Integrin-mediated Muscle Cell Spreading

THE ROLE OF PROTEIN KINASE C IN OUTSIDE-IN AND INSIDE-OUT SIGNALING AND EVIDENCE OF INTEGRIN CROSS-TALK*

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Marie-Hélène Disatnik and Thomas A. Rando‡

From the Department of Neurology and Neurological Sciences, Veterans Affairs Medical Center and Stanford University School of Medicine, Stanford, California 94305-5235

Muscle cell survival depends upon the presence of various integrins with affinities for different extracellular matrix proteins. The absence of either α5 or α7 integrins leads to degenerative disorders of skeletal muscle, muscular dystrophies. To understand the cell survival signals that are mediated by integrin engagement with matrix proteins, we studied the early signaling events initiated by the attachment of muscle cells to fibronectin, an interaction that is mediated primarily by α5 integrins. Cells that express α5 integrin rapidly spread on fibronectin, and this process is associated with the phosphorylation of focal adhesion kinase (FAK). Cells deficient in α5 integrin failed to spread or promote FAK phosphorylation when plated on fibronectin. For α5-expressing cells, both spreading and FAK phosphorylation could be blocked by inhibitors of protein kinase C (PKC), indicating that PKC is necessary for this “outside-in signaling” mediated by α5 integrin. Surprisingly, activators of PKC could promote spreading and FAK phosphorylation in α5-deficient muscle cells plated on fibronectin. This PKC-induced cell spreading appeared to be due to activation of α4 integrins (“inside-out signaling”) since it could be blocked by peptides that specifically inhibit α4 integrin binding to fibronectin. A model of integrin signaling in muscle cells is presented in which there is a positive feedback loop involving PKC in both outside-in and inside-out signaling, and the activation of this cycle is essential for cell spreading and downstream signaling to promote cell survival. In addition, the data indicate a cross-talk that occurs between integrins in which the outside-in signaling via one integrin can promote the activation of another integrin via inside-out signaling.

The interactions of cells with extracellular matrix proteins are mediated primarily by the integrin family of cell-surface receptors that function as heterodimers of α and β subunits. Such interactions are important in the regulation of cell proliferation and differentiation (1). Matrix proteins also function to promote survival of many cell types via integrins, as disruption of the binding of integrins to specific components of the extracellular matrix can lead to cell death (2, 3). The importance of integrin signaling in the survival of muscle cells is demonstrated by the recent reports of muscular degenerative disorders in mice with specific integrin deficiencies (4, 5). Mayer et al. (4) showed that mice that are homozygous null for the gene encoding α7 integrin (which is a receptor for laminin) develop normally, but begin to show signs of muscle cell death by several weeks after birth. The degenerative process continues throughout life such that by 100 days of age, over half of all myofibers in limb muscle show signs of previous cell death and subsequent regeneration. Similarly, Taverna et al. (5) demonstrated that mice with a deficiency of α5 integrin (which is a receptor for fibronectin) develop a degenerative disorder of muscle as well. Muscle cells deficient in α5 integrin have impaired survival both in vivo and in vitro (5). These reports together indicate that expression of both α5 and α7 integrins is necessary for long-term integrity of myofibers.

The intracellular signaling cascades that are activated when integrins bind to their extracellular ligands are varied (6). These biochemical changes indicate that integrins are true signaling molecules, transmitting information from the extracellular compartment into the cell, so-called “outside-in signaling” (1, 7). The specific pathways appear to differ depending on the specific integrin/ligand interaction and the type of cell. One of the earliest changes initiated by integrin engagement is clustering of integrins at focal adhesions and tyrosine phosphorylation of proteins such as paxillin, talin, and the cytosolic enzyme focal adhesion kinase (FAK) (8–11). FAK phosphorylation is considered to be a critical step in the downstream signaling that promotes cell spreading and cell survival. Although the details of these more distal events remain to be elucidated, there is evidence that the binding of integrins to their ligands may activate pathways that prevent apoptosis in a variety of cell types (3, 12).

Protein kinase C (PKC) appears to be one of the key intermediates in integrin-mediated signaling in many cell types (6, 7). In certain cell types, inhibition of PKC activity results in the inhibition of cell attachment and spreading as well as FAK phosphorylation (13, 14). Activation of PKC can promote the cellular changes mediated by integrin/matrix interactions (15–17). These results together demonstrate a specific role of PKC in integrin-mediated signal transduction.

Although integrin engagement leads to signal cascade activation, it is also clear that the process of cell attachment and spreading involves an “activation” of integrins themselves (“inside-out signaling”) such that there is increased affinity of the integrin for its extracellular matrix ligand. This activation promotes cell adhesion and may be an important step in the morphological changes that cells undergo when spreading on a...
solid substrate. Several studies have demonstrated that cell spreading is induced by PKC activation (15, 18). Vuori and Ruoslahti (15) reported that PKC activity increases preceding cell spreading on fibronectin, but not on polylysine, indicating that PKC-mediated cell spreading depends upon the nature of the substrate with which the cell is in contact, as would be expected for a specific ligand/receptor interaction.

In this report, we present evidence of outside-in signaling pathways that are initiated when muscle cells attach to fibronectin via $\alpha_5$ integrins (which are primarily, if not exclusively, $\alpha_5\beta_1$ heterodimers). As in other cells, FAK phosphorylation appears to be an early and critical event in integrin-mediated signaling that leads to cell spreading and cell survival. We found that PKC activation is necessary for cell spreading on fibronectin. Surprisingly, we also found that PKC activation is sufficient to induce cell spreading on fibronectin even in the absence of $\alpha_5$ integrins. This appears to be due to inside-out signaling and activation of $\alpha_5$ integrins, which can then mediate cell attachment and spreading and FAK phosphorylation. These results provide evidence of the involvement of PKC in both outside-in and inside-out integrin signaling in a positive feedback process and of cross-talk between integrins. A model of integrin signaling pathways involved in muscle cell spreading and survival is presented.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Cells deficient in $\alpha_5$ integrins were derived from limb muscles of neonatal mice that were chimeric for $\alpha_5$ integrin expression, as described (5). The muscles were cultured to isolate pure populations of myoblasts, and $\alpha_5$-deficient cells were selected by maintaining the cells in G418 for at least 2 weeks (5). Cells expressing $\alpha_5$ integrin were generated by retrovirus-mediated transfer of a human $\alpha_5$ cDNA into $\alpha_5$-deficient cells; as controls, $\alpha_5$-deficient cells were infected with a control retrovirus (5). For growth, all cells were plated on dishes coated with 5 $\mu$g/ml laminin (Life Technologies, Inc.) and maintained in growth medium consisting of Ham's F-10 medium (BioWhittaker, Walkersville, MD) supplemented with 20% fetal bovine serum (Sigma).

**Adhesion and Spreading**—For assessment of cell adhesion and spreading on different substrates, 60-mm dishes were coated with fibronectin (5 $\mu$g/ml; Life Technologies, Inc.), the GRGDNP (RGD) peptide (10–20 $\mu$g/ml; Life Technologies, Inc.), or the DELPQIVTLPHPR-LHGPEILDVPST (EILDV) peptide (20 $\mu$g/ml; Sigma) for 24 h at room temperature. One hour before plating, all dishes were coated with 1% bovine serum albumin (Sigma). Cells were trypsinized, treated as indicated, and then plated for 30 min. For study of peptide inhibition of spreading, the RGD or LDV peptide was preincubated with the cell in suspension for 20 min prior to plating on fibronectin in the presence of the respective substrate-coated dishes for 30 min. The cultures were assessed and photographed using a 40× phase-contrast immersion objective on a Zeiss Axioskop microscope.

**Immunoprecipitation and Western Blot Analysis**—After trypsinization, cells were plated on fibronectin or the indicated peptides for 30 min. For PKC activation, phorbol 12-myristate 13-acetate (PMA; Alexis Biochemicals Corp., San Diego, CA) was added to the cells in suspension for 10 min at the indicated concentrations. The cells were spun, and the plasma membrane was washed with protein G-agarose for 1 h. Proteins from total extracts or after immunoprecipitation were electrophoresed by 7.5% SDS-polyacrylamide gel electrophoresis, and phosphotyrosine-containing proteins were detected with a monoclonal anti-phosphotyrosine antibody (1:500; Sigma) followed by a horseradish peroxidase-linked anti-mouse secondary antibody (Amersham Pharmacia Biotech). Blots were also probed (or reprobed after stripping) with the anti-FAK polyclonal antibodies (1:500) followed by a horseradish peroxidase-linked anti-rabbit secondary antibody. Specific antibody binding was detected by an enhanced chemiluminescence system (Amersham Pharmacia Biotech). Where indicated, the bands were quantitated using a Bio-Rad Fluor-S MultiImager.

**RESULTS**

We have shown previously that $\alpha_5$-deficient cells survive poorly and undergo apoptotic cell death when plated on fibronectin as the sole substrate (5). The poor survival of $\alpha_5$-deficient cells is reflected by the absence of spreading on fibronectin, whereas cells that express $\alpha_5$ integrin attach and spread readily on fibronectin (Fig. 1A). In many cell types, the process of attachment and spreading is coincident with the formation of focal adhesions and the phosphorylation of FAK as well as other proteins that localize to focal adhesions (8, 9). Indeed, $\alpha_5$-expressing cells demonstrate robust FAK phosphorylation when plated on fibronectin (Fig. 1B). Consistent with
their inability to attach and spread, α5-deficient cells do not manifest any FAK phosphorylation on fibronectin (Fig. 1B). However, the machinery to attach, spread, and form focal adhesions is clearly present in α5-deficient cells as evidenced by both cell spreading (Fig. 1A) and robust FAK phosphorylation (Fig. 1B) when plated on laminin. Thus, the morphological and biochemical abnormalities of α5-deficient cells plated on fibronectin are not the result of a more generalized cellular defect.

As further evidence that α5β1 integrin is the primary fibronectin receptor in normal muscle cells that mediates cell spreading and focal adhesion formation, we inhibited the binding of α5 integrins to fibronectin. The RGD peptide represents the region of the fibronectin protein to which α5 integrins bind (19, 20). Of the other known fibronectin-binding integrins, only αvβ3 has been shown to be present in muscle (21); and αvβ5 binds to fibronectin via a different peptide domain, the EILDV domain (22). Thus, we used soluble RGD peptide to inhibit specifically the interaction of α5β1 integrin with fibronectin. In solution, the RGD peptide acts as an inhibitor of binding even though the peptide can itself serve as an effective binding site for the RGD peptide acts as an inhibitor of binding even though the peptide can itself serve as an effective binding site for fibronectin, PKC activation has been reported to be essential in cell spreading and focal adhesion formation on fibronectin.

Among the various signaling cascades that have been implicated in cell spreading and focal adhesion formation on fibronectin, PKC activation has been reported to be essential in several cell types (13, 15). To test whether the PKC pathway was necessary for spreading and FAK phosphorylation of myoblasts plated on fibronectin, we used two specific PKC inhibitors, calphostin C and bisindolyl maleimide I. Both inhibitors were effective in blocking cell spreading and FAK phosphorylation when α5-expressing myoblasts were plated on fibronectin. However, calphostin C, used in this experiment, was found to be more potent in blocking cell spreading. Fig. 3A shows that the attachment and spreading of myoblasts was inhibited in the presence of calphostin C at doses that have been shown to block PKC activity (15). We observed complete spreading and spreading without concurrent PKC activation.

Because PKC activity appears to be critical to α5 integrin-mediated muscle cell spreading and because PKC activation has been shown to promote and/or enhance cell spreading of different cell types (15, 18), we investigated the effect of PKC activation on both α5-expressing and α5-deficient myoblasts on fibronectin. As in other cells, activation of PKC with PMA led to more rapid spreading and FAK phosphorylation of α5-expressing cells (data not shown). FAK phosphorylation was quantitated to calculate the percentage of inhibition at the different RGD doses (as shown at the top of each lane). B, bound RGD peptide acts as an active substrate for α5 binding and signaling. Whereas soluble RGD peptide acted as an inhibitor of the interaction between α5 integrin and fibronectin (FN), the peptide was highly effective as a substrate for the integrin when coated onto tissue culture dishes (10 μg/ml). Cells expressing α5 integrins adhered as well to solid-phase RGD peptide as they did to fibronectin, and phosphorylation of FAK was indistinguishable between the two substrates as shown in this immunoblot analysis using an anti-phosphotyrosine antibody. FAK phosphorylation was analyzed 30 min after plating the cells in each case. Similar results were obtained in three separate experiments, and a representative result is shown here.

Fig. 2. α5β1 integrin is the major fibronectin receptor in muscle. A, soluble RGD peptide inhibits the α5/fibronectin interaction. Muscle cells expressing α5 integrins were preincubated with increasing concentrations of RGD peptide (as shown at the top of each lane) before and during plating on fibronectin. Thirty minutes after plating, the cells were collected and harvested in RIPA buffer, and the extent of FAK phosphorylation was determined by immunoblot analysis. Soluble RGD peptide inhibited the adhesion of the cells to the substrate in a dose-dependent manner, and this is reflected by the inhibition of FAK phosphorylation as shown here. Equal expression of FAK in each lane was confirmed by reprobing the blot with an anti-FAK antibody (data not shown). FAK phosphorylation was quantitated to calculate the percentage of inhibition at the different RGD doses (as shown at the top of each lane). B, bound RGD peptide acts as an active substrate for α5 binding and signaling. Whereas soluble RGD peptide acted as an inhibitor of the interaction between α5 integrin and fibronectin (FN), the peptide was highly effective as a substrate for the integrin when coated onto tissue culture dishes (10 μg/ml). Cells expressing α5 integrins adhered as well to solid-phase RGD peptide as they did to fibronectin, and phosphorylation of FAK was indistinguishable between the two substrates as shown in this immunoblot analysis using an anti-phosphotyrosine antibody. FAK phosphorylation was analyzed 30 min after plating the cells in each case. Similar results were obtained in three separate experiments, and a representative result is shown here.
mediated outside-in signaling (Fig. 3), but might also be acting in inside-out signaling to promote cell attachment to fibronectin through another fibronectin receptor. This action was specific for PKC activation because it was blocked by the PKC inhibitor bisindolylmaleimide I (Fig. 4C). Furthermore, activation of protein kinase A with dibutylryl cAMP had no effect (Fig. 4C), showing that it was not a nonspecific consequence of serine/threonine kinase activation. The puzzle was which fibronectin-binding protein might be mediating this interaction.

As mentioned previously, of the known fibronectin-binding integrins, the only one that has been shown to be expressed by muscle cells other than $\alpha_5\beta_1$ is $\alpha_4\beta_1$, albeit in low abundance (21). We hypothesized that PMA might increase the affinity of $\alpha_4$ integrins on the cell surface for fibronectin via an inside-out signaling pathway. Thus, when sufficiently activated, $\alpha_4$ integrins would then be competent to mediate cell spreading and focal adhesion formation on fibronectin. To test whether PKC-dependent spreading of $\alpha_5$-deficient cells on fibronectin was indeed mediated by $\alpha_4$ integrins, we blocked the binding of $\alpha_4$ integrins to fibronectin with a specific peptide inhibitor. The cells were treated in solution with the EILDV peptide, which, as mentioned above, represents the $\alpha_4$ integrin-binding domain on fibronectin. In the presence of the EILDV peptide, PMA treatment was ineffective in promoting spreading or FAK phosphorylation of $\alpha_5$-deficient cells on fibronectin (Fig. 5A).

As an additional test of the hypothesis that, upon PKC activation, the effective adhesion and spreading of $\alpha_5$-deficient cells on fibronectin are mediated by $\alpha_4$ integrin activation, we tested untreated and PMA-treated $\alpha_5$-deficient cells on different substrates (Fig. 5, B and C). As shown above, PMA stimulation promoted adhesion and FAK phosphorylation on fibronectin. Consistent with the idea that this interaction is mediated by $\alpha_4$ integrins, immobilized EILDV peptide was as effective as fibronectin in promoting spreading and FAK phosphorylation (Fig. 5C). Immobilized RGD peptide was ineffective as a substrate, indicating that other fibronectin-binding integrins that interact with the RGD domain of fibronectin (e.g. $\alpha_2\beta_1$ or $\alpha_5\beta_3$ (1)) either were not present or were not activated upon PMA stimulation. Thus, whereas we cannot conclude that $\alpha_4$ integrin is the only integrin activated by increased PKC activity in $\alpha_5$-deficient myoblasts, it certainly appears that $\alpha_4$ is the critical integrin that promotes spreading on fibronectin under these conditions.

**DISCUSSION**

Using both pharmacological and genetic approaches, we have demonstrated that $\alpha_5$ integrins mediate muscle cell spreading via PKC activation (outside-in signaling) and that PKC activation promotes muscle cell spreading on fibronectin by enhancing the binding of either $\alpha_5$ or $\alpha_4$ integrins (inside-out signaling). Below we present a model of integrin signaling that involves these distinct but interacting pathways (Fig. 6).

Upon integrin engagement with extracellular matrix proteins, there is a clustering of the integrins at focal adhesions (25). This aggregation leads in turn to the phosphorylation of various proteins, including FAK, talin, vinculin, and Paxillin, and also to their localization to focal adhesions (10, 23, 25). We have demonstrated that activation of PKC is necessary for the interaction of $\alpha_5$ integrin with fibronectin to promote FAK phosphorylation and spreading of muscle cells. This adds to the growing body of evidence of the importance of PKC in outside-in integrin signaling. Woods and Couchman (13) showed that kinase inhibitors prevent focal adhesion formation in fibroblasts plated on fibronectin. They also found that activation of PKC leads to the localization of proteins such as talin and vinculin to focal adhesions. Paxillin may in fact be a substrate for PKC, as it is phosphorylated on serine residues in a PKC-dependent manner (26, 27). Using specific PKC inhibitors, Haimovich et al. (14) showed that PKC plays a crucial role in integrin signaling and FAK phosphorylation in platelets. Haller et al. (18) showed that PKCe and PKCe translocate to nuclear structures and focal adhesions upon the binding of vascular smooth muscle cells to fibronectin. These results together suggest that PKC is an essential component in the proximal signaling pathway triggered by the binding of integrins to their ligands in certain cell types.

Inside-out signaling is considered to be the main mechanism by which cells regulate integrin function (1, 6, 7, 28, 29). Integrins in an inactive state have a low affinity for their ligands; activation of integrins in response to specific physiological
stimuli is associated with, or even defined as, an increased affinity for their ligands (29). Our studies with genetically deficient cells allow us to study inside-out and outside-in integrin signaling independently of one another and suggest that PKC activation can mediate inside-out signaling in muscle cells. Whereas α5-deficient cells failed to spread on fibronectin, treatment of the cells with FAK to activate PKC led to rapid and robust spreading and FAK phosphorylation. These events appear to have been mediated by the activation of α5 integrins since they were blocked by soluble EILDV peptide and promoted by immobilized EILDV peptide in plaques of fibronectin. The subsequent phosphorylation of FAK observed under these conditions would thus be due to the initiation of outside-in signaling via activated α5/fibronectin interactions. Several reports previously demonstrated enhancement of spreading on fibronectin by activation of PKC (15, 30, 31). Vuori and Ruoslahti (15) showed that activation of PKC activity is necessary for the adhesion, spreading, and migration of cells. Although T cells have integrins that bind fibronectin and laminin, it was shown that they will attach to these matrix molecules only upon PKC activation (32).

Positive Feedback Loop and Integrin Cross-talk—These data reveal two important characteristics of integrin signaling. First, because PKC activation is both necessary for outside-in signaling and sufficient to promote inside-out signaling, a positive feedback loop is created. 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. 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 (10, 15, 16). 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 the ligands into a complex matrix. A positive feedback loop is intrinsic to such a process.

The other interesting system characteristic that is highlighted by our data is cross-talk between integrins. In our cells, the consequences of outside-in signaling via α5 integrins lead to activation of α7 integrins. Lichtner et al. (33) provided evidence of negative cooperativity between integrins with regard to affinity for extracellular matrix proteins. Kolanus and Seed (34) pointed out that multiple signaling pathways, in particular PKC, not only converge from different integrins, but also modulate integrin affinity, thus supporting direct cross-talk between different integrins in the cell.

Model of Integrin Signaling—The model suggested by these data is a positive feedback loop of integrin engagement, signaling, and activation. In this model (Fig. 6), integrins exist in a dynamic equilibrium between an active state and an inactive state. 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 (inside-out signaling), and further outside-in signaling. This positive feedback loop promotes biochemical changes, including FAK phosphorylation and focal adhesion formation, which in turn lead to a downstream cascade of biochemical changes and changes in gene expression. The property of integrin cross-talk is demonstrated by the fact that initiation of the signaling pathway of one integrin can lead directly to the activation of another integrin. Clearly, these pathways would depend upon the specific cell type since different cells have different patterns of integrin expression and may have different balances between active and inactive states of those integrins. The use of genetically deficient cells in combination with specific integrin agonists and antagonists as we have used here will further reveal the details of these complex interactions.

Integrin Signaling and Muscle Cell Survival—We previously demonstrated that a deficiency of α5 integrin leads to apoptotic death of muscle cells (5). Vachon et al. (35) showed that blockade of the binding of β1 integrins leads to apoptotic death of myotubes in culture. Other investigators have likewise demonstrated apoptosis of cells in which the interactions between integrins and matrix proteins have been disrupted (2, 3, 12, 36, 37). These data suggest that integrin signaling induces cell survival pathways, whereas a relative deficiency of those signals may initiate cell death pathways.

The role of integrin signaling in muscle cell survival is most clearly demonstrated by the muscle degenerative disorders that develop in mice deficient in either α5 or α7 integrins (4, 5). A muscular degenerative disorder in humans was recently shown to be due to a mutation in the α7 integrin gene (38).
Clearly, muscle cells possess multiple integrins with different matrix binding capacities, and those integrins function to maintain the integrity of differentiated muscle fibers. It is thus easy to understand why a deficiency of a single integrin does not prevent muscle growth, differentiation, and maturation. However, over time, a deficiency of specific integrins can result...

**Fig. 5.** PKC-mediated inside-out signaling in $\alpha_5$-deficient muscle cells: activation of $\alpha_4$ integrin. A, soluble EILDV peptide inhibits FAK phosphorylation induced by PMA. $\alpha_5$-Deficient cells were treated in suspension with 200 $\mu$M EILDV (labeled LDV) peptide for 20 min and treated with 1 $\mu$M PMA for 10 min. The cells were plated on fibronectin in the presence of the EILDV peptide for 30 min and then lysed in RIPA buffer. Soluble EILDV peptide, which inhibits the binding of $\alpha_5$ integrins to immobilized fibronectin, blocked FAK phosphorylation, which was analyzed as described in the legend to Fig. 4. B, immobilized EILDV peptide is effective in allowing spreading of $\alpha_5$-deficient cells upon PKC stimulation. $\alpha_5$-deficient cells were treated with 1 $\mu$M PMA for 10 min and then plated on fibronectin (FN), the EILDV peptide, or the RGD peptide for 30 min. Both fibronectin and the EILDV peptide were effective in promoting the spreading of $\alpha_5$-deficient cells, whereas the RGD peptide was ineffective. The cells were photographed using a 40× objective. C, inside-out signaling mediates muscle cells spreading. $\alpha_5$-Deficient cells were treated for 10 min with 1 $\mu$M PMA and then plated on fibronectin, the EILDV peptide, or the RGD peptide as described for B. After 30 min, FAK phosphorylation was analyzed by Western blot analysis using an anti-phosphotyrosine antibody (anti-P-Tyr), and FAK protein content was analyzed using an anti-FAK antibody. Consistent with the results of cell spreading in A, activation of PKC promoted FAK phosphorylation on fibronectin and the EILDV peptide, but not on the RGD peptide. The experiments were carried out three to five times, and representative results are shown.

**Fig. 6.** Model of outside-in and inside-out integrin signaling in muscle cells. In this model (see “Discussion” for further details), integrins exist in an equilibrium between two states, an inactive state and an active state (indicated by asterisks). When in the activated state, there is higher affinity for the ligand, which is fibronectin in the case of $\alpha_5\beta_1$ and $\alpha_4\beta_1$ integrins. When activated integrin binds to its ligand, outside-in signaling is initiated, leading to phosphorylation of FAK and an increase in PKC activity. Increased PKC activity in turn leads to activation of more $\alpha_5\beta_1$ integrins (Positive Feedback Loop) and also to the activation of $\alpha_4\beta_1$ integrins (Integrin Cross-Talk). The cyclical processes are necessary for effective engagement of integrins to extracellular matrix proteins to lead to focal adhesion formation (and FAK phosphorylation) and cell spreading as well as downstream processes that promote cell survival. The effects of various pharmacological agents used in our studies (PMA, calphostin C, and bisindolylmaleimide 1) are indicated. Arrows in succession in the signaling pathways indicate multiple steps and not necessarily direct interactions.
in a gradual, progressive death of muscle cells (4, 5), as might be expected when there is a deficiency of one survival pathway amid many. The phenotype is not one of arrested development or immediate and widespread death. Rather, the phenotype represents a tilt in the balance between survival and death reflected in the gradual and stochastic loss of cells over time. Such a shift in balance in cellular signaling and gradual muscle cell loss may also be a model for the mechanism of muscle cell death in muscular dystrophies due to genetic defects in other membrane-associated proteins such merosin, dystrophin, and the sarcoglycans (39). Like the multicomponent complex of proteins associated with integrins, these proteins form a stable complex at the membrane with links to the extracellular matrix and cytoskeletal structures. Less is known about the possible cell survival signals mediated by these proteins, although the muscle-specific form of laminin, merosin, has been shown to promote muscle cell survival (40), and a reduction in α-dystroglycan leads to muscle cell apoptosis (41). Thus, muscular dystrophies due to abnormalities of integrin pathways may serve as a useful paradigm for similar investigations of pathogenetic mechanisms in other muscular dystrophies.

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