A Kinase Anchor Protein 75 Targets Regulatory (RII) Subunits of cAMP-dependent Protein Kinase II to the Cortical Actin Cytoskeleton in Non-neuronal Cells*

Ying Li‡, Christopher Ndubuka‡, and Charles S. Rubin§

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

Neuronal A kinase anchor protein (AKAP) homologs, such as AKAPs 75 and 150, tether cAMP-dependent protein kinase II (PKAII) isoforms to the postsynaptic cytoskeleton, thereby creating target sites for cAMP action. These AKAPs, which bind regulatory subunits (RIIs) of PKAII, are also expressed in certain non-neuronal cells. Non-neuronal cell lines that stably express wild type and mutant AKAP75 transgenes were generated to investigate the extraneuronal function of AKAPs. In non-neuronal cells, AKAP75 accumulates selectively in the actin-rich, cortical cytoskeleton in close proximity with the plasma membrane. AKAP75 efficiently sequesters cytoplasmic RIIα and RIIβ (PKAII isoforms) and translocates these polypeptides to the cell cortex. Two structural modules in AKAP75, T1 (residues 27–48), and T2 (residues 77–100), are essential for targeting AKAP75/RII complexes to the cortical cytoskeleton. Deletions or amino acid substitutions in T1 and/or T2 result in the dispersion of both AKAP75 and RII subunits throughout the cytoplasm. AKAP75 is co-localized with F-actin and fodrin in the cortical cytoskeleton. Incubation of cells with 5 μM cytochalasin D disrupts actin filaments and dissociates actin from the cell cortex. In contrast, the bulk of AKAP75 and fodrin remain associated with the cortical region of cytochalasin D-treated cells. Thus, targeting of AKAP75 does not depend upon direct binding with F-actin. Rather, AKAP75 (like fodrin) may be associated with a multiprotein complex that interacts with integral plasma membrane proteins.

The type IIβ isoform of cAMP-dependent protein kinase (PKAIIβ) is a major mediator of the actions of cAMP in the central nervous system of mammals (1–3). PKAIIβ is maximally expressed in forebrain neurons, where most of the enzyme is anchored in the cytoskeleton of dendrites (4). Tethering of PKAIIβ is due, in part, to the binding of the regulatory subunits (RIIβ) of the kinase with an A kinase anchor protein (AKAP). Homologous anchor proteins from bovine (AKAP75), human (AKAP79), and rat (AKAP150) brains avidly bind RIIβ and RIIα homodimers via a conserved, COOH-terminal domain that consists of ~20 contiguous amino acid residues (1, 2, 5–10). Anchor proteins sequester the PKAIIβ and PKAIIα holoenzymes and dissociated RII subunits with similar affinities (11, 12). In contrast, RIIα, RIIβ, and PKAI isoforms do not form complexes with AKAPs. Little is known about structural regions in AKAPs that mediate their attachment to the neuronal cytoskeleton. However, incorporation of AKAP-PKAII complexes into a Triton X-100-insoluble fraction of cultured cells is mediated by two targeting domains (T1 and T2) that are located near the AKAP NH2 terminus (8).

In neurons AKAP150 and PKAIIβ are enriched and co-localized along dendritic microtubules, in close proximity to the postsynaptic plasma membrane (4). A current model (reviewed in Ref. 9) proposes that this arrangement places a high concentration of PKAIIβ near a signal generator (neurotransmitter-activated adenylyl cyclase) and substrate/effecter proteins such as microtubule-associated proteins, ion channels, and G-protein-coupled neurotransmitter receptors. Phosphorylation of co-localized substrates could (a) facilitate signal transmission over substantial distances in neurons via interconnected proteins of the cytoskeletal network, (b) alter the electrical/ionic properties of the cell, and (c) desensitize active synapses to subsequent pulses of neurotransmitter. Thus, AKAP-PKAII complexes create specialized target sites for the efficient reception and propagation of signals carried by the second messenger cAMP. Dissociation of AKAP-RIG complexes in hippocampal neurons abrogates PKA-mediated regulation of glutamate-gated ion channels (13). Therefore, properly positioned AKAP-RII complexes are crucial for a normal neuronal function.

Expression of AKAP75/79/150 proteins (collectively designated AKAPs below) is not limited to neurons. Messenger RNAs encoding these AKAPs accumulate in kidney2 and thyroid (7). Moreover, AKAP150 and AKAP79 polypeptides are evident in particulate fractions of homogenates of rat lung (1) and human embryonic kidney cells (5, 8), respectively. Thus, AKAP-RII complexes may facilitate cAMP-mediated signaling in non-neuronal cells. This proposition raises fundamental questions that can be addressed in model systems. Do AKAPs accumulate at one major intracellular location, or are they dispersed in multiple cell compartments? Which structural domain(s) in AKAPs govern attachment to the cytoskeleton and/or organelles? What proportion of total RII subunits (PKAII isoforms) can be bound and anchored by AKAPs? Do AKAPs dock with microtubules or other structures in non-

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† These two authors contributed equally to these studies.

‡ 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@eecom.yu.edu.

1 The abbreviations used are PKA, protein kinase A; cAMP-dependent protein kinase; RI and RII, regulatory subunits of protein kinase A I and II, respectively; AKAP, A kinase anchor protein; S-AKAP, spermatic AKAP; T1 and T2, targeting domains corresponding to residues 27–48 and 77–100 in AKAP75, respectively; PBS, phosphate-buffered saline.

2 F. Dong, and C. S. Rubin, unpublished observations.
neuronal cells?

HEK 293 cells provide a good system for studying AKAP localization and function. The cells contain R11α, R11β, and AKAP79 (14). Moreover, the anchor protein is excluded from the cytoplasm. However, the content of endogenous anchor protein is low and only 5–8% of RII isosforms are captured in the particulate fraction of cell homogenates (14). Wild type and mutant AKAP75 proteins can be expressed at high levels (~15-fold greater than AKAP79) in stably transfected HEK 293 cells. As a result, intracellular targeting of AKAP75 and effects of AKAP75 on the compartmentalization of endogenous RII subunits (PKAII isoforms) can be analyzed in intact cells. Experiments were devised to (a) establish the exact subcellular location of AKAP75, (b) determine whether T1 and/or T2 mediate the immobilization of AKAP75, and (c) test the hypothesis that alterations in AKAP abundance can control the distribution of RII subunits (PKAII) between the cytoplasm and a specific anchoring site in intact cells.

We now report that AKAP75 and endogenous RII subunits accumulate selectively on the cytoplasmic side of the plasma membrane in the cortical actin cytoskeleton of transfected HEK 293 cells. AKAP75 mutants that have modified or deleted T1 or T2 domains fail to concentrate in the cortical cytoskeleton and are dispersed in the cytoplasm. Endogenous RII subunits (PKAII) are distributed in parallel with mutant AKAPs. Thus, multisite interactions, involving both the T1 and T2 domains, appear to be essential for targeting AKAP75-PKAII complexes to the cytoskeleton in non-neuronal cells. The generality of these observations was established by demonstrating that AKAP75 also anchors RII subunits in the cortical cytoskeleton of hamster AV12–664 cells. Wild type AKAP75 co-localizes with cortical F-actin. However, disruption of actin filaments with cytochalasin D did not alter the location of AKAP75 or fodrin, suggesting that the anchor protein (like fodrin) interacts with a protein complex that includes integral plasma membrane proteins.

EXPERIMENTAL PROCEDURES

Cell Culture—Cell lines derived from human embryonic kidney (HEK 293) and a hamster subcutaneous tumor (AV12–664) were obtained from the American Type Culture Collection. Stably transfected HEK 293 cells that overexpress wild type AKAP75 (H3 cells) were produced as described previously (14). Cells listed above and transfected HEK 293 and AV12 cells that express wild type and mutant AKAP75 appear to be essential for targeting AKAP75–PKAII complexes to the cytoskeleton in non-neuronal cells. The generality of these observations was established by demonstrating that AKAP75 also anchors RII subunits in the cortical cytoskeleton of hamster AV12–664 cells. Wild type AKAP75 co-localizes with cortical F-actin. However, disruption of actin filaments with cytochalasin D did not alter the location of AKAP75 or fodrin, suggesting that the anchor protein (like fodrin) interacts with a protein complex that includes integral plasma membrane proteins.

Mutagenesis and Construction of Expression Vectors—A segment of DNA that encodes AKAP75 was cloned in the eukaryotic expression plasmid pRCMV (Invitrogen, San Diego, CA) to generate pAKAP75 as described previously (5). The DNA insert (1.3 kilobase pairs) from pAKAP75 was excised by digestion with NcoI and XbaI, purified by electrophoresis, and subcloned into the plasmid pGEM5Zf (Promega), which was cut with the same restriction enzymes. The resulting recombinant plasmid was named pGAKAP75. Single-stranded template DNA was produced by transforming Escherichia coli with pGAKAP75 and infecting the transformed cells with M13K7 helper phage as described previously (8). Mutant AKAP75 polypeptides that contain amino acid substitutions or internal deletions were generated by the oligonucleotide-directed mutagenesis procedure of Kunkel (15) as modified by Liu et al. (16). Detailed descriptions of the mutagenesis procedure and the strategy used to design primers are provided in a previous publication (5). All mutants were verified by DNA sequencing as described previously (1, 2).

Mutated AKAP75 DNA inserts were excised from pGAKAP75 (see above) and cloned into the pRC/CMV expression plasmid that was digested with NotI and XbaI. In addition, wild type AKAP75 DNA was released from pAKAP75 by sequential digestion with XbaI and NotI. The XbaI overhang was made blunt by incubation with Klenow DNA polymerase prior to incubation with NotI. The DNA fragment was ligated into the mammalian expression vector pC2 (provided by C. Gorman, Genentech (17)), which had been cleaved with NotI and Hpal in its multiple cloning region. This placed the AKAP75 coding sequence downstream from a powerful cytomegalovirus promoter and upstream from a polyadenylation signal.

Production of Stably Transfected Cells—The pRC/CMV expression vector contains the bacterial neomycin gene under the regulation of a constitutive SV40 promoter. This permits the selection of stable transfectants with the drug G418 (Life Technologies, Inc.). HEK 293 cells were transfected, as described previously (5), with recombinant pRC/CMV plasmids that contain the mutant AKAP75 DNA inserts listed above. Stably transfected cells were selected with G418 and cloned as described by Nudukova et al. (14). The cloned cell lines could be maintained in the absence of G418 without the loss of AKAP75 expression or drug resistance. Cell lines derived from transfected HEK 293 cells are listed in Table I.

Hamster AV12 cells were transfected with a recombinant pC2 vector that contains wild type AKAP75 DNA downstream from a cytomegalovirus promoter. The transfection protocol was identical with that used for HEK 293 cells (5). Since the pC2 expression plasmid contains the dihydrofolate reductase gene under the regulation of a constitutive SV40 promoter (17) and (b) AV12 cells are killed by methotrexate (18), it was possible to select and clone stable transfected cells by adding 0.25 μM methotrexate to the culture medium. Thirteen independent clones that expressed similar levels of AKAP75 were obtained. Cells designated AV12–A75 cells were used for studies presented below.

Antisera—Antisera directed against AKAP75 and R11β were produced in rabbits and characterized as described previously (2, 4). Anti-RII IgG bind shared epitopes in RIIα and RIIβ. For immunofluorescence experiments anti-RII IgG were purified by affinity chromatography on a column of R11β-Sepharose 4B, using a methodology described in a previous paper (19). All results obtained with anti-RII IgG were independently reproduced with anti-RIIα IgG (data not shown), indicating that both RII isosforms were simultaneously bound and detected at the IgG concentrations used.

A mouse monoclonal antibody directed against P58, a protein located in the cytoplasmic and at the cytoplasmic surface of the Golgi apparatus (20), was obtained from Sigma. Rabbit anti-fodrin antibodies were generously provided by Dr. J. Morrow (Department of Pathology, Yale University School of Medicine). Affinity-purified fluorescein- or rhodamine-coupled secondary antibodies were obtained from Vector Labs (Burlingame, CA) or Zymed Laboratories (San Francisco, CA) (see below).

Immunofluorescence Analysis—Cells were grown on 20-mm glass
coverslips that were coated with poly-L-lysine. The coverslips were washed with 10 mM sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl (PBS). Next, cells were fixed by incubation with 4% paraformaldehyde in PBS for 10 min at 20 °C. After fixation and three washes with PBS, cells were permeabilized by incubation in acetone at −20 °C for 10 min. Further manipulations were performed at 20 °C. After washing three additional times in PBS, fixed/permeabilized cells were incubated in PBS containing 3% (w/v) albumin for 1 h. Subsequently, the cells were incubated with either anti-AKAP75 serum (1:100 in PBS) or affinity-purified, anti-RII β IgGs at 1:50 dilution (0.1 μg/ml) for 1 h. Following five washes with PBS containing 0.1% (w/v) Tween 20, the coverslips were incubated with 10 μg/ml fluorescein isothiocyanate-conjugated goat IgGs directed against rabbit immunoglobulins. Samples were incubated for 1 h with the secondary antibodies. Next, cells were washed three times in PBS containing 0.1% Tween 20. After air drying, 15 μl of 50% glycerol in PBS, containing 1 mg/ml p-phenylenediamine (antibleaching agent), was placed on the specimens, and the coverslips were mounted on slides.

The same procedure was used for staining with mouse anti-P58 (1:100) and rabbit anti-fodrin (1:100) IgGs. Fluorescein-tagged goat IgGs (10 μg/ml) directed against mouse immunoglobulins were used to detect antibody-P58 complexes. In one series of experiments (see Fig. 8), rhodamine-conjugated goat IgGs (20 μg/ml) directed against rabbit immunoglobulins were used to visualize RII-anti-RII IgG complexes.

Fluorescence signals corresponding to antigen-antibody complexes were examined with a Bio-Rad MRC 600 laser-scanning confocal microscope (Image Analysis Facility, Albert Einstein College of Medicine) as described previously (19). The fluorochromes fluorescein and rhodamine were excited at 488 and 568 nm, respectively. Emission filters for fluorescein and rhodamine were 622 and 605 nm. Sequential optical sections were recorded at 0.5-μm intervals. Results reported in this paper correspond to optical sections taken 1 μm above the surface of the coverslip.

For each series of immunofluorescence experiments, replicate samples of fixed/permeabilized cells were treated as described above except that antibodies directed against AKAP75 or RII were preincubated with saturating amounts of purified recombinant AKAP75 or RII polypeptides (22), respectively. No fluorescence signals were obtained from these samples.

Drug Treatment and Detection of F-actin—H3 cells were incubated with 5 μM cytochalasin D (Sigma) for 4 h at 37 °C to disrupt actin microfilaments. For visualization of F-actin, cells were processed as described above, except an incubation with 0.6 μM rhodamine-conjugated phalloidin (Sigma) in PBS was substituted for treatments with primary and secondary antibodies. The cells were then washed, mounted, and observed as described above.

Electrophoresis of Proteins—Cytosolic and particulate fractions of cells were prepared as described in Hirsch et al. (5). Samples (40–60 μg) of cytosolic and particulate proteins were denatured in gel loading buffer and subjected to electrophoresis in 9% or 10% polyacrylamide gels containing 0.1% SDS as described previously (1). Western blots were prepared by transferring the size-fractionated polypeptides and molecular weight standards to an Immobilon P (polyvinylidene difluoride) membrane (Millipore Corp.) as described by Bregman et al. (1).

Western Immunoblot Assays—Western blots of cytosolic and particulate proteins were blocked, incubated with anti-AKAP75 serum (1:3000) or affinity-purified anti-RII IgGs (1:1000 relative to serum), and washed as described previously (5, 8). AKAP75 and RII polypeptides were visualized by indirect chemiluminescence using an ECL kit from Amersham Corp. as previously reported (5, 14). Relative amounts of antigen were determined by scanning densitometry (Molecular Dynamics) of chemiluminescence signals on x-ray film. Standard curves were prepared with 0.5–100 ng of purified AKAP75 or RII. Amounts of AKAP75 and RII protein in experimental samples were obtained from the linear portion of the standard curves.

RESULTS

Determination of the Intracellular Location of AKAP75 in Non-neuronal Cells—Approximately 90–95% of total PKAIIα and PKAIIβ partitions with the cytosolic fraction of homogenates of HEK 293 cells (14). However, when an AKAP75 transgene is overexpressed in clonally derived transfectants 293 cells (designated H3 cells) >90% of total RII subunits and nearly all of the type II cAMP-stimulated kinase activity are recovered in a pellet fraction containing Triton X-100-insoluble proteins (14). In order to determine a candidate intracellular anchoring site(s) for RII subunits, permeabilized H3 cells were probed with anti-AKAP75 IgGs. Antigen-antibody complexes were visualized by confocal immunofluorescence microscopy. Intense fluorescence signals corresponding to AKAP75 immunoreactivity are evident near the plasma membrane at sites of intercellular contact (Fig. 1A). The striking “cobblestone” pattern of AKAP75 accumulation parallels results obtained for certain transmembrane proteins (e.g. cadherins) and polypeptides comprising the cortical actin cytoskeleton (e.g. zyxin, cingulin, fodrin, α-actinin) in several types of cells (22–26). Several considerations indicate that AKAP75 is not an integral component of the plasma membrane in H3 cells. AKAP75 has no predicted transmembrane domains, and in neurons, the anchor protein is located in the cytoskeleton near the cytoplasmic surface of the plasma membrane (4). No immune complexes are observed when intact H3 cells are treated serially with anti-AKAP75


AKAP75 Anchors RII in the Cell Cortex

The T1 and T2 Domains Are Essential for the Accumulation of AKAP75 in the Cortical Cytoskeleton—The T1 (amino acid residues 27-48) and T2 (residues 77-100) anchoring modules of AKAP75 were previously defined operationally, using low resolution subcellular fractionation procedures (14). Both T1 and T2 are required for the maximal accumulation of AKAP75 in a Triton X-100-insoluble fraction of cell homogenates. The discovery that AKAP75 accumulates in the cortical cytoskeleton (Fig. 1A) enabled a detailed analysis of the roles of T1 and/or T2 in the anchoring of RII-AKAP complexes at a specific site within intact cells.

AKAP75 cDNA was mutated to encode anchor proteins that either (a) lack the T1 or T2 domains or (b) contain multiple amino acid substitutions in the T1 or T2 regions. Wild type and mutant cDNAs were inserted downstream from a cytomegalovirus promoter in a mammalian expression vector. The expression plasmid also includes an SV40 promoter that drives the transcription of the neo6 gene. Several stably transfected lines of HEK 293 cells were selected (via G418) for each of the mutated versions of AKAP75 cDNA. The names of the cell lines and descriptions of the mutant AKAP75 polypeptides are presented in Table I. Expression of the variant anchor proteins was monitored by Western immunoblot analysis. Scanning densitometry revealed that mutant polypeptide accumulated to a level that was 10-20-fold higher than that of endogenous AKAP75. Examples are shown in Fig. 2. Typically, 60-95% of the wild type and mutant anchor proteins was recovered in particulate fractions from the cells listed in Table I. Only an AKAP75 mutant lacking the entire T2 domain was predominantly cytosolic (Fig. 2B, lanes 5 and 6).

Confocal immunofluorescence microscopy was used to determine the intracellular distribution of the mutant anchor proteins. All AKAP75 variants (Table I) that lack T1 or T2 (Fig. 3A) or contain amino acid substitutions in a targeting domain (Fig. 3B) were distributed throughout the cytoplasm (in a punctate fashion) and failed to accumulate in the cortical cytoskeleton (compare with Figs. 3C and 1A). In contrast, an AKAP75 mutant containing amino acid replacements in the segment of the polypeptide chain that links T1 and T2 (H3 linker, Table I) yielded a pattern of fluorescence identical to that shown in Figs. 1A and 3C (data not shown). Thus, intact T1 and T2 domains are essential for the selective anchoring of AKAP75 in the cortical cytoskeleton.

AKAP75 Mediates the Targeting of RII Subunits to the Cortical Cytoskeleton—The functional significance of the enrichment of AKAP75 in the cortical cytoskeleton was assessed by determining the intracellular distribution of the endogenous RII isoforms in nontransfected HEK 293 cells and H3 cells. Antibodies directed against common epitopes in RIIβ and RIIα were used for immunofluorescence analysis. In the absence of a high level of AKAP75, RII subunits (PKAII isoforms) were dispersed in the cytoplasm. (Fig. 4A). The distribution of a portion of the RII subunits is perinuclear and asymmetric, suggesting possible association with the Golgi apparatus. In contrast, a dramatic redistribution of RII subunits is evident in H3 cells (Fig. 4B). The CAMP-binding subunits of the PKAII isoforms are excluded from the cytoplasmic/perinuclear region and appear to be sequestered in cortical cytoskeleton. The overall pattern of RII-derived immunofluorescence is very similar to that observed for AKAP75 (Fig. 1A). Thus, an elevation in anchor protein content can elicit the efficient translocation of RII subunits from the cytoplasm to the cortical actin cytoskeleton.

Several experiments excluded the unlikely possibility that RII subunits were exported to the external surface of the plasma membrane. When intact (nonpermeabilized) H3 cells were incubated with anti-RII IgGs and then processed for the immunofluorescence assay, no signal was observed. RII was not detected in culture medium from H3 cells by Western blot analysis. Finally, intact HEK 293 and H3 cells were incubated with trypsin or chymotrypsin, and the amount and size of RII subunits in protease-treated and control cells were compared. The proteases had no effect on the apparent Mr or amount of RII in the cells.

An intense fluorescent spot that is observed within H3 cells, using anti-RII IgGs, is absent when the same cells are probed with anti-AKAP75 IgGs (compare Figs. 4B and 1A). This probably reflects the attachment of some RII subunits to centromeres via an anchor protein unrelated to AKAP75 (27, 28).

F-Actin Is Not Essential for the Association of AKAP75 with the Cortical Cytoskeleton—In the cell cortex, the ends of actin filaments are embedded in specialized “cytoskeletal lattice domains” (29-31). The lattice is composed of various actin-bind-
extracellular matrix (29–31). Thus, targeting of AKAP75 to the cortical cytoskeleton could be due to either (a) direct binding of the anchor protein with F-actin or (b) incorporation of the anchor protein in an assembly (lattice) of actin-binding proteins. Cytochalasin D, a drug that elicits the selective disruption of actin filaments, was used to distinguish between these possibilities. In H3 cells F-actin and AKAP75 co-accumulate at the same intracellular location (Fig. 3, A and B). After cells were incubated with 5 μM cytochalasin D, actin peeled away from the vicinity of the plasma membrane and coalesced into large, disorganized aggregates in the cytoplasm (Fig. 3, D). H3 cells assumed a more spherical morphology as a consequence of actin filament disorganization. Disruption of the actin cytoskeleton had little effect on the localization of AKAP75; a high proportion of the anchor protein remained clustered at or near the plasma membrane, in close apposition with cell-cell junctions (Fig. 3, C). Thus, incorporation of AKAP75 into the cortical cytoskeleton is not dependent on complex formation between the anchor protein and F-actin.

Fodrin is evident in both the cortical cytoskeleton and cytoplasm of H3 cells (Fig. 4, A). The subpopulation of fodrin molecules associated with intercellular junctions is unaffected by cytochalasin D (Fig. 4, B). Therefore, AKAP75 shares the properties of localization and cytochalasin resistance with fodrin, a well established component of the cortical cytoskeleton that is involved in transmitting signals between the extracellular matrix and the actin cytoskeleton (29–31).
The molar ratio of AKAP75:RII was estimated to be 6 when anchor protein and RII subunits were quantified by scanning densitometry (14). Moreover, ~90% of the AKAP75 and virtually all of the RII subunits were associated with the particulate fraction of AV12-A75 cells (Fig. 7).

AKAP75 is highly enriched at sites of contact between AV12-A75 cells, and anchor protein-antibody complexes yield a cobblestone pattern of fluorescence that closely resembles the pattern obtained for H3 cells (Figs. 1A and 3C). The abundance of endogenous RII in AV12-A75 cells permitted simultaneous immunostaining for two antigens. This enabled experiments that assessed both the ability of AKAP75 to mediate the intracellular targeting of RII subunits and the specificity of targeting. Intracellular distributions of RII and a protein called P58 were determined for AV12 cells that lack (Fig. 8, A and B) or express high levels of AKAP75 (AV12-A75 cells, Fig. 8, C and D). P58 is present in both Golgi membranes and the cytoplasm (20). The distribution patterns for P58 and RII in nontransfected cells are quite similar, although not identical (Fig. 8, A and B). Both proteins are dispersed in the cytoplasm, but intensely fluorescent spots corresponding to the Golgi/centrosome region suggest that a portion of each protein accumulates selectively at a unique intracellular site. Expression of AKAP75 in AV12-A75 cells causes a dramatic redistribution of RII subunits (Fig. 8D). The bulk of RII is selectively targeted to the cortical cytoskeleton (Fig. 8D), whereas the localization of P58 is not altered (Fig. 8C). Thus, AKAP75 mediates the efficient and highly specific tethering of RII polypeptides (and PKAII isoforms) to the cortical cytoskeleton of AV12 cells.

**DISCUSSION**

We elucidated the location and function of AKAP75 in non-neuronal cells. When an AKAP75 transgene is expressed in H3 and AV12-A75 cells, the anchor protein is concentrated in the actin-rich, cortical cytoskeleton (4) (Figs. 1, 3C, 5, and 8, and see "Results"). AKAP75 binds endogenous RII subunits in intact cells and mediates their depletion from cytoplasmic/perinuclear regions and concomitant accumulation in the cell cortex (Figs. 4 and 8). Moreover, RII subunits that are tethered to AKAP75 in H3 cells remain tightly associated with PKA catalytic subunits (14). Thus, PKAII holoenzymes are targeted to the cortical cytoskeleton of non-neuronal cells by AKAP75.

The role of AKAP75 in non-neuronal cells seems to be analogous to that postulated for AKAPs in neurons (9). AKAP75-mediated anchoring of RII in the cortical cytoskeleton of H3 and AV12-A75 cells places PKAII isoforms in proximity with

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*The intracellular distribution of endogenous AKAP79 is similar to that of AKAP75. The low abundance AKAP79 polypeptide yields weak fluorescence signals that make a negligible contribution to the results reported in this paper.*
showed that both T1 and T2 are required for maximal incorpo-
ration of signals carried by cAMP. However, neurons and non-neuro-
nal cells assemble target sites for cAMP at markedly different
compartments. Another possibility is that T1 and T2 bind to two different
sites in a cortical docking protein, thereby ensuring specific and
stable anchoring of PKAII isoforms at physiologically relevant
sites. It is not known whether the AKAP-calcineurin associa-
tion (a) principally reflects the tethering of the phosphatase or
(b) indicates a special role for calcineurin in promoting or
inhibiting anchoring of AKAP-RII complexes.

Prior to the discovery of AKAPs, Corbin et al. (34) reported that RII subunits were selectively associated with particulate fractions of heart homogenates. These investigators proposed that immobilized PKAII plays a key role in transmitting hormonal signals within specific intracellular compartments. Recent studies on non-neuronal RII binding proteins that are structurally unrelated to AKAP75 indicate that anchored PKAII accumulates in multiple cell types and intracellular
compartments (12, 35–39). Distinct RII binding proteins are detected in nuclei, Golgi membranes, endoplasmic reticulum, or other organelles. The structural basis for the localization of these proteins is not known. Novel RII tethering proteins were identified by their ability to bind RII subunits in vitro; however, their ability to sequester RII subunits and establish the localization of PKAII in intact cells remains to be documented. The discovery that AKAP75 accumulates selectively in the cortical cytoskeleton suggests that this compartment could play a role in receiving/disseminating cAMP signals. More importantly, our studies link the specific anchoring of RII (PKAII) in the physiologically relevant in situ localization of AKAP75 with the cortical cytoskeleton-specific anchoring of RII (PKAII) in the physiologically relevant milieu of intact cells.

Complexes of fodrin and other cortical proteins are often physically and functionally coupled to the transmembrane signaling proteins, cadherins and integrins (29, 30, 40–42). Fodrin, cadherins, and integrins exhibit patterns of intracellular distribution (22–26) that match the cobblestone pattern observed for AKAP75/RII complexes in non-neuronal cells (Figs. 1, 4, and 8). Like fodrin, AKAP75 (Fig. 5) and RII remain physically and functionally coupled to the transmembrane milieu of intact cells. Importantly, our studies link the specific anchoring of RII (PKAII) in the physiologically relevant in situ localization of AKAP75 with the cortical cytoskeleton-specific anchoring of RII (PKAII) in the physiologically relevant milieu of intact cells.

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