Development 125, 557-566 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
DEV1233

Shaker-1 mutations reveal roles for myosin VIIA in both development and function of cochlear hair cells

Tim Self[1], Mary Mahony[1], Jane Fleming[1], James Walsh[2], Steve D. M. Brown[2] and Karen P. Steel[1,*]

[1]MRC Institute of Hearing Research, University Park, Nottingham NG7 2RD, UK
[2]MRC Mammalian Genetics Unit and Mouse Genome Centre, Harwell, Didcot, Oxfordshire OX11 0RD, UK
*Author for correspondence (e-mail: karen@ihr.mrc.ac.uk)

Accepted 27 November 1997: published on WWW 22 January 1998

SUMMARY

The mouse *shaker-1* locus, *Myo7a*, encodes myosin VIIA and mutations in the orthologous gene in humans cause Usher syndrome type 1B or non-syndromic deafness. *Myo7a* is expressed very early in sensory hair cell development in the inner ear. We describe the effects of three mutations on cochlear hair cell development and function. In the *Myo7a^{816SB}* and *Myo7a^{6J}* mutants, stereocilia grow and form rows of graded heights as normal, but the bundles become progressively more disorganised. Most of these mutants show no gross electrophysiological responses, but some did show evidence of hair cell depolarisation despite the disorganisation of their bundles. In contrast, the original *shaker-1* mutants, *Myo7a^{sh1}*, had normal early development of stereocilia bundles, but still showed abnormal cochlear responses. These findings suggest that myosin VIIA is required for normal stereocilia bundle organisation and has a role in the function of cochlear hair cells.

Key words: *shaker-1*, Mouse mutant, Myosin VIIA, Cochlea, Hair cell, Stereocilia, Hearing, Usher syndrome

INTRODUCTION

Very little is known about the molecular basis of sensory hair cell function or development, partly because the small number of hair cells in each inner ear makes a biochemical approach impractical. We have adopted a genetic approach to identifying molecules with key functions in hair cells, using positional cloning of mouse mutations known to lead to inner ear defects. The first gene to be identified using this approach and affecting the sensory hair cells directly was the myosin VIIA gene (*Myo7a*) in the shaker-1 mouse mutant (Gibson et al., 1995).

The shaker-1 mouse mutant was first described in the 1920s (Lord and Gates 1929), and shows the typical shaker-waltzer type behaviour often associated with inner ear defects: deafness, hyperactivity, head-tossing and circling. Defects are first seen in the inner ear neuroepithelia by light microscopy, with progressive degeneration of the sensory hair cells of the organ of Corti and saccule (Deol, 1956; Kikuchi and Hilding, 1965; Grüneberg et al., 1940; Mikaelian et al., 1965; Shnerson et al., 1983). The mutants show some behavioural and physiological responses to loud sounds for a short period following the onset of auditory function at around two weeks after birth (Mikaelian and Ruben, 1964; Grüneberg et al., 1940; Steel and Harvey, 1992; Harvey, 1989). Previous reports of the rate of hearing loss and the organ of Corti abnormalities in the original shaker-1 mutants vary, which might be explained by differences in genetic background between the various studies (eg Emmerling and Sobkovicz, 1990; Steel and Harvey, 1992). The stria vascularis continues to function normally in generating a high resting endocochlear potential (Brown and Ruben, 1969).

We identified the gene affected by the shaker-1 mutations as encoding myosin VIIA, an unconventional myosin (Gibson et al., 1995). The same gene, MYO7A, is involved in Usher syndrome type 1B in humans, in which the congenital balance and hearing defects are accompanied by progressive retinitis pigmentosa (Weil et al., 1995; Weston et al., 1996; Lévy et al., 1997; Liu et al., 1997a), as well as in non-syndromic deafness (Liu et al., 1997b; Weil et al., 1997). The complete human (Chen et al., 1996; Weil et al., 1996; Kelley et al., 1997) and mouse (Mburu et al., 1997, accession number U81453) sequences for this large gene have been determined, and we have recently identified all seven available mutations at the shaker-1 locus (Mburu et al., 1997). Some of the mutations lead to premature stop codons early in the sequence, or are predicted to change highly conserved residues in functionally important parts of the motor head of the myosin; furthermore, at least two of the mutations in the mouse *Myo7a* gene are associated with very low levels (less than 1% of normal) of myosin VIIA protein, suggesting that these mutations are effectively null mutations (Hasson et al., 1997a).

The gene is expressed in the cochlea, eye, kidney, testis and lung, although there is no obvious kidney, testis or lung phenotype associated with myosin VIIA mutations (Gibson et al., 1995; Weil et al., 1995; Smith et al., 1994). Using antibodies to the myosin VIIA protein, Hasson and colleagues

(1995, 1997b) have localised the gene product to sensory hair cells of the inner ear and, in particular, to the stereocilia, cuticular plate and the pericuticular necklace, a vesicle-rich zone lying between the cuticular plate and the junctional complex surrounding the top of the hair cell (Hasson et al., 1997b; Kachar et al., 1997). We have recently shown that myosin VIIA is required for aminoglycoside accumulation in cochlear hair cells, although it does not appear to influence non-specific endocytosis at the apical surface of the hair cell (Richardson et al. 1997).

Little is known about the function of unconventional myosins such as myosin VIIA, although several have been implicated in vesicle transport in diverse cell types (eg Mermall et al., 1994; Durrbach et al., 1996; Hill et al., 1996; Govindan et al., 1995; Geli and Riezman, 1996; Mochida et al., 1994). The role of myosin VIIA in sensory hair cell function is unknown, so we have exploited the shaker-1 mouse mutants to perform a detailed study of the ultrastructural and electrophysiological development of cochlear hair cells. We have focussed on three of the mutations which are well-characterised and affect different parts of the 5′ end of the gene encoding the motor head of the molecule. Our findings suggest that myosin VIIA not only has a role in hair cell function, but also is essential for organising the developing stereocilia bundle (hair bundle) within the top of the hair cell.

MATERIALS AND METHODS

Mice

The original spontaneous shaker-1 (*Myo7a*^{sh1}) mutation was obtained from Harwell, UK, in the mid-1980s, backcrossed several times to the CBA/Ca inbred strain and is now maintained as a closed colony. *Myo7a*^{6J} was also a spontaneous mutation, occurring in the C57BL/6J strain and was kindly provided by Dr Wayne Frankel, The Jackson Laboratory, ME, on a mixed 25% BALBc, 75% C57BL/6J background (Letts et al., 1994). The *Myo7a*^{816SB} allele was derived from an ENU mutagenesis programme; the mutation was originally induced in a BALBc mouse, which was then repeatedly crossed to the BS inbred strain used at Oak Ridge National Laboratories, TN, and the stock was kindly provided by Dr Gene Rinchik (Rinchik et al., 1990). Mice were normally generated by heterozygote-to-homozygote matings to give segregating litters that included heterozygotes used as littermate controls. Timed pregnancies were used to generate prenatal stages, with the morning of the plug counted as 0.5 days post coitum (d.p.c.) for overnight matings, and the day of birth was 0 days. All mice were kept in full accordance with UK Home Office regulations.

Genotyping

Mice aged 12 days or older were classified as homozygous mutant or heterozygous control on the basis of the abnormal behaviour of the mutants. Mice younger than this were genotyped as follows. For the original *Myo7a*^{sh1} mutation, the primers CTGACAACCAGGAAGCACTG forward and ATCGATGAGGGAGATGACG reverse were used for PCR across the mutation, annealing at 51°C with 1 mM Mg^{2+}, followed by an *Msp*I digest to give a 65 bp product in homozygote mutants, and 65 bp, 37 bp and 28 bp products in heterozygous littermates. For the *Myo7a*^{816SB} mutation, the primers CAGGGCCTCCATCCCAGTAG forward and CGGATGCGGATCGTCTCCATC reverse were used for PCR across the mutation, annealing at 49°C with 4 mM Mg^{2+}, followed by an *Alu*I digestion to give 86 bp and 38 bp products in the homozygote mutants and 86 bp, 55 bp, 38 bp and 31 bp products in the heterozygote controls. The *Myo7a*^{6J} mutation was detected by using the primers GACAACTCTAGCCGCTTTGG forward and AGTGTGCTAACAGATGGCCC reverse for PCR, annealing at 57ºC with 2 mM Mg^{2+}, followed by restriction with *Afl*III, giving a 190 bp product in homozygous mutants and 190 bp, 99 bp and 91 bp products in heterozygotes.

In situ hybridisation

^{35}S-labelled RNA probes were made by standard techniques, using T7 (sense) or SP6 (antisense) RNA polymerase to transcribe from the plasmid template. The template was a myosin VIIA clone in pCRII (Invitrogen) covering bases 1 to 1350 of the gene, corresponding to the motor head of the myosin molecule. A total of 26 CBA/Ca mice were used at daily intervals from 14.5 d.p.c. to 19.5 d.p.c. plus newborn, 3 days and 6 days after birth, together with 8 BALBc mice ranging from 17 dpc to 6 days after birth. Specimens were fixed in 4% paraformaldehyde and embedded in paraffin wax, and serial sections through the entire inner ear were cut at 8 μm. Every tenth section (or more) was used to hybridise with the labelled sense strand as a control for non-specific hybridisation, while the rest were hybridised with labelled antisense strand overnight at 55°C, essentially as described before (Wilkinson et al., 1987; Steel et al., 1992).

Scanning electron microscopy

A total of 43 *Myo7a*^{6J} homozygotes and 36 littermate control heterozygotes were examined at 16.5 d.p.c., 18 d.p.c., newborn, 3 days, 12 days, 15 days and 20 days after birth. From the *Myo7a*^{816SB} stock, 5 homozygotes and 8 heterozygote controls were studied at 3 and 12 days of age, and from the *Myo7a*^{sh1} stock, 5 mutants and 8 littermate controls were studied at 3 and 15 days old. Inner ears were dissected under 2.5% glutaraldehyde, 0.25% tannic acid, pH 7.2, and fixed for 5 hours at 4ºC. The osmium tetroxide-thiocarbohydrazide (OTOTO) procedure adapted from Hunter-Duvar (1978) was used to stain prior to dehydrating and critical-point drying. Specimens were sputter coated with gold and examined in a Jeol 6400 Winsem at 20 kV.

Transmission electron microscopy

A total of 56 control hair cells and 82 mutant hair cells were examined at 18 d.p.c., newborn, 3 and 4 days after birth from the *Myo7a*^{6J} and *Myo7a*^{816SB} stocks. Cochleas were fixed in 2.5% glutaraldehyde, 1% paraformaldehyde, 0.25% tannic acid, 1% sucrose, pH 7.2 for 5 hours at 4ºC, postfixed in 1% osmium tetroxide, dehydrated, stained en bloc with 1% phosphotungstic acid and 1% uranyl acetate, and embedded in Araldite epoxy resin. Sections were stained with uranyl acetate and lead citrate and examined in a Jeol 1010 electron microscope at 80 kV.

Electrophysiology

Homozygous mutants and their littermate controls were anaesthetised with urethane, the middle ear was opened to expose the cochlea and a silver wire electrode was placed on the round window to record evoked cochlear potentials, as previously described (eg Steel and Smith, 1992). The following mice were studied: *Myo7a*^{816SB} stock, 13 mutants at 12, 15 and 20 days old; *Myo7a*^{6J} stock, 11 mutants at 12, 15 and 20 days old; *Myo7a*^{sh1} stock, 9 mutants at 15 and 20 days old, all with the same number of littermate controls.

RESULTS

Myosin VIIA is expressed very early in hair cell development

We have looked at expression of the myosin VIIA gene during normal development by in situ hybridisation using a clone of the gene covering part of the head domain that has previously

been shown by northern analysis to be specific for this gene (Gibson et al., 1995). Myosin VIIA was strongly expressed in sensory epithelia of the vestibular system (the saccular and utricular maculae and the three cristae) from the earliest stage we studied, 14.5 d.p.c. (data not shown). This is at about the time that sensory hair cells can be distinguished histologically (Mbiene et al., 1984). The labelling was located in the upper layer of the epithelia, suggesting it was restricted to developing sensory hair cells. In the cochlea, the first labelling was seen in the organ of Corti of the basal turn of the cochlear duct at 16.5 d.p.c. and, by 17.5 d.p.c., it was seen throughout the cochlea including the apex. This base-to-apex gradient in expression of myosin VIIA correlates with and pre-dates many other features of organ of Corti development which proceed in a base-to-apex direction (e.g. Rubel, 1978). Within the organ of Corti, the labelling appears to be localised to the region of the one row of inner hair cells and three rows of outer hair cells (Fig. 1), and no labelling above background levels was found in the developing supporting cells or the cochlear neurons. Myosin VIIA thus is expressed in cochlear hair cells a full day before we can detect the first signs of development of their characteristic ultrastructural features in mice of the same stock, suggesting that it may serve as a useful early marker for hair cell differentiation.

Mutants studied

We describe three shaker-1 mouse mutants here, with mutations affecting different parts of the motor head domain of the myosin VIIA molecule (Gibson et al., 1995, see Mburu et al., 1997 for detailed description of the mutations). The original shaker-1 (*Myo7a*^{sh1}) mutation is an arginine-to-proline missense mutation, located in a surface loop that has a relatively unconserved amino acid sequence. The *Myo7a*^{6J} mutation is also an arginine to proline missense mutation, but at a highly conserved position in the core of the motor domain, where it is expected to interact with a number of surrounding residues; this mutation is thus predicted to have a serious effect on protein stability and function. The third mutation examined here is the *Myo7a*^{816SB} mutation that changes a splice acceptor site sequence, leading to skipping of a 30-base exon and predicted deletion of ten amino acids from the core of the myosin head, close to the proposed hinge domain. Such a deletion would be expected to have a severe effect on protein structure and function. The *Myo7a*^{6J} and *Myo7a*^{816SB} mutations appear to affect protein stability, because mRNA levels are normal but myosin VIIA levels are reduced to 21% and 6% of normal, respectively (Hasson et al., 1997a). The phenotypic effects of these two mutations may be partly due to protein instability in addition to abnormal function of the remaining myosin VIIA molecules.

Stereocilia bundles develop abnormally in two of the shaker-1 mutants

We examined the development of the upper surface of cochlear hair cells by scanning electron microscopy from 16.5 d.p.c. to 20 days after birth. At 16.5 d.p.c. in our stocks, hair cells cannot be distinguished from surrounding supporting cells except by their position in relation to the greater and lesser epithelial ridges, and there were no obvious differences between mutants and controls (not shown).

In normal littermate controls, there was a dramatic maturation of stereocilia bundles developing from a uniform covering of microvilli. By 18 d.p.c., hair cells could be clearly distinguished by the regular array of microvilli, some of which on the lateral side of each hair cell were elongating to form a V-shaped array with a kinocilium at the pole and a flexible appearance (Fig. 2A). 2 days later, in the newborn mouse, the tallest microvilli were arranged in more ordered rows of graded heights, and appeared straighter, taller and more rigid than previously (not shown). By 3 days after birth, the stereocilia bundle is located in the centre of the hair cell top, with a kinocilium and a patch of microvillus-free membrane on the lateral side and remaining microvilli on the inner (modiolar) side (Fig. 2C). In the basal turn, which is more advanced in development, there are fewer of these microvilli, and they appear to be resorbed progressively over the next few days until there are only stereocilia and no microvilli remaining by around 12 days after birth (Fig. 2E shows a mature stereocilia bundle). The kinocilium also regresses and disappears during this same period.

In the *Myo7a*^{6J} mutants, the first signs of abnormalities can be detected as early as 18 d.p.c., when some of the growing microvilli show an irregular arrangement (Fig. 2B). Furthermore, the kinocilium is often eccentric in position, misplaced from its normal regular position at the lateral pole of each hair cell, and is often difficult to identify (Fig. 2B). By 3 days after birth, the formation of the stereocilia rows is very disordered with several small clumps of stereocilia appearing on the tops of some hair cells (Fig. 2D). As in normal mice, the basal turn develops ahead of the apical turn, and the disruption in development is thus more clearly seen in the base in the mutants. The disruption of the stereocilia rows and the erratic position of the kinocilium is more clearly seen in the outer hair cells, but is also present in the inner hair cells. The

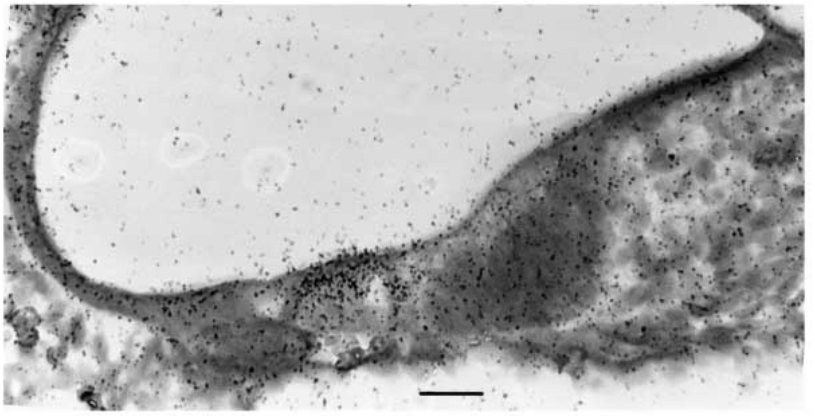

Fig. 1. In situ hybridisation showing myosin VIIA mRNA distribution in the normal developing inner ear. Organ of Corti at 19.5 d.p.c. with increased labelling (black grains) over inner hair cell (on the right) and outer hair cells (left). Although it is difficult to distinguish individual cells after processing for in situ hybridisation, the characteristic shape and staining pattern of the developing organ of Corti and the presence of the tunnel (a white triangle separating the inner and outer hair cells) are good indicators of the location of the hair cells. There is only background labelling elsewhere in the cochlear duct. Scale bar represents 20 μm.

stereocilia become more disorganised over the next few days, with many of them disappearing, possibly by falling off because stumps are often seen, until by 20 days after birth there are very few remaining (Fig. 2F).

In the *Myo7a*^{816SB} mutants at 3 days old, a similar disorganisation was seen as in *Myo7a*^{6J}, but the abnormalities were more extreme, with more hair cells showing several small clumps of stereocilia and fewer hair cells having a single clump (Fig. 3). The clumps of stereocilia each contained rows of graded heights, but the orientation of these rows was erratic, and there were often clumps oriented in opposite directions on a single hair cell.

In both these mutants, the stereocilia appear to have grown to their normal lengths and proportions, and have formed rows of graded heights as they normally would. However, the stereocilia do not appear to be positioned correctly within the top of the hair cell.

In both *Myo7a*^{6J} and *Myo7a*^{816SB} mutants at later stages, hair cells have deteriorated with many being lost or showing signs of degeneration. However, a few hair cells do remain at the stages used for electrophysiology (12, 15 and 20 days), and some of the best-preserved hair cells are shown in Fig. 4 to allow correlation with the physiological recordings.

Stereocilia bundles in the original shaker-1 mutant show only minor anomalies

In contrast to the clear stereocilia bundle defects seen in the *Myo7a*^{6J} and *Myo7a*^{816SB} mutants, in the original *Myo7a*^{sh1} mutants the stereocilia bundles look reasonably normal at 3 d.p.c. (Fig. 5). No gross disorganisation of the hair cells can be seen by scanning electron microscopy at 3 or 15 days after birth, although many of the outer hair cells at 15 days had only two rows of stereocilia instead of the usual three rows (Fig. 4).

Hair cell ultrastructure in shaker-1 mutants

We examined the hair cells of the two most severely affected mutants, *Myo7a*^{6J} and *Myo7a*^{816SB,} by transmission electron microscopy, focussing particularly on the cuticular plate, into which the stereocilia become anchored as they mature, and the synaptic regions. The cuticular plate at 18 d.p.c. was loosely organised, being represented by a clearing of intracellular organelles in some less-developed cells (e.g. apical turn hair cells) while, in more-developed hair cells, the cytoskeletal matrix comprising the cuticular plate was beginning to condense (Fig. 6A). By birth, all hair cells examined showed a significant condensation of cuticular plate material, which looked essentially mature (Fig. 6B). There were regions of vesiculation forming a network around the periphery of the cuticular plate (the pericuticular necklace) and the area immediately around the insertion point of the kinocilium was also filled with many vesicles. Densely stained rootlets were seen extending from the core of the stereocilia into the cuticular plate by birth, and the cylindrical shape of each stereocilium narrowed towards the insertion point, forming the ankle (Fig. 6B). Condensation

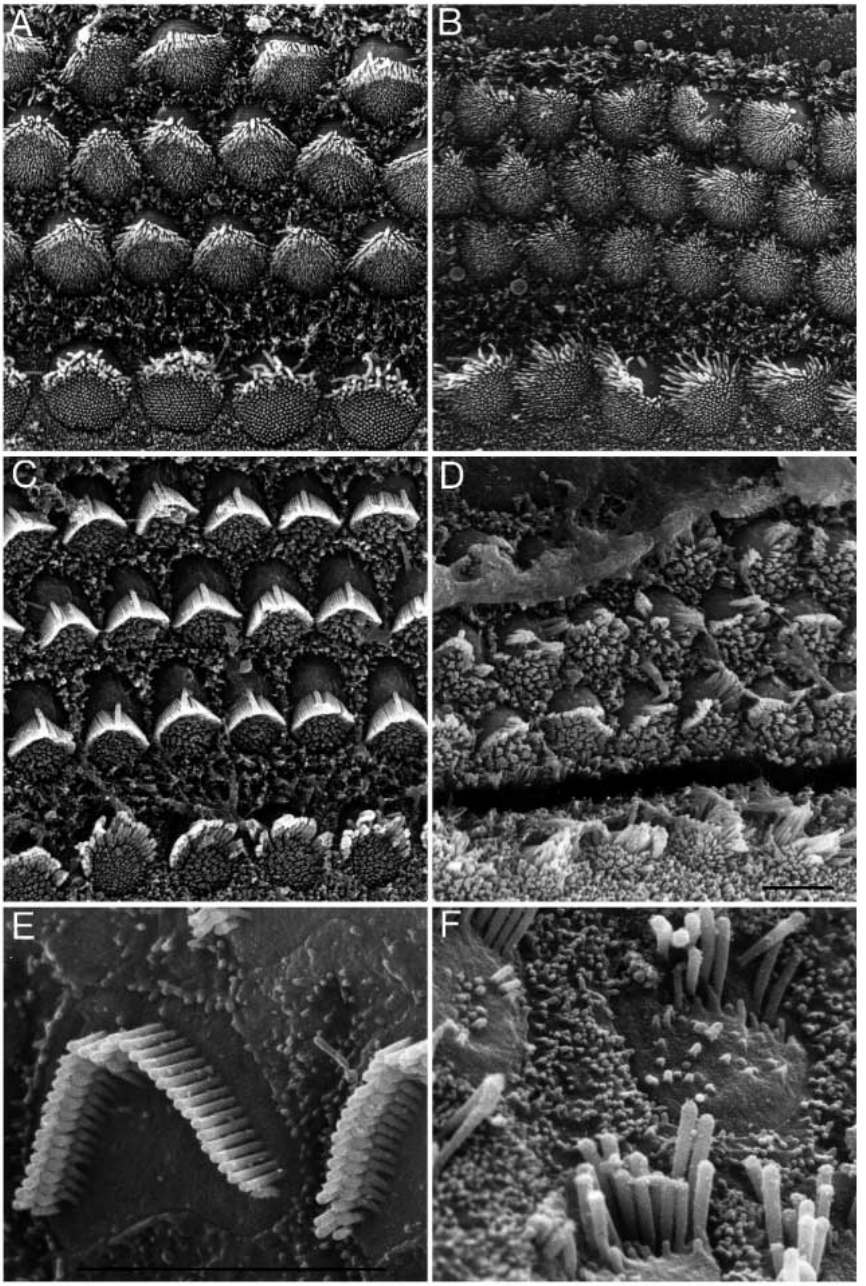

Fig. 2. Scanning electron micrographs of the surface of the organ of Corti of *Myo7a*^{6J} homozygotes (B,D,F) and littermate controls (A,C,E), showing widespread disorganisation of stereocilia bundles in the mutants. (A,B) 18 d.p.c.; (C,D) 3 days after birth; (E,F) 20 days after birth. (A-D) Same magnification, scale bar at bottom of D represents 5 μm. (E,F) Same magnification, scale bar at bottom of E represents 5 μm.

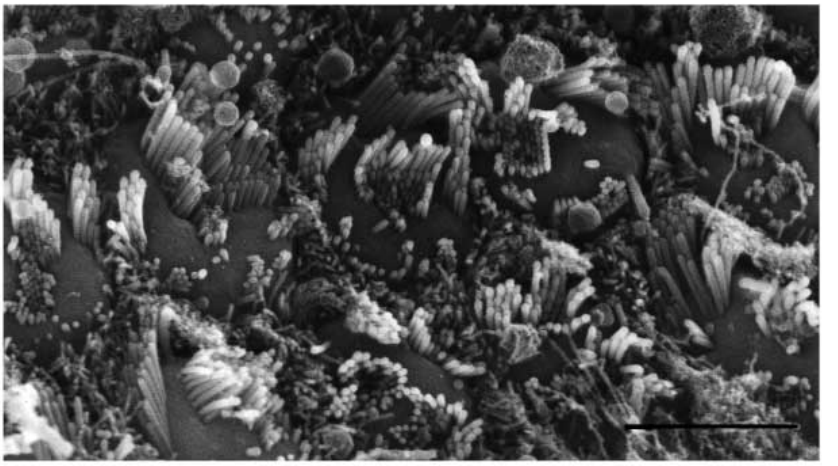

Fig. 3. Scanning electron micrographs of the organ of Corti of a *Myo7a*^{816SB} homozygous mutant at 3 days after birth, showing outer hair cells with extensive disruption of the stereocilia bundles. Scale bar represents 5 μm.

of the cuticular plate and formation of rootlets between 18 d.p.c. and birth must presumably play a role in anchoring the stereocilia.

In the mutants, there were no obvious abnormalities in the fine structure of the cuticular plate material and it condensed over the same period as in controls (Fig. 6A,B). Stereocilia structure, including rootlets and ankles, also developed normally in the mutants (Fig. 6C). However, there were more extensive areas of vesicle-rich cytoplasm beneath the upper surface of the mutant hair cells, including areas interspersed between patches of cuticular plate, which was never seen in controls (Fig. 6D). Furthermore, in some regions, stereocilia could be seen inserting into these vesiculated regions (Fig. 6C) in contrast to controls, in which all stereocilia inserted into cuticular plate. Irregular bulges in the cuticular plate were often observed in mutants and, as seen by scanning electron microscopy, the kinocilium could be mislocated amongst the stereocilia.

Ribbon-type synaptic vesicle arrays were seen in both

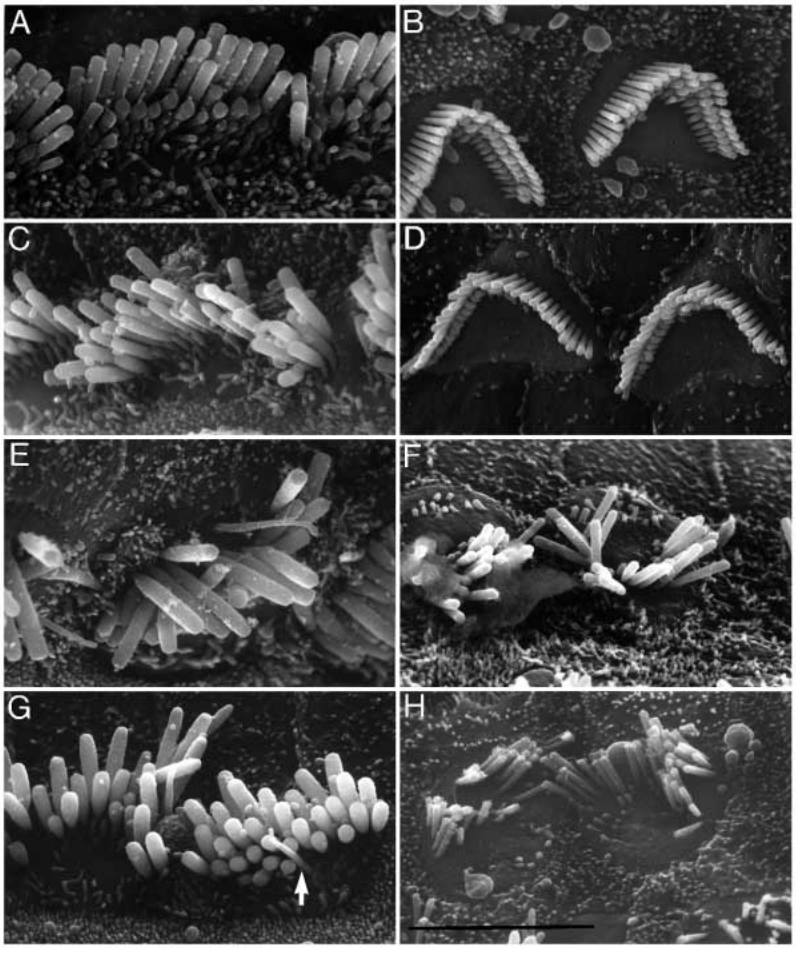

Fig. 4. Scanning electron micrographs of inner (left column) and outer (right column) hair cells from controls at 15days (A,B), *Myo7a*^{sh1} mutants at 15days (C,D), *Myo7a*^{6J} mutants at 15 days (E,F), and *Myo7a*^{816SB} mutants at 12 days (G,H). These cells are chosen to illustrate the best-preserved hair cells found at the ages used for electrophysiology, as these are the cells that are most likely to be giving any response seen, and many hair cells elsewhere in the cochlear duct are in later stages of degeneration. Only minor anomalies are seen in the *Myo7a*^{sh1} hair cells, such as the predominance of outer hair cells with only two rows of stereocilia instead of the more usual three (compare D with B). Both outer and inner hair cells are abnormal in the *Myo7a*^{6J} and *Myo7a*^{816SB} mutants (E-H). The kinocilium (arrow) is misplaced in both *Myo7a*^{816SB} inner hair cells shown, being on the side of the left hair cell and on the modiolar rather than lateral pole in the right hair cell (G). Note some tip links present on the left side of A. In all micrographs, lateral pole is at the top, and scale bar shown in H represents 5 μm.

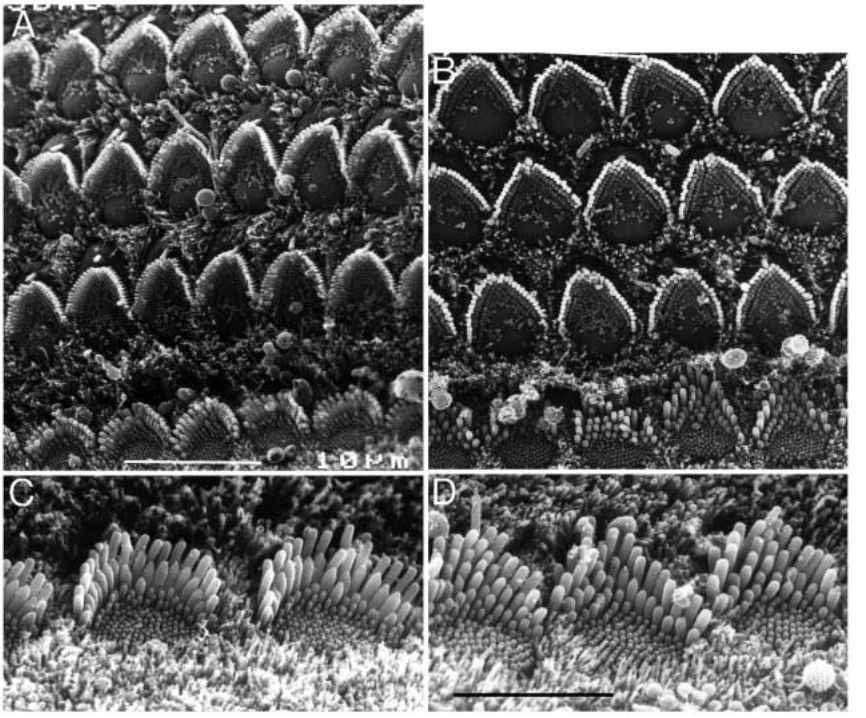

Fig. 5. Scanning electron micrographs of the inner hair cells of *Myo7a*^{sh1} homozygous mutant (B,D) and a littermate control (A,C) at 3 days after birth, showing no sign of gross abnormalities in stereocilia bundle organisation in the mutant. Scale bar in A represents 10 μm, in D 5μm.

mutants and controls, inner and outer hair cells, with no obvious differences between the genotypes (Fig. 7).

Cochlear dysfunction in shaker-1 mutants

Cochlear function was assessed using a recording electrode on the round window, a well-established method for assessing cochlear activity giving response thresholds close to those of single units (Johnstone et al., 1979). We looked at three responses, representing activity of different elements of the cochlea. Compound action potentials (CAP) consist of one or two sharp negative deflections at the start of the toneburst, representing synchronous firing of many cochlear neurons. Summating potentials (SP) are d.c. shifts in the waveform sustained for the duration of the toneburst stimulus and represent the gross counterpart of intracellular d.c. responses of sensory hair cells in the cochlea (eg Dallos et al., 1972; Dallos, 1986; Harvey and Steel, 1992). Cochlear microphonics (CM) are an a.c. response with a frequency identical to that of the stimulus and CM measured from the round window is thought to be primarily generated by the basal turn outer hair cells (e.g. Patuzzi et al., 1989, Dallos and Cheatham, 1976). In normal mice, CM and SP responses can be recorded from around 8-9 days using this approach but CAP responses are only detected from 11-12 days and responses gradually mature over the following few days (e.g. Harvey, 1989). We looked at mice during this period of maturation at 12, 15 and 20 days, to ensure that we did not miss any time window of activity.

The three different mutants showed different responses to sounds. Homozygotes for the *Myo7a*^{816SB} allele gave no CM, CAP or SP responses at all at any of the ages studied (12, 15 and 20 days after birth) up to the maximum sound intensities used (100 dB SPL for CM; up to 130 dB SPL depending on frequency for CAP and SP).

Of the eleven *Myo7a*^{6J} mutants examined, most showed no response to sound stimuli, but two of the mutants aged 20 days did show some response. These two mice gave an SP response, but only at very high intensities and only to a restricted range of frequencies (Fig. 8B). The responses are genuine SP responses despite the high thresholds because the waveforms are very similar in shape, size and latency to waveforms close to threshold in control mice and, furthermore, they could be measured repeatedly. These were the only responses that could be detected in these mutants.

The original shaker-1 mutant, *Myo7a*^{sh1}, shows more extensive responses than the other two alleles. The mutants showed CM responses with thresholds only slightly higher than in controls, indicating that outer hair cells can function at these ages (Fig. 8A). *Myo7a*^{sh1} homozygotes also show SP responses, albeit at raised thresholds compared with littermate controls (Fig. 8B), suggesting some sensory hair cells depolarise in response to sound stimulation. Finally, in this series, no CAP responses could be detected, although we have previously recorded severely abnormal CAPs in some but not all mutants (Harvey, 1989; Steel and Harvey, 1992).

DISCUSSION

We found a good correlation between the ultrastructure, physiological responses and the nature of the mutation in the three alleles of myosin VIIA studied here. The *Myo7a*^{816SB} mutation results in a 10-amino-acid deletion in the core of the motor head of the myosin molecule (Gibson et al., 1995; Mburu et al., 1997) and leads to the most severe disruption of development of stereocilia bundles seen among the mutants and a complete absence of stimulus-related cochlear potentials. The *Myo7a*^{6J} mutation is a missense mutation also within the core of the myosin VIIA head associated with abnormal

stereocilia bundle development and little or no cochlear responses. In contrast, the *Myo7a*^{sh1} mutation is a missense mutation located in a poorly conserved surface loop of the myosin head and this change is associated with the mildest of the pathological effects seen among the three mutants studied here: stereocilia appear to develop nearly normally and hair cells can generate CM and SP responses (albeit at raised thresholds).

Myosin VIIA clearly has a role in the development of stereocilia bundles. Stereocilia develop from the microvilli that cover the upper surface of the hair cell at early stages (16.5 d.p.c.), with lateral microvilli in a crescent-shaped array growing taller while microvilli on the modiolar (inner) side of the hair cell regress as the cell matures (Tilney et al., 1986, 1988, 1992; Kaltenbach et al., 1994). Myosin VIIA does not seem to be required for the initial establishment of this lateral-modiolar polarity of hair cells because this polarisation is clearly visible in the *Myo7a*^{6J} mutants at 18 d.p.c. despite early signs of disorganisation (Fig. 2B). Myosin VIIA also does not seem to be required for the ultrastructural maturation of stereocilia from the microvilli, which are their precursors, and the stereocilia in the mutants show apparently normal elongation to form rows of graded heights. However, myosin VIIA is required, either directly or indirectly, for maintaining the normal arrangement of the stereocilia in an ordered V-shaped array at the top of the hair cell, because in the *Myo7a*^{6J} and *Myo7a*^{816SB} mutants, the stereocilia form small clusters arranged in diverse patterns and orientations within the top of the hair cell. (See later for discussion of *Myo7a*^{sh1}.)

There are several possible explanations for the progressive disorganisation of the bundle. Firstly, myosin VIIA may be acting as an anchor for the stereocilia during early development. Myosin VIIA has been reported to be present in the cuticular plates of hair cells in the adult (Hasson et al., 1995, 1997b), which would support the suggestion of a role as an anchor molecule and immature hair cells in the frog saccule show particularly strong labelling for myosin VIIA. There is abundant actin within the mature cuticular plate (eg De Rosier and Tilney, 1989), which could interact with myosin in anchoring the rootlets of stereocilia. If myosin VIIA does act as an anchor, it seems unlikely to be the only molecule involved because a greater amount of stereocilia disorganisation might be expected if there was no effective anchor.

An alternative explanation for the disrupted stereocilia array is that myosin VIIA is required for cohesion of the cuticular plate, such that in the two most severely affected mutants, the cuticular plate does not form a single coherent mass but is interrupted by areas of vesicle-rich cytoplasm. This in turn might allow movement of the pieces of cuticular plate with

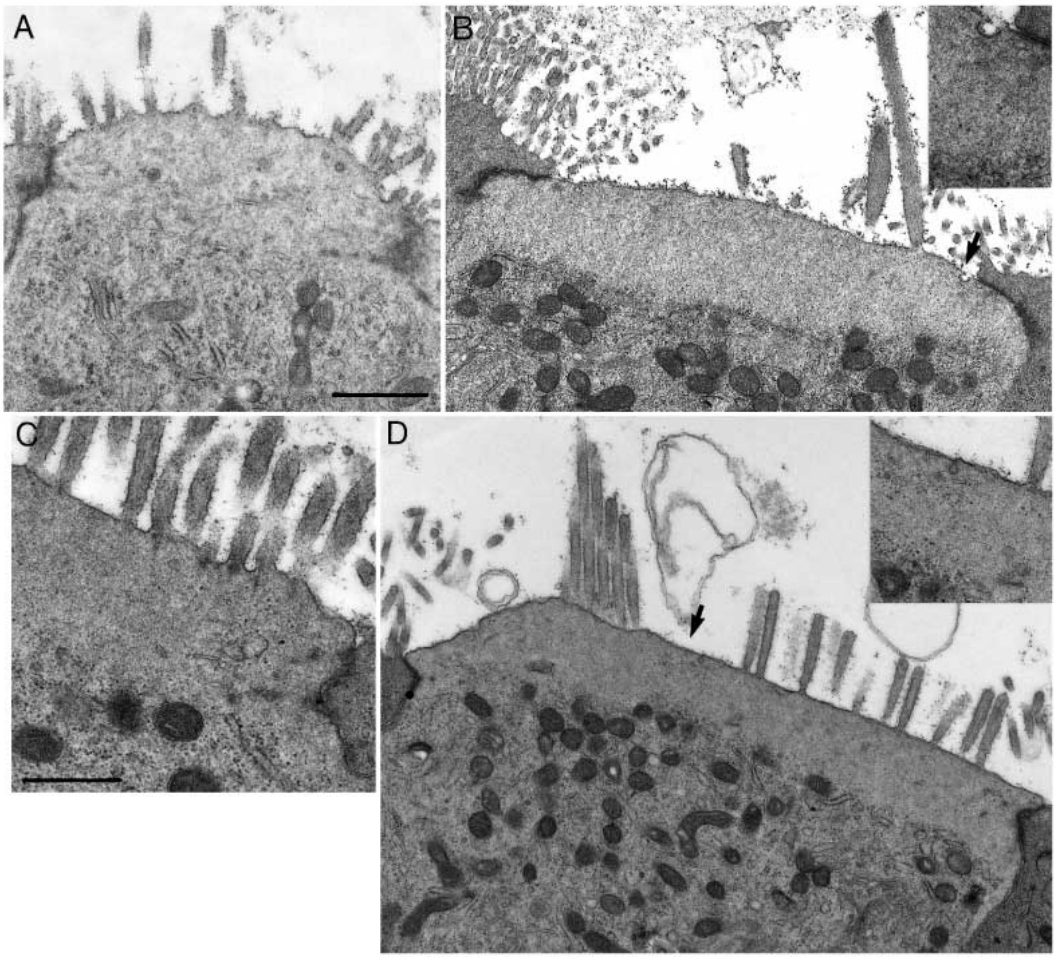

Fig. 6. Transmission electron micrographs of hair cells at (A) 18 d.p.c., (B-D) newborn. (A) The developing cuticular plate from an inner hair cell from a *Myo7a*^{6J} mutant, in which cuticular plate condensation is indistinguishable from that in controls. (B) Control inner hair cell cuticular plate 2 days later, at birth, shows extensive condensation of the matrix, and the vesicle-rich area close to the insertion of the kinocilium is shown (to the right in this view, arrow and inset). (C) *Myo7a*^{816SB} mutant outer hair cell at birth, showing insertion of stereocilia rootlets into a vesicle-rich area. Rootlet and ankle formation seen here are essentially the same as in controls, but rootlets always insert into cuticular plate in controls. (D) *Myo7a*^{816SB} mutant outer hair cell at birth, showing an interspersed vesicle-rich area (arrow and inset). The orientation of the section is mid-modiolar, and the position of the kinocilium inserting into a vesicle-rich area would normally be to the left of the view. The location of a vesicle-rich area between two patches of stereocilia in this view is abnormal. Scale bar A,B,D (marked on A) represents 1 μm. Scale bar on C represents 500 nm.

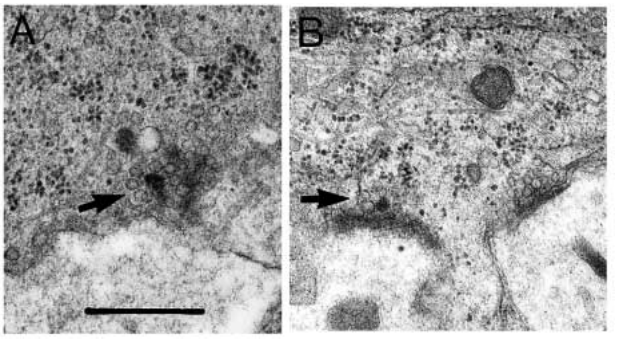

Fig. 7. (A) Synaptic vesicles arranged in a typical ribbon formation (arrow) at the base of a control outer hair cell at 3 days after birth. (B) Synaptic vesicles of a *Myo7a*^{6J} mutant inner hair cell look normal (arrow). Scale bar represents 500 nm.

attached stereocilia within the top of the hair cell. However, it seems just as likely that the abnormal presence of vesiculated regions within the cuticular plate is secondary to abnormal distribution of stereocilia, rather than being the cause of stereocilia disorganisation.

We were able to rule out a third possible explanation for the disorganisation: a delay in condensation of the cuticular plate in mutants, allowing a prolonged period of stereocilia mobility within the top of the hair cell. Our sections of hair cells during the period of condensation showed no such delay in mutants.

A fourth possible explanation for the abnormal organisation of stereocilia in the two mutants is that the kinocilium is primarily affected by the mutations and that this interferes with a putative role of the kinocilium in organising the stereocilia array (eg Sobkowicz et al., 1995; Kelley et al., 1992). A primary cilium defect has been proposed as an explanation for the link between retinal defects, vestibular dysfunction and hearing impairment in Usher syndrome, and ultrastructural defects have been observed in the photoreceptor cilia of three individuals with Usher syndrome type 2 (Barrong et al., 1992; Hunter et al., 1986; Berson and Adamian, 1992). In the *Myo7a*^{6J} and *Myo7a*^{816SB} mutants, in cells where the kinocilium could be clearly identified it was often some distance from its normal location (eg Fig. 4G), and there was no obvious relationship between the position of the kinocilium and the arrangement of the stereocilia. Thus, the observations in these two severely affected mutants suggest that, either the kinocilium is not involved in organising the stereocilia bundle in normal mice, or the kinocilium requires functional myosin VIIA to control stereocilia bundle organisation.

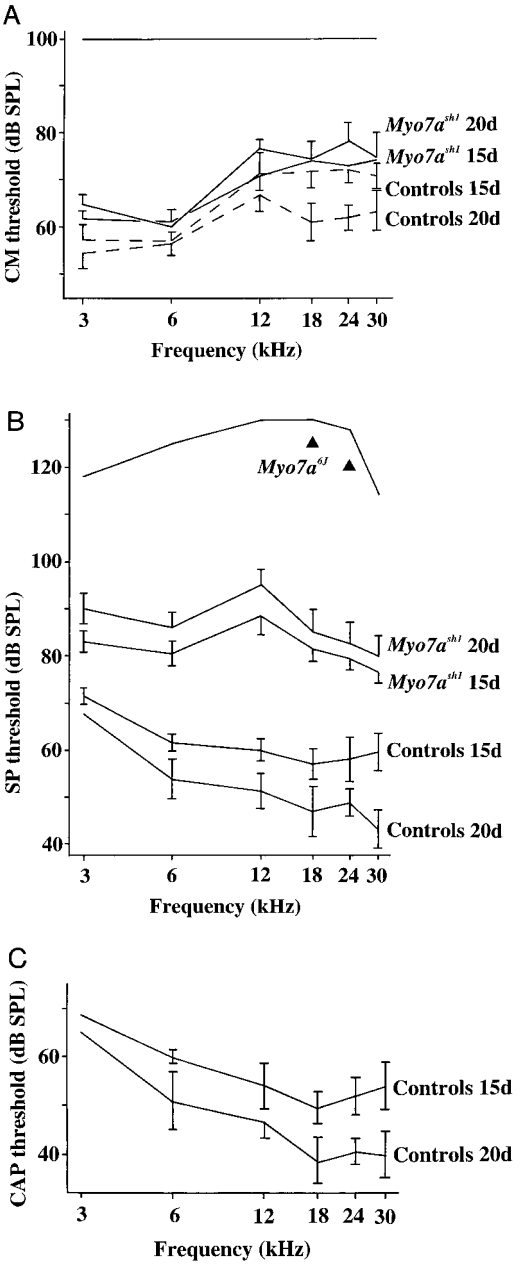

Fig. 8. Physiological responses of the cochlea. (A) Cochlear microphonic thresholds for a 2 μV response ±s.e.m. at a range of frequencies in controls (dashed lines) and *Myo7a*^{sh1} homozygotes at 15 and 20 days. Solid line at top represents maximum output of sound used, and *Myo7a*^{6J} and *Myo7a*^{816SB} homozygotes gave no CM responses up to this level. Thresholds for the *Myo7a*^{sh1} mutants are slightly higher than for the controls, and control thresholds improve between 15 and 20 days. CM amplitude was also analysed (not shown). Linear input/output functions are obtained from both genotypes, and the mean CM amplitude for the controls was slightly higher than for the mutants. CM responses at 3, 6, 12 and 18 kHz were analysed by analysis of variance for repeated measures: the test of between-subject effects showed no effect of age at any frequency (eg 6 kHz: f=0.39, df=15,1, P=0.544) and no significant effect of genotype at 12 and 18 kHz (12 kHz f=1.30, df=15,1, P=0.272; 18 kHz f=2.96, df=15,1, P=0.106), but a significant effect at the 5% level of genotype for 3 and 6 kHz stimuli (3 kHz: f=6.77, df=15,1, P=0.020; 6 kHz: f=5.69, df=1, P=0.031). Thus, there were significantly smaller CM amplitudes in the mutants at low frequencies but no significant differences at higher frequencies. (B) Thresholds (±s.e.m.) for visual detection of a summating potential response in the *Myo7a*^{sh1} mutants and littermate controls. Control thresholds improve from 15 to 20 days after birth, showing continuing maturation of the responses, but mutant thresholds deteriorate further during this time. Solid line at top represents the maximum sound intensity used, and all *Myo7a*^{816SB} and most *Myo7a*^{6J} homozygotes gave no SP response up to this level. Two *Myo7a*^{6J} mutants did give a clear SP response, but only at 18 or 24 kHz and with very raised thresholds; these are shown by the black triangles. (C) Compound action potential response thresholds ±s.e.m. in the control group. Thresholds improve between 15 and 20 days. No CAP responses were seen in any of the homozygote mutants studied here at any age, up to the maximum intensities used shown in B.

Apical membrane turnover in the hair cell may play a role in normal positioning of stereocilia, so a fifth possible explanation for the observed stereocilia disorganisation is that this process requires myosin VIIA. However, in a separate study (Richardson et al., 1997), we show that cationic ferritin uptake by hair cells of *Myo7a*^{6J} mutants is normal, suggesting that non-specific apical hair cell endocytosis is probably normal, arguing against this explanation for the stereocilia disorganisation.

Finally, Hasson et al. (1997b) suggested that myosin VIIA may be involved in crosslinks between the shafts of adjacent stereocilia. Abnormal crosslinks resulting from defective myosin VIIA might lead to a lack of cohesion of stereocilia within the bundle and consequent disruption of the bundle (*Myo7a*^{6J} and *Myo7a*^{816SB}) or excessive resorption of stereocilia that are not firmly linked to the rest of the stereocilia bundle (*Myo7a*^{sh1}). It would be interesting to know the ultrastructural localisation of myosin VIIA during development of stereocilia to assess this and the other possible explanations for the derangements observed.

The observations on the original shaker-1 mutation, *Myo7a*^{sh1}, were particularly interesting because they suggest that myosin VIIA may have a role in hair cell function in addition to its role in stereocilia bundle development. The mutant myosin VIIA molecule in these mutants appears to have sufficient activity to support normal early development of the stereocilia bundles, although there are some minor anomalies seen at 15 days. Outer hair cells seem to function moderately well judging by the nearly normal CM recorded in these mutants, despite many outer hair cells having only two rows of stereocilia instead of the more usual three. However, the raised thresholds for SP responses and grossly abnormal CAP responses suggest that hair cells require normal myosin VIIA activity for normal function, and that the missense mutation of the *Myo7a*^{sh1} allele interferes with this role. The nature of this requirement is not known. The simplest explanation is that myosin VIIA may be required for normal transduction, and the same process affected in the mild *Myo7a*^{sh1} mutant, be it stereocilia anchoring, stereocilia crosslinking, cuticular plate cohesion or some other process, is severely affected in the two more extreme alleles giving rise to much earlier signs of abnormality in the form of stereocilia disarray.

The progressive hearing loss observed in the *Myo7a*^{sh1} mutants suggests that some MYO7A mutations in humans may be involved with hearing impairments less severe than that seen in typical Usher syndrome type 1B and may lead to progressive hearing loss in some cases (Liu et al., 1997c).

The finding of an SP response in two of the *Myo7a*^{6J} mutants is particularly interesting, given the gross disorder of their stereocilia bundles. It suggests that some hair cells at 20 days after birth are able to depolarise in response to sound stimuli. We have recently shown that individual *Myo7a*^{6J} mutant hair cells in organ of Corti culture at the equivalent of 3 days after birth do produce transduction currents when their stereocilia bundles are stimulated (Richardson et al., 1997).

Finally, a disrupted pattern of stereocilia bundles has been described in one other mutant to date, the Jackson shaker (*js*) mutant (Kitamura et al., 1991a,b, 1992). The *js* gene product may be involved in the same process of stereocilia bundle organisation and may interact with myosinVIIA. Disorganised stereocilia bundles or multiple clumps of stereocilia have also been observed in presumed regenerating hair cells in the chick inner ear following damage (e.g. Cousillas and Rebillard, 1988; Hashino et al., 1991; Duckert and Rubel, 1993), indicating that the molecular or mechanical processes that lead to ordered development of the bundle in normal development are not always properly co-ordinated in regeneration and myosin VIIA may be involved in this process.

We thank Angela Pearce for help with the in situ hybridisation, David Marshall for the statistical analysis, Gene Rinchik and Wayne Frankel for access to the new *shaker-1* mutations, Guy Richardson for many useful discussions, and Ian Russell, Ed Rubel, Doug Cotanche and Jeff Corwin for their helpful suggestions. Supported by MRC, EU and Defeating Deafness.

REFERENCES

Barrong, S. D., Chaitin, M. H., Fliesler, S. J., Possin, D. E., Jacobson, S. G. and Milam, A. H. (1992). Ultrastructure of connecting cilia in different forms of retinitis pigmentosa. *Arch. Ophthalmol.* **110**,706-710.

Berson, E. L. and Adamian, M. (1992). Ultrastructural findings in an autopsy eye from a patient with Usher's syndrome type II. *Am. J. Ophthalmol.* **114**, 748-757.

Brown, P. G. and Ruben, R. J. (1969). The endocochlear potential in the shaker-1 (sh-1/sh-1) mouse. *Acta Otolaryng.* **68**, 14-20.

Chen, Z-Y., Hasson, T., Kelley, P. M., Schwender, B. J., Schwartz, M. F., Ramakrishnan, M., Kimberling, W. J., Mooseker, M. S., and Corey, D. P. (1996). Molecular cloning and domain structure of human myosin-VIIa, the gene product defective in Usher Syndrome 1B. *Genomics* **36**, 440-448.

Cousillas, H. and Rebillard, G. (1988). Morphological transformation of hair cells in the chick basilar papilla following an acoustic overstimulation. *Hear. Res.* **32**, 117-122.

Dallos, P. (1986). Neurobiology of cochlear inner and outer hair cells: intracellular recordings. *Hear. Res.* **22**, 185-198.

Dallos, P. and Cheatham, M. A. (1976). Production of cochlear potentials by inner and outer hair cells. *J. Acoust. Soc. Am.* **60**, 510-512.

Dallos, P., Schoeny, Z. G. and Cheatham, M. A. (1972). Cochlear summating potentials: descriptive aspects. *Acta Otolaryngol. Suppl.* **302**, 1-46.

DeRosier, D. J. and Tilney, L. G. (1989). The structure of the cuticular plate, an in vivo actin gel. *J. Cell Biol.* **109**, 2853-2867.

Deol, M. S. (1956). The anatomy and development of the mutants pirouette, shaker-1 and waltzer in the mouse. *Proc. Roy. Soc. Lond. B* **145**, 206-213.

Duckert, L. G. and Rubel, E. W. (1993). Morphological correlates of functional recovery in the chicken inner ear after gentamycin treatment. *J. Comp. Neurol.* **331**, 75-96.

Durrbach, A., Collins, K., Matsudaira, P., Louvard, D. and Coudrier, E. (1996). Brush border myosin-I truncated in the motor domain impairs the distribution and the function of endocytic compartments in an hepatoma cell line. *Proc. Natl. Acad. Sci. USA* **93**, 7053-7058.

Emmerling, M. R. and Sobkowicz, H. M. (1990). Acetylcholinesterase-positive innervation in cochleas from two strains of shaker-1 mice. *Hear. Res.* **47**, 25-38.

Geli, M. I. and Riezman, H. (1996). Role of Type I myosins in receptor-mediated endocytosis in yeast. *Science* **272**, 533-535.

Gibson, F., Walsh, J., Mburu, P., Varela, A., Brown, K. A., Antonio, M., Beisel, K. W., Steel, K. P. and Brown, S. D. M. (1995). A type VII myosin encoded by the mouse deafness gene *shaker-1*. *Nature* **374**, 62-64.

Govindan, B., Bowser, R. and Novick, P. (1995). The role of Myo2, a yeast class V myosin, in vesicular transport. *J. Cell Biol.* **128**, 1055-1068.

Grüneberg, H., Hallpike, C. S. and Ledoux, A. (1940). Observations on the structure, development and electrical reactions of the internal ear of the shaker-1 mouse (*Mus musculus*). *Proc. Roy. Soc. Lond. B* **129**, 154-173.

Harvey, D. (1989). Structural and functional development of the cochlea in normal (CBA/Ca) and hearing impaired shaker-1 (sh-1/sh-1) mice. PhD thesis, University of Nottingham, UK.

Harvey, D. and Steel, K. P. (1992). The development and interpretation of the summating potential response. *Hear. Res.* **61**, 137-146.

Hashino, E., Tanaka, Y. and Sokabe, M. (1991). Hair cell damage and recovery following chronic application of kanamycin in the chick cochlea. *Hear. Res.* **52**, 356-368.

Hasson, T., Heintzelman, M. B., Santos-Sacchi, J., Corey, D. P. and Mooseker, M. S. (1995). Expression in cochlea and retinal of myosin VIIa, the gene product defective in Usher syndrome type 1B. *Proc. Natl. Acad. Sci. USA* **92**, 9815-9819.

Hasson, T., Walsh, J., Cable, J., Mooseker, M. S., Brown, S. D. M. and Steel, K. P. (1997a). Effects of shaker-1 mutations on myosin-VIIa protein and mRNA expression. *Cell Motil. Cytoskel.* **37**, 127-138.

Hasson, T., Gillespie, P. G., Garcia, J. A., MacDonald, R. B., Zhao, Y., Yee, A. G., Mooseker, M. S. and Corey, D. P. (1997b). Unconventional myosins in inner-ear sensory epithelia. *J. Cell Biol.* **137**, 1287-1307.

Hill, K. L., Catlett, N. L. and Weisman, L. S. (1996). Actin and myosin function in directed vacuole movement during cell division in *Saccharomyces cerevisiae*. *J. Cell Biol.* **135**, 1535-1549.

Hunter, D. G., Fishman, G. A., Mehta, R. S. and Kretzer, F. L. (1986). Abnormal sperm and photoreceptor axonemes in Usher's syndrome. *Arch. Ophthalmol.* **104**, 385-389.

Hunter-Duvar, I. M. (1978). Electron microscopic assessment of the cochlea. Some techniques and results. *Acta Otolaryngol. Suppl.* **351**, 3-23.

Johnstone, J. R., Alder, V. A., Johnstone, B. M., Robertson, D. and Yates, G. K. (1979). Cochlear action potential threshold and single unit thresholds. *J. Acoust. Soc. Am.* **65**, 254-257.

Kachar, B., Battaglia, A. and Fex, J. (1997). Compartmentalized vesicular traffic around the hair cell cuticular plate. *Hear. Res.* **107**, 102-112.

Kaltenbach, J. A., Falzarano, P. R. and Simpson, T. H. (1994). Postnatal growth of the hamster cochlea. II. Growth and differentiation of stereocilia bundles. *J. Comp. Neurol.* **350**, 187-198.

Kelley, M. W., Ochiai, C. K. and Corwin, J. T. (1992). Maturation of kinocilia in amphibian hair cells: Growth and shortening related to kinociliary bulb formation. *Hear. Res.* **59**, 108-115.

Kelley, P. M., Weston, M. D., Chen, Z-Y., Orten, D. J., Hasson, T., Overbeck, L. D., Pinnt, J., Talmadge, C. B., Ing, P., Mooseker, M. S., Corey, D., Sumegi, J. and Kimberling, W. J. (1997). The genomic structure of the gene defective in Usher Syndrome Type Ib (MYO7A). *Genomics* **40**, 73-79.

Kikuchi, K. and Hilding, D. A. (1965). The defective organ of Corti in shaker-1 mice. *Acta Otolaryng.* **60**, 287-303.

Kitamura, K., Kakoi, H., Yoshikawa, Y. and Ochikubo, F. (1992). Ultrastructural findings in the inner ear of Jackson shaker mice. *Acta Otolaryng.* **112**, 622-627.

Kitamura, K., Nomura, Y., Yagi, M., Yoshikawa, Y. and Ochikubo, F. (1991a). Morphological changes of cochlea in a strain of new-mutant mice. *Acta Otolaryng.* **111**, 61-69.

Kitamura, K., Yoshikawa, Y. and Ochikubo, F. (1991b). An ultrastructural study on vestibular sensory cells in a new-mutant mouse. *Acta Otolaryng.* **111**, 1013-1020.

Letts, V. A., Gervais, J. L. M. and Frankel, W. N. (1994). Remutation at the shaker-1 locus. *Mouse Genome* **92**, 116.

Lévy, G., Levi-Acobas, F., Blanchard, S., Gerber, S., Larget-Piet, D., Chenal, V., Liu, X-Z., Newton, V., Steel, K. P., Brown, S. D. M., Munnich, A., Kaplan, J., Petit, C. and Weil, D. (1997). Myosin VIIA gene: heterogeneity of the mutations responsible for Usher syndrome type IB. *Hum. Molec. Genet.* **6**, 111-116.

Liu, X. -Z., Newton, V. E., Steel, K. P. and Brown, S. D. M. (1997a). Identification of a new mutation of the myosin VII head region in Usher syndrome type 1. *Hum. Mutat.* **10**, 168-170.

Liu, X. -Z., Walsh, J., Mburu, P., Kendrick-Jones, J., Cope, M. J. T. V., Steel, K. P. and Brown, S. D. M. (1997b). Mutations in the myosin VIIA gene cause non-syndromic recessive deafness. *Nature Genetics* **16**, 188-190.

Liu, X. -Z, Walsh, J., Tamagawa, Y., Kitamura, K., Nishizawa, M., Steel, K. P. and Brown, S. D. M. (1997c). Autosomal dominant non-syndromic deafness caused by a mutation in the myosin VIIA gene. *Nature Genetics*, **17**, 268-269.

Lord, E. M. and Gates, W. H. (1929). Shaker, a new mutation of the house mouse (*Mus musculus*). *Am. Naturalist* **63**, 435-442.

Mbiene, J-P., Favre, D. and Sans, A. (1984). The pattern of ciliary development in fetal mouse vestibular receptors. A qualitative and quantitative SEM study. *Anat. Embryol.* **170**, 229-238.

Mburu, P., Liu, X., Walsh, J., Saw, D. Jr., Cope, M. J. T. V., Gibson, F., Kendrick-Jones, J., Steel, K. P. and Brown, S. D. M. (1997). Mutation analysis of the mouse myosin VIIA deafness gene – a putative hybrid myosin-kinesin motor. *Genes Funct.*, **1**, 191-203.

Mermall, V., McNally, J. G. and Miller, K. G. (1994). Transport of cytoplasmic particles catalysed by an unconventional myosin in living *Drosophila* embroyos. *Nature* **369**, 560-562.

Mikaelian, D., Alford, B. R. and Ruben, R. J. (1965). Cochlear potentials and VIII nerve action potentials in normal and genetically deaf mice. *Ann. Otol. Rhinol. Laryngol.* **74**, 146-157.

Mikaelian, D. O. and Ruben, R. J. (1964). Hearing degeneration in shaker-1 mouse. *Arch. Otolaryngol.* **80**, 418-430.

Mochida, S., Kobayashi, H., Matsuda, Y., Yuda, Y., Muramoto, K. and Nonomura, Y. (1994). Myosin II is involved in transmitter release at synapses formed between rat sympathetic neurons in culture. *Neuron* **13**, 1131-1142.

Patuzzi, R. B., Yates, G. K. and Johnstone, B. M. (1989). The origin of the low-frequency microphonic in the first cochlear turn of guinea pig. *Hear. Res.* **39**, 177-188.

Richardson, G. P., Forge, A., Kros, C. J., Fleming, J., Brown, S. D. M. and Steel, K. P. (1997). Myosin VIIA is required for aminoglycoside accumulation in cochlear hair cells. *J. Neurosci.*, in the press.

Rinchik, E. M., Carpenter, D. A. and Selby, P. B. (1990). A strategy for fine structural analysis of a 6- to 11- centimorgan region of mouse chromosome 7 by high efficiency mutagenesis. *Proc. Natl. Acad. Sci. USA* **87**, 896-900.

Rubel, E. W. (1978). Ontogeny of structure and function in the vertebrate auditory system. In *Handbook of Sensory Physiology,* (ed. M. Jacobson) Vol. IX, pp. 135-237. Berlin: Springer Verlag.

Shnerson, A., Lenoir, M., Van De Water, T. R. and Pujol, R. (1983). The pattern of sensorineural degeneration in the cochlea of the deaf shaker-1 mouse: Ultrastructural observations. *Dev. Brain Res.* **9**, 305-315.

Smith, R. J. H., Berlin, C. I., Hejtmancik, J. F., Keats, B. J. B., Kimberling, W. J., Lewis, R. A., Möller, C. G., Pelias, M. Z. and Tranebjærg, L. (1994). Clinical diagnosis of the Usher syndromes. *Am. J. Med. Genet.* **50**, 32-38.

Sobkowicz, H. M., Slapnick, S. M. and August, B. K. (1995). The kinocilium of auditory hair cells and evidence for its morphogenetic role during the regeneration of stereocilia and cuticular plates. *J. Neurocytol.* B, 633-653.

Steel, K. P., Davidson, D. R. and Jackson, I. J. (1992). TRP-2/DT, a new early melanoblast marker, shows that steel growth factor (c-kit ligand) is a survival factor. *Development* **115**, 1111-1119.

Steel, K. P. and Harvey, D. (1992). Development of auditory function in mutant mice. In *Development of Auditory and Vestibular Systems 2* (ed. R. Romand), pp. 221-242. Amsterdam: Elsevier.

Steel, K. P. and Smith, R. J. H. (1992). Normal hearing in *Splotch* (*Sp*/+), the mouse homologue of Waardenburg syndrome type 1. *Nat. Genet.* **2**, 75-79.

Tilney, L. G., Tilney, M. S., Saunders, J. S. and DeRosier, D. J. (1986). Actin filaments, stereocilia, and hair cells of the bird cochlea. III. The development and differentiation of hair cells and stereocilia. *Dev. Biol.* **116**, 100-118.

Tilney, L. G., Tilney, M. S. and Cotanche, D. A. (1988). Actin filaments, stereocilia, and hair cells of the bird cochlea. V. How the staircase pattern of stereociliary lengths is generated. *J. Cell Biol.* **106**, 355-365.

Tilney, L. G., Cotanche, D. A. and Tilney, M. S. (1992). Actin filaments, stereocilia, and hair cells of the bird cochlea. VI. How the number and arrangement of stereocilia are determined. *Development* **116**, 213-226.

Weil, D., Blanchard, S., Kaplan, J., Guilford, P., Gibson, F., Walsh, J., Mburu, P., Varela, A., Levilliers, J., Weston, M. D., Kelley, P. M., Kimberling, W. J., Wagenaar, M., Levi-Acobas, F., Larget-Piet, D., Munnich, A., Steel, K. P., Brown, S. D. M. and Petit, C. (1995). Defective myosin VIIA gene responsible for Usher syndrome type 1B. *Nature* **374**, 60-61.

Weil, D., Lévy, G., Sahly, I., Lévi-Acobas, F., Blanchard, S., El-Amraoui, A., Crozet, F., Philippe, H., Abitbol, M. and Petit, C. (1996). Human myosin VIIA responsible for the Usher 1B syndrome: A predicted membrane-associated motor protein expressed in developing sensory epithelia. *Proc. Natl. Acad. Sci. USA* **93**, 3232-3237.

Weil, D., Küssel, P., Blanchard, S., Lévy, G., Levi-Acobas, F., Drira, M., Ayadi, H. and Petit, C. (1997). The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin-VIIA gene. *Nature Genetics* **16**, 191-193.

Weston, M. D., Kelley, P. M., Overbeck, L. D., Wagenaar, M., Orten, D. J., Hasson, T., Chen, Z-Y., Corey, D., Mooseker, M., Sumegi, J., Cremers, C., Möller, C., Jacobson, S. G., Gorin, M. G. and Kimberling, W. J. (1996). Myosin VIIA mutation screening in 189 Usher syndrome type 1 patients. *Am. J. Hum. Genet.* **59**, 1074-1083.

Wilkinson, D. G., Bailes, J. A. and McMahon, A. P. (1987). Expression of the proto-oncogene *int-1* is restricted to specific neural cells in the developing mouse embryo. *Cell* **50**, 79-88.