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MORPHOLOGICAL CORRELATES OF MECHANOTRANSDUCTION IN ACOUSTICOLATERAL HAIR CELLS

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Abstract

The development of ideas on mechanotransduction in acousticolateral hair cells is described, leading to the current idea that transduction depends on deflection of the bundle of stereocilia by a force parallel to the plane of the sensory epithelium. Electrophysiological experiments are summarised, suggesting that transduction depends on a shear between the different rows of stereocilia, and that the transducer channels are situated towards the tips of the stereocilia. Analysis of the ways that shear between the rows of stereocilia could be detected suggests that tip links are the structures which are most likely to transmit the stimulus-induced forces to the transducer channels on the membrane. The directional selectivity of mechanotransduction is associated with the position of the kinocilium and gradation in heights of the stereocilia; evidence is presented suggesting that in the lateral line these are partly determined by the mitosis giving rise to the hair cell. Tip links differentiate out of links which initially join the stereocilia in all directions, with their final spatial organisation, which sets the directional selectivity of mechanotransduction, probably being determined by the gradient in growth of the stereocilia.

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Introduction

Theories of hair-cell transduction

Hair-cell stereocilia were first described by Hensen (1863). He suggested that sensory transduction might be initiated by distortion of the hairs, produced when the hairs were squeezed between the reticular lamina and the tectorial membrane. The idea that transduction instead depended on deflection of the stereocilia primarily due to shearing forces *parallel* to the sensory epithelium was suggested by Breuer (1891) in the vestibular system, and later developed by ter Kuile (1900) in the cochlea. Nevertheless, there was no direct evidence on this mode of action, and other theories, including the suggestion that transduction might be initiated by direct compression of the hair cell body, were also proposed (Stevens and Davis, 1938).

The primacy of shearing forces leading to deflection of the stereocilia was later emphasised by von Bekesy's (1953) direct manipulations of the tectorial membrane in the mammalian cochlea, and by von Holst's (1950) investigations of behavioural responses to otolithic stimulation in the fish. These experiments were extended electrophysiologically by Jeilof et al. (1952) in the fish lateral line, and later by Flock (1965) who, by direct manipulation of the cupula overlying the hair cells, showed that cells produced their potentials in response to movement parallel to the sensory membrane, rather than to movement in other directions.

The mechanism of transduction remained controversial until the mid 1970's (e.g. Dallos, 1973), with theories suggesting, for instance, that the membranes might transfer electric charge directly, as in a piezoelectric crystal (e.g. Dohlman, 1960), or that the resistance of the hair cell membranes might be modulated (e.g. Davis, 1965).

Current ideas on hair-cell transduction

The mechanism of transduction became much clearer as a result of the experiments by Hudspeth and colleagues, who directly manipulated the bundles of hair cells in *in vitro* preparations of the bullfrog sacculus. They showed that deflection of the stereocilia was associated with a resistance change in the apical membrane of the hair cell, and that transduction depended on the stereocilia themselves rather than the kinocilium (Hudspeth and Corey, 1977; Hudspeth and Jacobs, 1979). Extracellular recording showed that the transducer currents were localised to the distal ends of the stereocilia (Hudspeth, 1982), suggesting that the mechanotransducer channels were at this site. However, this view has remained controversial, since Ohmori (1988), who measured Ca^{2+} influx by looking for fura-2 fluorescence within the hair cells, suggested that the great-

est changes in ion influx were found near the insertion of the bundle into the cuticular plate. Nevertheless, many of Ohmori's figures show that the greatest changes were found *within* the bundle (e.g. Fig. 5 of Ohmori, 1988), suggesting that the ions were in fact flowing down inside the stereocilia, in agreement with Hudspeth's hypothesis.

Any morphological theory of hair-cell transduction has to account for the following findings from direct electrophysiological experiments:

(i) The stereocilia on a hair cell are graded in height (Fig. 1). Deflection of the stereocilia in the direction of the tallest stereocilia in the bundle, and so in the direction of the kinocilium (if present), is associated with channel opening, intracellular depolarisation, and nerve excitation. Conversely, deflection in the opposite direction is associated with channel closing, hyperpolarisation, and nerve inhibition (Flock, 1965; Lowenstein and Wersäll, 1959; Hudspeth and Corey, 1977). Deflection at right angles to this axis has no effect (Shotwell et al., 1981).

(ii) Transduction is likely to be associated with a shear or sliding between the different rows of stereocilia, rather than a separation of the rows. Deflection of the stereocilia in the direction of the tallest, whether by pulling on a probe attached to the tallest stereocilia (Hudspeth, 1989), or by pushing on their shortest (Hudspeth, 1982), both produce excitation. However, as shown in Fig. 2, these two manipulations can be expected to produce opposite effects on the separation of the rows of stereocilia, and therefore anything depending on separation is unlikely to be responsible for transduction.

(iii) Measurement of the conductance change produced by stimulation (Russell, 1983), the variance of the transducer channel noise (Holton and Hudspeth, 1986), and compliance changes of the bundle during mechanical stimulation (Howard and Hudspeth, 1988), all suggest that there are only a very few, and perhaps only one, transducer channels per stereocilium.

(iv) Electrophysiological experiments suggest that the mechanical stimulus is transmitted to the transducer channel by means of an elastic link (Corey and Hudspeth, 1983). Moreover, channel opening and closing is likely to be directly and tightly coupled to the mechanics of the bundle, because channel opening and closing both have a very short latency (40 μs at 22 °C; Corey and Hudspeth, 1979). In addition, when the channels open and close, they pull back onto the stereocilia to produce measurable changes in the stiffness of the bundle as a whole (Howard and Hudspeth,

(v) There is likely to be only a single population of transducer channels, all with the same threshold, on each hair cell. All the channels are likely to be coupled into the mechanics of the stereocilia in a uniform way. This is suggested because no manipulation has ever succeeded in fractionating the response of a hair cell into subresponses of different sensitivity.

This paper will analyse current theories of the morphological basis of mechanotransduction, particularly with reference to the role of tip links in transduction (Pickles et al., 1984; Osborne et al., 1984; Pickles, 1985). It will give more recent information on the spatial organisation, fine structure, and development of the mechanotransducing areas of the hair cell.

Methods

Adult specimens were anaesthetised and perfused intracardially with fixative. In the case of embryonic chicks, the head was bisected, and the papilla opened under fixative. Fish were anaesthetised with MS222 in the bathing water, and the neuromasts dissected under fixative. Fixatives were 2.5% glutaraldehyde and 1% tannic acid in 0.1 M phosphate buffer, or 2.5% glutaraldehyde and 15% saturated picric acid in 0.1 M phosphate buffer, both at pH 7.4, or 4% paraformaldehyde in phosphate buffer (echidna only). Echidna temporal bones were decalcified for 3 months in EDTA before dissection of the cochlear duct. Specimens for SEM were dehydrated with acetone, dried by the critical-point technique with CO_2 (Emscope CPD 750), and sputter-coated with platinum in an Emscope SC500 or Bio-Rad 5400 sputter coater. Where no charging due to cracking was expected, specimens were coated with a nominal depth of coating of 25 nm, although the thickness of the finest structures indicated that the actual depth on those structures could be much less than this (e.g. 10 nm). Specimens were examined at 80 kV in a JEOL 120CX or 1200EX transmission microscope with scanning attachment, and images observed by means of a secondary electron detector. For TEM, the specimens were dehydrated in ethanol and embedded in Spurr's resin. Sections were cut on a Reichert Ultracut E microtome, picked up on mesh grids, stained in uranyl acetate and lead citrate, and examined in either a Philips 400 or Hitachi H-800 transmission electron microscope at 100 kV, or a Zeiss EM10 at 60 kV. In some cases, stereo pairs of the TEM sections were taken, with tilts of + and - 6° away from the normal position.

Results and Discussion

Cross-links between stereocilia and their relation to sensory transduction

The electrophysiological evidence clearly points to a shear between the different rows of stereocilia as being responsible for mechanotransduction, and suggests that stretch of some form of link (Hudspeth, 1985b, 1989) is most likely to operate the transducer channel. A number of sets of such links between stereocilia have been described morphologically. Some of these run between the stereocilia of the same row (i.e. between stereocilia of the same height on the bundle), and others between the stereocilia of the different rows (i.e. between stereocilia of different heights). The links connecting the stereocilia of the *same* row are not likely to be involved in transduction, since it is difficult to see how they could detect a shear produced *between* the different rows. Attention therefore is directed to the links running between the rows.

The links running between the rows of stereocilia can be divided into two groups. Links of the first group run parallel to the cuticular plate ("horizontally"). These join the stereocilia mainly just below their tips, and appear to hold the stereocilia in close apposition at their upper ends (Fig. 1b). Links of the second group (tip links) emerge from the tips of the shorter stereocilia in the bundle, and run nearly orthogonally to the cuticular plate ("vertically") to join the side-wall of the adjacent taller stereocilium (Figs 1a and b).

Are the horizontal links involved in transduction?

It is probably links of this group that Thurm (1981) suggested might be involved in transduction, although it is difficult to be sure because no micrograph was included. These links would be stretched by movements tending to produce separation of the stereocilia. We suggest that stretch of these links is unlikely to be involved in transduction, because, as shown in Fig. 2, two different manipulations which both produce channel opening would tend to produce opposite stretches of the links. In other words, if the bundle is moved in the direction of the tallest stereocilia, either by a pull on the tallest stereocilia (Fig. 2a; Hudspeth, 1989), or by a push on the shortest (Fig. 2b;
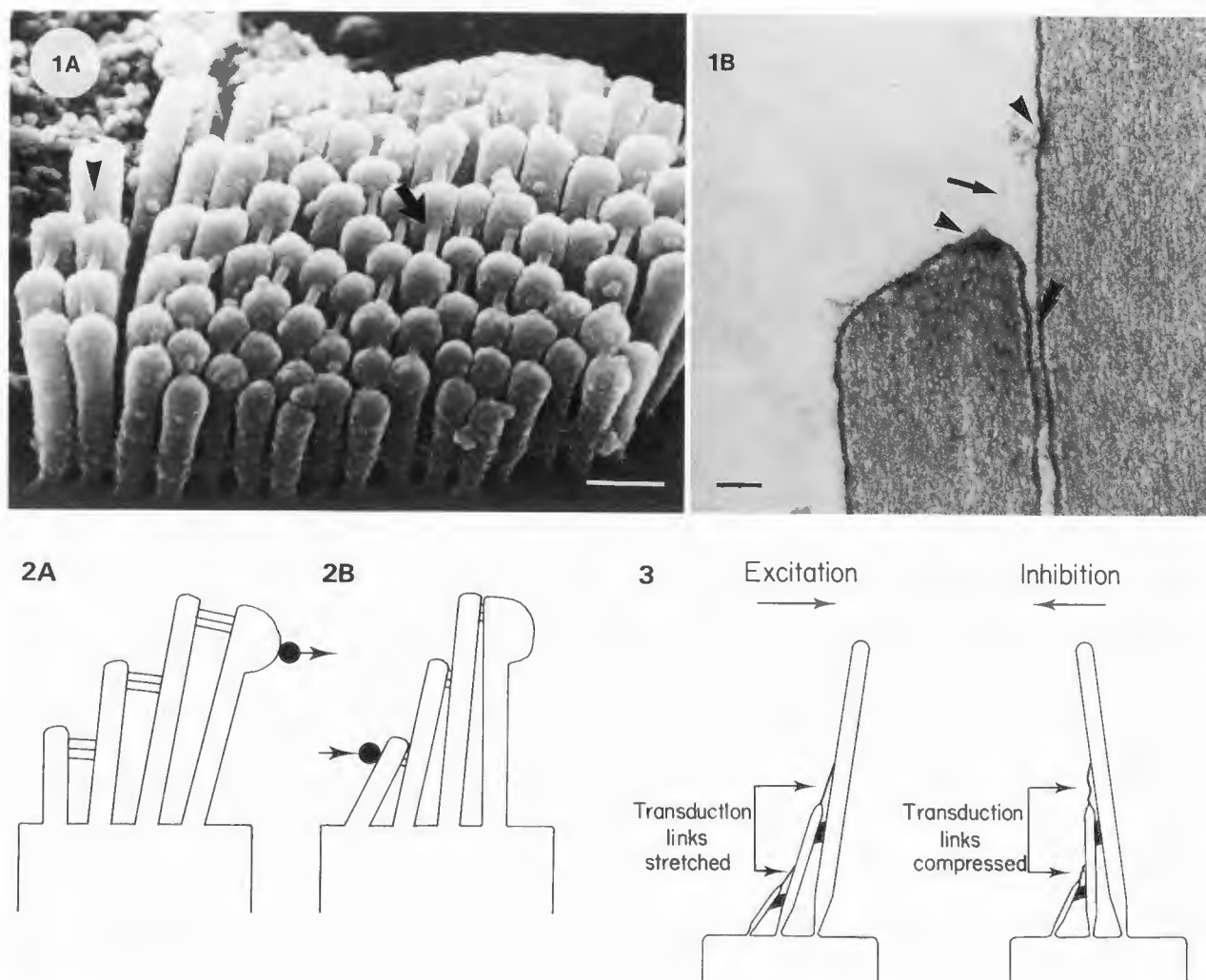
Figure 1a. Hair cell of the chick basilar papilla, showing gradation in heights of the stereocilia, and tip links (e.g. arrow). The stereocilium at the arrowhead receives tip links from two shorter stereocilia. Glutaraldehyde/picrate fixation. Scale bar: 500 nm.
Figure 1b. Tip link (arrow) between stereocilia of the chick basilar papilla, showing densities at upper and lower points of attachment (arrowheads), and band of links running laterally between the rows of stereocilia just below their tips (double arrowhead). Glutaraldehyde/tannic acid fixation. Scale bar: 100 nm.
Figure 2 a and b. Both these manipulations lead to channel opening, but have opposite effects on the separation of the stereocilia. Dark circle: probe used to manipulate the bundle. The kinocilium is shown with a bulb at the top. From Pickles et al., (1990), Fig. 1. Copyright © 1990 Alan R. Liss, Inc. Reprinted by permission of John Wiley & Sons, Inc.
Figure 3. On the tip link hypothesis of transduction, deflection of the stereocilia in the direction of the tallest stereocilia stretches the tip links, opening the transducer channels. The channels are partly open at rest, and deflection in the direction of the shortest stereocilia takes the resting tension off the links, closing the channels. From Pickles et al., (1984), Fig. 9.

Hudspeth, 1982), both manipulations give rise to channel opening. Two further arguments can be adduced as to why these links are unlikely to be involved in mechanotransduction: (i) As suggested by Hudspeth (1985a), if the horizontal links between two rows of stereocilia were to be stretched by deflection of the taller stereocilia, the stereocilia of the shorter row would have to be stiff enough to resist deflection. They would therefore be inefficient at transmitting the deflection to the next shorter row. In addition, input energy would be dissipated against the stiffness of the stereocilia rather than concentrated upon the transduction molecules. (ii) As shown in Fig. 1b, the sideways link is a broad band,

composed of many separate strands. However, the electrophysiological evidence shows that there are at most only a few transducer channels per stereocilium. This suggests that the great majority of strands are not involved in transduction. The presence of 'transducing' strands in parallel mechanically with 'non-transducing' strands would be energetically inefficient.

Are there ways in which the horizontal links might be able to detect shear or sliding movements between the different rows of stereocilia? To do so, the mechanotransducer channels, presumably at the points of attachment of the links, would have to be oppositely influenced by a

"downwards" and an "upwards" pull or rotation of the links. Such a mode of action, while presumably possible, does not fit with the kinetic models of Corey and Hudspeth (1983) and Howard and Hudspeth (1988), which suggest that channel opening is a monotonic function of the degree of stretch of the gating spring.

Are tip links involved in transduction?

The links emerging from the tips of the shorter stereocilia on the bundle (Figs 1a and b) are possible candidates for the "gating springs" (Hudspeth, 1985b) which transfer forces to the mechanotransducer channels. It is suggested that tension on the links pulls open the mechanotransducer channels, presumably at one or both of the points of attachment of the links to the stereocilia (Fig. 3). The authors do not know any way in which this hypothesis does not fit closely with the results, some very detailed, which have been obtained from electrophysiological investigations of mechanotransduction in hair cells.

The tip link theory has the following advantages:
(i) It explains why deflection of the stereocilia in the direction of the tallest is associated with channel opening, and deflection in the opposite direction is associated with channel closing (see Fig. 3).
(ii) It explains why a gradation in height of the stereocilia is seen universally in mechanotransducing acousticolateral hair cells, since this is required by the tip link hypothesis. However, lateral line electroreceptors, which are non-mechanotransducing hair cells but probably of similar phylogenetic origin, do not show a gradation (Szabo, 1974).
(iii) Tip links seem to be universally present in mechanosensory acousticolateral systems, having been seen in examples of all such systems, namely the cochlea and basilar papillae (Pickles et al., 1984; Pickles et al., 1989), vestibular apparatus (Little and Neugebauer, 1985; Jeffries et al., 1986) and the lateral line (Rouse and Pickles, 1991). They have been seen in all vertebrate classes, namely mammals (Pickles et al., 1984), birds and reptiles (Pickles et al., 1989), amphibia (Hudspeth, 1989), and fish (Little and Neugebauer, 1985; Rouse and Pickles, 1991).
(iv) Tip links explain the vector responses of hair cells to deflections of the bundle in different directions, since tip links run only parallel to the axis of mechanosensitivity (Figs 1a and 4). This is extremely important support for the tip links, since the stereocilia on a bundle are hexagonally packed, and logically it could be expected that tip links could run along any of the axes of the hexagon, rather than only one of them. The origin and development of this arrangement of the tip links will be discussed in more detail below.
(v) The hypothesis explains why transduction is associated with the stereocilia rather than the kinocilium, even in hair cells which possess a kinocilium (Hudspeth and Jacobs, 1979), since tip links are associated with the stereocilia rather than the kinocilium.
(vi) It explains why the mechanotransducing areas might be near the tips of the stereocilia.
(vii) The involvement of tip links in transduction fits exactly with the details of the electrophysiological evidence, and in particular, with the results of kinetic arguments which suggest that the channel is gated by an elastic link which is tensioned by movements in the excitatory direction (Corey and Hudspeth, 1983). The mechanical properties of the link can be calculated (Howard and Hudspeth, 1988), and are appropriate for a fibril with properties similar to resilient, elastic molecules such as elastin.
(viii) The estimate of how forces are distributed within the hair bundle, calculated from the geometry of the stereocilia and tip links, suggests that the force transmitted to each tip link in the bundle would be the same, even for tip links connecting different ranks of stereocilia (the authors, unpublished calculations). This is in agreement with the idea that each channel on the hair cell has the same threshold and input-output function.

It can be concluded that tip links are extremely strong candidates for the links which transfer the forces to the mechanotransducer channels. However, the arguments are all indirect, and it has not so far been possible to perform more direct experiments, for instance removing tip links selectively and seeing if the transducer currents disappear.

Hackney and Furness (1991) have reported a reaction to antibodies to amiloride-sensitive sodium channels in the tips of stereocilia in the guinea pig cochlea. While the transducer channel is not sodium-selective, it is known to be amiloride-sensitive (Jorgensen and Ohmori, 1988), and so may be antigenically related. At the electron-microscopic level, the reaction was seen in a number of sites around the upper ends of the stereocilia. Some of these, such as the tips of the tallest stereocilia on inner hair cells, are unlikely to be involved in transduction, since it is unlikely that the stimulus can be coupled to the site. It is possible that the reaction there may correspond to nonfunctional but immunologically reactive channels, such as are suspected to exist in the case of the amiloride-sensitive sodium channels of the kidney (Tousson et al., 1989). A fuller discussion of these interesting results awaits a more detailed publication.

Development of the spatial organisation of the stereocilia, the excitatory-inhibitory axis of the hair cell, and the tip links

Stereocilia emerge from the cuticular plate with a regular hexagonal packing, and the tip links run regularly along one of the axes of the hexagon, namely the axis parallel to the gradient in heights of the stereocilia (Fig. 4). If the hair cell has a kinocilium (not visible in Fig. 4), it is situated adjacent to the centre of the row of tallest stereocilia. The hair bundle is bilaterally symmetric around a line drawn between the kinocilium and the centre of the hair bundle. The tip links, and the axis of maximum sensitivity to deflection of the stereocilia, run parallel to this line.

How is this axis set within the hair cell? We have an answer in one hair cell system, namely hair cells in the neuromasts of the teleost lateral line. These hair cells show a great deal of turnover; SEM shows in neuromasts of mature species that perhaps 10% of hair cells are obviously degenerating, and that a similar number of apparently new hair cells have short, and presumably immature, stereocilia. We have noted in such systems that the immature bundles nearly always appear in pairs (Fig. 5). The two bundles always have the opposite polarity; that is, if the kinocilium of one cell is situated towards the left of the field of view, the kinocilium of the other is situated towards the right. In slightly older cells, where a gradation in heights of the stereocilia becomes apparent, the stereocilia are, as in other hair cells, tallest near the kinocilium. In the majority of the cases, the kinocilia are situated towards the centre of the pair. However, there is an interaction with the axis of the neuromast. Whatever the relative positions of the two cell bodies of the pair, the axis of bilateral symmetry of each individual cell always runs parallel to the length of the lateral line canal, i.e. parallel to the direction of the expected mechanical input. This is obviously appropriate for maximum mechanical sensitivity to the applied stimulus.

In most cases, a line joining the centres of the cell bodies of a pair is also parallel to the length of the lateral
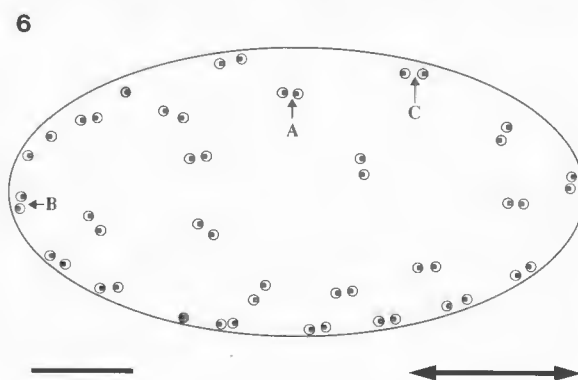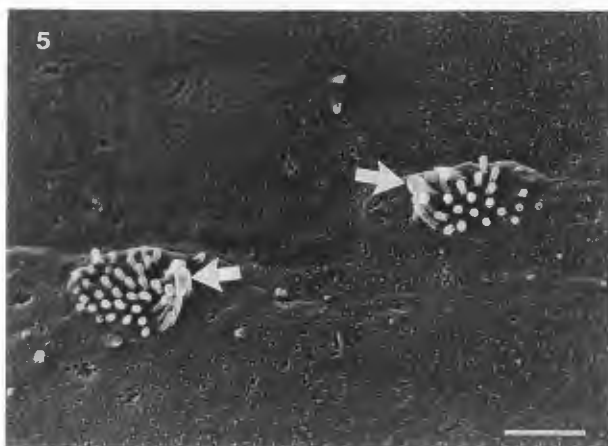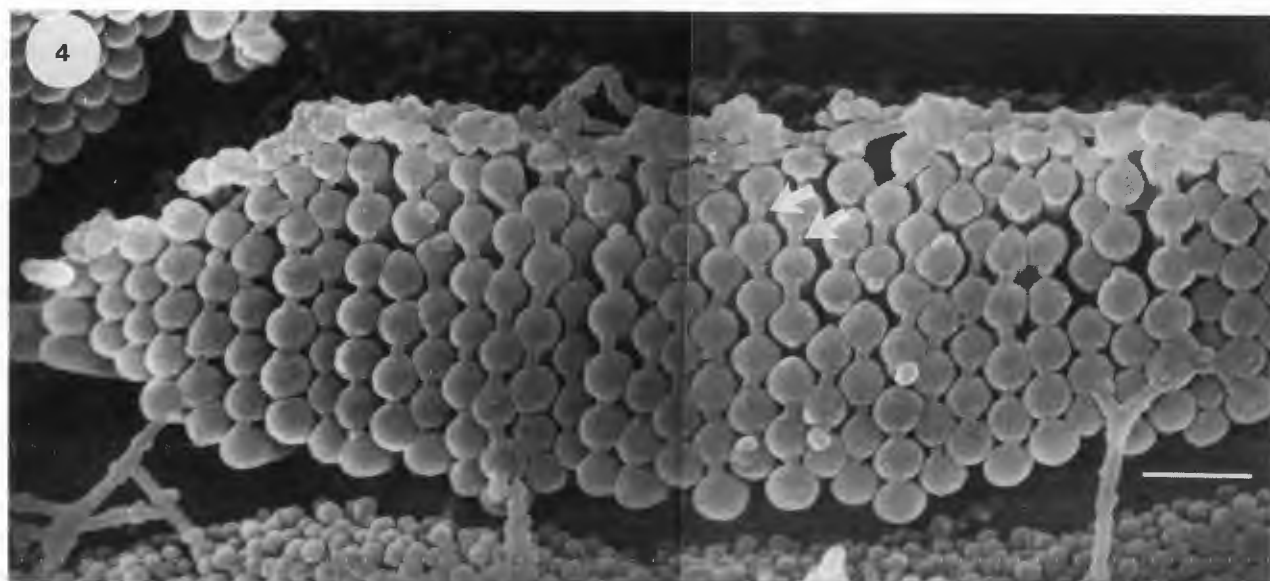
Figure 4. Top view of hair bundle in the starling basilar papilla, showing hexagonal packing of stereocilia. Tip links (e.g. arrows) run parallel to one of the axes of the hexagon. Tallest stereocilia are to the top of the micrograph. Some debris from the tectorial membrane remains attached to the tips of the tallest stereocilia. Glutaraldehyde/picrate fixation. From Pickles et al., (1990), Fig. 5. Scale bar: 500 nm.

Figure 5. Pair of immature hair cells in the canal neuromast of *Apogon cyanosoma*, showing opposing orientations of the bundles, indicated by the positions of the kinocilia (arrows). Glutaraldehyde/picrate fixation. Scale bar: 1 μm.

Figure 6. Plot of mandibular neuromast of *Apogon cyanosoma*, showing positions of immature hair cells (open circles), among the much more numerous mature hair cells (not shown for clarity). The positions of the kinocilia are shown by black dots, and indicate how immature hair cells appear in pairs of opposing polarity. Typical orientations of the pairs are shown for the centre of the neuromast (A), and the axial edge (B). An atypical pair is shown at C. There are also occasional single immature hair cells. For the purpose of this analysis, hair cells were marked as immature if the kinocilium was not more than 1.5 times taller than the tallest stereocilia. The large arrow shows the direction of water flow through the canal, parallel to the direction of the sensitive axes of the hair cells. Scale bar: 25 μm.

line canal (Fig. 6). In these cases, the cells generally have their kinocilia to the centre of the pair. However, and particularly along the short margins of the neuromast, the two bodies of the pair are aligned at an angle to the length of the canal. Here the kinocilia are situated to the sides of the pair (Fig. 6).

The close association of the two hair cells suggest that they result, directly or indirectly, from a single mitosis.

The fact that the kinocilia are generally situated towards the centre of the pair suggests that the axis of mitosis in some way determines the position of the kinocilium. This however is not simply a function of the mitosis alone, because of the way that the cell axes are aligned within the axis of the neuromast as a whole. It is suggested that a factor aligned with the neuromast axis interacts with a factor resulting from the mitosis to give the final hair cell orienta-
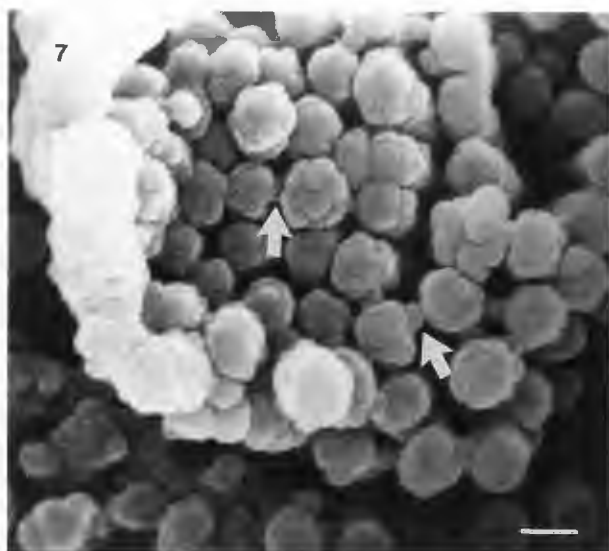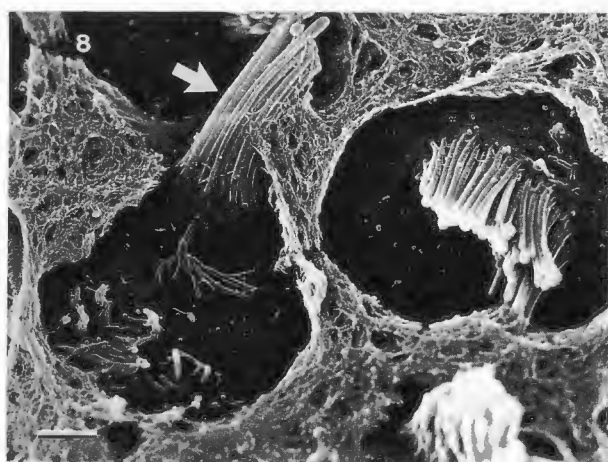
Figure 7. Immature hair cell in the basilar papilla of the embryonic chick. The majority of the stereocilia are not graded in height (a few on the left of the micrograph are longer). The immature stereocilia are connected by bands of material just below their tips, running in all directions without any apparent regularity (e.g. arrows). Glutaraldehyde/picrate fixation. Scale bar: 200 nm.
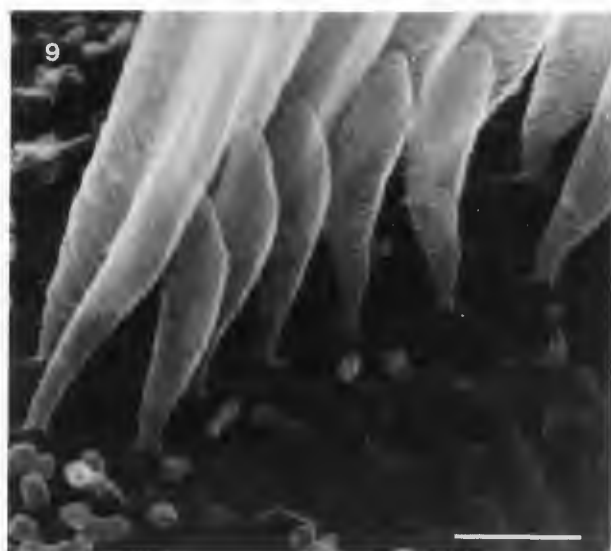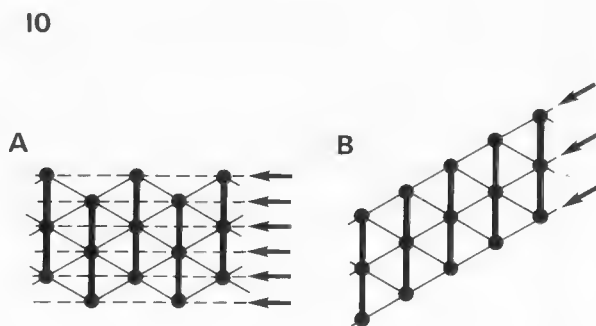

Figure 9. Inner hair cell from the echidna cochlea, with only two rows of stereocilia. This hair cell was situated in the third row of inner hair cells away from the centre of the arch of Corti. Tip links are missing, perhaps because of the extended decalcification used. Paraformaldehyde fixation. Scale bar: 500 nm.

Figure 8. Two hair cells from the basilar papilla of a 15-day (i.e. nearly mature) embryonic chick, treated with streptomycin on embryonic day 7. The hair bundle to the right is normal, the bundle to the left has one row of the tallest stereocilia (arrow), with shorter stereocilia scattered irregularly around the cuticular plate. Glutaraldehyde/tannic acid fixation. Scale bar: 1 μm.

Figure 10a. Packing of the stereocilia as in hair cells of the bird basilar papilla (a 'loose' bundle; Bägger-Sjöbäck and Takumida, 1988). **b**. Packing in a 'tight' bundle, as in guinea pig outer hair cells. Arrows point along rows of stereocilia of equal height. Thick lines: tip links.

tion. This analysis also suggests that the two products of mitosis are different, since the two hair cells end up oriented oppositely within the canal.

The lateral line is unique in that it has hair bundles of opposite orientation intermingled in the same region of the organ; in other systems, the hair cells of different orien-

tation are segregated. But in some species, e.g. the chick basilar papilla, the immature hair cells start off with a wide range of orientations in the young embryo, achieving their regular orientations only as the animal matures; we have not seen any sign that these immature cells initially appear in pairs of opposite orientation. In the mammalian cochlea,

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the orientations of early immature hair cells have not been described in detail, but from available descriptions in explants of the mouse cochlea the cells seem to have achieved their mature orientation as soon as a gradation in heights of the stereocilia becomes visible (Furness et al., 1989). Were the hair-cell axis to be set by the previous mitosis as in the fish, it suggests that these hair cells have a different lineage, possibly repeated mitoses from supporting cells or a stem cell line.

Sometimes hair cells in the mammalian cochlea show anomalous orientations of their bundles of stereocilia, with the 'V' shape of the bundle sometimes even being completely reversed from the normal orientation (Fujita and Orita, 1988; Comis et al., 1989). In the material of Comis et al., such anomalous hair cells tended to exist in groups situated radially across the cochlear duct. Perhaps these hair cells were influenced by a teratogen which was radially distributed in the cochlea. It is alternatively possible that the anomalous hair cells of a group may have all arisen from a common stem cell, and share a common anomaly for that reason.

The beautiful and regular orientation of tip links parallel to the hair cell axis of bilateral symmetry, as shown in Fig. 4, also deserves explanation. Before the stereocilia are graded in height, they are interconnected by links near their tips, with the interconnections running in all directions (Fig. 7). As the stereocilia become graded in height, some of the links are pulled to a more vertically-pointing position, to become similar to the tip links found in mature bundles. We suggest that the tip links are produced from the undifferentiated lateral links which exist in immature stereocilia, by the development in the gradient in heights of the stereocilia. However if this was the only process governing the differentiation into tip links, it is difficult to see how the very great degree of regularity seen in Fig. 4 would be the norm rather than the exception. The regularity of the bundle would be increased if each shorter stereocilium could give rise to only one tip link, although it could be allowed that taller stereocilia might receive more than one tip link. In cases where there are slight irregularities in the orientations of the tip links, this is the pattern seen (e.g. arrowhead, Fig. 1a).

The regular hexagonal packing of the stereocilia can be altered by giving ototoxic drugs during development. Chick embryos were given a single dose of streptomycin, of 166 mg/kg egg weight, at different times between the 7th and 11th day of incubation, and allowed to develop for 4 - 12 days. In these cases, the stereocilia were commonly broken up into small groups on the cuticular plate. In the hair cell illustrated in Fig. 8, what appear to be the tallest row of stereocilia (arrow) are separated from what appear to be the shorter stereocilia, which are scattered irregularly around the cuticular plate. It would appear that the cuticular plate has undergone irregular expansion between the stereocilia, although the cuticular plate itself is no bigger than in the adjacent normal cell (Fig. 8). The stereocilia themselves also show irregularities, often being reduced in number, and unusually thick, thin, or fused, and with irregular steps in width. The abnormalities in the cuticular plate and stereocilia could most easily be ascribed to altered actin polymerisation and bundling. Indeed, possible interactions between actin organisation and aminoglycosides have been described as a result of biochemical experiments *in vitro*. Someya and Tanaka (1979) showed that aminoglycoside antibiotics stimulated actin polymerisation. On the other hand, Schacht (1986) has suggested that aminoglycosides bind to phosphatidylinositol biphosphate, which can encourage actin polymerisation by means of an interaction with the actin capping and severing proteins gelsolin, profilin, and

gCap39, (Lassing and Lindberg, 1985; Janmey and Stossel, 1989; Yu et al., 1990).

Within the common plan of acousticolateral hair cells, with their graded rows of stereocilia, there exist a number of variations in, for instance, shapes and number of rows. We have found a 'champion' in managing to dispense with rows of stereocilia in the echidna, where the inner hair cells have only two, straight, rows of stereocilia. This of course is the minimum number needed on any theory of transduction that requires interactions between different rows of stereocilia. Sometimes there are vestigial remains of the stereocilia of a third row, although they may not make contact with the other stereocilia (Fig. 9). There are also more rows of inner hair cells themselves than on eutherian mammals: one of the three animals examined had four rows of inner hair cells, while other two had five. The variation in what in other mammals are tightly regulated features suggests some degeneracy in cochlear organisation. The large number of inner hair cells (four or five rows in a cochlea less than half the length of that in human beings, giving twice as many inner hair cells), may be a compensation for the small number of rows of stereocilia on each one. The outer hair cells, on the other hand, have several 'V'-shaped rows of stereocilia, with again 4 - 5 rows of cells.

Some of the differences in shapes of hair bundle can also be understood on the tip link hypothesis. Rootlets of stereocilia are hexagonally packed in the cuticular plate, and in most types of hair cell this arrangement is preserved in the packing of the stereocilia right up to their tips (see e.g. Fig. 4). In bundles such as those of the bird papilla (Figs 1a and 4), the axes of the hexagon, the tip links, and the rows of equal height are related as in Fig. 10a. In guinea pig outer hair cells on the other hand, the rows of equal height follow one of the principal axes of the hexagon (Fig. 10b), and the tip links follow one of the other axes. For this reason, the tip links cannot run orthogonal to the rows of equal height, and if the tip links are to run parallel to the expected direction of mechanical input, the rows of stereocilia must be set at an angle to the cochlear duct. It is suggested that this explains the 'V' or 'W' shape of the rows on many mammalian outer hair cells (Pickles et al., 1984; Comis et al., 1985). Mammalian inner hair cells, by contrast, have a looser arrangement, with the hexagonal packing at the rootlets of the stereocilia being lost towards the tips of the stereocilia. With this looser arrangement the tip links manage to run orthogonal to the rows of stereocilia of equal height, even though the rows of equal height follow one of the principal axes of the hexagon and run parallel to the length of the cochlear duct (Comis et al., 1985). It is suggested that the underlying difference between inner and outer hair bundles relates to the differences in the closeness of packing of the stereocilia, and that this might be related to a higher mechanical strength of outer hair cell bundles.
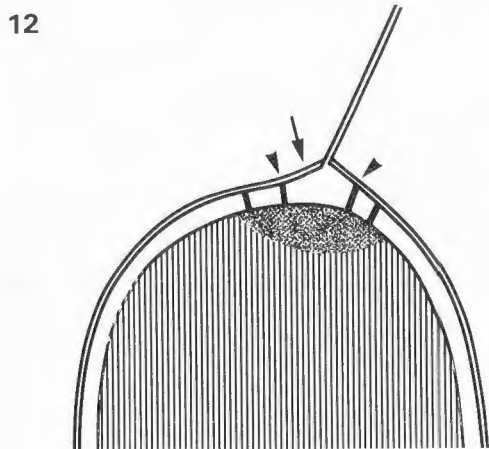
The fine structure of tip links

Investigations of the fine structure of tip links show that they have a fine central filament, approximately 6 nm in diameter, surrounded by a glycocalyx (Osborne et al., 1988). It is suggested that the central filament is proteinaceous, that it is responsible for the transferring the mechanical input to the mechanotransducer channels on the cell membrane. We have serially sectioned the tips of stereocilia in TEM, to make three-dimensional reconstructions of the way that the tip link attaches to the stereocilium. To give further information within the thickness of a section, stereo pairs of each section were taken. As shown in Fig. 1b, it was commonly found that the membrane at the lower
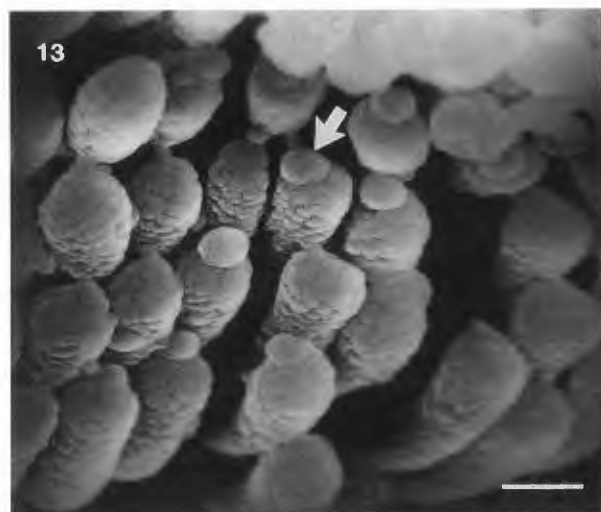
Figure 11. Tip of shorter stereocilium in the chick basilar papilla, showing extension of the membrane at the tip link insertion point (arrow). Glutaraldehyde/tannic acid fixation. Scale bar: 100 nm.

Figure 13. Blebs at the tip-link insertion points on the tips of the shorter stereocilia (e.g. arrow) in the chick basilar papilla. Glutaraldehyde/picrate fixation. Scale bar: 200 nm.

Figure 12. Suggested structure at the tip-link insertion point. The tip link attaches onto the stereociliar membrane or more likely onto connectors lying within it. These in turn are attached by strands (arrowheads) onto the density at the end of the actin paracrystal. The strain due to stimulus-induced forces produces a stretch of the membrane in this region (e.g. at arrow). This is an appropriate site for the mechanotransducer channel.

pen because there would then be no tendency for shrinkage to pull out one part of the tip link at the expense of other parts. We therefore suggest that the tip link is instead anchored onto structures at the level of the membrane, e.g. onto connectors running within the membrane, over which the membrane rides. The connectors are in turn are attached by strands to the dense spot in the actin paracrystal which underlies the attachment of the tip link (Fig. 1b). We have not so far been able to make absolutely unobstructed reconstructions of all the strands crossing the gap between the membrane and the actin paracrystal in this region, but estimate that there may be about four of them in a ring around the attachment site of the tip link, although the membrane is also attached to the dense spot by other strands further away. A suggested structure for this region is shown in Fig. 12. This arrangement would tend to stretch structures near where the tip link enters the membrane. If mechanotransduction involves pull on structures parallel to the plane of the membrane (Sachs, 1988), then this region forms the most appropriate site for the transducer channels. However, we have not seen any structures which could be candidates for the transducer channels themselves.

The membrane blebs seen in SEM probably represent sites of membrane weakness, and the lower attachment of the tip link is a favourite site for bleb formation (Fig. 13). This again suggests that forces arising from the tip link are concentrated on the membrane in this region.

The upper end of the tip link runs to a density on the side of the taller stereocilium (Pickles, 1985; Little and Neugebauer, 1985; Furness and Hackney, 1985; Osborne et al., 1988). Unlike the density underlying the lower end of the link, the density here makes a bridge between the actin paracrystal and the cell membrane, while membrane can be seen running over the surface of the density (Fig. 1b). In no case have we definitely seen the cell membrane being pulled out at this point. It is important to be able to distinguish between the central filament of the tip link, any possible pulled-out membrane, and the extracellular fragments which commonly attach at this point. We suggest that the link transfers the mechanical pull directly onto the

site of attachment of the tip link was pulled up into a point. In some cases, the extension was much more extreme than in Fig. 1b (e.g. Fig. 11). The simple interpretation is that such extensions are due to the membrane being pulled out by some form of shrinkage, probably during fixation. However, if the tip link inserted directly through the membrane onto the actin paracrystal below, and if it had the same extensibility at all points on its length, this would not hap-

density, which couples it onto the underlying actin paracrystal. We do not expect the force here to be coupled via the membrane; if there are membrane-bound channels here, the forces must in some way be redirected to them via a change in the conformation of the density.

Summary and Conclusion

The indirect arguments from electrophysiological investigations point to the tip links as being very strong candidates for the 'gating springs' that open the mechanotransducer channels. However, there has been no direct test of this hypothesis, nor do we have information as to which end of the tip link is the most likely site for the channels. The direction of mechanosensitivity is related to the spatial organisation of the stereocilia and the kinocilium; we have evidence from the fish lateral line suggesting that this is partly determined by the axis of previous mitosis of the hair cell. Once this is determined in development, we suggest that the gradient in the growth of the stereocilia produces the observed spatial organisation of the tip links, giving rise to the observed directional selectivity of the mechanotransducer process.

Acknowledgments

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the tip link is connected to the actin paracrystal via filaments, on which the membrane rides. Nevertheless, the microscopy data show that this is a region where membrane deformation does occur, and this suggests that there would be a corresponding stretch of the filaments on which the membrane rides. Since we have a deformation in the region of a membrane, it is possible that the filaments could transfer the forces onto channels within the plane of the membrane.

D.P. Corey: The speculation that streptomycin leads to irregular expansion of the cuticular plate, and does so by an effect on actin bundling, is really unsupported. Please comment.

D.N. Furness: In two respects the suggestion that the cuticular plate has expanded may be inappropriate. The visible features of the cell in SEM are the stereocilia and the apical membrane, not the cuticular plate itself. Therefore, it is not possible to suggest that the cuticular plate has expanded without using TEM to determine its limits. Moreover, since the authors cannot show that the dimensions of the cell are greater than adjacent ones, nor do they publish normative data from control material for comparison of size, then this interpretation is unsubstantiated. A simpler explanation of their images [sic] is that stereocilia have been lost or resorbed, leaving a reduced bundle and this could be readily substantiated by counting the stereocilia from equivalent areas in their controls, and comparing the numbers with the damaged hair bundle. Please comment.

Authors: A fuller paper on the effect of streptomycin is in press with Hearing Research (Pickles and Rouse, 1991). As shown in that paper, the apical surfaces of many cells are greatly enlarged with streptomycin, and TEM shows that the cuticular plate expands as well, although it is thinner than normal. When the apical surface is larger than normal, a reasonable explanation of wide gaps between the stereocilia (with the gaps being wider than the normal limits of a bundle's width), is that the apical surface has undergone irregular expansion. For a different type of pathology such as shown in Fig. 8, the explanation that stereocilia have merely been lost or resorbed is not appropriate, because stereocilia or remains of stereocilia are seen over most of the apical surface of the hair cell, and are not concentrated in a bundle on one side of the cell as in the normal cell to the right of Fig. 8. In many cells the stereocilia also are often of abnormally great length or irregular width. Since the cuticular plate contains actin, and the stereocilia contain actin (and the appearance of the actin cores can by TEM be shown to have changed), it is reasonable to suggest that something, directly or indirectly, has changed in the actin polymerisation and bundling. However of course we do not know if this is an early or late effect in the chain of abnormalities resulting from the streptomycin.

D.P. Corey: Suggesting that tip links derive from the lateral links seems awfully far out on a limb to me. Please comment.

D.N. Furness: New data are presented in the discussion of the development of the tip links and hair bundle with one figure (Fig. 7) which supports only one of the observations described, that links of random orientation occur in undeveloped bundles. This was first reported by Furness et al. (1989, text reference). Unfortunately, the other observations described by the authors here are not supported by micrographs. In particular the suggestion that "as the stereocilia become graded in height, some of the links are pulled to a more vertically-pointing position" is very important and should be substantiated. Therefore to include this material, further micrographs are needed which demonstrate adequa-

tely the authors' points. This is particularly important since the observations *do not agree with published data* from more than one source. Secondly, the published data should be discussed in the context of the authors' arguments. In particular, I would refer them to the paper on fish hair bundle development by Neugebauer (1986) in which the tip links are not present in developing bundles although some gradation in height is apparent. This is in agreement with Furness et al. (1989) who illustrate in organ culture that demonstrable tip links do not appear until after an initial gradient in stereociliary heights is established. Furness et al. then go on to suggest a hypothesis of how tip links of appropriate orientation may be 'selected' by auditory input in vivo. Please comment.

Authors: We have further evidence on this, mainly derived from TEM, and a paper discussing the observations in detail is in press with Hearing Research (Pickles et al., 1991). Our results, obtained in a closely-spaced sequence around the age at which the stereocilia start to develop their gradation in height, show that there is a continuity of appearance between (i) the band of links which connect immature stereocilia laterally just below their tips, (ii) the slightly upward-pointing links which are seen as soon as the stereocilia begin to develop their gradation in height, and (iii) the tip links seen in mature stereocilia. Although it is possible that these structures have nothing to do with each other, the most reasonable hypothesis is that they are related structures seen at different stages of development.

Preserving these fine extracellular structures is very difficult, particularly when they are immature, and it is possible that in the paper referred to, Neugebauer did not see tip links (or their precursors) for this reason. In the organ culture work of Furness et al. referred to, the argument becomes circular if a tip link is described as demonstrable only once a gradient in stereociliary heights has been established, since included in the definition of a tip link in SEM is that it runs "vertically" (i.e. nearly orthogonal to the cuticular plate), and so of necessity connects stereocilia of different height. The point made here, that there is a continuity of appearance between the links in different stages of development, suggests that tip links are a specialisation of lateral links. We suggest that the gradient in growth of the stereocilia induces this. With respect to the hypothesis of Furness et al. that auditory input plays a role in selecting tip link orientation, this is the full text of their discussion on that point: "This [the development of links] could occur during differentiation, perhaps in combination with functional selectivity, e.g. as a result of auditory input." (Furness et al., p. 107.) It is difficult for us to discuss the hypothesis when so few details are given. The operation of this mechanism is made unlikely by the patterns seen in anomalously oriented hair cells. They have tip links oriented in the normal direction with respect to the rows of stereocilia, but in an abnormal direction with respect to the adjacent normal outer hair cells, although we expect the two types of cell to share the same mechanical input (Comis et al., 1989, text reference).

D.N. Furness: The most compelling evidence presented in Ohmori (1988, text reference) that supports the idea that the transducer channels are located towards the base of the stereocilia, is from the Mn^{2+} - Fura-2 quenching experiment. This clearly shows a band of quenching associated with Mn^{2+} influx at the base of the bundle (Mn^{2+} can carry the transducer current). How would the authors account for these observations if the channels were at the tips of the stereocilia?

Authors: There are a number of unresolved points in the experiment of Ohmori (1988). The experiment with Mn^{2+}-

quenching, using a distant puff of Mn^{2+}-Ca^{2+} saline and presumably giving a small mechanical stimulus to the bundle, showed that the greatest changes in Ca^{2+} signal were commonly produced *within the bundle itself*. This is in agreement with his results from stimulation in Ca^{2+}-saline alone, and is compatible with the channels being within the hair bundle. The closer puff of Mn^{2+} saline produced the greatest changes in signal within the bundle and also at the level of the cuticular plate. It is possible, as suggested by Hudspeth (1989, text reference), that the greater range of effects in this case was due to intracellular diffusion of ions. In any case, Ohmori's results have more recently been contradicted by Huang and Corey (1990), who found the greatest Ca^{2+} influx at the tips of the stereocilia, by using the Ca^{2+} indicator dyes rhod-2/AM and fluo-3. The localisation of transducer channels to the tips of the stereocilia is also supported by Jaramillo and Hudspeth (1991), who found that transducer currents were most easily blocked by the extracellular application of channel blockers to the tips of the stereocilia, rather than elsewhere in the bundle.

D.N. Furness: Substantial numbers of intercellular links (connecting stereocilia of adjacent hair cells, especially inner hair cells) have been described (Hackney and Furness, 1986; Hackney et al., 1988). What are the implications of this for the nature of the links?

Authors: Because of the intercellular links that they found, Hackney et al. put forward the view that *all* links (even those between stereocilia on the same cell) were purely a fixation artefact, and nothing but a condensation of the extracellular glycocalyx. They also suggested that, being condensation artefacts, the tip links were likely to have nothing to do with transduction, and implied that they and the other links had no functional significance. This view has however not gained general acceptance. Applied to the interstereociliary links on the same cell, it does not account for the specific internal structure of the links, with a central filament (Osborne et al., 1988, text reference), for the fact that the links survive digestion by enzymes which attack glycocalyx but not those which attack protein (Osborne and Comis, 1990; Pickles et al., 1990b), and for the fact that links are associated with submembraneous densities (Fig. 1b; see also Osborne et al., 1988). Functionally, it would be difficult to see how in the unfixed state forces could be transferred between the different rows of stereocilia on a bundle if the links were just artefacts of fixation. Moreover the measurements of gating stiffness suggest that the channel is opened by a structure with the resilience of an elastic protein (Howard and Hudspeth, 1988, text reference). Of course, it is possible that artefactual condensation fragments can overlie the bundle (some are probably seen in extending towards the bottom of the picture in Fig. 4). One might possibly mistake these for linkages, although with reasonable care, involving an assessment of the number of such structures and the regularity of their appearance, there should be no possibility of error. Nevertheless, in reporting occasional 'supernumerary' or 'anomalous' links, the possibility of artefact should always be considered very carefully. The hypothesis for the formation of the tip and other links put forward by Pickles et al. (1991), suggests that material is secreted extracellularly by the stereociliar membrane, particularly at the tips, and condenses in the gaps between adjacent stereocilia to form links. In this way, links would tend to be formed between adjacent structures, with the main criterion being contiguity, and this may account for some of the links between hair cells mentioned above.

D.N. Furness: Tip links with abnormal orientations, and two-to-one tip links have been described (see e.g. Hackney

et al., 1988; Furness et al., 1990), which might argue against the precision of tip link orientation. Please comment.

Authors: We have given a systematic account of tip link orientation in our papers by Comis et al. (1985, text reference) and Pickles et al. (1989, text reference). In general, in the centre of the bundle, each taller stereocilium receives a tip link from only one shorter stereocilium. However, around the edges of the bundle, it is common for one taller stereocilium to receive tip links from two shorter stereocilia. An example has already been pointed out in Fig. 1A (arrowhead). This gives rise to a convergence of the tip link "columns" in the terminology of Pickles et al. (1989), and seems to be associated with the rounded shape of the edge of the bundle. Pickles et al. (1989) also described a minority of clearly anomalous cells which did not fit into this pattern, where there was convergence of tip link columns in the centre of the bundle, leading to large groups of tip links oriented in different directions in different parts of the bundle. These cells are however unusual; but, as in most biological systems, one should not be surprised if complete regularity is not always obtained. Concerning the papers by Hackney et al. and Furness et al. to which you referred: we are not sure about the implications of the points raised. In the paper by Furness et al. the bundle was clearly abnormal in other ways, being rotated with respect to the normal position, and with irregularly shaped rows. The two-to-one links were situated at the points where the rows changed direction. We suggest that the tip links reflect this anomalous organisation, particularly in view of our hypothesis that the direction of the tip links reflects the development in the gradient in heights of the stereocilia (see above). In the paper by Hackney et al., at least in the published micrographs available to us, we are not sure whether some of the structures referred to as tip links are tip links at all, rather than just fragments of material lying around the tips of the stereocilia (see particularly Fig. 2b of that paper, especially since the material identified as the link is adjacent to other similar-looking fragments of material which do not bridge between stereocilia). As pointed out above, it is very important to be sure that material identified as links are not artefactual. This can sometimes be very difficult, and we would prefer not to comment on cases which do not appear clear cut.

D.N. Furness: The diagram, Fig. 2, showing that stereociliary separation detected by horizontal links is not appropriate for gating the channels is not wholly convincing. If the tip links are drawn into this diagram, with fixed attachment points on the taller and shorter stereocilia, they are also *compressed* when the bundle is pushed and are *stretched* when it is pulled. Therefore if the stereocilia and the horizontal cross-links behave as in shown in Fig. 2, the tip links are equally poor candidates for gating the channels. Please comment.

Authors: The logic as we have presented it depends on the observation that the bundle is tapered, with the stereocilia of the different rows coming into close apposition at their tips (e.g. as drawn in Fig. 2b). The micrograph of Fig. 1b shows that the gap between the stereocilia at the tip is indeed narrow (about 30 nm in that case, although the exact figure must be treated with extreme caution). Therefore if the shorter stereocilia were pushed towards the taller, i.e. in the excitatory direction, the possibility for further closing of the stereocilia is limited (and it is likely that one of the functions of the glycocalyx is to keep the stereocilia apart; Flock et al., 1977). Pushing the stereocilia in the excitatory direction would for this reason tend to stretch and not compress the tip links, this being the critical direction for

the hypothesis presented.

D.N. Furness: The authors refer to unpublished calculations to support their argument that the forces transmitted to each tip link would be the same. What are these calculations?

Authors: Let a stereocilium make an angle α with the apical surface of the cell measured in the direction of gradation of the stereocilia, the upper end of the tip link insert at a distance u above the cuticular plate, the shorter stereocilium to which the same tip link is attached have a length l, the tip link continue in a direction with an angle β with respect to the long axis of the shorter stereocilium to which it is attached, the tip link have a length t, and the separation of the stereocilia at the base minus the spacing at the level of the tip of the shorter stereocilium be s, then when the taller stereocilium is deflected, if the separation of the upper ends of the stereocilia is kept constant by the lateral links, simple geometry shows that $dt/d\alpha$ (i.e. the stretch of the tip link per radian deflection of the taller stereocilium, for small deflections of the stereocilia) $= (us/l) \sin\alpha\cos\beta$. Simple geometry also shows that the shorter stereocilium is then deflected by an angle almost exactly the same as the taller one. The equivalent equation can therefore be applied to the tip link between it and the next shorter stereocilium and so on across the bundle. Measurements of the dimensions and angles of bundles in TEM cut accurately parallel to the plane of the gradation in heights of the stereocilia giving values of u, s, l, α and β show that $dt/d\alpha$ is nearly constant (to within a few percent) over the different ranks of tip links on a cell. This calculation assumes that the lateral links keep the tips of the stereocilia at a constant distance from each other; however, that assumption may not be necessary. Because the angle β is non-zero, tension on a tip link induces a turning moment on the shorter stereocilium, so transferring the deflection to the next row of stereocilia. The variation of β across the different rows of stereocilia in the bundle, together with the different distances of insertions of the tip links from the cuticular plate, are consistent with the hypothesis that a constant deflection is transferred to the different ranks of stereocilia on the bundle without any tendency for the rows to separate, even without any involvement of the side links. However, to establish this, one needs to know the actual values of ratio of the stiffness of the tip link to the stiffness of the stereociliar rootlets to deflection, which is at present not known.

D.N. Furness: The accelerating voltage quoted for SEM is rather high. Is this a typographical error?

Authors: We use 80 kV routinely, because we find that we get an increased spatial resolution which more than compensates for any possible loss of surface detail due to the increased electron interaction volume.

D.N. Furness: There is no discussion of the work of Tilney et al. (1988), who postulate the involvement of tip links in the development of the bundle, by causing the opening of channels and contributing to the control of actin filament growth. Please comment.

Authors: As pointed out previously (Pickles et al., 1988; Pickles et al., 1989, text reference), we have some results which agree with the hypothesis of Tilney et al. We showed in some bundles that the stereocilia in a single tip link column (i.e. a group of stereocilia graded in height and joined together by tip links) could sometimes be all a little shorter, or a little taller, than corresponding stereocilia in neighbouring columns. The obvious suggestion is that some factor, common to all the stereocilia in a single tip-link column, has affected their growth in a common way. This agrees with the hypothesis that the signal for growth is transmitted via the tip links. It should be added that the hypothesis of Tilney et al. has interestingly different implications depending on whether the transducer channels are at the upper or lower ends of the tip link. To restate their hypothesis: growth of the taller stereocilium (or, one would now add, the adaptation mechanism which keeps a resting tension on the tip link; Howard and Hudspeth, 1988, text reference), pulls on the tip link, opening the transducer channel at the lower end of the tip link, allowing Ca^{2+} to enter, and uncapping the actin filaments comprising the shorter stereocilium. In this way, a wave of growth would spread, as observed, from the taller stereocilia in the bundle to the shorter (Tilney et al., 1988). Similarly, if channels were at the top ends of the tip link, and Ca^{2+} instead *capped* actin filaments (as in some other systems; e.g. Yu et al., 1990), a signal for growth would also be transferred between stereocilia, although the wave of growth would now spread from the shorter stereocilia to the taller. On the other hand, no growth signal would be transferred (in either direction) along a tip-link column either if Ca^{2+} uncapped the actin filaments and the transducer channels were at the lower end of the tip link, or if Ca^{2+} capped actin filaments and the transducer channels were at the top end of the tip link. The observations described by Pickles et al. (1991) suggest that, in the initial phase of growth of stereocilia, when the tall stereocilia grow first and the shorter stereocilia grow later, there is no obvious columnar arrangement in the way that the growth is transferred across the bundle. On the other hand, in the final phase of lengthening described by Tilney et al. (1988), the shorter stereocilia stop growing first, and the taller stereocilia stop later. Could it be this final growth phase that gives rise to the small consistent variations in the height of tip-link columns? If so, it would suggest (i) that the transducer channel is at the top end of the tip link, and (ii) that ion entry (possibly Ca^{2+}) helps cap the actin filaments of the stereocilia.

D.N. Furness: Blebs can be seen in Fig. 13 at this location, even when a tip link is not apparent, and also in other places along the stereocilia (this has been reported in several other publications e.g. Osborne and Comis, 1990). This suggests that blebbing occurs at several weak points in stereocilia due to hypoxic damage, without any particular association with mechanical stress. Please comment.

Authors: Many factors could contribute to the membrane weakness which leads to blebbing, and we are suggesting that, particularly in the case of the material shown, mechanical stress is one of them. In cases where the tip links are missing, a link could have been expected to stress its attachment points as it broke during specimen preparation, and this could account for the presence of blebs at this site when the link was missing.

D.N. Furness: The description of tip links as being orthogonal to the cuticular plate is rather inaccurate. Firstly, the authors probably mean the surface of the cell (the cuticular plate underlies the cell membrane). If they wish to specify a relationship to the plate they should state clearly to which surface they are referring. Secondly, the tip links can be found running at many different angles to the apical surface of the cell and rarely are they truly orthogonal in EM preparations. It would be more accurate to describe them as having a 'vertical' component in order to distinguish them from horizontal links. Please comment.

Authors: The cuticular plate is parallel to the apical surface of the cell, and so either can be used as a reference plane with similar results. The tip links were described as running nearly orthogonal (not just orthogonal as stated in

the question), and this encompasses the range of angles seen. In using this terminology, we wanted to get away from describing the links as running "horizontally" and "vertically", because this depends on drawing the hair cell with the stereocilia pointing vertically, and this of course is a purely conventional orientation which bears no relation to the real orientation.

D.N. Furness: The authors are heavily dependent on physiological data published by Hudspeth and colleagues, with little or no reference to other major groups which have contributed substantially to this field. For example, Russell et al. (1986) present results on the mammalian system which show both similarities to, and differences from, Hudspeth's data. The work of Crawford, Fettiplace and other colleagues deserves more attention, in particular their work on adaptation and the effects on the transducer are relevant (Crawford et al., 1989), especially in relation to that of Hacohen et al. (1989) who gave detailed consideration of the tip-link hypothesis in discussing how the compliance changes with calcium. Morphological papers by Neugebauer (1986), Neugebauer and Thurm (1987), Furness and Hackney (1986) are also relevant. Please comment.

Authors: In order to encourage the readability of the review, we have only quoted those who made the initial and pioneering discoveries, not those whose work has been essentially confirmatory in relation to the points made here. For instance, the results of Russell et al. (1986) and those described in earlier papers, do not go beyond those of Hudspeth and Corey (1977, text reference) in so far as they relate to the basic mechanisms of transduction. We are not aware of any contradictions between these sets of results. The morphological papers of Neugebauer and Thurm, and Furness and Hackney, confirmed the presence of tip links, initially described by Pickles et al. (1984, text reference) and Osborne et al. (1984, text reference).

The papers by Hacohen et al., (1989) and Crawford et al. (1989) deal with a Ca^{2+}-sensitive adaptation of the transducer currents, following sustained deflection of the stereocilia. The results of Hacohen et al., and the earlier results of Howard and Hudspeth (1988, text reference), suggested that a mechanical reorganization of the bundle, most easily explained by a movement of the upper tip-link insertion point, explained adaptation. However Crawford et al. were able to turn off adaptation while still getting the mechanical shift of the bundle which Howard and Hudspeth and Hacohen et al. suggested was the basis for adaptation. Possibly, as Crawford et al. suggested, the mechanical changes due to the movement of the tip-link insertion point were too small to be detected. They also suggested that adaptation might be produced by Ca^{2+} directly affecting the operating point of the transducer channel. It should however be pointed out that there are some direct differences in the observations in the two systems. Adaptation in the bullfrog sacculus does not appear to change the slope of the input-output function, but does in the turtle cochlea. Moreover Crawford et al. did not detect any differences in the mechanical properties of the bundle associated with the gating stiffness (Howard and Hudspeth, 1988) in the cases where adaptation did and did not occur, suggesting that they were not able to detect mechanical changes associated with events at the level of the transducer. However it should be pointed out that these results relate only indirectly to the mechanisms of transduction and cannot be regarded as providing any closely direct evidence on its mechanism.

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