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Distribution of intermediate filaments in epithelial cells of the amphibian urinary bladder: An immunofluorescence study

Distribución de filamentos intermedios en células epiteliales de vejiga urinaria de anfibio

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The distribution of intermediate filaments in toad and frog urinary bladder was studied on frozen sections by indirect immunofluorescence microscopy using specific antiprekeratin antibodies.

Our results show that in both species, epithelial cells lining the urinary bladder are very rich in cytokeratin, organized as a filamentous network. In granular cells, the most abundant cells facing the urinary lumen, vasopressin promotes fusion of the membranous tubular structures located in the luminal cytoplasm with the apical cell membrane. A role for intermediate filaments in the membrane rearrangements induced by vasopressin in these cells, is proposed.

Amphibian urinary bladders have been extensively used as a model for studying the mechanism of action of vasopressin. In granular cells, the most abundant cells facing the urinary lumen (Rossier *et al.*, 1979), vasopressin induces an increase in water permeability by stimulating the fusion of tubular structures, located in the luminal cytoplasm, with the apical cell membrane. These tubular structures carry aggregates of intramembranous particles which seem to be the sites for vasopressin induced water flow (Chevalier *et al.*, 1974; Humbert *et al.*, 1977; Kachadorian *et al.*, 1977; Muller *et al.*, 1980; Pearl and Taylor, 1985).

Considerable experimental evidence shows that cytoskeletal elements are involved in the reorganization of the apical region of granular cells, that leads to an increase in water flow. The presence of microtubules, microfilaments and 10 nm filaments in the fibrillar network that frames the apical cytoplasm of these cells has been established by ultrastructural studies (Kachadorian *et al.*, 1979; Pearl and Taylor, 1983, 1985; Sasaki *et al.*, 1984). Immunofluorescence studies and decoration with heavy meromyosin have confirmed that actin microfilaments are important components of this network (Davis *et al.*, 1985; Pearl and Taylor, 1983). At the sale time, a role for actin microfilaments and microtubules in transcellular water flow has been established using pharmacological agents (Davis *et al.*, 1974; DeSouza *et al.*, 1974; Grosso *et al.*, 1978; Pearl and Taylor, 1985; Taylor *et al.*, 1973). On the other hand, although 10 nm filaments have been described in granular and basal cells (Davis *et al.*, 1985; Kraehenbuhl *et al.*, 1979; Pearl and Taylor, 1983; Sasaki *et al.*, 1984) no direct evidence of the presence and distribution of intermediate filaments (IF) in epithelial cells of amphibia urinary bladder has been presented so far. Thus, studies were undertaken to establish whether IF are present in toad and frog urinary bladder and their distribution. Since it has been shown that epithelial cells are characterized by IF containing proteins related to epidermal keratins (cytokeratins) (Lazarides, 1982; Steinert, 1985) indirect immunofluorescence microscopy using specific antiprekeratin antibodies was applied to study the organization of IF in these cells.

MATERIAL AND METHODS

Experiments were performed in toad (*Bufo spinulosus*) and clawed frog (*Xenopus laevis*) urinary bladders. Bladders were removed from

pithed animals and quickly frozen in liquid nitrogen after immersion in M-1 embedding matrix (Lipshaw). 5 μm cryostat sections were fixed and stained as previously described (Dabiké *et al.*, 1981), using specific antibodies against purified bovine hoof prekeratin. Fluorescein isothiocyanate (FITC)-labeled goat antibodies against rabbit IgG (Sigma) were used as second antibodies. Controls for indirect immunofluorescence staining included substituting the immune sera by preimmune sera, absorption of the immune sera with the purified antigen and staining of the cells with FITC-labeled antibody only.

RESULTS

At low magnification, the specific staining of both toad and frog urinary bladder epithelial cells was clearly visualized (Fig. 1 a-b). The underlying connective tissue and muscle cells, were not stained. At higher magnifications, the apical region of the cells lining the urinary space showed a strong decoration. The staining appeared framing a polygonal lattice (Fig. 2 a-b). The rim of cytoplasm adjacent to the lateral and basal borders of these cells was also stained. Tangential sections through this region of the cells showed that cytokeratin present in the lateral borders is also organized as a meshwork (Fig. 3 a-b). The perinuclear cytoplasm, visualized in sections traversing the center of the cells, appeared unstained (Fig. 4 a-b). In both species, the immunoreactivity of the basal cells appeared concentrated in the peripheral region (Fig. 5 a-b).

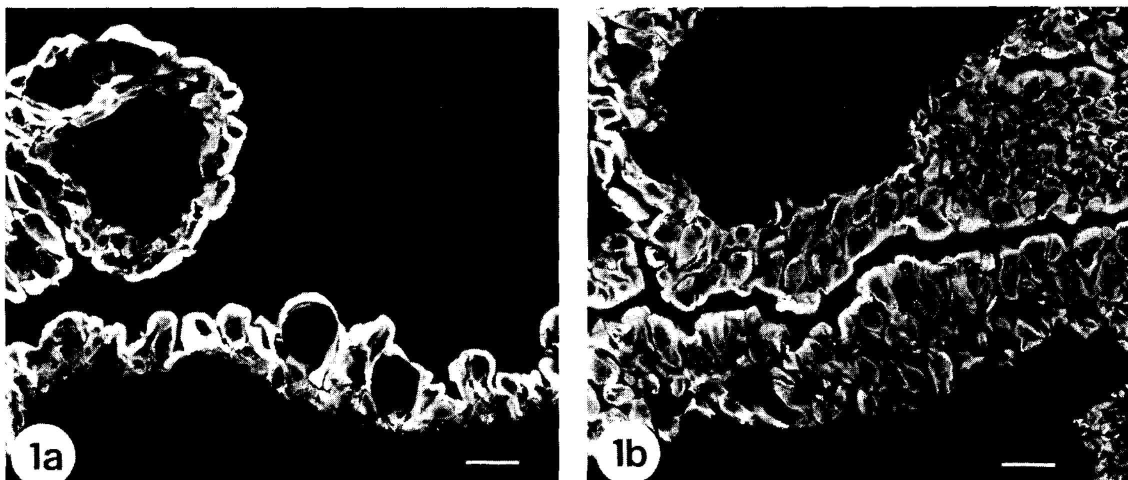

Fig. 1: Micrograph of a section of a) toad and b) frog urinary bladder. Specific decoration of the cells present in the epithelium that lines the urinary lumen, is observed. The underlying connective tissue and muscle cells do not stain. a: x 370; b: x 370. Bars represent 20 μm.

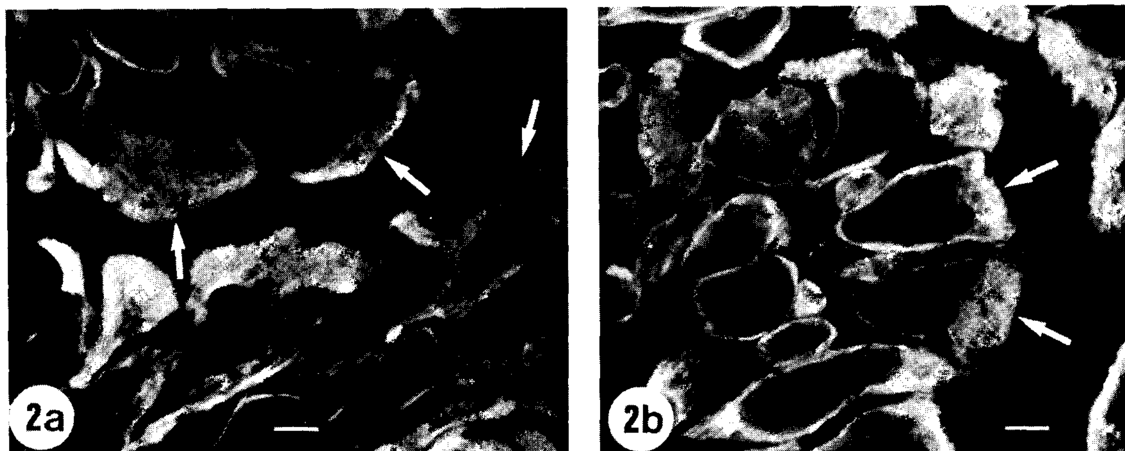

Fig. 2: Frozen sections of a) toad and b) frog urinary bladder. The meshwork-like organization of cytokeratin present in the apical region of the cells facing the urinary lumen (arrows), is clearly visualized. a: x 1400; b: x 1400. Bars represent 5 μm.

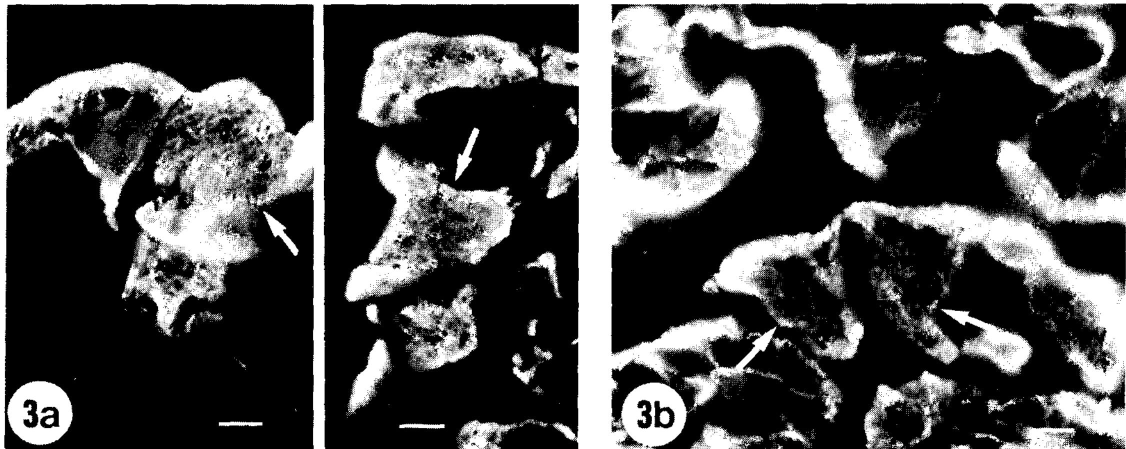

Fig. 3: Immunofluorescence staining of a) toad and b) frog urinary bladder with antiprekeratin antibodies. In tangential sections through the lateral border of cells linning the urinary lumen, the fine fibrillar polygonal lattice framed by cytokeratin (arrows), is clearly evidenced. The apical border of these cells appears strongly stained. a: x 1400; b: x 1400. Bars represent 5 μm.

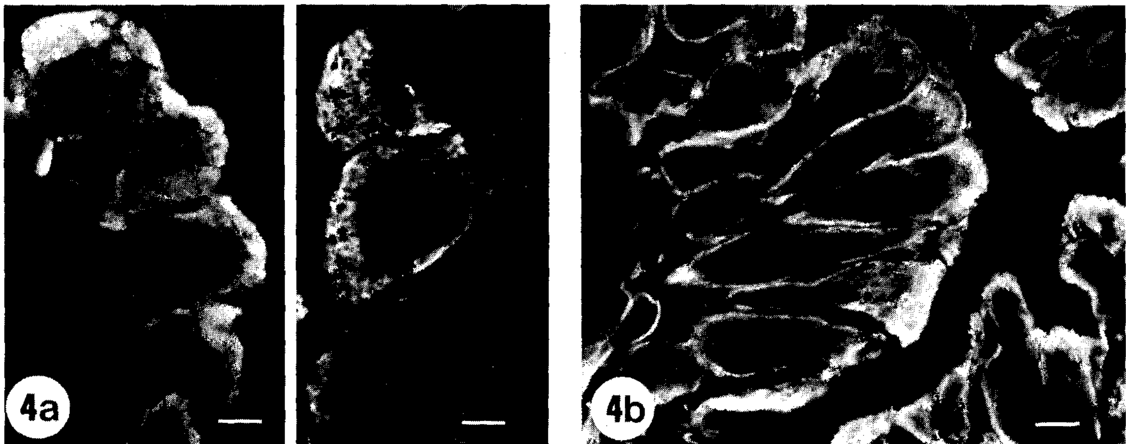

Fig. 4: Frozen sections of a) toad and b) frog urinary bladder. While the apical region of the cells facing the urinary lumen appears strongly decorated, the perinuclear cytoplasm does not stain. a: x 1400; b: x 1400. Bars represent 5 μm.

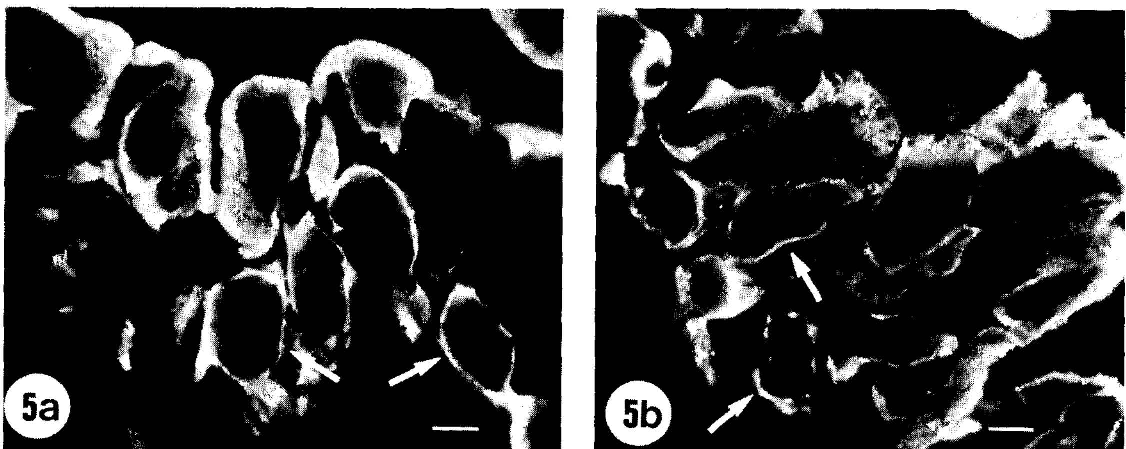

Fig. 5: Frozen sections of a) toad and b) frog urinary bladder. The lateral borders of the basal cells, facing the basal laminae, appear deeply stained (arrows). a: x 1400; b: x 1400. Bars represent 5 μm.

DISCUSSION

Our results show that epithelial cells lining the toad and frog urinary bladder are very rich in cytokeratin organized as a filamentous network. Studies performed in other amphibian species, have established that more than 50% of the cells that form the continuous monolayer that faces the urinary lumen correspond to granular cells (Rossier *et al.*, 1979). These cells contain a highly crosslinked network of actin microfilaments below the luminal plasma membrane (Kraehenbuhl *et al.*, 1979; Pearl and Taylor, 1983, 1985; Sasaki *et al.*, 1984). At the same time, prominent 10 nm filaments running along the subcortical cytoplasm have been described in this region of the cell (Davis *et al.*, 1985; Pearl and Taylor, 1983; Sasaki *et al.*, 1984). In microfilament-rich cells, the majority of the cells facing the basal laminae, bundles of 10 nm filaments that run parallel to the plane of the epithelium, have also been found (Kraehenbuhl *et al.*, 1979). The results presented here, confirm that cells present in the epithelium of amphibia urinary bladder contain abundant IF immunologically related to keratin. Though IF have been extensively studied during the last decade, they still represent structures whose biological function is unknown. In various H^+ secreting epithelia, cells that contain a system of endomembranes that carry specific channels or pumps held in reserve beneath the apical membrane, have been described. These are inserted into the luminal surface in response to appropriate stimuli. When the stimulus ceases, the vesicular membrane is removed to reform subplasmalemmal vesicles (Al-Awqati *et al.*, 1983; Diamond and Machen, 1983; Helander, 1981; Koenig, 1984; Lewis, 1983; Lewis and Moura, 1984; Madsen and Tisher, 1985; Vial and Orrego, 1960). Among these, hydrochloric acid secreting cells are the ones that experience the most striking changes in relation to secretion. In fact, vertebrate oxyntic cells undergo extensive membrane rearrangements in association with their capacity to secrete acid. During stimulation of secretory activity, the membranes of the tubular system fuse with the luminal membrane, incorporating the H^+–K^+ ATPase to the secretory surface (Koenig, 1984; Vial and Orrego, 1960). Microtubules and actin microfilaments have been implicated in the morphological changes experienced by oxyntic cells (Black *et al.*, 1982; Dabiké *et al.*, 1986; Davis *et al.*, 1974; Kasbekar and Gordon, 1979; Stewart and Kasbekar, 1981; Wolosin *et al.*, 1983). At the same time, a role for IF in these changes in shape has been suggested. It has been proposed that the developed system of IF present in oxyntic cells provides a fixed plane for the anchoring of actin microfilaments and associated proteins, probably responsible for the ultrastructural changes of the secretory pole (Dabiké *et al.*, 1981; Dabiké and Koenig, 1983). In epithelial cells that line the intestinal lumen ultrastructural studies have shown that IF form part of the elaborated terminal web that anchors the microvillar rootlets. These IF seem to frame a platform for the rest of the cytoskeletal elements probably related to microvillar movement (Hull and Staehelin, 1979; Matsudaira and Burgess, 1982). In amphibian urinary bladder granular cells, vasopressin stimulates the fusion of tubular structures, located in the luminal cytoplasm, with the cell membrane. These tubular structures carry aggregates of intramembranous particles which are exposed to the surface by fusion with the membrane (Humbert *et al.*, 1977; Kachadorian *et al.*, 1977; Muller *et al.*, 1980; Sasaki *et al.*, 1984). The importance of microtubules and microfilaments in the hydroosmotic action of the hormone has been established using cytoskeletal inhibitors. These prevent the appearance of particle aggregates in the apical membrane of granular cells and inhibit hormone-stimulated water flow (Chevalier *et al.*, 1974; Davis *et al.*, 1974; DeSouza *et al.*, 1974; Grosso *et al.*, 1978; Kachadorian *et al.*, 1979; Pearl and Taylor, 1983, 1985; Taylor *et al.*, 1973). The expansion of the apical pole of toad granular cells, induced by vasopressin, seems to be related with a relaxation of this region of the cell (DiBona, 1981 a, b). The

subluminal network of actin along with horizontally running filaments in the terminal web, would provide the basis for such an expansion. Freeze fracture studies have shown that in resting cells, the tubules are anchored in a cytoskeletal matrix and that they retain their anchorage after fusion induced by vasopressin. It has been suggested that this hormone induces an angulation of the tubules with an end undergoing fusion with the luminal membrane while the other end remains anchored to the matrix (Ding *et al.*, 1985; Sasaki *et al.*, 1984). Since IF are remarkably stable in physiological conditions, it is probable that this system of filaments anchors the tubules while they undergo the angulation process required for fusion. On the other hand, actin microfilaments assembled in response to antidiuretic hormone and/or microtubules (Davis *et al.*, 1985; Pearl and Taylor, 1985) would perform the work necessary for the displacement of the membranous elements.

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