activation, it is also clear that the process of muscle cell attachment and spreading involves an activation of integrins themselves, which then allows them to execute "inside-out signaling" (Disatnik et al, 1999). In this form of signaling, integrin has an increased affinity for its extracellular matrix ligand, such as laminin (figures 6 and 7). This activation of integrins by binding laminin promotes the cell adhesion that may be an important step in the morphological changes that cells undergo when spreading on a solid substrate (Disatnik et al, 1999). It turns out that PKC activation is sufficient to promote inside-out signaling and since it has already been shown that PKC is necessary for this signaling, a positive feedback loop is created (Disatnik et al, 1999). Indeed, the gradual morphological changes associated with cell spreading suggest a multistep process involving first the detection of the extracellular environment by the cell and then a progressive change of the cell membrane to interact with that environment (Disatnik et al, 1999). This is demonstrated most clearly by the fact that the changes do not occur when cells are plated in the absence of immobilized matrix proteins to which integrins can bind (Chen et al, 1994). The presence of such proteins initiates a signaling cascade inside the cells, and the cells in turn both alter their membrane properties to interact with the ligands and organize these ligands into a complex matrix (figure 6). A positive feedback loop is intrinsic to such a process (Disatnik et al, 1999) and this further refines the initial feedback model described earlier, thus completing the understanding of its significance in signal transduction.

The positive feedback loop of integrin engagement, signaling, and activation is shown in figure 7. Integrins propagate their activated signal to other cells, via a dynamic equilibrium between an active state and an inactive state (Disatnik et al, 1999). When there is a sufficient number of active integrins for effective engagement with their extracellular ligands, outside-in signaling is initiated, leading to an increase in PKC activity, a further increase in integrin activation and affinity (via inside-out signaling), and further outside-in signaling (Disatnik et al, 1999). This positive feedback loop promotes biochemical changes, including FAK phosphorylation and focal adhesion formation as seen in figure 6, which in turn lead to a downstream cascade of biochemical changes leading to gene expression as shown in figure 4. Inside-out signaling may also be effective in transducing a signal of interest to nearby or adjacent cells. The affinity of integrin to laminin, an extracellular matrix protein involved in cell to cell interactions, may allow for this to occur. As specific inactive integrins are recruited to become active, via the proposed positive-feedback mechanism, they may bind laminins and change conformation. This binding may cause a widespread agitation in the external environment in a cell, altering the orientation of the extracellular matrix surrounding neighboring cells. The fibronectin in these nearby cells may in turn be brought into closer proximity to the plasma membrane, thus facilitating the activation of transmembrane proteins, such as integrins, in the same way as that observed for the initial cell. By this process, integrins from completely different cells can "talk" to each other in a rather elaborate communication scheme, allowing for a more global response to the initiation of a MAPK signal transduction pathway.

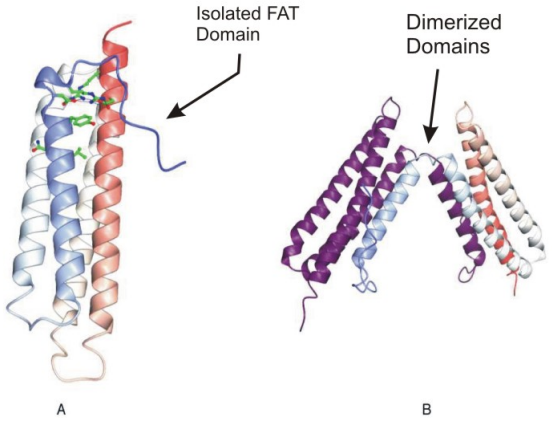

Figure 8. Two structures of the focal adhesion targeting domain of focal adhesion kinase

The localization of focal adhesion kinase (FAK) to sites of integrin clustering initiates downstream signaling. The C-terminal focal adhesion targeting (FAT) domain causes this localization by interacting with talin and paxillin (not shown). Isolated FAT folds into a four- helical bundle (A), which has the capacity to form domain-exchanged dimers in which the N-terminal (B). A structure-based alignment including these proteins and the vinculin tail domain reveals a conserved region which could play a role in focal adhesion targeting (Figure courtesy of Noble, et al).

Conclusion

MAPKs have been shown to be relatively ubiquitious in their activity, yet their activation is quite specific. This review article has introduced MAPKs in the conventional

light of signal transduction activation: the binding of a free ligand (i.e. EGF) to its specific receptor, and the subsequent downstream effects associated with such activation. To provide a more provocative contrast, the idea of mechanical stretching was introduced along with its role as a signal transducer. Albeit the binding of tangible elements from the extracellular matrix is involved in mechanotransduction, the overall induction of mechanically activated signal transduction pathways are initially employed by the intangible presence of tension and mechanical stress in the cell plasma membrane and associated proteins.

To further supplement this unconventional perspective on signal transduction, the hypothetical model of a positive feedback mechanism was discussed, involving the employment of calcium ions that originated from the activation of PKC and other cytosolic components. The final aspect that drove home the involvement of MAPKs in this feedback mechanism was the observation of cell spreading and cell growth, two events that are hallmarks of MAPK activation. While many of the mechanisms and processes discussed in this review are purely hypothetical, and should be treated as such, they are the products of the logical synthesis of concepts in MAPK signal transduction. Thus, in reality, a combination of events such as calcium activation, rearrangement of extracellular proteins, activation of transmembrane domains, and transcription at the level of the gene may all be occurring. Furthermore, these processes may be the result of other components not discussed in this review. Nonetheless, the models presented in this article do provide an integrative and innovative approach to signal transduction that may help in future discoveries.

Prospects

As is the case with most cell analysis, genetic models are always indispensable in the dissection of various signal transduction pathways. Although there has been a multitude of such genetic models from which to draw conclusions as to stress-activated MAPK pathway regulation, new emerging genetic models such as the dorsal closure pathway in *Drosophila*, coupled with the completion of the *C. elegans* and other genome sequencing projects, should make it possible to understand the epistatic relationships between MAPKs and their upstream activators (Tibbles et al, 1999).

Understanding these pathways in the context of human physiology and disease pathology is much more of a challenge. With regard to skeletal myocytes in particular, further study of knockout and transgenic mice may provide the link between signal transduction and muscular dystrophy, for example. A deficiency of the molecule a7 integrin has been positively linked to various cases of congenital muscular dystrophy. Muscular dystrophy, which causes a progressive deterioration of the muscle fiber architecture, seems to be slightly correlated to the insufficient presence of integrin. Since it is known that integrin helps establish the complex organization of the cell membrane, a lack of it must disrupt the integrity of the membrane leading to the onset of the genetic disease. Thus, a more fundamental understanding of integrin and related transmembrane proteins may help assist in elucidating possible treatment, or perhaps even a cure, for muscular dystrophy. However, paramount discoveries in the exact pathways of such debilitating muscle disorders can only be realized with a more aggressive, genetic approach to understanding signal transduction at its most fundamental core: the activation and initiation of a signaling pathway. Once this can be fully divulged for a particular skeletal muscle disease, then perhaps an inhibitory genetic mechanism can be proposed, in which symptoms can be fully repressed before they are manifested phenotypically.

At the current state of genetic engineering, the presence of specific genes in humans cannot be controlled. While this may at first seem like a major setback for the millions of people afflicted with skeletal muscle diseases and other genetic ailments alike, there appears to be a great deal of hope in fighting these diseases using knowledge of signal transduction. This is to say that while the transmission of a gene from one generation to another is difficult to control, the first major point of intervention may be at the level of transcription and translation of a gene, which is controlled by signal transduction. Gene therapy currently seems to be a promising solution for treatment of various genetic diseases. Gene therapy can be targeted to somatic (body) or germ (egg and sperm) cells. In somatic gene therapy the recipient's genome is changed, but the change is not passed along to the next generation. In germline gene therapy, the parents' egg and sperm cells are changed with the goal of passing on the changes to their offspring. Germline gene therapy is not being actively investigated, at least in larger animals and humans, although a lot of discussion is being conducted about its value and desirability. And while complete obliteration of genetic disease does not seem to be in the near future, regulating these genetic diseases before they get out of control is definitely within reach as more research is done on signal transduction.

About the Author

David A. Barron grew up in El Paso, TX, where he developed an early affinity for biomedical research through voluntary experiences in the local area medical center. He attends Rice University, where he is working towards completion of a B.A. in Biochemistry and Cell Biology. Although the majority of his coursework is completed at Rice, he has studied at Oxford University in Oxford, England and through the Baylor College of Medicine, in the Texas Medical Center, both institutions of which have enhanced his knowledge of the biosciences tremendously. He is currently engaged in a research project for the Department of Surgery at Baylor, examining the mechanical and structural properties of both desmin- and integrin-deficient mice as seen in biochemical assays and tissue stretching maneuvers. Past findings have led him into the realm of signal transduction, specifically investigating the influence of mechanical forces on the extracellular matrix. The research team he works with, led by Aladin Boriek, Ph.D., hopes to find a specific role of such mechanical signaling at the tissue level, with the long term prospect of developing a further understanding of debilitating muscular diseases resulting from genetic disorders.

Mr. Barron plans to continue his research during his final year at Rice, with the possibility of undertaking a joint Rice/Baylor research project in an attempt reach a synthesis of different ideas in his particular field of study. While still considering the possibility of pursuing an M.D./PhD. program, his career goals are primarily oriented towards earning an M.D. degree in order to apply his knowledge of biochemistry to patients in a health care setting. In either case, he plans to actively partake in research projects tailored toward dealing with human disease at both the macroscopic and microscopic level, so that he may ultimately examine disease states and pathologic patterns from both a scientific and clinical perspective.

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