

# Localization of Actin in the Mitochondria Rich Cells of Toad Skin

T. A. Whalen<sup>1</sup> and John W. Mills<sup>2</sup>

The epithelium of the toad is comprised of 7-8 cell layers. The outermost layer is the keratinized stratum corneum. Deep to this layer is the stratum granulosum, the outermost living cell layer, followed by several layers of the stratum spinosum. The basal layer of the epidermis is the stratum germinativum. These cells are referred to as the principal cells (PC) of the epidermis. The PCs regulate water absorption as well as ion transport. The cells of the s. granulosum are closely associated through tight junctions that establish a barrier to unregulated flux of water and ions. This allows the toad the ability to regulate the absorption of water solely through channels and pores. The PC also accomplish Na<sup>+</sup> transport. From the outermost living cell layer Na<sup>+</sup> can pass through to adjoining cells passively, through intercellular junctions, or be actively extruded to the basal side of the cell by means of a Na<sup>+</sup>-K-ATPase pump.

Mitochondria rich cells (MRC's) make up 1% - 5% of the epidermis and have a distinct flask shape. In addition to their shape, they are characterized by the large preponderance of mitochondria in their apical cytoplasm. Flask cells originate in the basal layer and migrate outwards. The apical surface of the mature flask cell is located at the level of the subcorneal space with its basolateral side located in the stratum spinosum. The MRC's regulate Cl<sup>-</sup> transport in the epithelium. This is accomplished through both passive and facilitated diffusion. The MRC utilizes a number of mechanisms to accomplish this transport. Through the hydrolysis of CO<sub>2</sub>, H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> are produced. H<sup>+</sup> is subsequently pumped out the apical side of the cell by means of a proton pump. Cl<sup>-</sup> is passively exchanged for HCO<sub>3</sub><sup>-</sup>. In addition, Cl<sup>-</sup> and Na<sup>+</sup> may enter passively or the Na<sup>+</sup> may enter by means of a Na<sup>+</sup> - H<sup>+</sup> exchanger.

In an attempt to isolate these MRC's from the rest of the epithelial cells it was inadvertently discovered that the flask cells retained their shape whereas the PC became rounded. Cell shape is thought to be a property of the actin filament system and cell isolation techniques are known to

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<sup>1</sup> Class of 2000, Biology, Poster Presentation

<sup>2</sup> Professor, Clarkson University

disrupt actin. Thus in MRC it may be that: 1. Actin is, uniquely, unaffected by the isolation technique, or 2. Some other protein in the MRC cytoskeleton is responsible for cell shape. In order to answer this question we first needed to determine the presence and organization of F-actin in MRC.

Using standard immunocytochemical procedures, fluorescein phalloidin and rhodamine DNase, were utilized in the localization of F-actin and G-actin, respectively. The localization of phalloidin revealed the presence of F-actin along all the membrane surfaces of the PC. This is a ubiquitous finding in epithelia. In MRC F-actin fluoresced as tuft like protrusions from the flask cells apical surface. The basolateral membrane was relatively devoid of F-actin. G-actin was also visible on the apical surface and diffusely distributed throughout the cytoplasm. These results indicate that the MRC is a unique epithelial cell in that F-actin is absent from the basolateral surface. The results also indicate that some other protein must play a role in maintenance of cell shape.