Nuclear compartmentalization of FAK and FRNK in cardiac myocytes

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Am J Physiol Heart Circ Physiol 290:H2509–H2515, 2006. First published December 22, 2005; doi:10.1152/ajpheart.00659.2005.—Focal adhesion kinase (FAK) and FAK-related non-kinase (FRNK) accumulate in the nucleus of cardiac myocytes during hypertensive hypertrophy. Nuclear FAK and FRNK are phosphorylated on different serines and form distinct bright spots. The subnuclear distribution of serine-phosphorylated FAK and FRNK was examined in this study by double labeling with fibrillarin, a component of nucleoli, and Sam68, a constituent of Sam68 nuclear bodies. We also investigated the role of protein kinase C (PKC)–mediated phosphorylation of FAK and FRNK on nuclear translocation. PKC activation by 12-O-tetradecanoylphorbol 13-acetate treatment increased serine phosphorylation of FAK and FRNK. Specifically, FAK was phosphorylated on serine 722 but not serine 910. On the other hand, FRNK was phosphorylated on serine 217, the equivalent site of FAK serine 910, but not serine 30, the homologous site of FAK serine 722. Serine-phosphorylated FAK and FRNK redistributed into the nucleus and formed distinct patterns. FAK with phosphorylation on serine 722 colocalized with Sam68 but not fibrillarin. On the contrary, FRNK phosphorylated on 217 coexisted with fibrillarin but not Sam68. Immunoprecipitation also confirmed that FAK associated with Sam68 and FRNK interacted with fibrillarin, respectively. These results suggest that FAK and FRNK target different nuclear subdomains by their association with distinct nuclear proteins.

focal adhesion kinase; focal adhesion kinase-related non-kinase; cardiac hypertrophy; hypertension; protein kinase C; nucleus

FOCAL ADHESION KINASE (FAK) is a key signaling molecule in integrin-mediated signal transduction (4). It is a structurally distinct kinase with a central catalytic domain between similarly sized NH2-terminal and COOH-terminal noncatalytic domains. In certain cell types, the COOH-terminal domain of FAK is expressed as a separate protein called FAK-related non-kinase (FRNK) with a molecular mass of approximately 41–43 kDa. Once activated, FAK undergoes phosphorylation on multiple tyrosines to cause conformational changes and create binding sites for other kinases, phosphatases, and cytoskeletal proteins (4, 19). Through multiple cascades of protein phosphorylation and interaction, signals are relayed to downstream targets.

FAK is activated by tyrosine phosphorylation during cardiac hypertrophy after acute pressure overload (6, 10). Our previous study also demonstrated that FAK was involved in mechanical signal transduction during cardiac remodeling in chronic hypertension (22). FAK was phosphorylated on tyrosine 397 and redistributed to the membrane cytoskeleton and intercalated disks in cardiac myocytes from 6-mo-old spontaneously hypertensive heart failure (SHHF) rats. FAK and FRNK were also phosphorylated on different serine residues, leading to their accumulation in the nucleus of hypertrophic cardiac myocytes with distinct distribution patterns (22). The nucleus is organized into many subdomains, such as Cajal bodies, Sam68 nuclear bodies, and nucleoli (15). These distinct nuclear compartments control RNA transcription, processing, and exporting. The morphology and distribution of nuclear FAK and FRNK are similar to Sam68 nuclear bodies and nucleoli, indicating their potential interaction and colocalization. The possible targeting of nuclear FAK and FRNK to Sam68 nuclear bodies and nucleoli suggests a role in hypertrophic gene expression during cardiac hypertrophy and failure.

Potential protein kinases involved in serine phosphorylation of FAK and FRNK remain to be defined. Protein kinase C (PKC), a family of serine/threonine protein kinases, plays a critical role in signal transduction of a variety of extracellular stimuli, and a large number of proteins have been shown to be phosphorylated by PKC in vivo and in vitro (16). The activation of PKC is identified in early pressure-overloaded right ventricle (11). More importantly, PKC is also activated in the heart of SHHF rats (8). The consensus phosphorylation sites for PKC are present in both FAK and FRNK. Thus both FAK and FRNK are potential substrates for PKC family kinases.

In the present study, we investigated the subnuclear localization of FAK and FRNK in cardiac myocytes from SHHF rats. We also examined the role of PKC activation by 12-O-tetradecanoylphorbol 13-acetate (TPA) treatment on serine phosphorylation and subnuclear localization of FAK as well as FRNK in cardiac myocytes from normotensive rats. Our results indicate that FAK with serine 722 phosphorylation associates and colocalizes with Sam68. On the other hand, FRNK phosphorylated on serine 217 binds to and coexists with fibrillarin. Furthermore, serine phosphorylation of FAK and FRNK by PKC activation leads to their nuclear accumulation in cardiac myocytes from normotensive rats.

MATERIALS AND METHODS

Animals. A total of 20 6-mo-old lean female SHHF and age-matched Wistar-Kyoto (WKY) rats was acquired from Charles River...
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(Wilmington, MA). Five animals from each group were randomly assigned for myocyte isolation or collection of intact tissue from the left ventricle (LV). Additional 3- to 4-month-old female WKY rats (n = 26) from Charles River were used for PKC activation by TPA treatment. Animals were equally divided into treatment or control group. Eight hearts from treatment or control group, respectively, were collected for immunoprecipitation and Western blot. The remaining five rats from each group were used for cell isolation. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (US Department of Health and Human Services, NIH Publication No. 85-23) and approved by the University of South Dakota Animal Care and Use Committee.

Antibodies. Rabbit polyclonal antibodies against Sam68, NH2 terminus (A-17), and COOH terminus (C-20) of FAK and their blocking peptide were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). A mouse monoclonal antibody against Sam68 and its blocking peptide were also obtained from Santa Cruz Biotechnology. Phosphorylation site-specific antibodies against FAK phosphoserine on 722 (2FAK-pSer722), FRNK phosphoserine on 217 (FRNK-pSer217 or FAK-pSer301), and their blocking peptides were acquired from Biosource International (Camarillo, CA). Serine 910 of FAK corresponds to serine 217 of FRNK, while serine 722 of FAK is the equivalent site of serine 30 in FRNK. Affinity-purified rabbit polyclonal antibodies against phosphoserine substrates modified by PKC or protein kinase A (PKA) were obtained from Cell Signaling Technology (Beverly, MA). Anti-fibrillarin monoclonal antibody was ordered from Cytoskeleton (Denver, CO). Alexa Fluor 488- or 568-conjugated goat anti-rabbit IgG or goat anti-mouse IgG antibodies for immunofluorescent labeling were obtained from Molecular Probes (Eugene, OR). Horseradish peroxidase-linked donkey anti-rabbit IgG antibody and goat anti-mouse IgG antibody for Western blots were from Amersham Pharmacia Biotech (Piscataway, NJ).

TPA stimulation of perfused intact hearts. Three- to four-month-old female WKY rats were intraperitoneally injected with heparin (50 U/100 g body wt) 30 min before they were anesthetized with isoflurane. The aorta was cannulated and retrogradely perfused with 1% Triton X-100, pH 7.4 containing phosphatase inhibitors (0.1 mM Na2VO4, 30 mM NaF, and 50 mM NaF) and proteinase inhibitors (10 mg/ml PMSF and 1 μg/ml aprotinin). The homogenates were centrifuged at 15,000 g for 15 min, and the supernatant was quantified with BCA protein assay (Pierce). An equal amount of total protein was loaded to each lane and separated by Laemmli SDS-PAGE. Western blots were quantified as described with actin as an internal control to ensure equal loading.

Myocyte isolation, immunofluorescent labeling, and confocal microscopy. Cardiac myocytes were isolated through retrograde perfusion of 0.1% collagenase as described previously (12, 21). The aorta was cannulated for retrograde perfusion with oxygenated calcium-free Joklik’s media (Sigma, St. Louis, MO) containing 0.1% collagenase at 37°C for 8–10 min. The LV was dissected free and minced in calcium-free Joklik’s media. The individual cardiac myocytes were filtered through a nylon mesh (250 μm) into 8% paraformaldehyde solution and fixed at a final concentration of 4% for 10 min.

Labeled cardiac myocytes were resuspended in PBS and aliquoted onto positively charged slides. The membrane of cardiac myocytes was permeated with 0.5% Triton X-100 for 30 min at room temperature. A primary antibody was added for overnight incubation at 4°C after incubation with 1% bovine serum albumin to block nonspecific binding. After the primary antibody was removed and washed with PBS, the myocytes were incubated with the fluorescent conjugated secondary antibody for 1 h at room temperature. Incubation with a second set of antibodies was performed for double labeling. Propidium iodide was used to stain the nucleus. The slides were mounted in glycerol and sealed with nail polish for observation using an Olympus Fluoview 300 Confocal Laser Scanning Microscope System (Olympus America, Melville, NY). Blocking peptides were used to test the specificity of antibodies against Sam68, FAK, phosphorylated FAK, and FRNK. The antibody solution was incubated with specific blocking peptides for 1 h before the antibody was used for immunolabeling. Fluorescent labeling of cardiac myocytes with neutralized primary antibody was negative throughout. For fibrillarin, negative controls were incubated by the omission of the primary antibody or the use of nonspecific mouse serum under the same conditions.

Statistics. Data are expressed as means ± SE. A paired two-sample t-test was performed to compare the density of Western blots between TPA-treated and control groups. A P value <0.05 was regarded as statistically significant.

RESULTS

Subnuclear localization of FAK and FRNK in cardiac myocytes. Our previous study showed that FAK formed a weak striated pattern in cardiac myocytes of both SHHF and WKY rats (22). FAK and FRNK redistributed into the nucleus of cardiac myocytes in SHHF but not WKY rats. Nuclear FAK and FRNK in SHHF rats were phosphorylated on serine 722 and 217, respectively. Consistent with our previous report, serine-phosphorylated FAK and FRNK in the nucleus demonstrated several distinct bright dots when labeled with phosphorylation-specific antibodies against serine-phosphorylated FAK on 722 and FRNK on 217 in addition to diffuse nuclear staining (Fig. 1). Fibrillarin and Sam68 are nuclear proteins forming nucleoli and Sam68 nuclear bodies (15). To determine subnuclear localization of phosphorylated FAK and FRNK and their possible colocalization with Sam68 and fibrillarin, double immunolabeling was performed. The bright spot of FAK phosphorylated on serine 722 (Fig. 1, A–F) had a distinct pattern different from the nuclear dot of FRNK with phosphorylation on 217 (Fig. 1, G–L). Serine phosphorylation of FAK on 722 colocalized with Sam68 (Fig. 1C) but not fibrillarin (Fig. 1F).

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On the other hand, FRNK phosphorylation on 217 co-distributed with fibrillarin (Fig. 1) but not Sam68 (Fig. 1).

Protein substrates phosphorylated by PKC were increased in the LV of SHHF rats and WKY rats treated with TPA. FAK phosphorylation on serine 722 and FRNK on serine 217 were increased in cardiac myocytes of SHHF rats (22). To identify possible kinases involved in the serine phosphorylation of FAK and FRNK, we employed phosphorylation-specific antibodies against substrates of a specific kinase. The antibody specific to a particular kinase substrate is a useful means to determine kinase activity and identify new potential kinases acting on substrates. Phosphoserine/threonine PKC or PKA substrate antibodies can recognize phosphorylated serine and/or threonine within the context of protein motifs that are phosphorylated by PKC or PKA (16).

It has been shown in a previous report that FAK and FRNK could be phosphorylated by PKA (18). We tried to identify PKA substrates in the LV of SHHF rats with an antibody specific to proteins phosphorylated by PKA. Only weak background signal was obtained, and no difference was observed between SHHF and WKY rats. On the basis of the published sequence, FAK also has consensus phosphorylation sites for PKC. As demonstrated by Western blots using the antibody specific to proteins phosphorylated by PKC, several proteins were phosphorylated by PKC with migration at 125, 97 to 116, 66, and 41 to 43 kDa in the LV from both SHHF and WKY rats (Fig. 2A). Apparently, signals at 125 and 41/43 kDa were increased in the LV of SHHF rats (Fig. 2A), indicating that FAK and FRNK are possible PKC substrates.

To test the hypothesis that PKC may phosphorylate serines on FAK and FRNK, we perfused intact hearts from normotensive WKY rats with or without TPA, a PKC activator. Several proteins ranging from 41 to 125 kDa were increasingly phosphorylated by TPA treatment as revealed by Western blots with the antibody specific to proteins phosphorylated by PKC (Fig. 2B). Similar to results obtained from the heart of SHHF rats (Fig. 2A), PKC substrate phosphorylation was increased at 125 and 41/43 kDa (Fig. 2B) after TPA treatment.

TPA promoted serine phosphorylation of FAK and FRNK in vitro. Serines 722 and 910 of FAK (serines 30 and 217 of FRNK) are two major sites of serine phosphorylation in the COOH terminus of FAK in interphase cells (14) and hypertrophic myocardial cells (22). FAK-pSer722 antibody recognizes...
specific phosphoserine 722 on FAK and equivalent phosphoserine 30 on FRNK. Similarly, FRNK-pSer217 detects phosphoserine 217 on FRNK and homologous phosphoserine 910 on FAK. The specificity of FAK-pSer722 and FRNK-pSer217 was confirmed by mutating these serine sites (14). In the rat heart, however, FAK-pSer722 specifically reacted with FAK but not FRNK, indicating only FAK was phosphorylated on serine 722 (Fig. 3 A). On the other hand, FRNK-pSer217 detected a single band around 41/43 kDa, suggesting only FRNK was phosphorylated on this serine (Fig. 3 A). Using an antibody against COOH terminus (FAK-C20) shared by both FAK and FRNK, we detected two bands representing FAK and FRNK (Fig. 3 A) in the rat heart. Total content of FAK and FRNK showed no change in the heart of WKY rats before and after TPA treatment. FAK phosphorylation on serine 722 was increased in cardiac myocytes after TPA treatment (Fig. 3, A and B). Similarly FRNK phosphorylated on serine 217 was elevated after PKC activation (Fig. 3, A and B).

**TPA stimulation promoted nuclear relocation of FAK in vitro.** Cardiac myocytes treated with and without TPA were immunofluorescently labeled with FAK (A-17), FAK-pSer722, and FRNK-pSer217 antibodies, respectively. As demonstrated previously (22) and above by Western blots, only FAK was phosphorylated on serine 722 and detected by FAK-pSer722 antibody. On the other hand, FRNK was phosphorylated on serine 217 and reacted with FRNK-pSer217. In contrast to weak nuclear fluorescence without TPA treatment (Fig. 4 A), bright fluorescence of FAK was observed in the nucleus of cardiac myocytes treated with TPA (Fig. 4B). Similarly, strong labeling of FAK-pSer722 and FRNK-pSer217 was present in

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**Fig. 3.** Representative Western blots with FAK-pSer722, FRNK-p217, and FAK C-20 antibodies. **A:** FAK-pSer722 (left) detected FAK at 125 kDa but not FRNK at 41/43 kDa. On the other hand, FRNK-pSer217 (middle) reacted with FRNK at 41/43 kDa but not FAK at 125 kDa. FAK C-20 Western blots (right) demonstrated both FAK and FRNK. FAK phosphorylation on serine 722 and FRNK on serine 217 increased significantly in the LV after TPA treatment. **B:** densitometry analysis also demonstrated a significant increase in FAK-pSer722 content in TPA-treated WKY rats compared with untreated controls (CTL: 120.0 ± 10.9 vs. 21.6 ± 2.7; P < 0.001 and n = 8). Similarly, a significant increase in FRNK-pSer217 content was present in TPA-treated WKY rats compared with untreated control (105.8 ± 3.9 vs. 30.7 ± 1.6; P < 0.001 and n = 8). Total FAK (107.9 ± 4.8 vs. 112.4 ± 4.3; P = 0.50 and n = 8) and FRNK (53.3 ± 2.3 vs. 59.4 ± 2.3; P = 0.087 and n = 8), however, showed no change between control and treated groups. CTL, no TPA treatment. *P < 0.05 between TPA-treated and control rats.

**Fig. 4.** Representative confocal images of cardiac myocytes isolated from 5 WKY rats with (B, D, F) or without TPA treatment (A, C, E) and labeled with FAK-A-17 (A, B), FAK-pSer722 (C, D), and FRNK-pSer217 (E, F). A weak diffuse green fluorescence of FAK distributed in the cytoplasm of myocytes from both control (A) and TPA-treated groups (B), but strong green fluorescence of FAK accumulated in the nucleus of myocytes treated with TPA (B) in contrast to weak fluorescence in the nucleus of control myocytes (A). Unlike weak fluorescence in the nucleus of control myocytes (C), FAK-pSer722 labeling showed that evidently bright green fluorescence accumulated in the nucleus of myocytes treated with TPA (D). Weak fluorescence of FAK-pSer722 also distributed diffusely in the cytoplasm of myocytes from both controls (C) and TPA-treated groups (D). FRNK-pSer217 labeling revealed that weak signal distributed diffusely in the cytoplasm of myocytes from both controls (E) and TPA-treated groups (F), but evidently bright green fluorescence accumulated in the nucleus of myocyte from TPA-treated group (F) compared with weak fluorescence in the nucleus of myocyte from controls (E). Scale bar, 50 μm.
the nucleus of TPA-treated myocytes (Fig. 4, D and F) compared with the weak signal of untreated controls (Fig. 4, C and E). These results suggest that FAK and FRNK redistributed to the nucleus after TPA treatment. The nuclear distributional pattern of phosphorylated FAK and FRNK after TPA treatment was identical to that seen in cardiac myocytes from SHHF rats (Fig. 1).

Interaction of FAK phosphorylated on 722 with Sam68. Our double-immunolabeling study revealed that FAK phosphorylated on 722 colocalizes with Sam68 but not fibrillarin (Fig. 1). To confirm the physical association of FAK with Sam68, we performed immunoprecipitation and Western blot with FAK and Sam68 antibodies. Sam68 immunoprecipitation probed with C-20 revealed that Sam68 associated with FAK at 125 kDa but not with FRNK at 41/43 kDa (Fig. 5A). In addition, immunoprecipitation with an antibody against NH2 terminus (A-17) of FAK pulled down Sam68 but not fibrillarin (Fig. 5B). Both Sam68 and fibrillarin (Fig. 5C) were detected after immunoprecipitation by using an antibody against FAK COOH terminus (C-20), which is shared by both FAK and FRNK. The association of Sam68 with FAK increased significantly after TPA treatment (Fig. 5, A–C). Furthermore, Western blot with phosphorylation-specific antibodies after Sam68 immunoprecipitation confirmed that Sam68 associated with FAK phosphorylated on serine 722 but not FRNK phosphorylated on serine 217 (Fig. 5D). The interaction of FAK-pSer722 with Sam68 was dramatically increased in TPA-treated hearts compared with untreated controls (Fig. 5D).

Fig. 5. Representative Western blots (WB) after immunoprecipitation (IP) demonstrating association of Sam68 and fibrillarin with FAK and FRNK, respectively. A: immunoprecipitation with Sam68 antibody pulled down similar amounts of Sam68 in rat hearts with or without TPA treatment. Western blots with FAK A-17 and C-20 revealed that Sam68 associated with FAK at 125 kDa but not with FRNK at 41/43 kDa. The association of Sam68 with FAK increased significantly after TPA treatment. B: immunoprecipitation with FAK A-17 showed that FAK interacted with Sam68 but not fibrillarin. TPA treatment dramatically increased their interaction. C: immunoprecipitation with FAK C-20, which binds to both FAK and FRNK, pulled down both Sam68 and fibrillarin. Their interaction was obviously increased in TPA-treated hearts compared with controls. D: Western blots with FAK-pSer722 and FRNK-pSer217 after immunoprecipitation with Sam68 demonstrated that Sam68 associated with FAK phosphorylated on serine 722 but not FRNK phosphorylated on serine 217. FAK-pSer722 association with Sam68 was dramatically increased in TPA-treated compared with untreated hearts. E: similar amount of fibrillarin was immunoprecipitated with fibrillarin antibody in rat hearts with or without TPA treatment. Western blots with FAK A-17 and C-20 antibodies revealed that fibrillarin associated with FRNK at 41/43 kDa but not FAK at 125 kDa. Their association was dramatically increased by TPA-treated compared with control. F: Western blots with FAK-pSer722, and FRNK-pSer217 after immunoprecipitation with fibrillarin antibody confirmed that fibrillarin associated with FRNK phosphorylated on serine 217 but not FAK with serine 722 phosphorylation. More FRNK-pSer217 was pulled down by fibrillarin antibody in TPA-treated hearts compared with controls. Quantification of above Western blots by densitometry is presented as an online supplement available at http://ajpheart.physiology.org/cgi/content/full/00659.2005/DC1. CTL, TPA-untreated control; TPA, TPA treatment.

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** Interaction of FRNK phosphorylated on 217 with fibrillarin. **

FRNK phosphorylated on serine 217 overlapped with fibrillarin but not Sam68 (Fig. 1). Only FAK COOH-terminal antibody, which reacts with both FAK and FRNK, but not FAK NH2-terminal antibody, precipitated fibrillarin (Fig. 5, C and B). In addition, fibrillarin immunoprecipitation probed with C-20 also confirmed that fibrillarin associated with FRNK at 41/43 kDa but not FAK at 125 kDa (Fig. 5E). Their association was dramatically increased by TPA-treated hearts compared with controls (Fig. 5E). Western blots with FAK-pSer722 and FRNK-pSer217 after immunoprecipitation with fibrillarin antibody further showed that fibrillarin associated with FRNK phosphorylated on serine 217 but not FAK showing serine 722 phosphorylation (Fig. 5F). Increased amounts of FRNK-pSer217 were pulled down by fibrillarin antibody in TPA-treated rats compared with controls without treatment (Fig. 5F).

** DISCUSSION **

In the present and our previous studies (22), we have clearly demonstrated the presence of FAK and FRNK in the nucleus of cardiac myocytes. Through in vitro stimulation with a PKC activator TPA, we further revealed that the nuclear accumulation of FAK and FRNK in cardiac myocytes was increased after PKC activation by TPA treatment. Additionally, phosphorylation of FAK on serine 722 and FRNK on serine 217 corresponding to FAK serine 910 was significantly increased after TPA treatment. As in cardiac myocytes of SHHF rats, serine-phosphorylated FAK and FRNK accumulated in the nucleus after TPA treatment. Furthermore, we showed that phosphorylated FAK and FRNK localized to specific nuclear regions. Fonseca et al. (5) have shown recently that FAK translocates to the nucleus immediately after acute pressure overload. In COS-7 and NIH-3T3 cells, FAK also accumulates in the nucleus after sumoylation (9). On the basis of these findings, there seems no doubt that FAK is present in the nucleus. The role of FAK in focal adhesion formation and cytoskeletal remodeling has been intensively investigated, but its function in the nucleus remains to be investigated.

During cell adhesion-mediated signal transduction, multiple cascades of protein phosphorylation result in activation of transcriptional machinery in the nucleus (4, 19). Recent progress has revealed that many cell adhesion-associated proteins shuttle into the nucleus, which indicates that more direct communication and interaction exists between cell adhesion and the nucleus (2). Actin and its binding proteins are enriched in the nucleus (3). In addition, focal adhesion-associated proteins zyxin and paxillin shuttle between the focal adhesion and the nucleus. Furthermore, cell-cell adhesion-associated proteins catenins and ZO-1 also target to the nucleus (2). These proteins often contain no traditional nuclear localization sequences (NLSs). How they get into the nucleus is currently unclear. It is assumed that they translocate into the nucleus with other proteins containing NLSs (19). Once in the nucleus, they are involved in the regulation of chromatin structure, RNA transcription, processing, and exporting.

Nuclear proteins are compartmentalized into distinct subdomains involved in RNA transcription and processing. Many nuclear substructures, such as Cajal bodies, Sam68 nuclear bodies, and nucleoli, have been discovered (15). Sam68 family proteins contain SH2/SH3 binding domains acting as nuclear adaptor proteins. They bind to both RNA and signaling molecules such as Src family kinases, Grb2, and phospholipase Cγ-1, assembling nuclear signaling complexes similar to other adaptor proteins in the focal adhesion (17). Here we have clearly revealed serine-phosphorylated FAK associates and colocalizes with Sam68. On the other hand, serine-phosphorylated FRNK binds to and coexists with fibrillarin, a major protein of the nucleolus. The association of different nuclear proteins with FAK and FRNK may help to shuttle them into the nucleus and also point out that they might have distinct nuclear functions.

FAK activation involves multiple tyrosine phosphorylations. FAK and FRNK are also phosphorylated on multiple serines (14). Phosphorylation of serine 722 affects its binding to the SH3 domain of the adapter protein p130Cas, modulating signal transduction at the focal adhesion (20). Serine phosphorylation of FAK and FRNK may release them from the focal adhesion and create new protein binding sites for their nuclear partners. Four major serine phosphorylation sites are present on the COOH terminus of FAK (14). Serine 148 of FRNK (serine 842 on FAK) is phosphorylated by PKA while serine 151 (serine 845 on FAK) is phosphorylated by casein kinase after PKA-mediated phosphorylation of serine 148. Serine 30 and serine 217 of FRNK (serine 722 and serine 910 of FAK) are two other major sites of serine phosphorylation in the COOH terminus of FAK in interphase cells and hypertrophic myocardial cells (14, 22). The kinases involved in these two serine phosphorylations are not clear. Both PKC and PKA can phosphorylate serines (16). PKA prefers serine preceded by a lysine or an arginine. On the other hand, PKC favors serine followed by a lysine or an arginine. Serine 722 of FAK is followed by an arginine and could be potentially phosphorylated by PKC. Serine 910 has arginines on both sides and could be phosphorylated by either PKA or PKC.

PKC family members of protein serine/threonine kinases play crucial roles in a variety of intracellular signaling (13, 16). A large number of proteins, such as G protein-coupled receptor kinases, growth factor receptors, transcription factors, and translation factors, have been shown to be phosphorylated by PKC both in vivo and in vitro (7, 16, 21). PKC has been shown to be activated in pressure-overloaded myocardium (11). More importantly, FAK can be activated by TPA treatment (13). In SHHF rats, PKC isoforms are activated in the hypertrophic myocardium (8). In the present study we have revealed that protein substrates phosphorylated by PKC, but not PKA, are elevated in the hearts of SHHF rats. Furthermore, PKC activation by TPA treatment in the hearts of normotensive rats increases FAK and FRNK serine phosphorylation, specifically on FAK serine 722 and FRNK serine 217. These changes of FAK and FRNK in TPA-treated cardiac myocytes in vitro resemble those demonstrated by hypertrophic cardiac myocytes of SHHF rats. Taken together, our data suggest that PKC is activated in the LV of SHHF rats, resulting in serine phosphorylation and nuclear translocation of FAK and FRNK.

In summary, we have demonstrated that FAK and FRNK were differentially phosphorylated on serines in the hearts of both SHHF and TPA-treated WKY rats. Serine-phosphorylated FAK and FRNK associated with Sam68 and fibrillarin, respectively, and targeted distinct nuclear subdomains. Further studies with FAK and FRNK mutated in these serine sites will
confirm the role of serine phosphorylation in the nuclear translocation and compartmentalization of FAK and FRNK.

REFERENCES


