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The sensory hair cell

A RE-EXAMINATION OF A HAIR CELL ORGANELLE IN THE CUTICULAR PLATE REGION AND ITS POSSIBLE RELATION TO ACTIVE PROCESSES IN THE COCHLEA

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A striated body (Friedmann body) has been consistently observed in the infracuticular plate region of inner hair cells of normal chinchillas. The dark and light banded bodies are fibrillar and may be composed of membranous lamellae surrounded by electron dense material. The dark membranous bands appear continuous with the smooth endoplasmic reticulum and the electron dense material lining the junctional complex at the periphery of the cell. The light bands of fibrillar material appear continuous with the substance of the cuticular plate. The striated bodies line the cuticle free region in the area of the basal body and follow the contours of the cuticular plate. They are associated with the plasma membrane, mitochondria and microtubules. A similar organelle has not been identified in outer hair cells. The structure found in the inner hair cells is compared with similar striated structures reported in vestibular and cochlear hair cells of other animals as well as with striated structures observed in various other systems. The function of the striated body in the inner hair cell of chinchilla is unknown, but possibilities include structural support and active participation in inner hair cell function.

Key words: cochlea; mechanoreceptors; contractile proteins; hair cells.

INTRODUCTION

The recent finding of Flock that actin is present in the stereocilia of hair cells in the inner ear [10] has resulted in a renewed interest in the ultrastructure of the apical region of these cells. We report here the consistent occurrence of Friedmann bodies [12], or striated organelles, in the infracuticular plate region of the inner hair cells of the normal chinchilla cochlea. Similar striated bodies have been found below the cuticular plate in normal [8,21] and pathological [11,13,21] vestibular hair cells. Their presence has also been noted in the cochlear hair cells of the normal cat [25,39], squirrel monkey [8] and the deaf white mink [17]. The consistent occurrence of the striated organelle in the inner hair cells of the normal chinchilla suggests that the striated body is a normal structural component of the inner hair cells and that it may play an active role in inner hair cell function.

MATERIALS AND METHODS

Both cochleas from five normal chinchillas, 3–7 years old, were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, containing 0–10 mM magnesium chloride. The cochleas were washed with phosphate buffer, post-fixed in 1% osmium tetroxide in

0.1 M phosphate buffer, washed with phosphate buffer and dehydrated through ethanol and propylene oxide. The cochleas were embedded in Araldite, trimmed and serially sectioned. Sections were stained with 2% uranyl acetate and lead citrate and examined with a Siemens 1A electron microscope.

RESULTS

A striated organelle was found in each of 40 inner hair cells that were sectioned. It was never observed in any of the outer hair cells although three times as many outer hair cells were examined. Fig. 1a shows a micrograph of a radial section of the organ of Corti, through the long axis of an inner hair cell. The striated organelle can be seen in the region below the cuticular plate where it curves to follow the inferior surface of the cuticular plate The body is composed of electron dense bands separated by spaces of less electron dense material. The dark bands appear to have the same width and to have a periodicity of 50–60 nm. In some instances the striated body is separated from the cuticular plate and is surrounded by cytoplasm and endoplasmic reticulum. It is closely associated with the mitochondria and at times they appear to be in contact.

The striated body appears to be associated with the plasma membrane of the inner hair cell In radial sections it can be seen attached to the sides of the inner hair cells at the level where tight junctions, intermediate junctions and desmosomes connect the hair cells with adjacent supporting cells. The electron dense material which lines the junctional complex and forms a shelf for the cuticular plate [8] appears to merge with the electron dense bands of the striated organelle.

The striated body has a complex morphology and at times it may be seen to project into the cytoplasm of the inner hair cell toward the centrally placed nucleus. It can be found in close association with the striated ciliary rootlet of the basal body that projects below the level of the cuticular plate into the cytoplasm. When the striated body is sectioned in a plane perpendicular to that of the striations (Figs 1b and c), the electron dense bands have a substructure which suggests that the organelle may be composed of stacks of membranous lamellae and the lighter parts of the striated body appear fibrillar.

If the organ of Corti is sectioned in a plane parallel to the basilar membrane, many inner hair cells can be observed at one time. Fig 2 shows a micrograph of an inner hair cell sectioned at a level below the cuticular plate. The striated body again appears to line the cuticular plate at its contact with the cytoplasm. However, when the organelle is cut in this plane, the direction and pattern of the striations differ. The most frequent orientation of the banding pattern is in a radial direction, with striations running from the area close to the inner pillar cell in the direction of the modiolus. In some areas, the striated body retains the banding pattern shown in Fig. 1a, but in other areas the banding is composed of thick electron dense bands, alternating with thinner electron dense bands, separated one from the other by spaces of less electron dense material. When the striated body assumes this alternate banding pattern, the periodicity of the individual adjacent bands increases to 70–80 nm, while the periodicity of the alternating thicker electron dense bands is 150–160 nm. In this configuration the body has the more characteristic appearance of a Friedmann body.

The striated body has been observed in contact with the junctional complex material

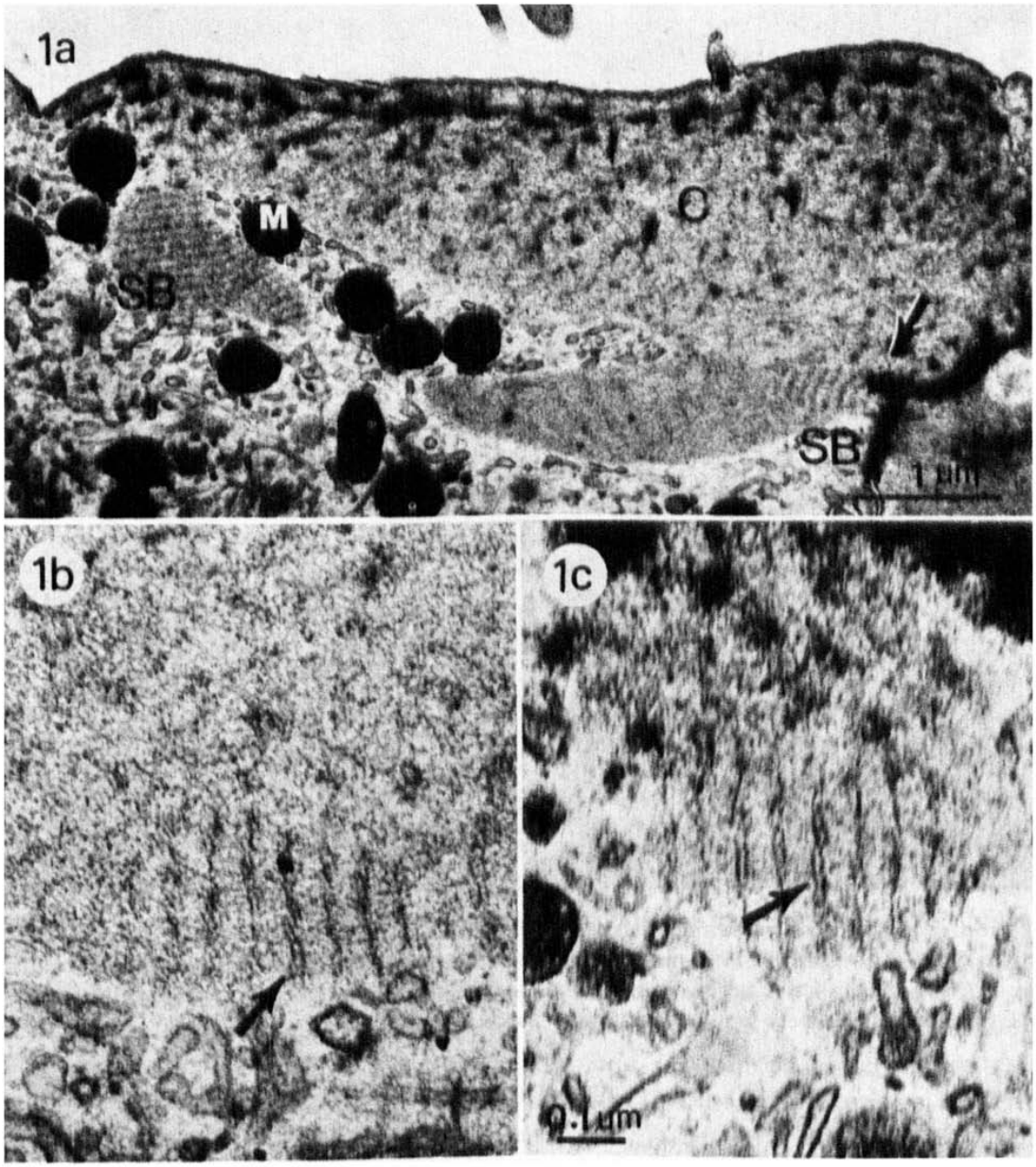

Fig. 1. Radial sections through inner hair cells showing location and morphological characteristics of the striated body. (1a) The striated body (SB) is in the infracuticular plate region where it follows the inferior surface of the cuticular plate (C) and is in close association with mitochondria (M). Arrow indicates where the electron dense material lining the junctional complex appears to merge with dense bands of the body (1b and 1c) The striated body is sectioned perpendicular to the plane of striations. Arrows indicate lamellar substructure of dense bands while less dense spaces appear fibrillar and continuous with the cuticular plate material

at the circumference of the cell and surrounding the cuticle free region where the basal body is normally found. It is associated with the microtubules that line the cuticular plate region and also with the smooth endoplasmic reticulum. In some cases elaborations of the smooth endoplasmic reticulum appear to be continuous with the electron dense bands of the striated body.

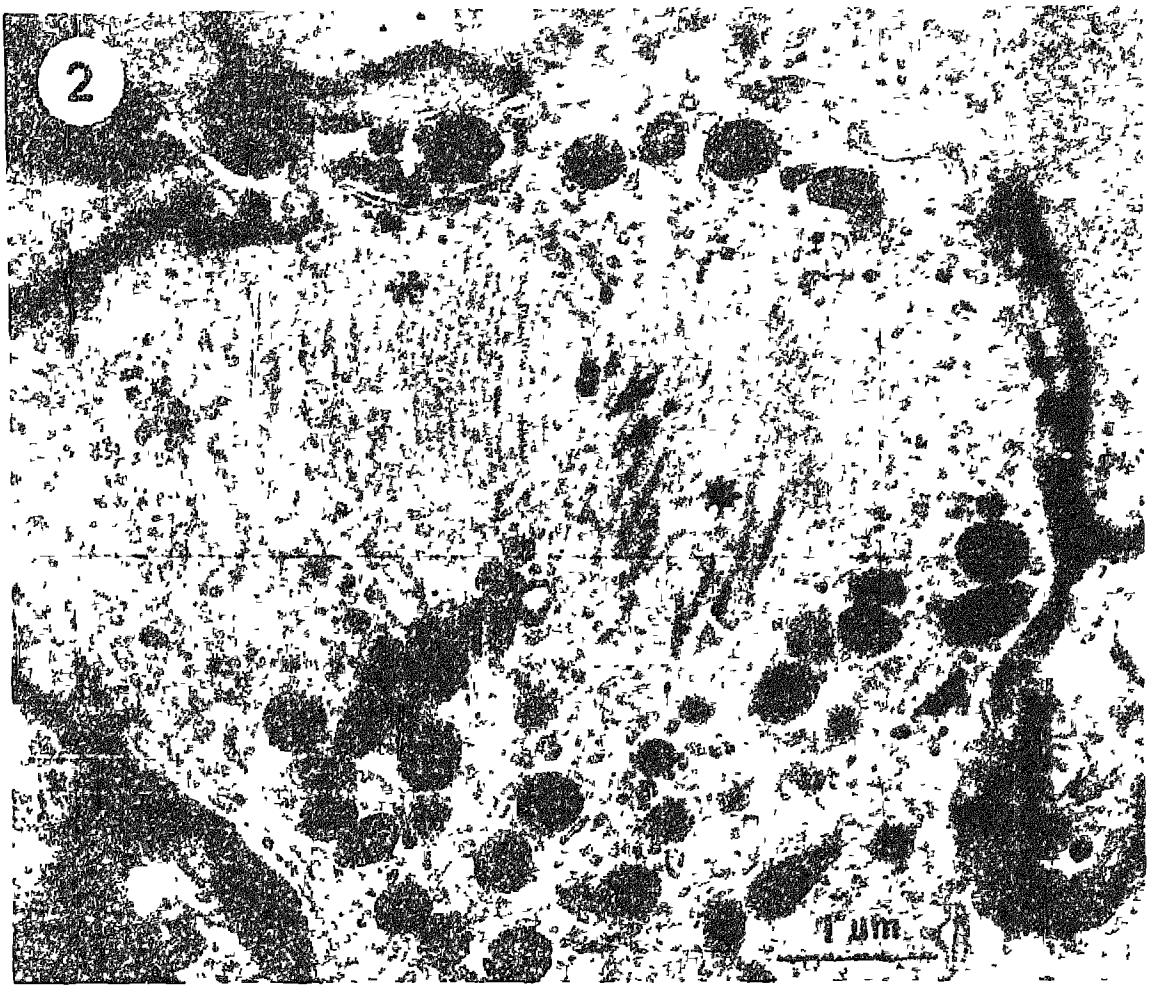

Fig 2 Horizontal section through inner hair cell showing location and morphological characteristics of the striated body. Asterisk indicates portion of striated body where banding pattern is similar to that in Fig 1 Star indicates alternate banding pattern Arrows indicate where elaborations of the smooth endoplasmic reticulum appear to merge with dense bands.

DISCUSSION

The form of the striated body found in the inner hair cells of the normal chinchilla cochlea is similar in appearance to striated structures found in systems other than the labyrinth. Although inferences deduced solely on the grounds of ultrastructural morphology from thin sections must be viewed with caution, a possible clue to the role of this organelle may be obtained by comparing the striated structure with these similar structures present in other systems. While its appearance is immediately reminiscent of the contractile apparatus found in striated muscle, the striated body seems more like the leptomeric organelles [22] or microladders [23] that have been found in muscle cells. These organelles have been found running parallel to the sarcomeres and associated with Z-line material and because the periodicity of the leptomeric organelle changes with con-

traction and relaxation of the muscle fibers [27], there has been speculation that they may be contractile

Periodic organization of contractile proteins has also been observed in smooth muscle cells [1] and in the microfilament bundles or stress fibers of non-muscle cells known to undergo changes in cell shape [3,4] and to be contractile [6,34]. Longitudinal actin filaments are collected into sets by dense bodies and anchored to the cell membrane at the junctional complex by intermediate filaments and dense material [38]. Both the dense bodies and the junctional material are thought to be composed of alpha-actinin [4] and to play a role in these cells analogous to the role played by Z-lines in striated muscle Contraction is believed to be mediated by the interaction of the longitudinal filaments of actin with thick filaments of myosin distributed throughout the thin filaments [1].

Thus in the leptomeric organelles, smooth muscle cells and non-muscle cells, all of which are systems believed to be contractile, the interaction of actin is not only with myosin but may be with other proteins. The striations observed in these systems may be periodic insertions of these proteins, capable of polymerizing the actin monomers into filamentous actin [20] or organizing the web of actin filaments into bands of filaments [36]. The contractile proteins in these systems may be organized into primitive sarcomere-like units which would facilitate the interactions among these proteins and result in various degrees of cell motility and contraction Since the appearance of striated organelles in various cells has been correlated with contractile events known to occur in these systems, the presence of a morphologically similar striated organelle in the inner hair cell may indicate the possibility that active contractile processes may occur in the cochlea.

Because of its periodicity, proximity to the basal body and association with microtubules, the striated body may alternatively be considered a type of ciliary rootlet It is common for basal bodies of ciliated and nonciliated sensory cells to have striated structures associated with them [2,32], which vary in size, shape and periodicity [28,37] They project from an area adjacent to the basal body and have been observed to extend to the plasma membane [27] and/or toward the nucleus [40]. Recent evidence shows that such structures may be contractile [35] and play a role in the transduction of sensory information [15,19,27]. In light of the fact that the cochlear sensory cells of the cat do not have basal bodies [39], the basal body itself is probably not the essential excitable structure in the hair cell as previously suggested [7] If the striated organelle found in the inner hair cell of normal chinchillas is similar to the ciliary rootlet structures such as the rhizoplast, it may be related to the microtubule system found in the apical portion of the hair cell, lining the cuticular plate and surrounding the cuticle free region It may be that it is the striated organelle in association with this microtubule system (requiring only the presence of a microtubule organizing center and not a basal body [31,41,42]) that initiates or modulates the transduction process

Moreover, in searching for a role for the striated organelle it is of interest to compare the ultrastructure of the sensory cells of the ear with epithelial cells of the intestine These brush border cells, like hair cells, have an organized array of microvilli extending out from the cell surface. In the brush border cells actin filaments anchored at the tip run the length of the microvilli and extend into the cell as a dense core. These core filaments splay out to interact with the actin filaments from neighbouring microvilli

[30] as well as with filaments extending horizontally into the cytoplasm from the cell periphery [3,33]. Thus, the cytoplasmic filaments of this so called terminal web display a high degree of structural organization and form a broad band across the cytoplasm at the level of the junctional complex [18]. In the current models to explain contraction of microvilli, core actin filaments of one polarity and peripheral actin filaments of opposite polarity are thought to interact with a common myosin polymer and slide toward one another in a manner similar to the interaction of actin and myosin in striated muscle [3,29,33].

Flock has shown that actin filaments are present in the stereocilia of vestibular [10] and cochlear [9] hair cells of the inner ear and that a system of cytoplasmic filaments similar to the organization of the terminal web fibers in the brush border cells of the intestine is present in the cuticular plate region of vestibular hair cells [9]. Perhaps the organization of the cuticular plate region in the hair cells of the mammalian cochlea is also similar. The striated structures observed in the hair cells of the chinchilla may, like the terminal web, play a role both in organizing the stereocilia rootlet filaments at the base of the cuticular plate region and in anchoring the filaments to the cell membrane along the circumference of the cell through the junctional complex material.

It is interesting that in the brush border cell, two types of contraction have been experimentally induced by the addition of calcium and ATP. In the first type, bundles of core microfilaments move into and through the terminal web causing a shortening of the microvilli [29]. In the second type, there is a circumferential constriction of the apical portion of the cells in the region of the junctional complex [33]. Although we have no anatomical evidence for the shortening of stereocilia in the cochlear sensory cells, there is some evidence that the circumferential contraction may occur. If the organ of Corti is sectioned in a tangential plane, and inner and outer hair cells examined, there are differences in the way that the stereocilia insert into the cuticular plate. Fig. 3a shows the outer hair cell stereocilia insert into the cell, perpendicular to the cuticular plate, while Fig. 3b shows that the stereocilia from the inner hair cell can insert into the cuticular plate at an angle. The tips of the inner hair cell stereocilia fan out into the endolymph while the rootlets point toward the center of the cell. This is what might be expected to occur if there is circumferential 'pinching in', similar to the effects of a contractile ring around the periphery of the cell just below the cuticular plate. Thus we may speculate that if actin is responsible for active processes in the cochlea, perhaps the contractile proteins and the active processes may be similar to the current models for microvillar motility.

Another possibility may be considered if the striated organelle has a substructure consisting of membranous lamellae that are continuous with the smooth endoplasmic reticulum. In light of the recent evidence that smooth endoplasmic reticulum, like sarcoplasmic reticulum, may act as an intracellular compartment capable of releasing and sequestering calcium [5,16], one may speculate that the striated organelle may play a role in regulating calcium levels in an area of the hair cell where contractile proteins and microtubules are known to be present. Controlled increases of calcium may be used to modulate active processes in the cell such as actomyosin interactions, nonfilamentous to filamentous actin transformations and microtubule assembly and disassembly. In almost all eucaryotic cells, these proteins are present and play an important role in regulating tension, cytoplasmic viscosity and cell shape.

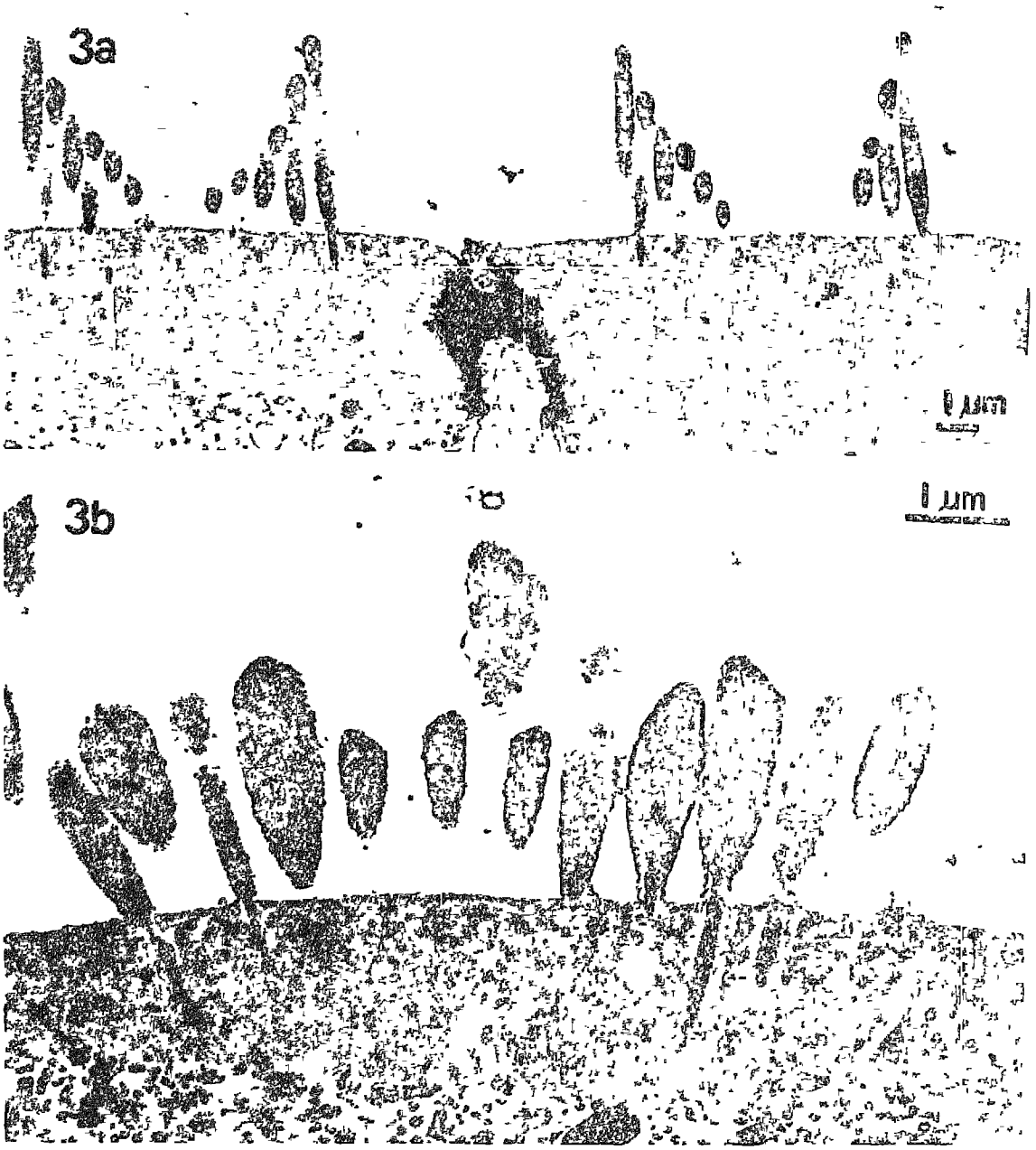

Fig. 3. Tangential sections through hair cells. (3a) Section through two outer hair cells where stereocilia project into the cuticular plate perpendicular to the reticular lamina. (3b) Section through an inner hair cell where stereocilia project into the cuticular plate at an angle and the reticular lamina appears to bulge out into the endolymph.

The recent discovery of echoes emanating from the cochlea following stimulation [24] coupled with the demonstration of contractile proteins in the hair cells [10] has resulted in much discussion of active processes in the cochlea. The location and morphological characteristics of the striated structure observed in the inner hair cells from the normal chinchilla suggest that these structures may play some role in these processes.

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