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Amiloride-sensitive sodium channel is linked to the cytoskeleton in renal epithelial cells

(ankyrin/fodrin/actin/polarity/protein biochemistry)

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ABSTRACT **Amiloride-sensitive sodium channels are localized to the microvillar domain of apical membranes in sodium-transporting renal epithelial cells. To elucidate the elements that maintain sodium channel distribution at the apical membrane, we searched for specific proteins associating with the channel. Triton X-100 extraction of A6 epithelial cells reveals that sodium channels are associated with detergent-insoluble and assembled cytoskeleton. Indirect immunofluorescence and confocal microscopy show that sodium channels are segregated to the apical microvillar membrane and colocalize with ankyrin, fodrin, and actin. We document by immunoblot analysis that ankyrin and fodrin remain associated with sodium channels after isolation and purification from bovine renal papillae. ^{125}I-labeled ankyrin can be precipitated by anti-sodium-channel antibodies only in the presence of purified bovine sodium-channel complex. Direct binding of ^{125}I-labeled ankyrin shows ankyrin binds to the 150-kDa subunit of the channel. Fluorescence photobleach lateral-diffusion measurements indicate sodium channels are severely restricted in their lateral mobility. We conclude that ankyrin links the amiloride-sensitive sodium channel to the underlying cytoskeleton and this association may sequester sodium channels at apical microvilli and maintain their polarized distribution in renal epithelial cells.**

Most Na^+-reabsorbing epithelia contain amiloride-blockable Na^+-specific channels situated within their plasma membranes. With antibodies generated against the amiloride-sensitive Na^+ channel isolated from bovine renal papillary collecting duct, this Na^+ channel has been shown to be localized to the microvillar domain of apical membranes (1, 2). Maintenance of this polarized distribution of Na^+ channels in the apical membrane is essential for electrogenic Na^+ transport. However, mechanisms that maintain the channel distribution within the apical plasma membrane are presently unknown.

Four mechanisms have been suggested to maintain integral membrane proteins within the apical-membrane domain of polarized epithelial cells: (*i*) diffusion-mediated trapping (3, 4); (*ii*) tight junctions (4, 5); (*iii*) anchoring of integral membrane glycoproteins to the membrane via covalent linkage to a glycophospholipid—i.e., glycosylphosphatidylinositol (6, 7); and (*iv*) anchoring of integral membrane proteins to submembranous cytoskeleton (4, 7). With respect to the latter, specific isoforms of the cytoskeletal proteins ankyrin and spectrin (fodrin), which are expressed on the apical membranes of polarized epithelial cells, may contribute to the maintenance of specific proteins at the apical-membrane surface (4, 7). In several cells, ankyrin appears to function as a linking protein that directly mediates the interaction between the integral membrane protein and the actin-binding protein spectrin (4, 7–9), thus forming an integral membrane protein–ankyrin–spectrin–actin complex. To date, ankyrin and spectrin have been shown to associate with the anion exchanger (band 3) of erythrocytes, voltage-dependent Na^+ channels of neurons, lymphocyte adhesion antigen Pgp-1, ankyrin-binding glycoprotein 205 from brain, Na^+/K^+-ATPase, and the anion exchanger (band 3 analog) on the basolateral membranes of transporting epithelial cells (for review, see ref. 8). However, association of ankyrin with an apically situated integral membrane protein in polarized epithelial cells has not yet, to our knowledge, been reported.

The occurrence of apically associated isoforms of ankyrin and spectrin (fodrin) in renal epithelial cells (10, 11) suggests that the amiloride-blockable Na^+ channel may be maintained in the apical membrane domain of Na^+-reabsorbing epithelia via ankyrin and fodrin. In this study, we document that ankyrin and fodrin copurify with the amiloride-sensitive Na^+ channel isolated from bovine renal papillae and that ankyrin binds directly to the 150-kDa amiloride-binding subunit of the channel. Based upon these observations, we propose that association of ankyrin and fodrin with the amiloride-sensitive Na^+ channel contributes to the maintenance of the polarized distribution of the Na^+ channel to the apical domain of Na^+-reabsorbing epithelia.

MATERIALS AND METHODS

Materials. Chemicals were obtained from the following sources. Carrier-free $Na^{125}I$ and ^{125}I-labeled protein A were from New England Nuclear; ^{125}I-labeled Bolton–Hunter reagent (≈2000 Ci/mmol; 1 Ci = 37 GBq) was from Amersham; fluorescein isothiocyanate-conjugated goat anti-rabbit IgG was from The Jackson Laboratory; goat anti-rabbit IgG conjugated to colloidal gold was from Janssen; goat anti-rabbit and anti-rat IgG conjugated to alkaline phosphatase were from The Jackson Laboratory; monoclonal anti-actin antibody was from ICN; protein A-bearing *Staphylococci* (Pansorbin cells) were from Calbiochem; and all culture components were from GIBCO. All reagents and standards used in gel electrophoresis and immunoblotting were purchased from Bio-Rad. All other chemicals used were of the highest purity available.

Cell Culture. The A6 cell line, clone A6 2F3, derived from the *Xenopus laevis* kidney, was provided by B. Rossier (University of Lausanne, Lausanne, Switzerland). Stocks were cultured as described (12, 13).

Protein Purification. Isolation and purification of epithelial Na^+ channels were as described (12, 13); however, wheat germ agglutinin affinity chromatography was omitted before size-exclusion HPLC. Ankyrin was purified from Triton X-100-extracted human erythrocyte ghosts by ion-exchange chromatography on DEAE-cellulose and by sedimentation through sucrose gradients (14, 15). Fodrin was purified from pig brain by using low-salt extraction procedures (15) and then gel filtration.

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Preparation of Antibodies. Preparation of rabbit polyclonal antibodies directed against amiloride-sensitive Na^+ channel purified from bovine renal papillae has been described (16). Rat polyclonal anti-amiloride-sensitive Na^+-channel antibodies were prepared by splenic injection of male Wistar rats with amiloride-sensitive Na^+ channel purified from bovine renal papillae. Anti-erythrocyte ankyrin and anti-brain fodrin antibodies were prepared in New Zealand White rabbits. All antibodies were characterized by immunoblotting, immunoprecipitation, and immunocytochemistry.

Cell-Membrane Protein Extraction. Filter-grown A6 cell monolayers were washed twice with the cytoskeletal extraction buffer of Fey *et al.* (17). After being washed, cells were incubated for 5 min in extraction buffer containing 0.5% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride. For immunoelectron microscopic localization of Na^+ channels, preparations were scraped from filters and processed by the protocol of Tousson *et al.* (1). Cytoskeletal residues and solubilized proteins for immunoblot analyses were prepared by the protocol of Nelson and Veshnock (18).

Immunocytochemistry. Confluent A6 cell monolayers were fixed in 3% paraformaldehyde/phosphate-buffered saline (PBS), pH 7.4. Cells were subsequently incubated in PBS/2% bovine serum albumin for 50 min; primary antibodies were diluted 1:20 in PBS/bovine serum albumin for 2 hr, washed in PBS/bovine serum albumin (five times), and then incubated in fluorescein isothiocyanate-conjugated goat anti-rabbit IgG diluted 1:200 (The Jackson Laboratory) in PBS/bovine serum albumin for 1 hr. Cells were washed in PBS (six times at 5 min each) and mounted in glycerol/PBS, 9:1 (vol/vol) containing 0.1% phenylene diamine. For labeling of actin, A6 cell monolayers were incubated in rhodamine–phalloidin diluted 1:20 in PBS for 20 min before final washes and mounting. Monolayers were examined by using a Nikon Diaphot inverted microscope equipped for epifluorescence or by confocal microscopy on a Nikon microscope fitted with a Phoibos 1000 (Sartros, Ypsilanti, MI) confocal laser-scanning microscopy system.

Gel Electrophoresis and Immunoblotting. SDS/PAGE (7% or 8% gels) was accomplished by the method of Laemmli (19). For immunoblotting, proteins were transferred to either nitrocellulose or Immobilon (Millipore) by the method of Towbin *et al.* (20) and probed with antibodies. Immune complexes were detected as described (16).

Coprecipitation of Ankyrin and Sodium Channel. Seven to 15 μg of ankyrin, purified as above and ^{125}I-labeled with Bolton–Hunter reagent (15), was incubated with 5–10 μg of purified Na^+ channel at 4°C for 16 hr. After incubation, either 10 μg of rabbit polyclonal anti-sodium channel antibody or nonimmune rabbit IgG (The Jackson Laboratory) was added, and the samples were mixed at 25°C for 1 hr. One hundred microliters of a 10% suspension of protein A-bearing *Staphylococci* (Pansorbin cells) was then added. After 3 hr, samples were centrifuged for 2 min at 12,000 × *g*, and the resulting pellets were washed, dissolved in electrophoresis sample buffer, and applied onto a 7% or 8% gel. After SDS/PAGE, gels were autoradiographed by using Kodak X-Omat AR film.

Binding of ^{125}I-Labeled Ankyrin to the Na^+ Channel. To examine binding of native ankyrin to the Na^+ channel, the gel-overlay procedure of Morrow *et al.* (21) was used. After separation on SDS/PAGE, Na^+-channel protein was transferred to nitrocellulose, blocked, and incubated with 75 nM purified soluble ^{125}I-labeled ankyrin (2.2×10^7 cpm/nmol) in buffer. After extensive washing, binding of ^{125}I-labeled ankyrin was detected by autoradiography. Control experiments with irrelevant proteins revealed no binding whatsoever.

Preparation of Fluorochrome-Conjugated Antibody. Fifty micrograms of anti-Na^+-channel antibody was labeled with the succinidmidyl ester of 5-(and 6-)carboxytetramethylrhodamine (Molecular Probes). Unbound dye was separated from the conjugate by gel filtration on Sephadex G-50. For preparation of fluorophore-labeled Fab' fragment, the column eluate was concentrated and absorbed to immobilized papain (Pierce); the eluant was recovered and applied to an immobilized protein A column. Labeled Fab' fragments were recovered in the void volume. Concentration and labeling was determined by measuring absorbance at 280 nm and 560 nm.

Fluorescence Photobleach Recovery. A6-bearing coverslips were equilibrated with fluorescently labeled anti-Na^+ channel Fab' at 25°C, and coverslips were washed with isotonic buffer. Lateral-diffusion coefficients (D_L) and mobile fractions (f) of fluorescently labeled sodium channels were measured by the spot photobleaching method on a described instrument (22). Lateral-diffusion coefficients and the mobile fraction were determined by curve-fitting procedures (22). Incomplete recovery of fluorescence on the time scale of the experiments was interpreted as an immobile fraction of fluorophores ($D \leq 1.0 \times 10^{-12}$ cm^2/s). Appropriate controls for nonspecific fluorescence were done by measuring areas devoid of cells, with unlabeled cells, or in cellular areas remote from the apical surface. A sufficiently large number of recordings (>40) were used to analyze and evaluate statistically the differences in measurements between various regions on the A6 epithelial cell surface.

RESULTS

Antibody Characterization. The antibody directed against human erythrocyte ankyrin recognizes partially purified ankyrin, as well as a 210-kDa protein, on immunoblots of crude erythrocyte homogenates. Anti-pig brain fodrin antibody detected fodrin bands on immunoblots at molecular masses of 240 and 235 kDa, which correspond to α- and β-fodrin, as well as a 150-kDa band that represents a proteolytic breakdown product of α-fodrin (23). Rabbit antiserum generated against the bovine amiloride-sensitive Na^+-channel protein recognizes three subunits of the channel—namely, the 315-, 150-, and 95-kDa subunits, and a single subunit of the Na^+ channel purified from A6 cells, the 150-kDa subunit (1, 16). The rat polyclonal anti-Na^+-channel antiserum recognizes the 95- to 110-kDa subunit of the purified A6 channel in immunoblots and is capable of immunoprecipitating the Na^+-channel complex from solubilized A6 membranes. The Na^+-channel antibodies do not show reactivity against purified erythrocyte ankyrin, brain fodrin, or actin (data not shown).

Association of Na^+ Channels with the Cytoskeleton of A6 Cells After Detergent Extraction. In this study, we sought information on the mechanism by which Na^+ channels are localized to the apical membrane. To examine whether the Na^+ channel is associated with the cortical cytoskeleton, A6 epithelial cells were treated with 0.5% Triton X-100 in cytoskeletal extraction buffer under conditions that have been demonstrated to preserve the cortical cytoskeleton and those associated membrane proteins (17, 18, 24, 25). Fig. 1 *A* and *B* show that immunoelectron microscopy of A6 cells labeled with anti-Na^+-channel antibody and colloidal gold-conjugated secondary antibody reveals that, even after extraction with Triton X-100, Na^+ channels remain associated with the cytoskeleton. Immunoblotting of both Triton X-100-insoluble cytoskeleton fraction (Fig. 1*C*) and Triton X-100-solubilized proteins (Fig. 1*D*) with rat polyclonal anti-Na^+-channel antibody indicates that nearly all Na^+ channels are associated with the detergent-insoluble cytoskeleton.

Cosegregation of Na^+ Channel and Cytoskeletal Proteins in A6 Epithelial Cells. Although differential detergent extraction and immunolocalization of Na^+ channels with the detergent-

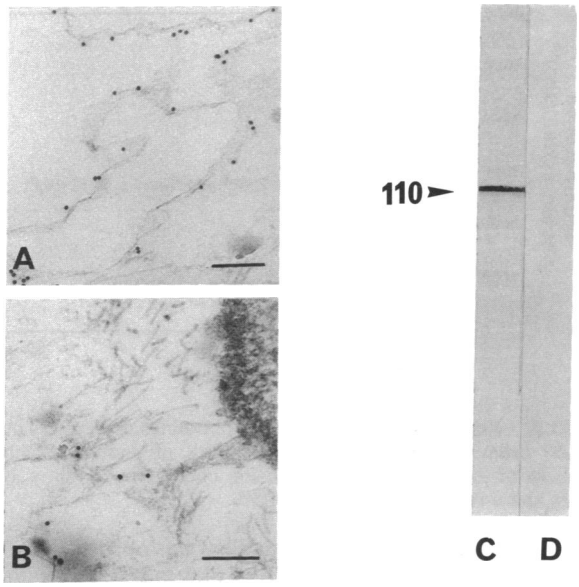

FIG. 1. Transmission electron micrographs of A6 epithelial cells after extraction with 0.5% Triton X-100 and incubation with rabbit anti-Na^+-channel antibody and 10-nm colloidal gold particles conjugated to goat anti-rabbit IgG. (*A* and *B*) Na^+ channels remain associated with detergent-insoluble membrane proteins. (*C* and *D*) Immunoblot analysis of A6 detergent–insoluble and soluble proteins, respectively (10 μg each lane), with rat polyclonal anti-Na^+-channel antibodies; these antibodies only recognize the 110-kDa subunit of the channel.

insoluble fraction suggests an association with the cytoskeleton, we sought to identify specific proteins that cosegregate with the Na^+ channel. The distribution of ankyrin, fodrin, and actin within A6 epithelial cells was examined by indirect immunofluorescence microscopy to determine which are segregated to the apical surface and may colocalize with the Na^+ channel. Staining of A6 cells with rabbit polyclonal antibodies directed against the Na^+ channel shows it is predominantly localized to the apical microvilli (Fig. 2*A*). As shown in Fig. 2*B*, anti-ankyrin antibody stains the apical membrane and microvilli. This antibody also stains the basolateral margins of the cells, which agrees with previous observations for polarized renal epithelial cells (10, 11). Anti-fodrin immunoreactivity is present in the apical membrane and associated microvilli (Fig. 2*C*). Fig. 2*D* shows rhodamine–phallodin staining of F-actin situated in the apical microvilli. The occurrence of ankyrin, fodrin, and actin in the apical microvilli of A6 cells is consistent with previous reports on the distribution of these cytoskeletal proteins in renal epithelia (10, 11, 26). Confocal imaging further showed that an immunoreactive isoform of ankyrin is localized to the apical cell surface as well as to the base of the cells (data not shown).

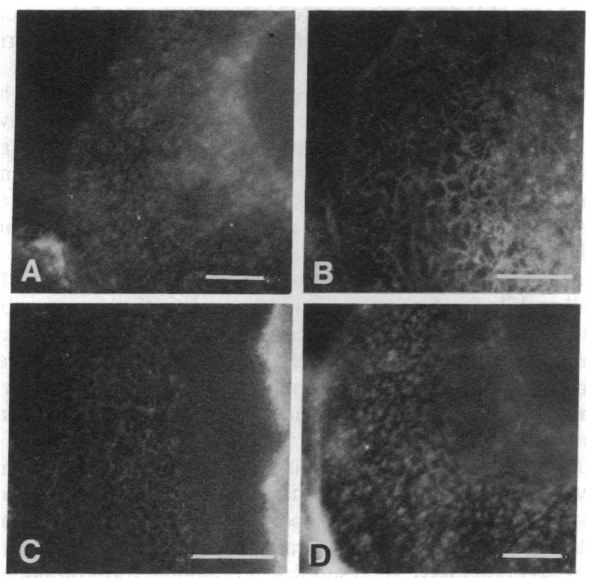

FIG. 2. Immunofluorescence localization of amiloride-sensitive Na^+ channels (*A*), ankyrin (*B*), fodrin (*C*), and F-actin (*D*) in A6 renal epithelial cells. Rhodamine–phalloidin was used to visualize F-actin. In all cases, staining is predominately localized to the apical microvilli. Perinuclear staining is nonspecific, as it is seen with the secondary antibody alone (data not shown). (Bars = 10 μm.)

Immunoblot Analysis of Purified Bovine Na^+ Channel. To determine whether ankyrin, fodrin, or actin copurify with the Na^+ channel, solubilized, partially purified bovine renal Na^+-channel preparations were examined by immunoblotting with anti-ankyrin, anti-fodrin, and anti-actin antibodies. Immunoblot analysis of HPLC-purified bovine renal Na^+ channel with these specific probes identifies ankyrin, fodrin, and actin (Fig. 3). The antibody directed against erythrocyte isoform of ankyrin recognizes a 120-kDa proteolytic product of brain ankyrin in the channel preparation (Fig. 3, lane 2), whereas anti-brain fodrin antiserum identifies the 240-kDa α subunit as well as its 150- and 70-kDa proteolytic products (23, 27) (Fig. 3, lane 3). Examination of channel preparations with a monoclonal anti-actin antibody reveals that actin also coisolates with the bovine Na^+ channel (Fig. 3, lane 4). Immunoblot analysis of amiloride-sensitive Na^+ channel purified from A6 cells also reveals the same M_r polypeptides as the bovine Na^+ channel (data not shown). Inclusion of wheat germ agglutinin chromatography in the purification of the bovine renal Na^+ channel before HPLC chromatography dissociates actin from fodrin, suggesting that actin is not likely directly associated with the channel.

Coprecipitation of Ankyrin with the Na^+ Channel. Although immunoblot analysis suggests that these proteins coisolate with the Na^+ channel, we examined whether ankyrin is directly associated with the channel by immunoprecipitation of the complex with anti-Na^+-channel antibodies. Purified native Na^+-channel protein was incubated with ^{125}I-labeled

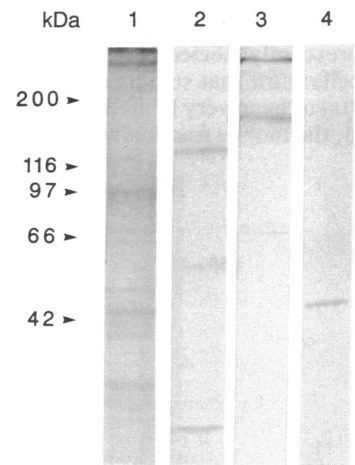

FIG. 3. SDS/PAGE and immunoblotting of partially purified bovine renal papilla amiloride-sensitive Na^+-channel preparation (15 μg of Na^+ channel per lane, SDS/8% PAGE). Lanes: 1, Coomassie blue-stained partially purified Na^+ channel; 2, anti-ankyrin antibody (1:20 dilution) recognizes the 120-kDa and a 29-kDa subunit; 3, anti-fodrin antibody (1:20 dilution) recognizes the 240-kDa α subunit and the 150- and 70-kDa proteolytic fragments of fodrin; 4, monoclonal anti-actin antibody (1:1000 dilution) recognizes only actin (43 kDa).

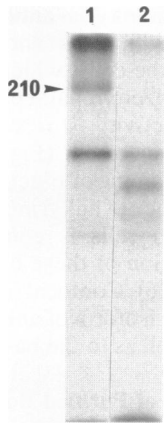

FIG. 4. Immunoprecipitation of ^{125}I-labeled purified ankyrin bound to bovine Na^+-channel complex by anti-Na^+ channel antibodies. ^{125}I-labeled ankyrin was incubated with partially purified bovine Na^+ channel for 16 hr, and the ^{125}I-labeled ankyrin–channel complex was immunoprecipitated with anti-Na^+-channel antibodies and Pansorbin cells (SDS/8% PAGE). Autoradiograph shows the ^{125}I-labeled ankyrin (210 kDa) coprecipitated by the anti-Na^+ channel antibody (lane 1). Lane 2 shows an autoradiograph of partially purified bovine Na^+ channel incubated with ^{125}I-labeled ankyrin and immunoprecipitated with nonimmune IgG and Pansorbin cells.

purified, nondenatured erythrocyte ankyrin followed by immunoprecipitation with rabbit anti-Na^+-channel antibody. Indeed, only with Na^+-channel protein was ^{125}I-labeled ankyrin (210-kDa protein) immunoprecipitated (Fig. 4, lane 1). Control experiments showed that ankyrin was not precipitated with either nonimmune IgG (Fig. 4, lane 2) or when Na^+ channel was omitted (data not shown).

Ankyrin Binds Directly to the Amiloride-Binding Subunit of the Na^+ Channel. Copurification and coprecipitation of ankyrin with the Na^+ channel suggests that ankyrin associates with the channel. To determine which subunit of the Na^+-channel protein participates in the association, we examined binding of ^{125}I-labeled native erythrocyte ankyrin to purified Na^+ channel. Fig. 5 shows that when purified Na^+ channel was overlaid with ^{125}I-labeled ankyrin, ankyrin bound only to the 150-kDa polypeptide, which corresponds to the amiloride-binding subunit (28).

Na^+-Channel Lateral Mobility. Measurements of the lateral mobility of fluorescently labeled Na^+ channels on the apical surface of A6 cells show that sodium channels are immobile ($D < 10^{-12}$ cm^2/s) or have very limited mobility. In almost all cases examined, the mobile fraction was very low (12 ± 5%,

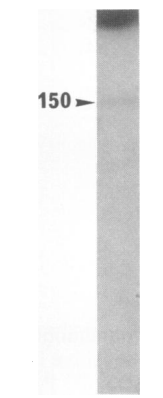

FIG. 5. Autoradiograph of direct binding of ^{125}I-labeled purified, native ankyrin to partially purified bovine Na^+-channel protein. Fifteen micrograms of Na^+ channel was subjected to SDS/8% PAGE, transferred to nitrocellulose, and incubated with ^{125}I-labeled ankyrin. Ankyrin binds only to the 150-kDa subunit of the channel.

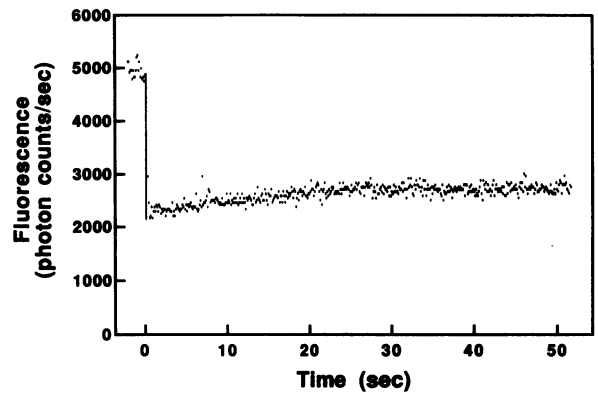

FIG. 6. Representative fluorescence photobleach recovery curve on A6 cells, demonstrating slow mobility of sodium channels. Analysis of the curve yields a diffusion coefficient of 6.8×10^{-11} cm^2/s and a recoverable fraction of 5%.

$n = 21$). In those cases where mobility was observed, rate of lateral mobility was also slow [$D_L = (4.3 \pm 2) \times 10^{-11}$ cm^2/s, $n = 21$; Fig. 6]. This mobility does not reflect the geometrical properties of the apical cell membrane because the mobilities of fluorescently labeled phospholipids have unrestricted lateral mobility in the same domain (10^{-8} cm^2/s). Thus, it appears that some elements are responsible for the immobility of sodium channels at the apical cell surface.

We also examined the effects of colchicine (10^{-5} M) and cytochalasin B (10 μg/ml), agents known to affect cytoplasmic microtubules and microfilaments. There were no differences in either the measured diffusion coefficient or fractional recovery in any region, indicating that these elements are probably not directly associated or involved in controlling sodium channel lateral mobility.

DISCUSSION

We present evidence that the Na^+ channel remains associated with the detergent-insoluble cytoskeleton and may serve to localize and maintain the channel at the apical membrane. Further, the Na^+ channel and erythroid form of ankyrin and brain form of fodrin cosegregate to the microvilli of the apical membrane domain in renal epithelial cells, and both ankyrin and fodrin copurify with Na^+ channel. This interaction is specific and is mediated through the 150-kDa subunit of the Na^+ channel. These data suggest that the amiloride-sensitive Na^+ channel interacts with ankyrin and fodrin to maintain its polarized distribution to the microvilli of the apical membrane domain in polarized renal epithelial cells.

Measurements of the lateral mobility by fluorescence photobleach recovery of fluorescently labeled Na^+ channels in A6 cells show that these channels have limited mobility. The small mobile fraction seen could be due to either a reversible equilibrium of bound and free channels or channels that are confined within the microdomain of the underlying cytoskeletal matrix. Thus, some elements like fodrin and ankyrin apparently serve to restrict the lateral mobility of the Na^+ channel.

Our studies have shown that there is a direct association between ankyrin and the amiloride-sensitive Na^+ channel. Previous studies in polarized transporting epithelial cells with antibodies generated against preparations of ankyrin from erythrocytes and brain have reported that a brain-like form of ankyrin can be found at both apical and basolateral membranes, whereas an erythrocyte-like form is localized primarily in the basolateral membrane (29, 30). Recent information from cloning of erythrocyte ankyrin has shown that, in addition to there being two immunologically distinct ankyrins of different molecular masses, alternative splicing generates several more forms expressed in

the same cell (31, 32). Even though the antibody used in this study was generated against erythrocyte ankyrin, it reacts strongly and with restricted specificity with neuronal cells (K.J.A., unpublished work). Our observations of an apical distribution of ankyrin in epithelial cells, in addition to the recognition of erythrocyte ankyrin in immunoblots, suggest that this antibody recognizes a specific epitope expressed by an apically restricted isoform of ankyrin.

In preparations of partially purified Na^+ channel, the 210-kDa polypeptide of ankyrin was not detected in immunoblots, even though protease inhibitors were included in the purification of the Na^+ channel. However, we consistently observed the 120-kDa proteolytic product (11). We believe that this result is significant because the proteolytically processed 120- to 140-kDa form of ankyrin has a substantially higher affinity to Na^+/K^+-ATPase and other proteins. Indeed, this form may be the form preferentially localized and segregated to the apical domain and revealed by anti-ankyrin immunocytochemistry.

Although immunoblotting does not prove that ankyrin directly associates with the channel, we have shown that native ankyrin binds directly to the channel by: (*i*) coimmunoprecipitation of ^{125}I-labeled ankyrin with the Na^+ channel via anti-Na^+ channel antibodies, and (*ii*) binding of ^{125}I-labeled ankyrin only to the 150-kDa subunit of purified Na^+ channel. Among the channel subunits, the 150-kDa subunit is a glycosylated, transmembrane polypeptide that binds amiloride (28) and may have ion-conductance properties (33).

Linkage of the epithelial Na^+ channel to ankyrin, fodrin, and actin suggests a possible mechanism to maintain the polarized distribution of Na^+ channels to the microvilli of the apical membrane in polarized renal cells. The findings of this study also have broader implications with respect to the maintenance of epithelial polarity, as they provide evidence supporting a role of ankyrin and fodrin in maintaining the distribution of an integral-membrane protein to the apical domain of polarized epithelial cells (7). Although an association between the actin component of the cytoskeleton and apically situated integral membrane proteins in renal epithelial cells has been documented (24, 25), proteins that link actin to apically situated integral membrane proteins were heretofore unknown.

As has been proposed for Na^+/K^+-ATPase (4) and the voltage-dependent Na^+ channel (34), the amiloride-sensitive channel becomes part of a large interlinked molecular complex through its interactions with ankyrin and fodrin and, thus, may provide a mechanism to sequester and accumulate the Na^+ channel at the apical surface and to regulate its lateral mobility. In light of the direct interactions between the Na^+ channel and the cytoskeleton, it is plausible that this interaction also plays a role in the biogenesis of the polarized distribution of the channel in the apical membrane.

In summary, we present evidence that the amiloride-sensitive Na^+ channel is linked to the apical cytoskeleton in renal epithelial cells through the binding of ankyrin to the 150-kDa subunit of the channel. Maintenance of amiloride-sensitive Na^+ channels in the apical membrane is an essential feature for electrogenic Na^+ transport in Na^+-reabsorbing epithelia.

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