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Regulation of Cytoskeleton Organization and Paxillin Dephosphorylation by cAMP

STUDIES ON MURINE Y1 ADRENAL CELLS*

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Jing-Dong Han and Charles S. Rubin‡

From the Department of Molecular Pharmacology, Atran Laboratories, Albert Einstein College of Medicine, Bronx, New York 10461

Cyclic AMP induces corticosteroid production, differential gene transcription, and cell cycle arrest in adrenal cortex-derived Y1 cells. These responses follow a cAMP-controlled transformation in Y1 cell morphology: the conversion of flat epithelial cells into rounded, highly refractile cells with short processes. Little is known about effector proteins and mechanisms that link activated protein kinase A to the alteration in cell shape. We now report that cAMP causes rapid (1 min) and selective tyrosine dephosphorylation of paxillin, a focal adhesion protein. Paxillin is maximally dephosphorylated before other physiological effects of cAMP are detected in Y1 cells. Dephosphopaxillin translocates from focal adhesions to the cytoplasm as stress fibers vanish and F-actin accumulates in membrane ruffles and cytoplasmic aggregates. Remnants of focal adhesion complexes dissociate from the cell cortex and coalesce into large structures that contain aggregated F-actin. Pervanadate, an inhibitor of protein-tyrosine phosphatases, abrogates all effects of cAMP. Conversely, genistein-sensitive protein-tyrosine kinase activity is essential for establishing epithelial morphology and reversing effects of cAMP in Y1 cells. Thus, cAMP/protein kinase A (PKA) actions are initially targeted to focal adhesions and cortical actin cytoskeleton; paxillin is an early and unexpected downstream target in a PKA-mediated signaling pathway, and protein-tyrosine phosphatase activity provides an essential link between PKA activation and the control of cell shape.

The second messenger cAMP and its effector, protein kinase A (PKA),[1] mediate the regulation of many processes including glycogenolysis, ion transport, gene transcription, and cell proliferation and differentiation (1–4). Increased intracellular levels of cAMP also cause a reorganization of the actin cytoskeleton in REF52 fibroblasts (5) and a marked alteration in the shape of epithelial cells (6). Hormone-induced changes in cell shape often precede alterations in cell physiology and/or differentiation. An example is provided by murine Y1 cells. The cells have a flat epithelioid morphology and contain receptors for adrenocorticotropic hormone (ACTH) that activate adenylate cyclase (7). Increases in cAMP content elicit a rapid morphological transition that yields rounded refractile cells with short processes (6–8). This cell shape transition is completed before other responses to cAMP are detected. Slower responses are: synthesis and secretion of corticosteroids, induction of cytochrome P-450 steroid hydroxylase genes, and withdrawal of Y1 cells from the cell cycle (7–10). The cited responses to cAMP produce a phenotype in Y1 cells that mimics normal secretory cells of the zona fasciculata of the adrenal cortex. Furthermore, the change in shape of Y1 cells appears to be required for the subsequent appearance of this differentiated phenotype (11).

The abrupt rounding of ACTH-treated Y1 cells apparently reflects a reorganization of the actin cytoskeleton (7, 8, 11). Although this morphological transition facilitates subsequent cAMP signaling in Y1 cells, little is known about effector proteins and mechanisms that are involved in coupling activation of PKA to the control of cell shape. Likewise, the possibility that early downstream mediators of PKA action accumulate at specific loci within the Y1 cytoskeleton has not been investigated. Thus, PKA-regulated effector proteins may be anchored and enriched at sites along lengthy microfilaments (stress fibers) or concentrated in the F-actin-enriched cell cortex, which is contiguous with the inner surface of the plasma membrane. To begin an analysis of these topics we addressed several basic questions: Is the cortical actin cytoskeleton a proximal site of cAMP/PKA action? If so, are focal adhesion protein complexes (which anchor actin filaments in the cell cortex) early downstream targets in the cAMP signaling pathway? Are the effects of cAMP on cell shape solely the result of PKA-catalyzed Ser/Thr phosphorylation of effector proteins, or are other signaling pathways involved?

We demonstrate that cAMP causes the rapid tyrosine dephosphorylation of the focal adhesion protein paxillin in Y1 cells. Subsequently, paxillin translocates to the cytoplasm, stress fibers vanish, and F-actin accumulates in cytoplasmic aggregates and membrane ruffles. Remnants of multiple focal adhesions coalesce into large complexes that include aggregated F-actin. Pervanadate inhibits these processes, whereas genistein prevents their reversal. Thus, protein-tyrosine phosphatase activity couples activated PKA to regulation of actin cytoskeleton organization in Y1 cells.

EXPERIMENTAL PROCEDURES

Cell Culture—Murine Y1 cells were cloned from an explanted adrenal cortex tumor (6). Y1 cells were grown in Ham's F-10 medium containing 12.5% fetal bovine serum in an atmosphere of 7.5% CO_2, 92.5% air (37 °C). Fed, firmly adherent, 50–75% confluent Y1 cells were used as a standardized system for investigating the effects of cAMP on

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‡ To whom correspondence should be addressed: Dept. of Molecular Pharmacology, F-229, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461. Tel.: 718-430-2505; Fax: 718-430-8922; E-mail: rubin@aecom.yu.edu.

[1] The abbreviations used are: PKA, protein kinase A; ACTH, adrenocorticotropic hormone; CPT-cAMP, 8-(4-chlorophenylthio)-cAMP; FITC, fluorescein isothiocyanate; FAK125, focal adhesion kinase of M_r 125,000; PY, phosphotyrosine; PTP, protein-tyrosine phosphatase; PBS, phosphate-buffered saline; PIPES, 1,4-piperazinediethanesulfonic acid.

the organization of a pre-existing actin cytoskeleton and its associated focal adhesion complexes.

Preparation of Cell Extracts and Western Immunoblot Analysis—Y1 cells were washed twice with 10 mM sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl (PBS). Next, cells were lysed at 0 °C by the addition of 0.5 ml of PEMS buffer (35 mM PIPES, pH 7.1, 5 mM EGTA, 5 mM $MgCl_2$, 0.2 M sucrose, 0.5% (v/v) Triton X-100, 2 mM Na_3VO_4, 0.1 M NaF, 5 μg/ml aprotinin, 5 μg/ml leupeptin, and 5 μg/ml pepstatin) per 10-cm culture dish. After scraping, lysates were vortexed for 15 s and incubated on ice for 5 min. Samples of total lysate protein (0.1 mg) were mixed with SDS loading buffer (12) and heated for 10 min at 95 °C. Proteins were size-fractionated by SDS-polyacrylamide gel electrophoresis (9% gel) and transferred to an Immobilon P membrane (Millipore Corp.) as described previously (12). Membranes were probed with anti-phosphotyrosine (anti-PY) IgG (1:1000, Transduction Laboratories) or anti-paxillin IgG (1:10,000, Transduction Laboratories) as described previously (13). Antigen-antibody complexes were visualized on x-ray film by enhanced chemiluminescence methodology (Amersham Corp.) (13).

Immunoprecipitation Analysis—Cell lysates were centrifuged at 12,000 × *g* for 10 min, and supernatant fractions were collected. Solubilized proteins (0.4 mg) were incubated with either 1.5 μg of anti-paxillin IgG or 3 μg of anti-PY IgG that was immobilized on protein A-Sepharose 4B beads (Zymed). After 1 h at 0 °C beads were harvested by centrifugation at 7,000 × *g* for 2 min. Protein A-Sepharose containing IgG-antigen complexes was washed five times with 0.3 ml of 50 mM HEPES buffer, pH 7.4, containing 0.15 M NaCl, 2 mM Na_3VO_4, 50 mM NaF, 1 mM dithiothreitol and 1% Triton X-100. Proteins were released from the beads by the addition of 60 μl of SDS loading buffer and incubation at 95 °C for 10 min. Immunoprecipitated and nonprecipitated (supernatant) proteins were fractionated by denaturing electrophoresis and transferred to an Immobilon P membrane. Replicate blots were probed with anti-paxillin or anti-PY IgGs and developed as indicated above.

Immunofluorescence Analysis—Y1 cells were grown to ~60% confluency on glass coverslips. Cells were fixed (4% paraformaldehyde) permeabilized (0.2% Triton X-100) and blocked (3% albumin) in PBS. Next, cells were incubated sequentially with anti-PY IgG (1:50 dilution), and 10 μg/ml fluorescein isothiocyanate (FITC) conjugated goat IgGs directed against mouse immunoglobulins. Cells were washed with PBS/0.2% Triton X-100 between incubations. Cells were co-stained for F-actin by using 0.6 μM rhodamine-conjugated phalloidin (Sigma). A second batch of Y1 cells was incubated with anti-paxillin IgG (1:500) and FITC-conjugated secondary antibodies and then co-stained with rhodamine phalloidin. Y1 cells were co-stained for paxillin and PY-containing proteins by using the primary monoclonal antibodies described above in combination with either rhodamine-conjugated secondary antibodies directed against mouse IgG_1 (the anti-paxillin IgG isotype) or FITC-tagged secondary antibodies that bind mouse IgG_{2b} (the anti-PY IgG isotype). Fluorescence signals corresponding to antigen-antibody or F-actin-phalloidin complexes were collected with a Bio-Rad MRC 600 laser scanning confocal microscope as described previously (14).

Materials—Genistein and tyrphostin 25A were purchased from LC Laboratories. Phenylarsene oxide, albumin, Na_3VO_4, forskolin, 8-(4-chlorophenylthio)-cAMP, and ACTH were obtained from Sigma. Pervanadate was generated as described by Evans *et al.* (15).

RESULTS

Y1 epithelial cells firmly adhere to uncoated plastic dishes or glass coverslips (Fig. 1*A*, *a*). Treatment with 0.2 mM 8-(4-chlorophenylthio)-cAMP (CPT-cAMP), a cell-permeable analog of cAMP, alters cell morphology. Within 3 min some cells begin to retract their membranes and assume a "rounder" shape. After 6 min, ~10% of the cells display a highly refractile central region that gives rise to short, incompletely retracted processes (Fig. 1*A*, *b*). Approximately 50% of the cells exhibit this shape after 10 min, and virtually the entire population completes the morphological transition by 30 min (Fig. 1*A*, *c*). Incubation with 20 nM ACTH or 20 μM forskolin, an adenylate cyclase activator, yields the same results. The speed with which Y1 cells alter their cytoskeleton suggests that post-translational modifications of proteins mediate the response to cAMP.

Proteins with apparent molecular weights of 130,000 and 76,000 account for most of the PY residues in Y1 cells (Fig. 1*B*, *lane 1*). Treatment of Y1 cells with ACTH, forskolin or CPT-cAMP resulted in dephosphorylation of the 76-kDa polypeptide within 1 min (Fig. 1*B*, *lanes 3*, *4*, *7*, *8*, *11*, and *12*). The 130-kDa protein was not markedly dephosphorylated in ACTH and forskolin-treated cells. Dephosphorylation of the 76-kDa protein precedes the cell shape transition and identifies this protein as an early downstream target of cAMP/PKA action.

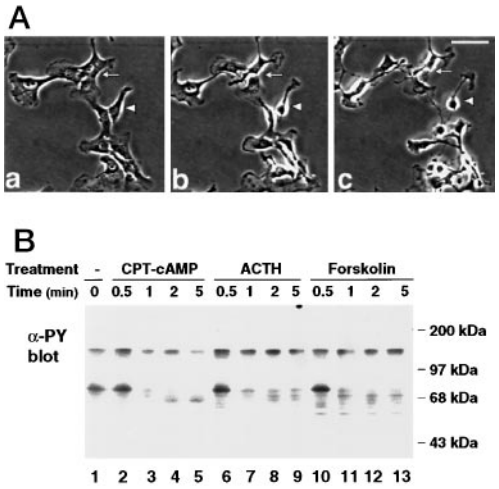

FIG. 1. **Cyclic AMP provokes a rapid change in cell shape that is preceded by the dephosphorylation of a 76-kDa protein.** *A*, *a–c* are phase contrast micrographs showing a typical field of Y1 cells 0, 6, and 30 min, respectively, after addition of 0.2 mM CPT-cAMP. Cells marked with the *arrow* and the *arrowhead* undergo the morphological transition in a slightly asynchronous manner. *Bar*, 50 μm. *B*, Y1 cells were incubated with 0.2 mM CPT-cAMP, 20 nM ACTH, or 20 μM forskolin for the indicated time periods. Subsequently, cells were lysed in a buffer containing 0.5% Triton X-100. Proteins from cell lysates were fractionated by denaturing electrophoresis and transferred to an Immobilon P membrane as described under "Experimental Procedures." The membrane was probed with anti-PY IgG, and antigen-antibody complexes were visualized on x-ray film by chemiluminescence as described under "Experimental Procedures."

The size and PY content of the 76-kDa protein suggested that it might be paxillin, a focal adhesion phosphoprotein (16, 17). Anti-paxillin IgG quantitatively precipitated a broad band of polypeptides with M_r values in the range of 68,000–76,000 (Fig. 2*A*, *lanes 2–7*). This pattern is typical for paxillin (17). Densitometry revealed that ~20% of the protein in the broad band was bound by anti-PY IgGs (Fig. 2, *A* and *B*, *lanes 8* and *9*). A similar percentage of paxillin molecules are phosphorylated on tyrosine during focal adhesion formation (16). The apparent M_r of the phosphoprotein precipitated by anti-PY IgG was ~76,000 (Fig. 2, *A*, *lane 9*, and *B*, *lanes 3* and *9*), indicating that the upper portion of the band obtained with anti-paxillin IgG (Fig. 2*A*, compare *lanes 3*, *5*, *9*, and *10*) corresponds to tyrosine phosphorylated forms of paxillin. Paxillin was dephosphorylated when cells were treated with forskolin or CPT-cAMP (Fig. 2*B*, *lanes 2–13*). Because the 76-kDa PY immunoreactivity observed in Figs. 1*B* (*lanes 1*, *2*, *6*, and *10*) and 2 (*lane 9*) is quantitatively precipitated by anti-paxillin IgG, we conclude that paxillin is a downstream target for PKA-mediated signaling in Y1 cells.

Antibodies directed against FAK125 (a focal adhesion tyrosine protein kinase (18)) neither precipitated solubilized 130-kDa phosphoprotein nor bound the 130-kDa polypeptide on a Western blot (data not shown). Y1 cells express a low level of FAK125, and its content of PY is negligible.[2]

Neither 0.3 mM genistein (Fig. 3*A*, *lane 7*) nor 50 μM tyrphostin A25 affected cell shape or PY content of paxillin over a 7-h

[2] J.-D. Han and C. S. Rubin, unpublished observations.

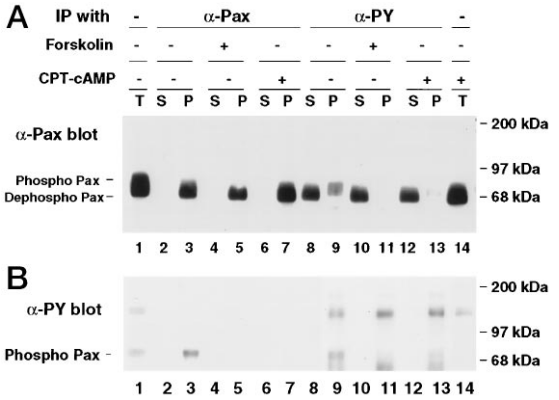

FIG. 2. **Cyclic AMP stimulates the dephosphorylation of tyrosine residues in the focal adhesion protein paxillin.** Control Y1 cells and cells incubated for 10 min with either 20 μM forskolin or 0.1 mM CPT-cAMP were solubilized with PEMS lysis buffer. Samples of solubilized proteins were incubated with either anti-paxillin IgG (*lanes 2–7*) or anti-PY IgG (*lanes 8–13*) that was immobilized on protein A-Sepharose 4B beads (see "Experimental Procedures"). Proteins precipitated with the beads (*P*), and polypeptides remaining in the supernatant solution (*S*) were size-fractionated by denaturing electrophoresis (9% gel) and transferred to an Immobilon P (*IP*) membrane. *Lanes 1* and *14* received total proteins (*T*) from control and CPT-cAMP-treated Y1 cells, respectively. Replicate Western blots were probed with anti-paxillin (*α-Pax*) (*A*) and anti-PY (*α-PY*) IgGs. *B*, immunocomplexes were visualized by enhanced chemiluminescence methodology. Only the relevant portion of each immunoblot is shown. *Phospho Pax*, phosphopaxillin.

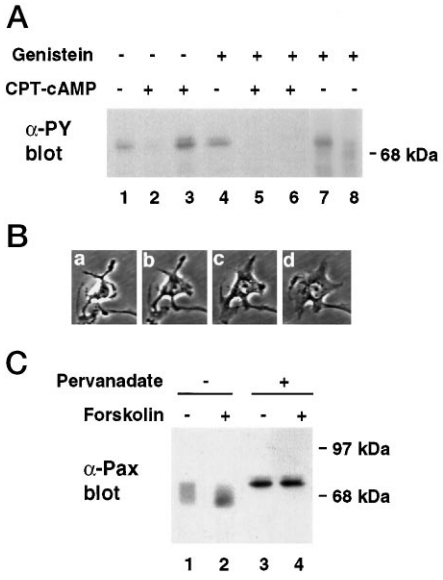

FIG. 3. **PTP activity is required for the cAMP-regulated rearrangement of the cytoskeleton, whereas tyrosine protein kinase is essential for the restoration of epithelial morphology and the basal state of paxillin phosphorylation.** *A*, Y1 cells were incubated with or without 0.2 mM CPT-cAMP for 10 min. Replicate plates of CPT-cAMP-treated cells were either processed immediately after exposure to the cAMP analog or incubated for an additional 20 min in medium lacking CPT-cAMP. Cell proteins (0.1 mg) were size-fractionated in an SDS-polyacrylamide gel (9%) and transferred to an Immobilon P membrane (see "Experimental Procedures"). The blot was probed with anti-PY IgG. *Lanes 1* and *4* received proteins from Y1 cells that were not exposed to CPT-cAMP; *lanes 2* and *5* contained proteins from cells harvested immediately after exposure to CPT-cAMP; and *lanes 3* and *6* received proteins from cells that were incubated in the absence of CPT-cAMP for 20 min after a 10-min exposure to the cAMP analog. Proteins analyzed in *lanes 4–6* were obtained from cells that were maintained in the presence of 0.3 mM genistein (60 min of preincubation plus experimental manipulation in the presence of the drug). Proteins in *lanes 7* and *8* were derived from Y1 cells that were chronically maintained in medium supplemented with 0.3 mM genistein for 7 and 14 h, respectively. *B*, Y1 cells were preincubated with CPT-cAMP for 30 min to generate refractile cells with neurite-like processes. The morphology of a typical cell was monitored by real time video microscopy after feeding the cells with fresh medium lacking the cAMP analog. Frames obtained at 0, 6, 10, and 20 min (*a–d*, respectively) after removing CPT-cAMP document the rapid restoration of epithelial morphology. *C*, Y1 cells were preincubated 10 min with or without 0.2 mM pervanadate. Cells were then incubated in the presence or the absence of 20 μM forskolin for 10 min as indicated. Protein samples were prepared and analyzed as described under "Experimental Procedures." The blot was probed with anti-paxillin IgG.

period. However, treatment with genistein for 8 h produced a rounded phenotype in 20% of Y1 cells; after 14 h of incubation with genistein nearly 90% of Y1 cells resembled cells treated with CPT-cAMP for 30 min (Fig. 1*A*, *c*). The PY content of paxillin was slightly diminished at 8 h (not shown), whereas the protein was predominantly dephosphorylated after incubation with genistein for 14 h (Fig. 3*A*, *lanes 4*, *7*, and *8*). These data suggest that PY on paxillin is hydrolyzed very slowly in unstimulated cells. Thus, a paxillin tyrosine kinase is not a likely target for rapid PKA-mediated signaling.

Paxillin is phosphorylated (Fig. 3*A*, *lane 3*), and Y1 cells revert to their epithelial morphology (Fig. 3*B*) 20 min after the removal of CPT-cAMP. However, genistein blocked both processes (Fig. 3*A*, *lanes 4–6*; cell shape identical to that shown in Fig. 3*B*, *a*). Thus, phosphorylation of paxillin may be a requisite step in the re-establishment of the epithelial phenotype of Y1 cells.

Pervanadate, which potently inhibits PTPs (19, 20), suppressed cAMP-stimulated paxillin dephosphorylation in short (Fig. 3*C*) and long term (tested up to 1 h) experiments. Cells did not alter their shape (Fig. 4 *g–h′*), and paxillin became a sharp (maximally phosphorylated) band on Western immunoblots (Fig. 3*C*, *lanes 3* and *4*). Although pervanadate may partially activate certain tyrosine kinases (19, 20), results obtained with genistein (see above) suggest that increased tyrosine phosphorylation is not required for the cAMP-controlled morphological transition. Moreover, phenylarsene oxide, a PTP inhibitor that does not activate tyrosine kinases, also blocked effects of cAMP in short term experiments.[2]

Y1 cells contain actin stress fibers (Fig. 4, *upper panel*, *a′* and *b′*) and focal adhesions that stain with antibodies directed against either PY or paxillin (Fig. 4, *upper panel*, *a* and *b*). CPT-cAMP promotes the rapid translocation of a substantial proportion of paxillin from focal adhesions to the cytoplasm (Fig. 4, *d* and *f*). As paxillin redistributes, stress fibers become disorganized and F-actin is concentrated in membrane ruffles and intracellular aggregates (Fig. 4, *c′* and *d′*), although remnants of focal adhesions are evident (Fig. 4*c*). After 30 min most of the paxillin is diffusely dispersed in the cytoplasm (Fig. 4*f*). In contrast, paxillin-depleted focal adhesion complexes (which still contain protein linked PY and a low level of paxillin) coalesce in co-aggregates with actin (Fig. 4, *e* and *e′*). Double immunostaining analysis directly verified that most of the paxillin separates from focal adhesion complexes and accumulates in cytoplasm when Y1 cells are incubated with CPT-cAMP (Fig. 4, *lower panel*). This phenotype is stable for at least 90 min. Similar results were obtained with ACTH and forskolin. cAMP-regulated changes in cytoskeleton were prevented by pervanadate (Fig. 4, *g*, *g′*, *h*, and *h′*) or phenylarsene oxide.

DISCUSSION

Shape and functions of Y1 adrenal cells are controlled by hormones that activate PKA (6–10). When the cAMP concentration is low, Y1 cells have a flat epithelial shape that receives structural support from a network of actin filaments (Fig. 4, *upper panel*, *a′* and *b′*). At the cell cortex, the filaments are anchored by multi-protein complexes known as focal adhesions (Fig. 4, *upper panel*, *a* and *b*) (21, 22). Focal adhesions mediate

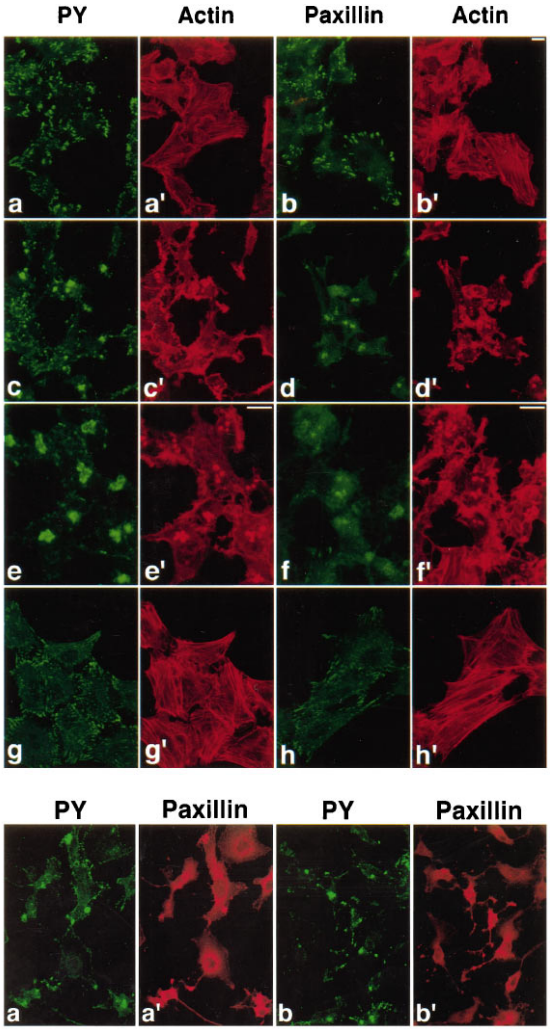

FIG. 4. **Cyclic AMP induces the translocation of paxillin, aggregation of PY-containing proteins, and rearrangement of the actin cytoskeleton in Y1 cells.** *Upper panel*, Y1 cells were grown on coverslips and treated with 0.2 mM CPT-cAMP for 0 (*a*, *a′*, *b*, *b′*, *g*, and *g′*), 10 (*c*, *c′*, *d*, *d′*, *h*, and *h′*), or 30 min (*e*, *e′*, *f*, and *f′*). Cells shown in *g*, *g′*, *h*, and *h′* were preincubated with 0.2 mM pervanadate for 10 min, and the same concentration of pervanadate was used during a subsequent 10-min experimental incubation with CPT-cAMP. Cells were then fixed, permeabilized, and blocked with 3% albumin. Y1 cells in *a*, *c*, and *e* were incubated sequentially with anti-PY IgG and 10 μg/ml FITC-conjugated, goat IgGs directed against mouse immunoglobulins. The same cells (*a′*, *c′*, and *e′*) were co-stained for F-actin by using rhodamine-conjugated phalloidin. Y1 cells in *b*, *d*, *f*, *g*, and *h* were incubated with anti-paxillin and FITC-conjugated secondary antibodies and then co-stained with rhodamine-tagged phalloidin (*b′*, *d′*, *f′*, *g′* and *h′*). Fluorescence signals corresponding to antigen-antibody (*green*) or F-actin-phalloidin (*red*) complexes were collected with a Bio-Rad MRC 600 laser scanning confocal microscope (see "Experimental Procedures"). *Bar*, 10 μM. *Lower panel*, Y1 cells were co-stained for proteins containing PY (*green*) and paxillin (*red*) after incubation with 0.2 mM CPT-cAMP for 10 (*a* and *a′*) or 30 min (*b* and *b′*).

bidirectional signaling between the actin cytoskeleton and extracellular matrix by coupling with integrins. Tensin, FAK125 and paxillin are constituents of focal adhesions (16, 21, 22). Ligand-activated integrins stimulate (indirectly) the phosphorylation of these proteins on Tyr. Moreover, phosphorylation of paxillin is tightly coupled to the *de novo* assembly of F-actin-focal adhesion-integrin complexes (16, 17, 21).

We observed that an increase in cAMP content (and activation of PKA (7, 23)) triggers the rapid (≤1 min) and selective dephosphorylation of tyrosine residues on paxillin in Y1 cells. The dephosphorylation reaction precedes depletion of paxillin from focal adhesions, disassembly of adhesion plaques, dissolution of stress fibers, and rearrangement of the actin cytoskeleton. Thus, cAMP/PKA actions appear to be initially targeted to the actin cytoskeleton and focal adhesions in the cell cortex. Paxillin is a previously unappreciated, early downstream target in a PKA-mediated signaling pathway.

Acute regulation of paxillin phosphorylation is not exerted at the level of tyrosine kinase(s). Rather, cAMP elicits an apparent increase in PTP activity. Perhaps PKA directly phosphorylates and activates a PTP. Ser^{39} of PTP-PEST is phosphorylated by PKA *in vitro* and in cells (24). However, this modification reduced PTP-PEST activity by ~30% in cell extracts. Although the exact regulatory significance of serine phosphorylation in PTP-PEST and other PTPs remains to be determined, the data indicate that PTPs can be direct targets for PKA in intact cells. Alternatively, PKA may phosphorylate paxillin within the consensus substrate sequence ^{496}RRGSL^{500} (25). If PKA-catalyzed phosphorylation alters the conformation of paxillin, its accessibility to regulated or constitutively active PTPs may be increased. More complex mechanisms involving phosphorylation of activators or inhibitors of PTPs are also possible (26).

Although exact mechanisms remain to be determined, it is evident that cAMP (PKA) regulates disassembly of focal adhesions and dissolution of actin filaments as well as the PY content of paxillin. Effects of cAMP on Y1 cells are blocked by PTP inhibitors. Moreover, tyrosine protein kinase activity is essential for reassembly of F-actin-focal adhesion complexes, regeneration and recruitment of phosphopaxillin to the cell cortex, and restoration of epithelial shape of Y1 cells when cAMP levels decline. Because injection of recombinant PTPs into Y1 cells produces the phenotype shown in Figs. 1*A* (*c*) and 4 (*c–f*),[2] it appears that PTP activation and/or translocation is a necessary and sufficient step in the regulation of actin cytoskeleton organization and cell shape. Coordinated control of paxillin dephosphorylation and cell morphology via PKA-mediated signaling is not restricted to Y1 cells. Similar results were obtained with immortalized forebrain neurons that rapidly extend long neurites in response to isoproterenol, 8-bromo-cAMP, or forskolin.[3]

Paxillin subserves intertwined scaffolding and signaling functions in focal adhesions via distinct domains that bind ≥6 effector proteins (16, 21, 22). Csk, Crk, and tensin contain SH2 domains that bind PY-containing segments of paxillin. Engagement of integrins with extracellular matrix stimulates (indirectly) tyrosine phosphorylation of paxillin, thereby enhancing multi-protein complex formation (16, 17). cAMP-controlled tyrosine dephosphorylation of paxillin would generate incomplete (possibly unstable) focal adhesion complexes and compromise signaling between integrins and effectors in the actin cytoskeleton. A logical speculation is that changes in the quality or intensity of such signals coupled with a co-operative weakening in focal adhesion structure due to the loss of SH2 binding sites results in the displacement of stress fibers from the cell cortex and the redistribution of F-actin.

In summary, our results suggest that a PTP and paxillin could be early, target/effector proteins in a cAMP-controlled signaling pathway that regulates cell shape. Y1 cells provide a system for further evaluation of this proposition.

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