DISTRIBUTION OF F-ACTIN IN THE TUBULAR CELLS OF THE DOGFISH (SQUALUS ACANTHIAS) RECTAL GLAND. John W. Mills, Elise Saks, and Arnost Kleinzeller, Dept. of Anatomy, Dartmouth Medical School, Hanover, New Hampshire, and Dept. of Physiology, University of Pennsylvania, Philadelphia, Pennsylvania.

When exposed to a high K^+ media dogfish rectal gland cells swell and undergo a marked alteration in morphology. Upon return to standard Na^+-containing media the cells recover normal volume and morphology. Microfilaments appear to play a role in morphological recovery since cells treated with cytochalasins throughout the swelling and recovery periods, although returning to a normal volume, are markedly vesiculated (Mills and Kleinzeller, Bull. Mt. Desert Isl. Biol. Lab. 25:50-53, 1985). This vesiculation process appears similar to that reported in rat liver cells after recovery from swelling at 1°C (van Rossum and Russo, J. Membrane Biol. 59:191-209, 1981).

In order to further investigate the role of actin-containing microfilaments in cellular morphology and volume control processes, it was first important to determine the distribution of actin within the shark rectal gland cell. To do this we used the F-actin specific probe nitrobenzoxadiazole-phallacidin (NBD-PH). Phallacidin has a high affinity for filamentous actin and when coupled to nitrobenzoxadiazole the binding sites can be observed in the fluorescent microscope. Rectal gland slices were fixed in shark Ringer's containing 4% formaldehyde. The slices were washed in shark Ringer's and then frozen onto a cryostat specimen holder. Six micron sections were cut at -15°C and picked up on gelatin-coated coverslips and then incubated for twenty minutes in Ringer's containing 33ng/ml of NBD-PH. The sections were washed for five minutes and mounted on glass slides in a 1:1 mixture of PBS and glycerol. The tissue sections were viewed and photographed in a Zeiss photomicroscope equipped with epifluorescent optics. The following controls for specificity were also run. 1) Sections were exposed to unlabelled phalloidin (200ng/ml) for twenty minutes prior to exposure to NBD-PH. 2) The NBD-PH was first exposed to purified F-actin isolated from skeletal muscle and then the sections were incubated in this solution for twenty minutes.

The distribution of F-actin, as revealed by NBD-PH fluorescence, is shown in Figure 1. The brightest areas are along the apical membrane and the basolateral membranes up to the area of the tight junctions. The cytoplasm, when seen relatively far from the extensive membrane folds, showed little fluorescence above background. The localization to the basolateral membrane can best be seen in Figure 1b where a section has been cut tangential to the base of a rectal gland tubule. Here the many folds of the basolateral membrane are highlighted by the binding of NBD-PH. The membrane-associated fluorescence was eliminated by both of the controls for specificity.

Ultrastructural analysis of the rectal gland reveals that microfilaments can be seen in the microvilli that are present on the apical surface of the tubular cells (Fig. 2a). Resolution of microfilaments along the basolateral membranes was difficult to obtain. However, in some cases where the lateral folds of adjacent cells were highly ordered (Fig. 2b), there was an indication of filamentous material.

These results show that F-actin has a specific distribution within the tubular cells of the shark rectal gland. The localization close to both the apical and basolateral plasma membrane surfaces supports the hypothesis that this cytoskeletal element may play a role in membrane-associated events such as ion transport and vesicle insertion and retrieval.

Figure 1. Fluorescent micrographs showing the distribution of NBD-PH in the tubule cells of the shark rectal gland. X640. (a) Cross-section through tubule showing both apical and basolateral surfaces. Bright fluorescence can be seen on the apical membrane lining the lumen. Also notice intense fluorescence along lateral membranes from just below the area of the light junction to the base of the cell (arrowheads). Brightly fluorescent granules (arrow) are autofluorescent cellular inclusions. (b) Section through tubule that includes basolateral surface (right side of micrograph). Basolateral folds of the plasma membrane are highlighted by the NBD-PH fluorescence (arrowhead).

Figure 2. Electron micrographs of shark rectal gland cells. (a) Apical surface. Microvilli contain core filaments (arrow). X48,000. (b) Basolateral folds of adjacent cells. Filamentous material can be observed in some of the folds (arrow). X25,000.