The genes for α- and β-tectorin encode the major non-collagenous proteins of the tectorial membrane. Recently, a targeted deletion of the mouse α-tectorin gene was found to cause loss of cochlear sensitivity (1). Here we describe that mRNA levels for β-tectorin, but not α-tectorin, are significantly reduced in the cochlear epithelium under constant hypothyroid conditions and that levels of β-tectorin protein in the tectorial membrane are lower. A delay in the onset of thyroid hormone supply prior to onset of hearing, recently described to result in permanent hearing defects and loss of active cochlear mechanics (2), can also lead to permanently reduced β-tectorin protein levels in the tectorial membrane, while Tectorin protein levels remain low in the tectorial membrane up to one year after the onset of thyroid hormone supply has been delayed until postnatal day 8 or later and are associated with an abnormally structured tectorial membrane and the loss of active cochlear function. These data indicate that a simple delay in thyroid hormone supply during a critical period of development can lead to low β-tectorin levels in the tectorial membrane and suggest for the first time that β-tectorin may be required for development of normal hearing.

It has been known for many years that thyroid hormone (TH) is necessary for normal development of the auditory system (3–5). Both genetic and acquired neonatal TH deficiency may result in a profound mental disability that is often accompanied by deafness. The existence of various TH-sensitive periods during inner ear development and the success of corrective TH treatment has recently been investigated (2, 6). These studies revealed that maternal TH prior to hearing function has defects in tectorin expression, as well as TH supply beyond the onset of hearing at postnatal day 12 (P12), was not critical for the development of normal hearing in rats (2, 6). However, within the crucial period of time any delay in the rise of TH plasma levels (transient hypothyroidism) leads to permanent hearing defects, though the organ of Corti develops to an organ without obvious structural or neuronal abnormalities (2). Analysis of distortion product otoacoustic emissions revealed that the active cochlear process was TH-dependent and was permanently lost following the induction of a transient TH-free period between E17 and >P8 (2). Distortion product otoacoustic emissions are sounds that emanate from the ear and are believed to be produced by the interaction of actively amplified traveling waves on the basilar membrane (7–9). The tectorial membrane plays a crucial role in this active process, because it couples transverse, sound-induced, basilar membrane motion to a radial deflection of the sensory hair bundles. In former studies with rodents (10, 11) it has been observed that the tectorial membrane is distorted as a result of constant hypothyroidism. The major non-collagenous components of the tectorial membrane, α- and β-tectorin, were identified recently as the products of single copy genes (12). Missense mutations in the α-tectorin gene were found in five families with non-syndromic hearing impairment (13–16). In mice, a targeted deletion in α-tectorin revealed a loss of cochlear amplification (1). As yet it is not known whether β-tectorin is essential for the development of normal hearing.

To investigate whether animals with transient TH deficiency prior to hearing function have defects in tectorin expression that cause hearing impairment and the loss of active cochlear mechanics, we analyzed the TH dependence of tectorin mRNA and protein expression in the rat cochlea of animals constantly deprived of TH in comparison to animals that had been subjected to only a short period of TH deficiency prior to the onset of hearing and had thereby lost their active cochlear function (2). The effects on the structure of the tectorial membrane were investigated also.

**EXPERIMENTAL PROCEDURES**

**Animals and Drug Administration**—Wistar rats were purchased from Interfauna (Tuttlingen, Germany). The anti-thyroid drug methylmercaptoimidazol (MMI; 0.02%), described to safely suppress plasma-TH levels in both animals and humans (17, 18), was administered in the drinking water of the dams prior to E17 after conception. This treatment was not critical for the development of normal hearing in rats (2, 6).

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continued after birth until sacrifice of the animal and dissection of the Cochlea (constant hypothyroid group) or stopped at various time points between P3 and P12 (transient hypothyroid group). After discontinuation of MMI treatment, plasma levels of T₄ and T₃ returned to normal (>5 μg/dl T₄ and >75 ng/dl T₃) within 2–4 days, and the pups developed under standard conditions (2).

T₄/T₃ Determination—Blood from the pups was collected when the animals were sacrificed for isolation of the cochlea, whereas blood from pregnant dams was collected upon retrobulbar puncture. Serum was collected, and T₄ or T₃ levels were determined as described (2).

Preparation of α- and β-Tectorin Antiserum—Chick α-tectorin and the α-1-subunit of chick α-tectorin were purified by preparative gel electrophoresis and used to produce polyclonal sera in rabbits. In brief, tectorial membranes were dissected from cochlear ducts of 2-day post-hatch chicks, collected in phosphate-buffered saline containing 0.1% Triton X-100, and frozen at −80 °C for storage until sufficient tissue had been collected for each immunization. Tectorial membrane samples were thawed, washed sequentially by resuspension and centrifugation (15000 × g × 5 min) in high salt (1 m NaCl, 0.1% Triton X-100, 10 mM Tris-HCl, pH 7.4, 0.1% Triton X-100), low salt (10 mM Tris-HCl, pH 7.4, 0.1% Triton X-100), and non-ionic detergent (1% Triton X-100/10 mM Tris-HCl, pH 7.4) buffers, and then solubilized by boiling in non-reducing SDS-polyacrylamide gel electrophoresis sample buffer. Solubilized tectorial membrane proteins were separated on preparative 7.5% minigels. Strips of the gels containing the α-tectorin (Mₙ 196,000) and β-tectorin (Mₙ 41,000) bands were cut out, and the proteins were eluted with 0.1% SDS in 50 mM ammonium bicarbonate. After dialysis against water the samples were lyophilized. Protein from the α-tectorin band was subsequently solubilized in reducing SDS-polyacrylamide gel electrophoresis sample buffer and subjected to a second round of gel electrophoresis, and protein was eluted and recovered from the region of the gel containing the α-tectorin band (Mₙ 146,000). Rabbits were immunized with gel-purified tectorins that were resuspended in phosphate-buffered saline and emulsified with Freund’s complete adjuvant for the first injection and Freund’s incomplete adjuvant for the subsequent boosts. Protein from 400 tectorial membranes was used for each injection. Rabbit antiserum to chick α-tectorin (R7) was obtained from a rabbit that was immunized with protein obtained from ~2000 tectorial membranes. Rabbit antiserum to chick α-tectorin subunit (R9) was obtained from a rabbit that was immunized with protein obtained from a total of 1200 tectorial membranes.

Immunohistochemical Staining for Fluorescence and Laser Scanning Confocal Microscopy—For immunohistochemistry, rat cochlear sections from control or hypothyroid rats, respectively, from the offspring of untreated (control) or MMI-treated dams were stained as described (2). For double labeling studies, both antibodies were incubated simultaneously—untreated (control) or MMI-treated dams were stained as described (2). Templates for in vitro transcription were a nearly full-length cDNA plasmid clone for mouse α-tectorin constructed by ligating a NarI to KhoI fragment of clone A2 into NarI/KhoI double digested clone A3 (2) and an almost full-length β-tectorin clone (clone B3) (2). Plasmids were linearized with EcoRI for in vitro transcription of antisense probes and with KhoI for in vitro transcription of sense probes. Complementary strands for sense and antisense probes were transcribed either from T3 or T7 promoter sites in the presence of digoxigenin-UTP (Roche Molecular Biochemicals). Human glyceraldehyde-3-phosphate dehydrogenase cDNA was purchased from CLONTECH. Labeled antisense RNA probes were prepared as described by the manufacturer.

Northern blot analysis was performed as described recently (6, 22, 27). The effect of TH on mRNA levels was evaluated and semiquantified using mRNAs isolated from a similar number of cochleae as described previously (22). Using the ImageMaster VDS system (Amersham Pharmacia Biotech), Northern blots were digitized, and relative intensities were calculated using the ImageMaster 1D prime software (Amersham Pharmacia Biotech).

RESULTS

The structure of the organ of Corti of animals that were analyzed for tectorin expression in the present study is illustrated schematically in Fig. 1. The organ of Corti of constant hypothyroid adult animals, in which TH levels were suppressed from the onset of fetal thyroid gland function onwards, persists at least until the 3rd or 4th postnatal week, an immature developmental stage resembling a P5 organ of Corti. Here, the multicellular layer of the greater epithelia ridge (GER) persists, and the tectorial membrane remains attached to the GER and the inner sulcus does not form (Fig. 1A). In analogy, the organ of Corti of a transient hypothyroid animal also persists at the immature developmental stage of a P5 organ of Corti until

![Fig. 1. Schematic illustration of the organ of Corti of animals analyzed in the present study. A, the organ of Corti of an adult constant hypothyroid animal looks similar to the immature organ of Corti of a P5 animal. B, the organ of Corti of a transient hypothyroid animal prior to the stop of goitrogen (here, e.g. P10) appears similar to the immature organ of Corti of a P5 animal. C, the organ of Corti of an adult transient hypothyroid animal looks comparable with an untreated adult control with no obvious structural or neuronal abnormalities. I Sule, inner sulcus; Lim, limbus; OHC, outer hair cells; P, pillar cell; Tec, tectorial membrane (red color).](Image)
the day of the stop of goitrogen, in the present study until P10 or P12 (Fig. 1B). After stop of the goitrogen, the organ of Corti recovers within few days and shows in adults no obvious structural abnormalities from untreated control animals; despite the temporal delay the multicellular epithelial layer of the GER recovers within few days and shows in adults no obvious structural abnormalities from untreated control animals; despite the temporal delay the multicellular epithelial layer of the GER transforms to a monocellular layer leading to a detached tectorial membrane and typical inner sulcus formation (Fig. 1C).

The distribution of α- and β-tectorin mRNA in sections of the early postnatal rat cochlea was revealed using non-radioactive in situ hybridization. For α-tectorin at P5, a broad band of expression was observed in the greater epithelial ridge (Fig. 2A, GER), and a narrower band was seen in the supporting cells that lie alongside the outer hair cells and are probably Hensen’s cells (Fig. 2A, open arrow, H). Low levels of α-tectorin mRNA were also found in the phalangeal processes of Deiters’ cells (Fig. 2A, open arrow, D) and in the pillar cells (closed arrow, P). β-Tectorin mRNA was found in a narrow region of the GER, as well as in a row of supporting cells, probably the 3rd row of Deiters’ cells (open arrow, D). Lim, limbus; OHC, outer hair cells; P, pillar cell. Bar, 10 μm.

To analyze the consequences of altered tectorin mRNA expression caused by a reduction in TH supply, we examined the distribution of tectorin protein in the tectorial membrane of control and hypothyroid animals using antibodies specific to α- and β-tectorin. The microvillar marker ezrin was used to visualize the interface between the surface of the greater epithelial ridge and the matrix of the tectorial membrane. A severe reduction of β-tectorin protein in the tectorial membrane of hypothryoid animals was observed using laser scanning confocal microscopy as shown for the midbasal cochlear turn at P6 (Fig. 6, Control and Hypo), whereas the tectorial membrane was stained in a similar manner in control and hypothyroid rats by antibodies to α-tectorin (not shown).

These findings suggested that a reduction in β-tectorin mRNA expression should be considered as a cause of hearing loss in animals transiently deprived of TH (2). We therefore first followed the expression of β-tectorin mRNA after a period of transient TH deficiency lasting from E17 until P10, a condition known to lead to hearing impairment in these animals (2). As in previous studies we stopped the hypothyroid condition in young rats by withdrawing MMI treatment at P10. Prior to this stage, low levels of β-tectorin mRNA were noted in the GER (Fig. 4B, Hypo, P8), and low levels of β-tectorin protein were detected in the tectorial membrane (Fig. 6, Hypo P6). Within 5
Tectorin and Thyroid Hormone

**Fig. 4.** Distribution of α-tectorin mRNA (A) and β-tectorin mRNA (B) in the middle or apical turn of untreated (Control) and hypothyroid (Hypo) animals at the indicated postnatal ages as detected by in situ hybridization. A, α-tectorin mRNA expression is expressed already at P1 in untreated animals, declines toward P8, and totally disappears by P42, when the inner sulcus is formed. In the absence of TH, α-tectorin mRNA expression is slightly enhanced at P8 and remains elevated until 6 weeks after birth, when in the apical turn small amounts of mRNA can be detected in presumptive still existing GER cells (Hypo, P42). B, β-tectorin mRNA in untreated animals is rapidly up-regulated between P1 and P8. Consistently weaker expression of β-tectorin is noted under hypothyroid conditions, and reduced β-tectorin mRNA levels persist even in the apical turn 6 weeks after birth (Hypo, P42). I Sulc, inner sulcus; Lim, limbus; OHC, outer hair cells. Bar, 10 μm.

**Fig. 5.** Effect of perinatal hypothyroidism on α- and β-tectorin mRNA levels in the rat cochlea. Northern blot of cochlear mRNA from untreated (C) and hypothyroid (H) rats of the ages indicated. The α-tectorin RNA probe hybridizes with a single mRNA of 7.5 kb, whereas the β-tectorin probe hybridizes with mRNAs of 2.9 and 2.6 kb. A glyceraldehyde-3-phosphate dehydrogenase probe was cohybridized and detected a 1.9-kb mRNA. The α-tectorin mRNA level is slightly enhanced at P8 in hypothyroid animals, and low mRNA levels remain detectable under hypothyroid conditions until P22. The typical rapid developmental rise of the 2.9- and 2.6-kb β-tectorin mRNA observed in controls (C) is significantly reduced in the absence of TH (H).

**Fig. 6.** Localization of β-tectorin in control and hypothyroid animals (Hypo) at P6 using confocal microscopy. Ezrin is used to demarcate the apical surface of the cochlear epithelium (green). Note a significant difference in the intensity of anti-β-tectorin protein staining in hypothyroid animals. Lim, limbus; OHC, outer hair cells; Tec, tectorial membrane. Bar, 10 μm.

**Fig. 7.** Analysis of β-tectorin mRNA (A) and β-tectorin protein (B) in the organs of Corti of hypothyroid rats 5 days after the cessation of hypothyroid conditions (Hypo P10 + 5 days) and 8 days after cessation of hypothyroid conditions (Hypo P10 + 8 days). Upon cessation of MMI-treatment, β-tectorin mRNA was rapidly up-regulated to peak levels within 5 days (A, Hypo P10 + 5 days) and declined within the next 3 days (A, Hypo P10 + 8 days); concomitant with the formation of the inner sulcus (I Sulc). B, during the same period the β-tectorin protein level in the tectorial membrane remained reduced as illustrated for cochleae at 5 (B, Hypo P10 + 5 days) and 8 days (B, Hypo P10 + 8 days) after cessation of MMI. Note that the decalcification of cochleae in samples older than P10 enabled the visualization of β-tectorin antigen within the cells of the GER (B, Hypo P10 + 5 days, GER). Lim, limbus; OHC, outer hair cells; Tec, tectorial membrane. Bar, 10 μm.

Days of withdrawing MMI treatment, the cochlear epithelium invaginated rapidly to form an inner sulcus (Fig. 7A, Hypo P10 + 5, I Sulc) leading to the detachment of the tectorial membrane from the cells of the GER (Fig. 7B, Hypo P10 + 5, Tec). β-Tectorin mRNA levels rose rapidly as inner sulcus formation recovered, peaked within 5 days (Fig. 7A, Hypo P10 + 5, GER), and then declined completely within the next 3 days when the adult morphology was nearly reached (Fig. 7A, Hypo P10 + 8). Although β-tectorin antibodies (I2) did not typically recognize newly synthesized intracellular β-tectorin proteins in the cochlear epithelium and only β-tectorin in the tectorial membrane, the decalcification of cochlear bones required for sectioning at stages later than P10 enabled the β-tectorin antigen to be visualized in the cochlear epithelium. Thus, it became evident that within a short time period after the cessation of hypothyroid conditions, β-tectorin protein could also be visualized in the cochlear epithelium (Fig. 7B, Hypo P10 + 5, GER). How-
however, the β-tectorin protein level in the tectorial membrane stayed at a reduced level at both 5 (Fig. 7B, Hypo P10 + 5, Tec) and 8 days after cessation of hypothyroid conditions (Fig. 7B, Hypo P10 + 8, Tec).

The functional importance of this finding was analyzed in animals 1 year after they had endured a transient hypothyroid period from the normal onset of thyroid gland function (at E17) until P10. β-Tectorin protein levels were detected in the cochleae using scanning confocal microscopy (Fig. 8). Again, ezrin was used as a marker protein to indicate the apical surface of the epithelium (Fig. 8). We observed a strongly reduced β-tectorin protein level in the tectorial membrane of transiently TH-deprived animals (Fig. 8, Hypo P10 + 1 year) in contrast to that in the tectorial membrane of untreated controls of similar age (Fig. 8A, Control (1 year)).

Semiquantification of the β-tectorin protein level in the tectorial membrane of 1-year-old transiently TH-deprived animals was performed using Western blots (Fig. 9). As in recent studies we stopped hypothyroid conditions in young rats by halting MMI treatment of the dams at P8, P10, and P12 (2). Additionally, we used untreated animals (controls) or offspring of dams treated with MMI until P3, P4, or P6 (2). Following hearing analysis (2), 1-year-old animals were sacrificed, and cochleae were prepared as described. Ezrin was codetected to confirm the loading of approximately equivalent amounts of protein. In two independent experiments, a 43-kDa β-tectorin polypeptide band was detectable in controls (Fig. 9, A and B, Control), which was significantly reduced if the animals had experienced a short period of TH deficiency until P10 (Fig. 9A, β-Tec I, Hypo or P12 (Fig. 9B, β-Tec I, Hypo). A polypeptide of ~75 kDa was noted occasionally and was presumed to be a β-tectorin dimer (Fig. 9B, open arrow). During these experiments, a 47-kDa polypeptide band was also found to cross-react with β-tectorin antibodies (Fig. 9A, A and B, β-Tec II). We therefore examined the size of β-tectorin polypeptides that are produced during early postnatal development (Fig. 9C) and compared it with β-tectorin polypeptides that result when the onset of TH supply is delayed during the early postnatal period. On Western blots we noted that the β-tectorin protein size underwent a change in apparent molecular mass from an early “immature” 47-kDa polypeptide at P0 and P4 (Fig. 9C, P0, P4, β-Tec II) to a “mature” 43-kDa polypeptide at P20 (Fig. 9C, P20, β-Tec I). At intermediate stages, P12, two polypeptides of 47 and 43 kDa were noted (Fig. 9C, P12). In 1-year-old animals with normal hearing (Fig. 9D, Co) or 1-year-old animals that had undergone transient TH-deficiency until P3 (Fig. 9D, H3) or P6 (not shown), β-tectorin polypeptides of the mature 43-kDa size were noted predominantly. However, a delay in the onset of TH supply beyond P8, P10, or P12 led to the nearly total disappearance of this 43-kDa β-tectorin polypeptide (Fig. 9D, H8, H10, H12, β-Tec I), suggesting loss of the mature β-tectorin isoform.

The structure of the tectorial membrane matrix in animals that had been deprived of TH was examined by transmission electron microscopy. In control animals, the structure of the tectorial membrane was similar to that described in mice (29). Within the central core of the tectorial membrane, the region overlying the inner sulcus and the organ of Corti, bundles of 20-nm-diameter collagen fibrils were observed imbedded in a striated sheet matrix composed of alternating, light and dark staining, cross-linked, 7–9-nm-diameter filaments (Fig. 10A). These filaments are interpreted to be the tectorins (12). Dense areas of matrix, within which it is hard to visualize any structural details, typically form the coverent fibrils, Hensen’s stripe, Kimura’s membrane, and the marginal band of control tectorial membranes (Fig. 10B). In the hypothyroid animals, the matrix within which the collagen fibrils are imbedded was of a dense appearance and did not possess the normal regular appearance of the striated sheet matrix (Fig. 10C). Within the coverent, and in proximity to Hensen’s stripe, unusual 9-nm-diameter fibrils packed in paracrystalline arrays were noted in the hypothyroid animals (Fig. 10D).

DISCUSSION

The major non-collagenous proteins of the mammalian tectorial membrane, α- and β-tectorins, have been identified as the products of two single genes (12). The α-tectorin gene maps to mouse chromosome 9 and human chromosome 11q22–29 (29), and β-tectorin maps to mouse chromosome 19, in a region that shows synteny with human 10q25–26 (30). α-Tectorin comprises an NH2-terminal region with similarity to the globular G3 domain of entactin, a large central region with three full and two partial repeats homologous to the D domain of prepro-von Willebrand factor and a C-terminal zona pellucida
domain (12). In contrast, β-tectorin comprises only a single zona pellucida domain (12). The zona pellucida domain (31) is a feature shared by a number of different proteins, all of which are capable of forming filament-based matrices or gels (32–38). The presence of a common domain in both α- and β-tectorin has led to the suggestion that these two proteins may either self-associate to form homomeric filaments or interact with each other to form heteromeric filaments via their zona pellucida domains and that these filaments correspond to the light and dark staining filaments that are visible in the collagenase-insensitive, striated-sheet matrix of the tectorial membrane (12). Analysis of the human α-tectorin gene in two families with autosomal dominant non-syndromic hearing impairment revealed missense mutations replacing conserved amino acid residues within the zona pellucida domain of α-tectorin (13). The hearing impairment in both families, which is prelingual in onset, is therefore related presumably to a disruption of the interactions involving the zona pellucida domain of the α-tectorin and, as a consequence, a disruption in tectorial membrane structure (13). However, there are as yet no mouse models available for any of the human tectorin mutations that have been described, and the effects that mutations in the zona pellucida domain of α-tectorin may have on matrix structure remain unknown.

In the present study suppressed β-tectorin expression is described in constant hypothyroid animals, whereas transient hypothyroid conditions prior to onset of hearing lead to recovery of β-tectorin mRNA in the greater epithelial ridge but to a permanently reduced β-tectorin protein level in the tectorial membrane.

There is no doubt that constant hypothyroidism may cause multiple subtle effects on the organ of Corti because of a combination of both direct and indirect effects (4, 5, 11). Though many of these effects resolve by later stages, even beyond the 4th postnatal week abnormal inner sulcus formation and retarded innervation was noted and was causally related with hearing loss (4, 5, 11). The consequences of a short transient period of hypothyroidism for less than 10 days may be less complex, in particular as we detected rapid recovery of inner sulcus formation and innervation under these conditions, leading to an organ of Corti without any obvious structural or neuronal abnormalities within less than 1 week after stop of goitrogen (2). Though we cannot exclude that transient hypothyroidism may influence the post-translational processing or glycosylation of β-tectorin, a delay of TH supply may lead to a simple mismatch in timing of important developmental processes. When TH supply is restored following transient hypothyroidism, the inner sulcus rapidly begins to form whereas β-tectorin mRNA production gradually increases. Translation of β-tectorin mRNA levels therefore occurs as the sulcus is forming and as the tectorial membrane is already detaching from the epithelial surface, rather than prior to tectorial membrane detachment as is the case in control animals. This mismatch in the timing of developmental events may not only lead to retarded β-tectorin translation but as a consequence also to retarded β-tectorin protein secretion into a tectorial membrane. The β-tectorin protein may thereby be released in the endolymphatic space instead of into the tectorial membrane, resulting in a tectorial membrane that has a low β-tectorin protein content. In line with such a concept we note that transient hypothyroidism leads to depletion of the presumptive mature, processed β-tectorin isoform. The available evidence (12, 39, 40) suggests the tectorins are synthesized as glycosylphosphatidylinositol-linked, membrane-bound precursors that are released from the plasma membrane by the action of an endopeptidase. The glycosylphosphatidylinositol anchors are thought to direct the tectorins to the apical surface of the epithelium, and the tectorins may be either proteolytically released from the membranes of secretory granules while they are on route to the apical surface of the epithelium or released from the surface of the microvilli into the tectorial membrane matrix after their arrival at the apical surface of the cochlear epithelium. The shift to lower molecular mass observed for β-tectorin on Western blots during development shown in this study may reflect the relative abundance of membrane bound and/or processed, presumptively released, forms of β-tectorin protein. Longer transient hypothyroid periods prior to hearing (cessation of MMI at P8 and P12) but not shorter hypothyroid periods (cessation of MMI at P4 or P6) lead to a significant reduction in the lower molecular mass β-tectorin polypeptide chain, suggesting β-tectorin processing may be abrogated, or processed β-tectorin proteins may be lost in the endolymphatic space when hypothyroid conditions extend beyond a critical time. It is noteworthy in this context that the critical hypothyroid period beyond β-tectorin protein levels is reduced in the tectorial membrane, is precisely overlapping with the critical hypothyroid period beyond loss of active cochlear mechanics, and is observed in the same animals (2).

In conclusion, the data presented indicate strongly that TH supply during the correct time frame prior to onset of hearing is essential to guarantee that sufficient amounts of β-tectorin protein are incorporated into the tectorial membrane. Though we cannot exclude from the present data that other processes on, for example, the outer hair cell level show high sensitivity for transient TH-deficient periods prior to hearing onset, the change in tectorial membrane matrix structure that accompanies the reduction in β-tectorin protein content may be sufficient to cause the loss of active cochlear mechanics (2). Tecto-
rrial membrane development in humans occurs prenatally, so 
TH deficiency for short periods during early pregnancy, for 
example because of iodine deficiency, could have an adverse 
effect on hearing. A loss of β-tectorin may underlie some of 
the hearing impairments observed in genetic and acquired diseases 
related to thyroid dysfunction (18, 41–45). It will be interesting 
to study thyroid hormone receptor mutants (46) for normal 
β-tectorin expression as a cause of deafness (46, 47). Further-
more, a β-tectorin null mutant mouse will be helpful in future 
studies to establish the role played by β-tectorin in the forma-
tion of the non-collagenous matrix of the tectorial membrane.

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