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An electronmicroscopic study of microtubules in the development of marginal cells of the mouse stria vascularis *

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This study provides an ultrastructural evaluation of cytoplasmic microtubules during the maturation of marginal cells of the mouse stria vascularis. Postnatal marginal cells are cuboidal in structure and over a period of days develop the numerous cellular processes typical of transporting epithelia. Immediately after birth, marginal cells contain numerous microtubules randomly oriented throughout the cytoplasm. Golgi bodies and vesicles are also abundant. The initiation of cellular extension is characterized by the presence of sheet-like extensions of plasma membrane about areas of the cell periphery. Subsequently, large organelle-containing processes form, whose plasmalemma appear pleated due to the presence of the above mentioned sheet-like extensions. Typically, these processes contain microtubules oriented parallel to the direction of their extension. Within these processes, microtubules are closely associated with organelles, such as mitochondria, and may function to displace and stratify these organelles within the processes. The number and length of microtubules increase as the processes grow larger. In the adult mouse, the strial marginal cell processes are attenuated and contain almost exclusively microtubules and mitochondria. The membrane pleats unfold, apparently to provide plasma membrane for cellular extension. The data strongly implicate microtubules in strial development. Furthermore, it is suggested that the depolymerization of microtubules in the adult may underlie strial atrophy.

Key words: strial marginal cells; microtubules; development.

Introduction

Cell surface folding is the single common anatomic feature of transporting epithelia [1]. Such folding greatly expands the cell membrane surface area, thereby providing an enhanced ability for membrane transport. The stria vascularis displays numerous interdigitations among its cell components [7,15]. The establishment of this adult strial architecture is prerequisite for the development of the endolymph's ionic composition and electrical potential and, in turn, for normal cochlear function [2,5,8]. Kikuchi and Hilding [9] have shown that the adult stria evolves from a single

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layer of cuboidal marginal cells separated from underlying fibroblasts by a basement membrane. After varying periods of time, depending upon the position along the length of the cochlear duct, the numerous interdigitations characteristic of the adult stria are formed. However, the basement membrane is absent from beneath the marginal cells. The fully differentiated form of the stria vascularis is dependent upon a process of cellular extension.

Recently, Santos-Sacchi [13] noted the abundance of microtubules within the cytoplasm of adult mouse marginal cells and suggested a possible role for microtubules in strial maturation. Microtubules have been shown to be involved in cell shape development. For example, Heywood et al. [6] provided morphologic evidence that microtubules influence cell shape during in vitro development of vestibular hair cells. The present study offers a morphologic evaluation of marginal cell cytoplasmic microtubules during the development of strial architecture.

Methods

The preservation of microtubules necessitates the use of aldehyde fixatives; unfortunately, glutaraldehyde fixation of strial tissue induces artifacts such as mitochondrial vacuolation [10,14].

Mice (Balb/cJ) at varying stages from newborn (0, 2, 4, 6, 8, 10 days postnatal) to adult were decapitated, the cochleae dissected out, fractured open and immersed in either 2% or 6% glutaraldehyde buffered with cacodylate (0.1 M), pH 7.4, at room temperature. After 2 h primary fixation, strial tissue from various cochlear turns was microdissected free and post-fixed in cacodylate-buffered 1% osmium tetroxide, pH 7.4, at 0–4°C for 2 h. Samples were rinsed with buffer, rapidly dehydrated in a graded series of acetone, and embedded in Epon at 60°C overnight. Thick sections were cut and stained with toluidine blue for orientation purposes. Gray-silver sections (50–80 nm) were cut with a diamond knife on an LKB Ultratome, stained with lead citrate, and examined with a Zeiss 9S-2 or Philips 300 electron microscope.

Results

Although the onset of marginal cell development proceeds from base to apex over an extended period of time [9], the cellular mechanisms involved are the same regardless of location along the cochlear duct. Areas of transition between developing and immature marginal cells along the duct were chosen for study. As such, marginal cells are characterized by the degree of maturation and not by their location along the duct or by the age of the particular animal.

Prior to the evolution of adult strial structure, marginal cells can be distinguished within the cochlear duct by virtue of their dark cytoplasm. Whereas future intermediate and basal cells generally contain few organelles at this time, marginal cells possess numerous structural elements. Particularly prominent are the Golgi bodies (Fig 1). Microtubules are present in immature cuboidal marginal cells at birth. They

are randomly distributed within the cytoplasm (Fig. 1). This random orientation is preserved in the apical cytoplasm through adulthood, although occasionally microtubules are found perpendicular to the apical plasma membrane in the adult [13].

The initiation of marginal cell development is characterized by the production of small cellular extensions, about 60 nm in width, within which microfilaments may sometimes be distinguished. These extensions occur laterally as well as basally (Figs. 2, 3). Serial sectioning demonstrates that they are sheet-like. Subsequently, large organelle-containing cell processes extend basally (Figs. 2, 3). The plasmalemma of these large processes often appears pleated due to the presence of the smaller sheet-like extensions which decorate the perimeter of the large processes (Fig. 3). Within the processes microtubules are invariably found, frequently oriented parallel to the longitudinal axes of the processes. As the processes increase in size, the number and length of microtubules increase. In fact, microtubules can often be observed coursing for several micrometers within developing cell elongations.

The basement membrane which is present between the cuboidal marginal cells

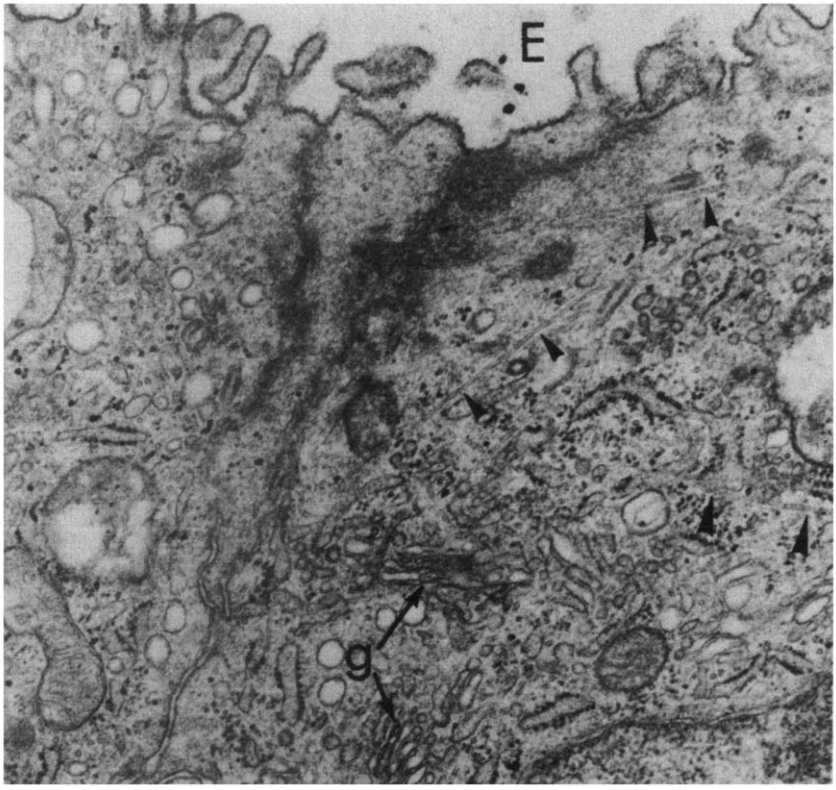

Fig. 1. Cytoplasm of immature marginal cells displaying numerous Golgi bodies (g), vesicles and endoplasmic reticulum. Arrows indicate randomly oriented microtubules. E, endolymphatic space. ×20000.

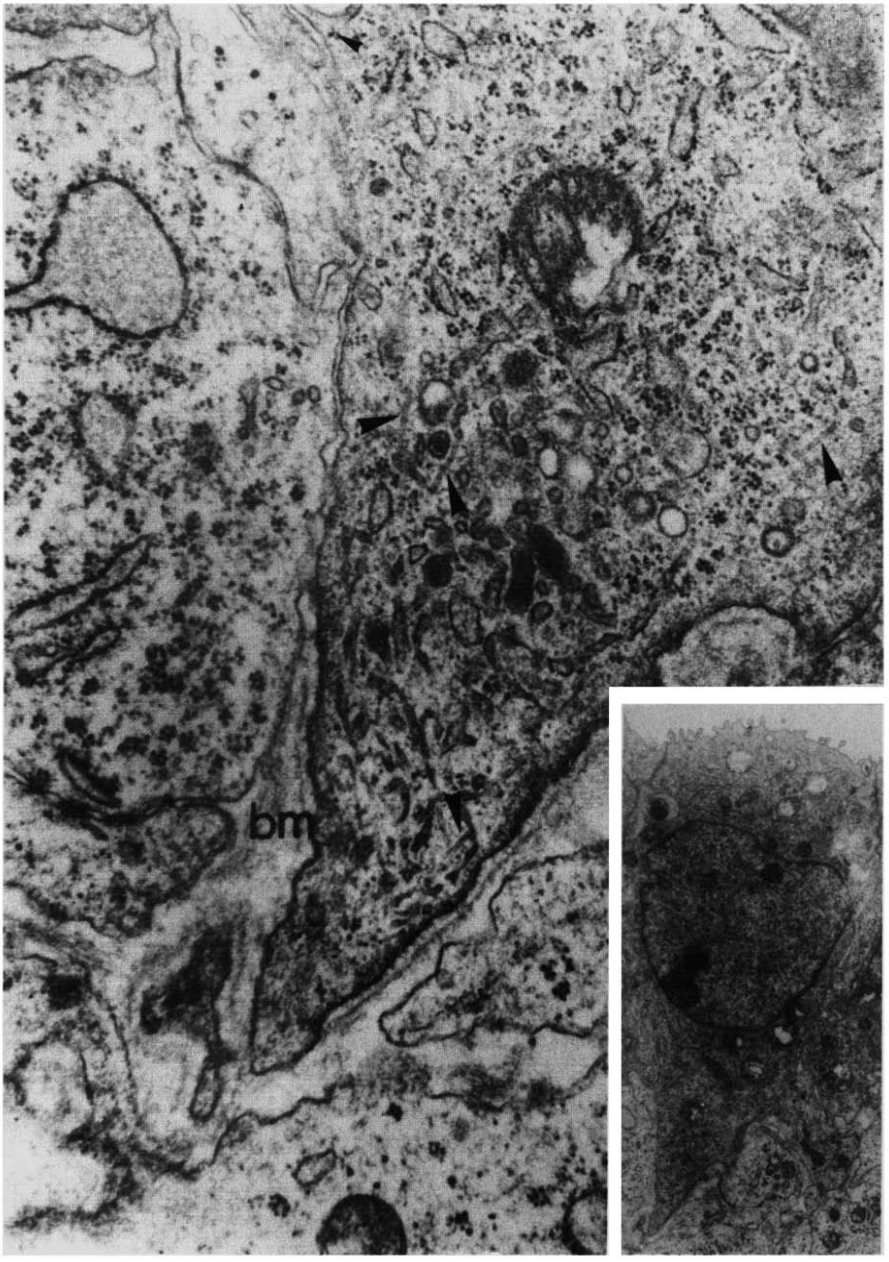

Fig. 2. Early developing cell process of marginal cell. Large arrows indicate microtubules. Small arrow indicates lateral sheet-like extension. bm, basement membrane. ×32000. Insert: Low-power electron-micrograph of same marginal cell. ×4200.

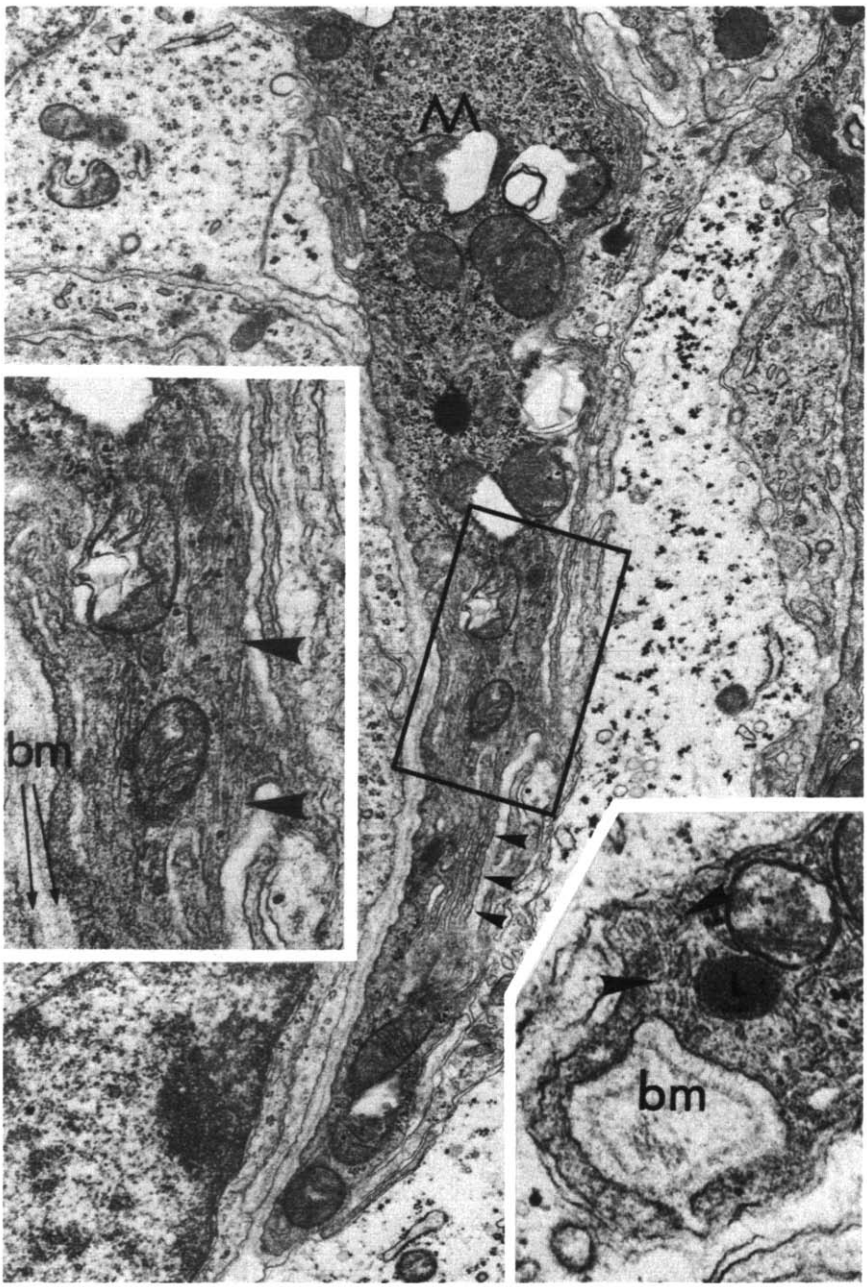

Fig. 3. Cell process (M) of maturing marginal cell abutting strial capillary. Small arrows indicate pleating of plasmalemma of large process by small sheet-like extensions. Insert at left: enlargement of boxed-in area. Note longitudinally oriented microtubules (arrows) and double basement membrane (bm) adjacent to capillary. Insert at lower right shows tip of a different maturing marginal cell process engulfing basement membrane. Note microtubules and lysosome (L). ×17000. Left insert: ×34000. Right insert: ×40000.

and deeper cell layers of the immature stria disappears during maturation. Initially, the basement membrane extends with the developing marginal cell processes (Figs. 2, 3), and occasionally it can be observed within marginal cell invaginations, as if being phagocytized (Fig. 3, insert lower right). Despite this, the absence of a basement membrane in the adult is probably largely due to dispersion and thinning of this structure as a result of the expansive increase of plasma membrane surface area.

Discussion

Numerous researchers have presented evidence that microtubules are important for cellular elongation [3,12]. In addition, microtubules have been implicated in the process of organelle displacement. For example, Raine et al. [11] reported close lateral associations between mitochondria and microtubules within mammalian axons and suggested that microtubules may provide a mechanism for mitochondrial displacement. The close proximity of microtubules to organelles, especially mitochondria, in marginal cell processes may be indicative of such a function. Indeed, such associations apparently promote the stratification of mitochondria within the cell processes, thereby providing the spatial arrangement of energy production sites requisite for fluid transport.

The dramatic increase in cell surface area which accompanies cellular extension necessitates the manufacture and incorporation of new plasma membrane. Accordingly, immature cuboidal marginal cells often contain large amounts of smooth endoplasmic reticulum, Golgi bodies, and vesicles. It is interesting to note that in the adult mouse the typical marginal cell process is extremely attenuated, contains almost exclusively mitochondria and microtubules, and the membrane pleats characteristic of immature cell processes are largely absent [13]. Apparently, as processes extend, membrane pleats unfold. In addition, the number of apical microvilli is drastically reduced in the adult marginal cell, and this process may provide another source of plasma membrane for the rapidly expanding cell surface.

The data presented here indicate an important role for microtubules in strial development. Microtubular integrity may also be required for the morphologic stability of the adult stria. It is known that disruption of microtubules by anti-tubulins can induce the retraction of cell processes [16]. The strial atrophy induced by a number of experimental manipulations, including intermixing of endolymph and perilymph [4] may be related to microtubule effects. Although microtubules have not been studied under such circumstances, electron micrographs of atrophied striae show extensive loss of cellular processes. The study of microtubular integrity under conditions of advancing strial atrophy is certainly warranted.

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