A Role for Focal Adhesion Kinase in Phenylephrine-induced Hypertrophy of Rat Ventricular Cardiomyocytes*

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A variety of agonists including phenylephrine (PE) induce hypertrophy in neonatal ventricular cardiomyocytes. Here we report that signals provided by extracellular matrix proteins (ECM) augment the PE-induced hypertrophic response of cardiomyocytes and provide evidence that ECM-dependent signaling is mediated in part by the protein tyrosine kinase, focal adhesion kinase (FAK). Addition of PE to cultured neonatal cardiomyocytes stimulated sarcomeric organization, increased cell size, and induced atrial natriuretic factor in cardiomyocytes plated on the ECM protein laminin or fibronectin. In contrast, cardiomyocytes plated on the non-adhesive substrate gelatin exhibited a reduced capacity to undergo these PE-stimulated hypertrophic changes. In cardiomyocytes cultured on ECM, PE stimulated a rapid increase in tyrosine phosphorylation of focal adhesion proteins including FAK, paxillin, and p130 Crk-associated substrate and subsequent formation of peripheral focal complexes. Inhibition of the PE-induced hypertrophic response by genistein and herbimycin-A indicated a requirement for protein tyrosine kinases in PE signaling. To determine whether activation of FAK is required for PE-induced hypertrophy, a dominant-interfering mutant form of FAK, termed FRNK (FAK-related non-kinase), was ectopically expressed in cardiomyocytes using a replication-defective adenovirus expression system. FRNK expression attenuated PE-stimulated hypertrophy as assessed by cell size, sarcomeric organization, and induction of atrial natriuretic factor. These data indicate that the signal transduction pathways leading to cardiomyocyte hypertrophy are strongly influenced by and/or dependent upon an integrin-mediated signaling process requiring FAK.

Cardiomyocyte hypertrophy, characterized by increased volume and myofibrillar protein content, is important for the normal developmental growth of the heart. However, in the fully developed adult heart, pressure or volume overload, myocardial infarction, or hormonal imbalance can lead to chronic pathological hypertrophy characterized by induction of immediate early genes, re-activation of an embryonic gene program, and reorganization of myocyte cytoskeletal architecture.

The ability of integrins, extracellular matrix receptors, to regulate cytoskeletal architecture has been well characterized, and integrin signaling has clearly been implicated in hormone and growth factor-induced alterations in gene transcription in variety of cell types (2). Integrins are a family of heterodimeric transmembrane receptors (composed of \(\alpha\) and \(\beta\) subunits) containing extracellular ligand binding domains that show binding specificity for extracellular matrix (ECM) components and a short cytoplasmic domain that serves to couple integrins with the actin cytoskeleton (3). Several studies have shown that cardiac hypertrophy is accompanied by alterations in ECM components and cardiac integrin expression (4–6). Specifically, enhanced expression of collagen III, fibronectin, osteopontin, and \(\beta_1\), \(\alpha_7\), and \(\alpha_5\) integrin subunits correlates with advancement of hypertrophy (4–6). A direct role for integrins in the development of hypertrophy has been demonstrated by transgenic studies in which cardiac specific expression of activated \(\alpha_5\) integrin leads to profound ventricular hypertrophy (7). Taken together, these observations support a model whereby alterations in signaling pathways responsive to ECM may influence the progression of cardiac hypertrophy.

Integrin signaling in many cell types involves activation of focal adhesion kinase (FAK), a cytoplasmic non-receptor protein tyrosine kinase. Clustering of integrins leads to the recruitment of FAK to the newly formed focal adhesion and results in autophosphorylation and concomitant activation of FAK. Tyrosine phosphorylation of FAK at Tyr-397 (proximal to the kinase domain) creates a binding site for the SH2 domain of another non-receptor tyrosine kinase, Src, resulting in formation of a bipartite complex of two tyrosine kinases (8). The formation of the FAK-Src complex results in the activation of Src and the subsequent activation of downstream signals leading to the regulation of cellular processes such as growth, migration, and apoptosis (2, 3, 9).

The C-terminal domain of FAK contains binding sites for a number of signaling molecules including phosphoinositide 3-kinase, p130 Crk-associated substrate (CAS), GRB2, paxillin, and the GTPase regulator associated with FAK (Graf, Ref. 10). The recruitment and activation of these molecules are thought to be

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The abbreviations used are: ECM, extracellular matrix; MAP, mitogen-activated protein; EGF, epidermal growth factor; GRB2, growth receptor-bound protein-2; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; RIPA, radioimmunoprecipitation assay buffer; Ab, antibody; HRP, horseradish peroxidase; PE, phenylephrine; FAK, focal adhesion kinase; ANF, atrial natriuretic factor; FRNK, FAK related non-kinase; CAS, p130 Crk-associated substrate; ERK, extracellular signal regulated kinase; DMEM, Dulbecco’s modified Eagle’s medium; GFP, green fluorescent protein; FN, fibronectin; LAM, laminin; m.o.i., multiplicity of infection; FITC, fluorescein isothiocyanate.
FAK Regulates Myocyte Hypertrophy

important for the subsequent activation of other downstream signaling pathways. For example, association of FAK with CAS is required for integrin-mediated activation of ERK2 (11). In some cells, the C-terminal domain of FAK is expressed as a separate protein termed FRNK (FAK-related non-kinase), whose overexpression attenuates integrin-mediated tyrosine phosphorylation of FAK and paxillin (12, 13) and results in the inhibition of the rate of cell spreading and cell migration (12, 13). These data indicate that FRNK can act as a biological inhibitor of FAK signaling and highlight the importance of FAK in ECM-mediated signaling events.

In addition to providing specific signaling from ECM, recent evidence suggests that integrin engagement is required to mediate efficient growth factor and hormonal-mediated signaling. When fibroblasts are held in suspension, EGF-, platelet-derived growth factor-, and lysophosphatidic acid-stimulated ERK2 activity is markedly reduced compared with cells plated on fibronectin (14, 15). Importantly, the inhibition observed in suspended cells can be rescued by overexpression of activated FAK (16). These data indicate that growth factors (or hormones) synergize with cell adhesion signals to stimulate cell growth and survival.

We report herein that PE-induced hypertrophy in isolated neonatal cardiomyocytes requires signaling through FAK. Plating cells on the integrin-dependent ECM proteins laminin or fibronectin supports the PE-induced hypertrophic response of the cultured cardiomyocytes, whereas plating cells on the integrin-independent substrate gelatin fails to sustain cellular hypertrophy. Addition of PE stimulates tyrosine phosphorylation of focal adhesion proteins including FAK, paxillin, and CAS and alters the subcellular localization of paxillin in cardiomyocytes plated on ECM. In addition, we show that ectopic expression of FRNK, a dominant-interfering mutant for FAK, attenuates PE-induced hypertrophy. These data support a role for integrin-mediated, FAK-dependent signaling for induction of cardiomyocyte hypertrophy.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—Antibody (9E10) to the Myc epitope tag was purchased from Santa Cruz Biotechnology, the anti-ANF antibody (JHC9103) was from Peninsula Laboratories; the 4G10 phosphotyrosine-specific antibody was from Upstate Biotechnology, Inc.; the anti-paxillin antibody was from Transduction Laboratories; and the anti-GFP antibody was from CLONTECH. An anti-FAK tyrosine 397-specific antibody was purchased from BIOSOURCE International, and an anti-human FAK antibody was purchased from Upstate Biotechnology, Inc. The ERK1/2 antibodies used include a phosphorylation-specific antibody purchased from Calbiochem, and polyclonal (TR10) and monoclonal (1B3B9) anti- antibodies were generously provided by Dr. Michael Weber (University of Virginia). CAS was detected using a mixture of two polyclonal antibodies, whose overexpression attenuates integrin-mediated tyrosine phosphorylation of FAK and paxillin, and CAS and alters the subcellular localization of paxillin in cardiomyocytes plated on ECM. In addition, we show that ectopic expression of FRNK, a dominant-interfering mutant for FAK, attenuates PE-induced hypertrophy. These data support a role for integrin-mediated, FAK-dependent signaling for induction of cardiomyocyte hypertrophy.

**Expression Constructs and Adenovirus Production**—The cDNA constructs encoding the N-terminal Myc- and GFP-tagged variants of FRNK were generated by cloning chicken FRNK into the BamHI/NotI or BglII/EcoRI sites of the mammalian expression vectors pCMV-Neo-BamHI or pEGFP-C1, respectively. For adenovirus production, GFP and GFP-tagged FRNK were generated by PCR from pEGFP-C1 and pEGFP-C1-FRNK, respectively, using primers that added 5′ and 3′ BamHI restriction sites. The resultant PCR products were digested with BamHI and ligated with BamHI-digested pAd-lox (an adenoviral shuttle vector generously provided by Stephen Hardy, Somatix Therapy Corp., Alameda, CA). Correct orientations of all reading frames were confirmed by sequencing and Western blot analysis of expressed proteins. The GFP-pA-lox and GFP-FRNK-pA-lox constructs were subsequently transfected into replication-defective NSE virus into HEK293 cells that produce stable overexpression of the Cre-recombinase. After recombination, plaque-purified virus (1 × 10^6 plaque-forming units/ml) was generated and purified by cesium-chloride gradients as described elsewhere (18).

**Cell Culture, Infection, and DNA Transfection**—Ventricular cardiomyocytes were isolated from neonatal rats (1–3 days old) by trypsin and collagenase digestion and purified as described previously (19). The cells were resuspended in DMEM:Media 199 (4:1) containing 10% fetal calf serum and 1% penicillin-streptomycycin and plated on tissue culture plastic for two consecutive 1-h periods to remove non-cardiomyocyte cells. For the biochemical experiments, cardiomyocytes were treated with 100 μM 5-bromo-2′-deoxyuridine as described previously to obtain cultures with >95% myocytes (20). The cardiomyocytes were then plated on tissue culture dishes or chamber slides pre-coated with ECM or gelatin as required. Cardiomyocytes were plated at low density (2 × 10^6 cells/ml) for immunofluorescent studies and at high density (1 × 10^6 cells/ml) for biochemical studies. After 18–24 h the cells were rinsed in serum-free DMEM:Media 199 (4:1) and plated in serum-free DMEM:Media 199 (4:1) containing 1% penicillin-streptomycycin. After a 24-h incubation period, cells were treated with vehicle or PE for the indicated times.

For adenoviral infection, cells were infected in serum-free media with either GFP or GFP-FRNK (4 m.o.i.) for 12 h prior to treatment with PE. In some experiments cardiomyocytes were transfected with a Myc-FRNK expression vector using the FuGene transfection reagent (Roche Molecular Biochemicals). Typically 3 μg of DNA/20-mm chamber slide was combined with 6 μl of FuGene and incubated with the cells in serum-free DMEM:Media 199 (4:1) for 24 h prior to treatment with vehicle or PE.

**Immunocytochemistry**—For immunofluorescent staining, cells were washed three times with phosphate-buffered saline (PBS, calcium- and magnesium-free) and fixed using 4% paraformaldehyde in PBS for 20 min at room temperature. Cells were washed three times in PBS and permeabilized with 0.4% Triton X-100 in PBS for 3 min at room temperature. Slides were then washed three times in PBS to remove the detergent and incubated with either the primary anti-Myc monoclonal antibody, 9E10 (1 μg/ml), or the polyclonal anti-ANF Ab (1:500, Peninsu- lara Laboratories) for 1 h. Cells were washed three times in PBS and incubated with Cascade Blue-conjugated goat anti-mouse Ab (2 μg/ml) and/or Texas Red-conjugated donkey anti-rabbit Ab (2 μg/ml) for 1 h. In some experiments, Texas Red- or FITC-conjugated phalloidin (1:1000; Molecular Probes) was used to visualize filamentous actin.

**Immunoprecipitation and Western Blots**—Cardiomyocytes were lysed by homogenization in a modified RIPA buffer (50 mM Hepes, 0.15 M NaCl, 2 mM EDTA, 0.1% Nonidet P-40, and 0.05% sodium deoxycholate, pH 7.2) containing 1 mM Na_2VO_4, 40 mM NaF, 10 mM Na_2 pyrophosphate. Paxillin or CAS or ERK1/2 was immunoprecipitated with incubation of 1–2 mg of cell extract with 5 μg of the appropriate antibody for 2 h at 4 °C, followed by a 1-h incubation with protein A-Sepharose-conjugated beads (Amersham Pharmacia Biotech). For paxillin immunoprecipitation, the beads were pre-coupled to anti-mouse anti-body (10 μg/ml, Jackson Laboratories). The immune complexes were collected by centrifugation, and the beads were washed three times with RIPA buffer and once with Tris-buffered saline (0.2 M NaCl and 50 mM NaCl, pH 7.4), boiled in sample buffer, and the proteins resolved by 10% SDS-PAGE. Proteins were transferred to nitrocellulose, and Western blots were performed using the appropriate primary antibody at a 1:1000 dilution followed by incubation with either horseradish peroxi-dase-conjugated rabbit anti-mouse Ab or horseradish peroxidase-conju gated protein A-Sepharose (Amersham Pharmacia Biotech) at a 1:1000 dilution. Blots were visualized after incubation with chemiluminescence reagents (ECL, Amersham Pharmacia Biotech).

**Immune Complex Kinase Assay**—ERK was immunoprecipitated by incubation of cardiomyocyte extracts (100 μg) from GFP or GFP-FRNK-infected cells with a polyclonal antibody specific for ERK1 and ERK2 (TR10) as described above. The immune complexes were resuspended in MAP kinase reaction buffer (25 mM Hepes, pH 7.5, 10 mM magnesium acetate, and 50 μM unlabeled ATP) containing myelin basic protein (2 μg) and γ-[32P]ATP (1 μCi, 6,000 Ci/mmol) and incubated at 30 °C for 20 min at room temperature. Reaction mixtures were boiled in SDS sample buffer, resolved by 15% SDS-PAGE, and visualized by autoradiography.

**ANF Secretion**—Cardiomyocytes were isolated as described previously and plated at a density of 2.5 × 10^6 cells/well in a 24-well plate. Cells were treated with either GFP virus or GFP-FRNK virus (4 m.o.i.) or vehicle control in serum-free media (300 μl/well). 12 h after infection, cells were treated with either vehicle alone or 100 μM PE for 24 h. The conditioned media were collected and centrifuged to pellet any possible debris. The concentration of ANF in the conditioned media was then determined using a peptide enzyme immunoassay (Peninsula Labora-tories, San Carlos, CA).
RESULTS

Many features of pathological hypertrophy can be reproduced in isolated neonatal cardiomyocytes by treatment with a variety of agents including phenylephrine (PE), angiotensin II, and endothelin-1 (21). These cells respond to an increase in cell size, sarcomeric organization, increased expression of immediate early genes (c-fos, c-jun, Egr-1, jun-B, and Nur-7), and re-expression of embryonic genes (atrial natriuretic factor (ANF); skeletal a-actin, smooth muscle a-actin, and b-myosin heavy chain; see Ref. 22). By using this experimental paradigm, we examined to what extent integrin-mediated signaling is involved in the hypertrophic response in vitro.

Initially, we examined the ability of PE to stimulate hypertrophy in neonatal cardiomyocytes plated on integrin-dependent (e.g. laminin, fibronectin, and collagen type I) or integrin-independent (e.g. gelatin) substrates. PE treatment of cardiomyocytes plated on laminin (Fig. 1A, middle panels) stimulated sarcomeric organization and increased cell size and ANF production compared with the control vehicle-treated cells (Fig. 1A, top panels). In contrast, cardiomyocytes plated on gelatin exhibited a reduced capacity to undergo PE-stimulated hypertrophic changes (Fig. 1A, bottom panels).

To quantitate the extent of hypertrophy on different matrices, a total of 150–200 cells in each treatment group (representing cells from three separate experiments) were scored for sarcomeric organization and ANF induction using fluorescent microscopy (Fig. 1B). Sarcomeric reorganization was observed in approximately 90% of PE-treated cells plated on laminin versus 35% of the cells plated on gelatin. Cells not treated with PE showed little actin reorganization whether plated on laminin or gelatin (Fig. 1B, left panel). ANF production was observed in approximately 70% of PE-treated cells plated on laminin compared with 15% of the cells plated on gelatin. Fibronectin supported PE-stimulated sarcomeric reorganization and ANF production to a similar extent as laminin, whereas type I collagen was far less efficient in supporting these hypertrophic responses (Fig. 1B). Thus, these initial experiments provide support for the role of ECM-directed integrin signaling in PE-induced hypertrophy.

Since maximal PE-induced cardiomyocyte hypertrophy required ECM, we examined whether components of the integrin-signaling cascade were altered after PE stimulation. First we examined whether PE treatment altered the subcellular localization of focal adhesion proteins during the hypertrophic response. Fig. 2 shows immunostaining of actin stress fibers and the focal adhesion protein paxillin during the progression of PE-induced hypertrophy in cells plated on laminin. Fibronectin supported PE-stimulated sarcomeric reorganization and ANF production to a similar extent as laminin, whereas type I collagen was far less efficient in supporting these hypertrophic responses (Fig. 1B). Thus, these initial experiments provide support for the role of ECM-directed integrin signaling in PE-induced hypertrophy.
organization was virtually complete by 4 h. The recruitment of the focal adhesion protein paxillin to complexes at the periphery of cells appeared to be concomitant with the enhanced sarcomeric reorganization (Fig. 2B). Paxillin staining was generally dispersed in untreated cells, but after 1 h of PE treatment, staining appeared more focal in nature, localized to the periphery of cells. At 4 h of PE treatment paxillin appeared in distinct complexes at the termini of actin fibers. A similar staining pattern was observed with antibodies directed against another prominent focal adhesion protein, vinculin (data not shown). The PE-stimulated paxillin complexes observed in cardiomyocytes are reminiscent (albeit smaller than) of classical focal adhesions observed following plating of fibroblasts or epithelial cells on ECM proteins and suggest that PE treatment is giving rise to a rearrangement of the ECM adhesion structures.

Tyrosine phosphorylation is a critical covalent modification driving protein-protein interactions required for cytoskeletal reorganization and focal adhesion assembly (10). As shown in Fig. 2B, PE treatment of cultured cardiomyocytes induced the formation of phosphotyrosine-rich focal adhesion-like complexes at the periphery of cells similar to that observed for paxillin immunostaining. To determine the kinetics and extent of tyrosine phosphorylation of focal adhesion-associated proteins, FAK and CAS were immunoprecipitated from PE-treated cells and analyzed by Western blotting with anti-phosphotyrosine (PTyr) antibodies. As shown in Fig. 3, cells plated on fibronectin, but not gelatin, exhibited a PE-stimulated, time-dependent increase in tyrosine phosphorylation of FAK (Fig. 3A). Typically, an increase in FAK Tyr(P) is observed within 5–10 min after treatment with PE and is sustained for at least 30 min. A similar PE-induced tyrosine phosphorylation of the FAK-binding partners, CAS and paxillin, were also observed when cells were plated on fibronectin (Fig. 3, B and C) but not gelatin (data not shown). Importantly, as shown in Fig. 3D, PE-induced activation of the downstream signaling molecule ERK2 is also dependent on ECM interactions. Although variability is observed in the onset of ERK phosphorylation (ranging from 5 to 10 min) a consistent correlation is observed between the time course for PE-induced FAK and ERK phosphorylation within each experiment. These observations mirror data from numerous studies showing enhanced tyrosine phosphorylation of focal adhesion proteins after integrin ligation induced by plating cells on ECM proteins and are consistent with integrin signaling being a component of PE-induced hypertrophy.

Previous studies have shown that tyrosine phosphorylation is central to integrin-stimulated focal adhesion formation and downstream signaling. Therefore, we examined the requirement for tyrosine phosphorylation in the induction of PE-induced hypertrophy. Fig. 4 shows that the protein tyrosine kinase inhibitors genistein (100 μg/ml) or herbimycin A (875 nM) markedly inhibited PE-induced myofibrillar organization and expression of endogenous ANF in cells plated on ECM. However, these inhibitors did not alter viability or morphology of
vehicle-treated cardiomyocytes (data not shown). These studies corroborate a previous report indicating that induction of an ANF-reporter construct was attenuated by genistein (23). The requirement for tyrosine phosphorylation and the ability of PE to stimulate FAK tyrosine phosphorylation in cardiomyocytes plated on ECM suggested the possibility that FAK is a downstream mediator of PE-stimulated hypertrophy (12, 13). Since this dominant-negative strategy required a high percentage of FRNK-expressing cells in order to observe population changes in PE-induced signaling, we generated replication-defective adenovirus encoding GFP-tagged FRNK (GFRNK) or GFP alone as a control. GFP and GFRNK were efficiently expressed in 90–100% of the cardiomyocyte population as determined by immunofluorescence (data not shown). As shown in Fig. 5A, both GFP and GFRNK proteins are expressed in cardiomyocytes within 12 h after adenoviral infection. Importantly, infection of cardiomyocytes with GFP-FRNK but not GFP alone attenuates PE-stimulated FAK activation as assessed by immunoblotting with antibodies specific for FAK Tyr(P) at position 397 (Fig. 5B). These data indicate that infection of cardiomyocytes with GFRNK is an effective means of inhibiting FAK signaling in these cells.

To determine whether FAK activity was required for PE-stimulated hypertrophy, cardiomyocytes plated on fibronectin were infected with GFP or GFRNK virus prior to PE stimulation. Fig. 6 shows that PE stimulates actin rearrangement and ANF production in GFP-infected cardiomyocytes, indicating that adenovirus alone does not interfere with PE-stimulated hypertrophy and is not cytotoxic to these cells. However, infection of cardiomyocytes with GFRNK resulted in a near-complete inhibition of PE-stimulated hypertrophy as assessed by cell size, sarcomeric reorganization, and endogenous ANF staining (Fig. 6). Lack of PE-stimulated ANF production in GFRNK-infected cells compared with those infected with GFP or uninfected control cells was confirmed by quantitating the amount of ANF secretion in these cells using a peptide enzyme immunoassay. PE stimulated a 3.2–6.0-fold increase in ANF secretion over vehicle-treated uninfected control cells (n = 5; p < 0.05) and a 2.6–4.0-fold increase in GFP-infected cells (n = 5, p < 0.05). Basal ANF secretion was not significantly altered by infection with either GFP or GFRNK viruses (n = 5, p > 0.05). A similar inhibition of PE-stimulated hypertrophy was observed when cells were transfected (rather than infected) with a Myc-tagged FRNK cDNA construct and scored visually for organized actin structures or ANF production (data not shown). Taken together these data indicate that activation of FAK is critical for maximal PE-stimulated hypertrophy in isolated cardiomyocytes.
It is possible that the requirement for functional integrin signaling through FAK in PE-stimulated hypertrophy may be due to the ability of FAK to activate cooperatively an essential PE-stimulated pathway. One candidate PE-stimulated pathway involves signaling from Ras to ERK. Activation of ERK2 has been implicated in the regulation of hypertrophic gene expression, and as shown above, PE-stimulated ERK2 activity is dependent on ECM. As shown in Fig. 7, PE stimulated a time-dependent increase in ERK1/2 activity in GFP-infected cardiomyocytes. However, in cardiomyocytes infected with GFRNK, no increase in ERK1/2 activity was observed following PE treatment. These data indicate that PE-induced signaling to ERK is uncoupled by expression of FRNK and suggest that ERK activation may be a critical point of convergence between FAK and PE signaling.

DISCUSSION

We have shown that PE-induced hypertrophy in isolated neonatal cardiomyocytes requires ECM signaling through FAK. Specifically, plating cells on laminin or fibronectin supports maximal hypertrophic growth, whereas plating on gelatin or type I collagen does not. In addition, in cardiomyocytes plated on laminin or fibronectin, PE enhances the tyrosine phosphorylation of the focal adhesion proteins FAK and CAS and the subsequent formation of focal adhesion complexes. Furthermore, inhibition of signaling through FAK by ectopic expression of a dominant-interfering mutant attenuates PE-stimulated hypertrophy in isolated cardiomyocytes.

The observation that collagen type I is less efficient in supporting PE-induced hypertrophy is consistent with data which demonstrate that neonatal cardiomyocytes have a reduced affinity for type I collagen compared with other ECM such as laminin, fibronectin, and type III or IV collagen (6). Our data presented above are also consistent with a recent report showing that PE-mediated ANF production was observed in cardiomyocytes plated on laminin but not in cells plated on the non-adhesive substrate, bovine serum albumin (24). Others, however, have reported agonist-induced hypertrophic responses in cardiomyocytes plated on gelatin (25, 26). It is possible that the increased cardiomyocyte plating density and the increased duration of the agonist treatment in these latter experiments led to modifications in the ECM environment (e.g., the secretion of ECM proteins) by the myocytes during culture, allowing for the support of hypertrophic growth.

The studies described above point to a critical role for integrin signaling and specifically the activation of FAK in cardiomyocyte hypertrophy. Our work confirms and extends that
models (28–30). The signaling molecules downstream of the Gq-coupled receptor responsible for activation of hypertrophy are reasonably delineated and appear to involve signaling through the small molecular weight G protein, Ras. Importantly, the involvement of Gαq and Ras in the hypertrophic response has been shown not only in vitro but also in vivo in several elegant transgenic mouse models (28–30). The signaling molecules downstream of the Gαq-coupled receptor responsible for activation of hypertrophy are reasonably delineated and appear to involve signaling through the small molecular weight G protein Ras to the ERK, Jun N-terminal kinase, and p38 MAP kinase pathways, each of which has been implicated in hypertrophic signal transduction (32–34). In certain cell types, association of FAK with CAS is required for integrin-dependent ERK2 activation (11). Future experiments will address whether this association is required for PE-stimulated hypertrophy. Interestingly, deletion of CAS by homologous recombination results in embryonic lethality an event likely due in part to abnormal development of the heart (36). Histological examination of hearts from CAS-deficient embryos revealed a thin myocardial wall accompanied by disorganized myofibrils and disrupted Z-disks in the ventricular cardiomyocytes, indicating that CAS may be involved in cardiomyocyte growth and possibly hypertrophy (36).

An alternative explanation for the reduced extent of hypertrophy in the absence of FAK signaling is that the cardiomyocytes are undergoing apoptosis. There is a growing body of evidence indicating that the switch from hypertrophy to cardiac failure may involve an apoptotic pathway (34, 37, 38). For instance, aortic banding of mice with heart-restricted deletion of the gp130 gene results in dilated cardiomyopathy accompanied by cellular apoptosis, an event presumably due to lack of activation of a critical survival signaling pathway during the hypertrophic process (37). Therefore, it is possible that FAK (like gp130) may activate an essential survival pathway that is critical to observe PE-stimulated hypertrophy. Indeed, in other cell types, activation of FAK has been shown to be important for cell survival, and attenuating FAK signaling can lead to apoptosis (39). However, to date we have found no evidence of FRNK-induced apoptosis in cardiomyocytes in our experiments as assessed by DNA content using flow cytometry.²

We have shown that PE signaling in cardiomyocytes activates FAK and that activation of FAK is required for PE-stimulated hypertrophy of cultured cells. Since angiotensin II, prostaglandin growth factor 2α, and hypo-osmotic stress also enhance tyrosine phosphorylation of FAK in myocytes, it is

² J. M. Taylor, J. D. Rovin, and J. T. Parsons, unpublished observations.
possible that activation of FAK may be a common requirement for a variety of hypertrophy-inducing agents (40–42). The idea that FAK activation may play a role in the development of hypertrophy in vivo is supported by a recent report that demonstrates enhanced cytoskeletal association of β3 integrin, FAK, and Src in pressure-overloaded hypertrophying hearts (43). Taken together, these data support a role for integrin-mediated signaling through FAK in the development of cardiac hypertrophy. Future studies will determine the utility of targeting FAK signaling through FAK in the development of cardiac hypertrophy.

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