Mutations affecting development of the zebrafish inner ear and lateral line

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SUMMARY

Mutations giving rise to anatomical defects in the inner ear have been isolated in a large scale screen for mutations causing visible abnormalities in the zebrafish embryo (Haffter, P., Granato, M., Brand, M. et al. (1996) Development 123, 1-36). 58 mutants have been classified as having a primary ear phenotype; these fall into several phenotypic classes, affecting presence or size of the otoliths, size and shape of the otic vesicle and formation of the semicircular canals, and define at least 20 complementation groups. Mutations in seven genes cause loss of one or both otoliths, but do not appear to affect development of other structures within the ear. Mutations in seven genes affect morphology and patterning of the inner ear epithelium, including formation of the semicircular canals and, in some, development of sensory patches (maculae and cristae). Within this class, dog-eared mutants show abnormal development of semicircular canals and lack cristae within the ear, while in van gogh, semicircular canals fail to form altogether, resulting in a tiny otic vesicle containing a single sensory patch. Both these mutants show defects in the expression of homeobox genes within the otic vesicle. In a further class of mutants, ear size is affected while patterning appears to be relatively normal; mutations in three genes cause expansion of the otic vesicle, while in little ears and microtic, the ear is abnormally small, but still contains all five sensory patches, as in the wild type. Many of the ear and otolith mutants show an expected behavioural phenotype: embryos fail to balance correctly, and may swim on their sides, upside down, or in circles. Several mutants with similar balance defects have also been isolated that have no obvious structural ear defect, but that may include mutants with vestibular dysfunction of the inner ear (Granato, M., van Eeden, F. J. M., Schach, U. et al. (1996) Development, 123, 399-413.). Mutations in 19 genes causing primary defects in other structures also show an ear defect. In particular, ear phenotypes are often found in conjunction with defects of neural crest derivatives (pigment cells and/or cartilaginous elements of the jaw). At least one mutant, dog-eared, shows defects in both the ear and another placodally derived sensory system, the lateral line, while hypersensitive mutants have additional trunk lateral line organs.

Key words: inner ear, otic vesicle, otolith, semicircular canal, mechanosensory hair cell, lateral line, hearing, balance, zebrafish, Danio rerio, mutant

INTRODUCTION

The vertebrate inner ear is an intricate and self-contained model system for developmental studies. An organ of initially simple and finally complex three-dimensional structure, it illustrates almost all the developmental concepts and problems usually assigned to an embryo as a whole: induction, positional information, neurogenesis, epithelial to mesenchymal transition and interaction, axonal guidance, morphogenesis and cell-cell signalling. The mature organ, found in all jawed vertebrates, has two functions: it serves as an auditory system, which detects sound waves, and as a vestibular system, which detects linear and angular accelerations, enabling the organism to maintain balance. The membranous labyrinth of the inner ear, a series of fluid-filled chambers surrounded by a continuous epithelium, is complex in structure. Three semicircular canals are arranged orthogonally to each other; these detect angular accelerations. The canals open into a series of chambers that contain the detectors for linear accelerations and sound. Fish do not possess a cochlea, the specialised hearing organ of amniotes, nor are their inner ears linked to a middle and outer ear system. Instead, the macular organs of the sacculus, lagena and macula neglecta detect sound waves, and in Otoophysan species (including zebrafish), the gas bladder
(swimbladder) is used to enhance auditory stimulation of the inner ear (reviewed in Popper and Fay, 1993). While details of inner ear structure vary between vertebrates, all use the same basic cell type to detect both vestibular and auditory sensory stimulation - the mechanosensory hair cell (Corwin and Warchol, 1991; Hudspeth, 1989). Hair cells show exquisite precision of differentiation in a reproducible and position-specific manner (Tilney et al., 1986, 1992; Tilney and Saunders, 1983) in thickened patches of sensory epithelium within the ear, known as maculae or cristae.

Studies of both the development and adult structure of the zebrafish inner ear suggest that it is broadly representative of the inner ears of other teleosts and of vertebrates in general (Haddon and Lewis, 1995; Platt, 1993; Waterman and Bell, 1984). The otic placode, visible by early somite stages, gives rise to the otic vesicle by thickening and cavitation, rather than invagination. Neuroblasts, fated to form neurons of the vestibulocochlear (VIIIth) ganglion, delaminate from the otic epithelium between 22 and 42 hours of development. Sensory patches and semicircular canals arise at precise and stereotyped positions in the otic vesicle. Sensory patches arise ventrally, hair cells differentiating first in two maculae (Haddon and Lewis, 1995). Each macula is overlain by an otolith, a dense crystalline deposit of calcium carbonate; shear forces on the hair cells result from movement or inertia of the otolith relative to the epithelium (caused by sound waves or gravity, for example). Hair cells in the three cristae, the sensory patches for the semicircular canals, appear after those in the maculae. Semicircular canal formation begins by ingrowth of epithelial projections from the walls of the vesicle, which fuse to span the ear lumen (Waterman and Bell, 1984). A photograph and drawing of the zebrafish inner ear at 96 hours of development are shown in Fig. 1; photographs of wild-type ear development up to this stage are also shown in Fig. 2.

The lateral line is a sensory system found in fishes, amphibian larvae and the adults of aquatic amphibia. It is responsible for detecting water motion close to the surface of the organism, and mediates diverse behaviours such as shoaling, prey detection, and predator and obstacle avoidance (Coombs et al., 1989). The structure of lateral line organs (neuromasts) resembles that of the sensory patches within the inner ear; each neuromast contains hair and supporting cells. Neuromasts, and the afferent neurons that innervate them, arise, like the inner ear, from placodal ectoderm (for a description of lateral line development in zebrafish, see Metcalfe, 1989, and references within). Although neuromast defects were not explicitly screened for, at least one of the inner ear mutations also affects the lateral line, and one mutation apparently affecting the lateral line but not the ear was found during complementation analysis. Both sensory systems become functional within the time of the present study (120 hours); fish orient themselves with respect to gravity and show a startle response at 96 hours, which may be mediated by both vestibulocochlear and lateral line nerve activity impinging on the Mauthner cell (Eaton and Farley, 1975, and references within).

This paper presents an overview of all the zebrafish mutants showing defects in inner ear development isolated in a large scale mutagenesis screen (Haffter et al., 1996). 58 mutations were classified as having a primary phenotype in the inner ear. 48 of these have no obvious defects in other structures; the remaining ten have defects in addition to the ear phenotype. A further 37 mutations (in 19 genes) have been classified as causing a secondary or tertiary defect in the ear, with the primary defect in a different structure (see references in Table 3). While many of the ear mutants show an expected balance defect, a group of 17 mutants have been identified in which balance is defective, but for which the ear was judged to be morphologically normal. These may include mutants with vestibular dysfunction of the inner ear, and are discussed in the accompanying paper on those mutations broadly affecting motility (Granato et al., 1996).

MATERIALS AND METHODS

Isolation of mutant lines

Mutagenesis, maintenance and breeding of stocks, identification of mutant phenotypes, and complementation testing are described by Haffter et al. (1996).

Fluorescein-phalloidin stain for actin in hair cell stereocilia

Larvae were fixed in 4% formaldehyde in PBS overnight, and rinsed in several changes of 2% Triton X-100 in PBS, over several days, to permeabilise the tissue and dissolve the otoliths. Larvae were then stained with 2.5 μg/ml fluorescein-labelled phallloidin (Sigma) in PBS for 2 hours, and rinsed several times with PBS over 2 hours. All fixing, staining and rinsing was done at 4°C. Dissection was necessary before observation, as other tissues expressing large amounts of actin (e.g. muscle) also fluoresce brightly, obscuring the inner ear hair cells. The skin of the head, and the eyes, brain and jaw were removed with forceps in that order, leaving the two ears attached to the trunk, and connected to each other by a horizontal plate of cartilage. This plate was then pulled off the trunk, with the two ears attached, and mounted in Citifluor (AF-1; Citifluor Ltd.) for observation.

Whole-mount in situ hybridisation

Synthesis of digoxigenin-labelled RNA probes was performed according to Boehringer or Stratagene instructions. Whole-mount in situ hybridisation was performed essentially as described by Oxtoby and Jowett (1993), with some minor modifications.

DASPEI live staining of lateral line hair cells

120-hour old larvae were immersed in 1 mM DASPEI (2-(4-dimethylaminostyryl)-N-ethyl pyridinium iodide; Molecular Probes, Oregon) in E3 (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl2, 0.33 mM MgSO4) for 20 minutes, as in Balak et al. (1990). They were rinsed thoroughly in E3, anaesthetised with MESAB (0.5 mM 3-aminozenoic acid ethyl ester, 2 mM Na2HPO4), and mounted in methylocellulose in a depression slide for observation using a Leitz I-2 filter block.

RESULTS

58 mutants with a primary ear phenotype were isolated and kept in the Tübingen large-scale zebrafish screen, representing 4.8% of all mutants kept (Haffter et al., 1995). Inner ear phenotypes were identified on a morphological basis; the major anatomical features screened for were size and shape of the otic vesicle, size, shape and number of otoliths, and the morphology of the semicircular canals (Figs 1 and 2A-D). The ear is a large and easily visible organ in the embryo; in particular, any defect of otoliths is easy to see. This may mean that certain classes of ear phenotypes (such as those affecting otoliths - see
sensory patch or macula. Kinocilia of the crista hair cells (kc) are long, and project into the canal lumens. Kinocilia of the maculae are shorter and the otoliths appear to sit directly on the stereociliary bundles (sc) of the macular hair cells. The smaller (anterior) otolith lies in a lateral position; the larger (posterior) otolith lies medially. dls, dorsolateral septum. Scale bar, 50 μm.

Molecular probes were used to characterise the mutants further. Fluorescein-tagged phalloidin reveals the bundles of actin-rich stereocilia on each hair cell in the sensory patches (see Fig. 3 and compare with Fig. 1). Five sensory patches are evident at this stage: three cristae, one for each of the semicircular canals, and two larger maculae, one for each otolith. The vital dye DASPEI stains hair cells in lateral line neuromasts (Fig. 6). Gene expression patterns were also examined in the mutants. The homeobox gene mshC is expressed in three distinct patches in the wild-type ear at 48 hours, which almost certainly correspond to the developing cristae (Fig. 4A; Ekker et al., 1992).

**otx1** is another homeobox gene, which shows strong expression in a ventral, lateral and posterior domain of the otic vesicle from 18 hours to beyond 48 hours, and is also expressed in regions of the fore- and midbrain (Fig. 5A,B; Li et al., 1994). The paired box gene paxb is expressed strongly on the medial side of the otic vesicle, where it is closely apposed to the hindbrain, in addition to its striking expression in the midbrain, optic stalks, nephritic primordium and isolated cells of the hindbrain and spinal cord (Krauss et al., 1991).

### Table 1. Mutations affecting development of otoliths, with no obvious morphological defects of the ear epithelium

<table>
<thead>
<tr>
<th>Phenotypic class</th>
<th>Gene name</th>
<th>Allele names</th>
<th>Additional phenotype</th>
<th>L/V A M O P N Cross reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>One otolith at all stages</td>
<td>einstein, eis</td>
<td>ta29, tc7c, tp17, tc27a, td204g, tc296f, tg205d, td258c, td272c, to228, to236, ts284, t272b, tw224, ts256, ti230c (lost), ts209 (lost)</td>
<td>Balance, circling in some alleles</td>
<td>v n n n n n a</td>
</tr>
<tr>
<td>Initially no otoliths, or very tiny otoliths; sometimes one or two otoliths present later</td>
<td>menhir, men</td>
<td>tm121</td>
<td>sv n n</td>
<td></td>
</tr>
<tr>
<td>Initially no otoliths, or very tiny otoliths; sometimes one or two otoliths present later</td>
<td>half stoned, hst</td>
<td>ta229a, ta51d</td>
<td>Balance</td>
<td>sv n n</td>
</tr>
<tr>
<td>cup und bein</td>
<td>stein und bein, sub</td>
<td>tg289a</td>
<td>Pelvic fin missing in 75% of adults; balance (in embryo)</td>
<td>v n b</td>
</tr>
<tr>
<td>Otoliths absent at all stages</td>
<td>what’s up?, wup</td>
<td>tj241b</td>
<td>Balance, circling</td>
<td>l n n n n</td>
</tr>
<tr>
<td>Loose otoliths</td>
<td>keinstein, kei</td>
<td>ts242a, tj229f, ts276e, db231b, tc242a</td>
<td>Balance, circling</td>
<td>l n a n a</td>
</tr>
<tr>
<td>Loose otoliths</td>
<td>backstroke, bks</td>
<td>tm317d</td>
<td>Balance, circling</td>
<td>l n n</td>
</tr>
<tr>
<td>Loose otoliths</td>
<td>rolling stones, rst</td>
<td>tlv0e</td>
<td>v n</td>
<td></td>
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</table>

L/V: viability, l, lethal; v, viable; sv, semiviable. A, fluorescein-phalloidin stain for stereocilia of inner ear hair cells; M, mshC expression in ear at 48 hours; O, otx1 expression in ear at 24 or 48 hours; P, paxb expression in ear at 24 hours; N, neuromasts present (as seen under microscope or stained with DASPEI). n, normal; a, abnormal. A blank entry indicates ‘not done’. See text for details.

The Cross reference column indicates descriptions of other aspects of the mutant phenotype: a, Malicki et al. (1996); b, van Eeden et al. (1996b). Total number of unresolved otolith mutations: k: tg234, tg225, td254e, td276, td279c, ts218d, ts259b, ts242d.

*Boston alleles: Malicki et al. (1996). Cross-complementation with ear mutants from the zebrafish screen in Boston has only been done so far for einstein and keinstein.*
The mutants classified as having a primary phenotype in the ear can be divided into two broad classes, according to which structures in the ear are affected by the mutation – those affecting the otoliths only, with no other ear defect (Table 1), and those affecting epithelial morphology of the ear, many of which also affect otoliths (Table 2). Structural criteria have been used for classification rather than temporal ones, as the exact temporal point of action of each mutation has not yet been determined. Table 3 lists those mutants having a secondary or tertiary defect in the ear, with the primary defect affecting a different structure. Within each table, further subdivisions are given according to the exact nature and severity of the phenotype in the ear, and presence of other defects in the embryo.

**Mutations affecting otoliths only, with no other ear defect**

One otolith phenotypes

Presence, number, size and shape of the otoliths were scored for in the screen. The most common phenotype seen is a single

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**Fig. 2.** DIC images showing the ear phenotypes of live mutants. (A-D) Lateral views of wild-type (wt) ears, for comparison with the mutant phenotypes. Ages are given in hours (h) for each image. From 72-120 hours, the appearance of the ear does not change much, although the vesicle and otoliths increase in size (see Fig. 1 for appearance of the ear at 96 hours, with explanatory diagram). (B-P) Selected examples of the mutant phenotypes. Gene names and ages are shown for each image; see text for details. Arrowheads indicate otoliths; arrows mark epithelial projections in the ear which form the semicircular canals. Asterisks indicate the anterior sensory macula. pc, posterior crista. Scale bars, 50 μm.
245 Zebrafish ear mutants

Otolith instead of the normal two. 17 mutants with this phenotype are alleles of a single gene, einstein (eis; Table 1). In most eis alleles, one otolith in each ear is missing from the earliest stages of otolith development (Fig. 2E). In some alleles, it is clear which otolith is missing (usually the anterior, more lateral one), but in others, the remaining otolith is in an

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**Fig. 3.** Fluorescein-phalloidin staining of hair cells in sensory patches. All panels are dorsal views of ears at 120 hours; anterior is to the left, medial is up, and lateral is down. Compare with diagram in Fig. 1A. Gene names are given for each panel. (A,B) Photographs of wild-type (wt) ears taken in different planes of focus to show all five sensory patches: the two maculae (m) and three cristae (c). In B, hair cells of a neuromast (arrowhead) on the surface of the ear are stained. Muscle (mu) also fluoresces brightly. (C-F) Otolith mutants in which sensory patches and hair cell patterning appear normal. (G,H) dog: cristae are absent, and hair cells in the maculae are reduced in number. (I) vgo: only a single sensory patch is present. (J-L) Mutants in which ear size is abnormal but sensory patches appear unaffected. See text for details. Scale bars, 50 μm. (M,N) Maculae of ty85e compared with wild type; M,M', medial macula; N,N', anterior macula. Hair cell number is decreased and hair cell patterning is disrupted in the mutant, especially in the anterior macula. Scale bars, 25 μm.
abnormal position or appears to be of abnormal size or shape for its position (data not shown). This may be because the otolith is not tethered correctly within the ear and has rolled to a new position (compare with \textit{rst}, below). At 120 hours, \textit{eis} has a normal complement of sensory patches, as stained with fluorescein-phalloidin (Fig. 3C). Thus, hair cells in the maculae appear to differentiate normally despite the lack of an otolith overlying one of these sensory patches. It is not known whether supporting cells in the patches also develop normally, but the hair cells appear correctly patterned (Fig. 3C). Five \textit{eis} alleles tested all show normal expression of the genes \textit{mshC} (Fig. 4B) and \textit{otx1} (data not shown). One \textit{eis} allele tested also showed normal \textit{paxb} expression in the ear (data not shown). Lateral line development in \textit{eis} is normal, as judged by presence of neuromasts under the dissecting and compound microscopes (data not shown). \textit{eis} embryos initially fail to balance properly, and at 120 hours, may swim on their sides or even upside down. The mutation, however, is homozygous viable; adult fish recover a normal posture, and are fertile. The ability of \textit{eis} adults to balance correctly appears to be via the use of visual cues rather than a recovery of vestibular function, as they will lean over towards a unidirectional light source presented from

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{\textit{mshC} expression in ears of the mutants. In situ hybridisations to \textit{mshC} mRNA in ears at 48 hours of development. Gene names are given for each panel; see text for details. Lateral views; anterior to the left. (A) Wild type (wt). Note the strong expression in three patches (anterior, lateral and posterior; arrowheads), thought to represent the developing cristae. The maculae do not appear to express \textit{mshC}. (C-E) Expression in \textit{kei} embryos is delocalised. D and E are views of the same ear at different focal levels, to show limited localisation to three patches (arrowheads) in addition to delocalised staining. (F) Close up of \textit{kei} anterior patch of expression. The asterisk marks the developing anterior macula, which does not express \textit{mshC}. (J-L) \textit{dog}: in I, the allele \textit{dog}^\textit{tp85b} does not show any expression, while in \textit{dog}^\textit{to15b}, delocalised and weak expression is seen. (K) \textit{vgo}: no expression is evident. Asterisk marks the single macula, with nuclei in two distinct layers, as in the wild type. L, \textit{cls}: only two patches of \textit{mshC} expression are evident. Scale bars, 50 \textmu m.}
\end{figure}
the side, whereas wild-type fish do not do this to the same extent (S. Massey, unpublished data).

Mutations in the gene menhir (men) give rise to a similar phenotype to eis (Table 1). A mutation causing a one-otolith phenotype has also been described from a postmeiotic mutagenesis screen in zebrafish (Riley and Grunwald, 1995). It is not yet known if this is an allele of one of the complementation groups in Table 1.

No otoliths or tiny otoliths
Ten mutants display a phenotype where the otoliths are lacking entirely, or where they are initially absent, with small or single otoliths developing later. Five of these fall into a complementation group, keinstein (kei). The remaining mutations fall into four smaller groups: half stoned (hst), stein und bein (sub), what’s up? (wup) and backstroke (bks) (Table 1).

kei mutants lack otoliths at all stages of development (Fig. 2F), but the otic vesicle otherwise appears to develop normally, although it may appear slightly larger than wild type at early stages. Semicircular canals form correctly, and all five sensory patches are present at 120 hours (Fig. 3D). kei nevertheless shows abnormal expression of mshC in the ear; expression is delocalised rather than restricted to three strong patches as in the wild type (Fig. 4C-F). However, in most individuals, within the background of delocalised expression, small patches of slightly higher intensity stain can be distinguished, which

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**Table 2. Mutations affecting epithelial ear morphology**

<table>
<thead>
<tr>
<th>Phenotypic class</th>
<th>Gene name (number of alleles)</th>
<th>Allele names</th>
<th>Additional phenotype</th>
<th>L/V</th>
<th>A</th>
<th>M</th>
<th>O</th>
<th>P</th>
<th>N</th>
<th>Cross reference</th>
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<tr>
<td>Expanded ears, normal otoliths</td>
<td>big ears, bge (2)</td>
<td>tr216e, tb233c</td>
<td></td>
<td>v</td>
<td>n</td>
<td></td>
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<td></td>
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<td></td>
<td>headphones, hph (1)</td>
<td>tr15</td>
<td></td>
<td>v</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td></td>
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<tr>
<td></td>
<td>lauscher, lau (1)</td>
<td>tk256a</td>
<td></td>
<td>v</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small ears, otoliths relatively normal</td>
<td>little ears, lre (2)</td>
<td>tg414b, to27d</td>
<td>Balance</td>
<td>l</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td></td>
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<tr>
<td></td>
<td>microtic, mtc (1)</td>
<td>ta221</td>
<td>Jaw, circling, retina, motility, pectoral fins</td>
<td>l</td>
<td>n</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Abnormal ear morphology, otoliths relatively normal</td>
<td>boxed ears, bxe (1)</td>
<td>tc214c</td>
<td></td>
<td>l</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td>esselhohr, eso (1)</td>
<td>tp85d</td>
<td></td>
<td>v</td>
<td>n</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>earache, era (1)</td>
<td>tc288e</td>
<td></td>
<td>v</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>van gogh, vgo (2)</td>
<td>tm208, tu285</td>
<td>Jaw, circling</td>
<td>l</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>n</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>Abnormal ear morphology, small otoliths</td>
<td>spock, spk (1)</td>
<td>tc311</td>
<td></td>
<td>v</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>ear plugs, eps (1)</td>
<td>tc286c</td>
<td>Circling</td>
<td>l</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>dog-eared, dog (4)</td>
<td>tc85b, to15b, tm90b, tc237e</td>
<td>Jaw and lateral line retarded; circling</td>
<td>l</td>
<td>a</td>
<td>a</td>
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</table>

See legend to Table 1.
Total number of unresolved ear morphology mutations: 3: tm136, tp219e, ty85e.

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**Fig. 5. otx1 expression in ears of the mutants.** (A) Wild-type and vgo sibling embryos at 24 hours; lateral views. Arrowhead, domain of otx1 in the wild-type ear. Scale bar, 200 μm. (B, B’) albino (wt) and cls embryos at 48 hours; dorsal views. Scale bars, 100 μm.
probably correspond to the developing cristae (Fig. 4D-F). These become more evident with longer staining times.

The mutant bks has a similar phenotype to kei (a lack of otoliths at all stages), and like kei, has a normal complement of hair cells in cristae and maculae. Unlike kei, however, the expression pattern of mshC in the bks ear at 48 hours is indistinguishable from wild type (data not shown). In sub, both otoliths are missing at 24 hours of development, but from 48 hours onwards, the otic vesicle contains a single otolith. sub is homozygous viable; the adults show an incompletely penetrant visible fin phenotype, which may be due to the same mutation, or to one which is closely linked (van Eeden et al., 1995b). The mutants hst and wup also show some otolith development; in wup, tiny otoliths are visible (Fig. 2G), while in hst, as in sub, the ear initially has no otoliths, but some individuals later develop one or two otoliths (data not shown). wup embryos show normal expression of the genes mshC (Fig. 4G) and otx1 (data not shown). All mutants in this class appear to have normal sensory patches at 120 hours (e.g. wup; Fig. 3E).

Most mutants lacking one or both otoliths show an expected behavioural phenotype, and fail to orient themselves correctly while swimming or at rest. In some mutants (e.g. kei, bks, and some eis alleles), larvae sometimes swim in tight circles, in the same horizontal plane or in vertical loops. Circling behaviour is also seen in other classes of the zebrafish ear mutants (see Table 2), and in many mouse mutants with inner ear dysfunction (see Discussion).

Small otoliths
This is a heterogeneous class of mutant phenotypes, all of which show abnormally small otoliths at some stage of development. There are eight mutants of this class with no other obvious defect in the morphology of the ear (Table 1; unresolved). Some show small otoliths from 24 hours; in others the phenotype only becomes apparent at 96 hours. As yet, none of these have been found to fall into a complementation group, but it is not yet known whether this is because saturation for this phenotypic class was not achieved, or because these mutations represent weak alleles of the classes above. Six of the small otolith mutants have been stained for actin; all these have normal sensory patches (data not shown), and two tested for mshC expression (tg225, th276) are both indistinguishable from wild type (Fig. 4H).

Loose otoliths
In rolling stones (rst), the ear develops normally until about 96 hours, when the otoliths, although of normal size, are found at odd positions in the ear, usually lying close together (Fig. 2H). On inverting anaesthetised rst larvae, the otoliths are found to be loose within the ear cavity, and may even become lodged in the dorsal portion of the ear; in the wild type they stay fixed over the maculae when the fish is turned upside down. Otoliths are normally embedded in a gelatinous matrix found over each macula, the otolithic membrane. It is likely that rst embryos lack a component of this matrix. Unless the fish are inverted, the phenotype is not evident in 25% of the progeny of a cross, and most are able to swim and balance normally, presumably because their otoliths remain in normal positions, despite being loose. Hair cells in all sensory patches of rst embryos appear normal (Fig. 3F).

Mutations affecting morphogenesis of the inner ear
This class is subdivided according to apparent severity of the phenotype, and according to the structures affected within the ear (Table 2). The most subtle phenotype is that of eselsohr
(eso), in which the dorsal portion of the anterior canal appears to be collapsed or missing at 96 hours (Fig. 2I; arrow). The sensory patches and otoliths of eso develop normally (data not shown), and this mutation is homozygous viable. Homozygotes have no balance problems, as judged by their swimming behaviour in normal lighting conditions.

The mutant dog-eared (dog) (Table 2) shows a severe phenotype, in which both ear morphology and the otoliths are affected. Epithelial projections to form semicircular canals are present, but are disorganised, and the otoliths are small (Fig. 2J). The dog phenotype is just visible morphologically at about 60 hours, but can be distinguished in doggp85b at 48 hours by the lack of mshC expression in the ear (Fig. 4I). In another allele, dogmol5b, mshC appears to be expressed very weakly and shows no localisation to distinct patches (Fig. 4J). Expression of mshC appears to be normal elsewhere in the embryo. By 120 hours, ears show variable abnormal development of the sensory patches. Cristae are absent, as judged by actin staining (Fig. 3G,H). For doggp85b, ears have been examined at 72, 96 and 120 hours of development, but no crista hair cells were seen at any stage (data not shown). In the wild type, crista hair cells are already present by 72 hours. This suggests that lack of crista hair cells at 120 hours is due to a lack of formation or differentiation of these cells, rather than being due to hair cell degeneration, although cell death has not been examined in these mutants. Macular hair cells are also abnormal, showing decreased numbers and defective arrangement (Fig. 3G,H). These features are variable between alleles and between individuals; in particular, the disrupted hair cell patterning in Fig. 3H may be due to degeneration of the sensory patch, as other individuals do not show the same phenotype.

Development of the jaw and the lateral line in dog are also abnormal (Fig. 6A,B). The jaw is an abnormal shape (arrow in Fig. 6A), but staining with Alcian blue reveals that all elements of the jaw and gill arches are present, although they appear smaller than normal (data not shown). Although the dog lateral line phenotype varies between individuals, there are consistently fewer neuromasts in the tail than in wild type. Almost all dog larvae lack the characteristic wild-type set of neuromasts at the tail tip, the most caudal of which is normally beyond the last myotome (Fig. 6A,B). It is not yet known whether this phenotype is autonomous to the lateral line cells or whether it reflects a defect in the pathway of migration of the lateral line primordium.

Other mutations that affect development of the semicircular canals, but which have not yet been analysed in detail, are boxed ears (bxe) (Fig. 2K), spock (spk), which has a pointed-shaped ear and small otoliths at 72 hours, earache (era), and ear plugs (eps) (Table 2; data not shown).

Abnormal ear and jaw morphology
Mutations in three genes that cause an ear abnormality give

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**Table 3. Mutations in which there is a secondary ear phenotype**

<table>
<thead>
<tr>
<th>Primary phenotype</th>
<th>Gene name (alleles)</th>
<th>Ear phenotype</th>
<th>Other phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jaw, pectoral fins, retinotectal projection</td>
<td>dackel, dak (4)</td>
<td>Expanded, no clear internal structures</td>
<td></td>
<td>a, b, c</td>
</tr>
<tr>
<td>Pigment: all three cell types (melanophores, xanthophores and iridophores) affected</td>
<td>boxen, box (8)</td>
<td>Expanded</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>stonewashed, stw (1)</td>
<td>Retarded ear and otoliths</td>
<td></td>
<td>d</td>
</tr>
<tr>
<td>Pigment: xanthophores only affected</td>
<td>blanced, beh (1)</td>
<td>Small otoliths</td>
<td>Eye pigment, general retardation</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td>bleached, bIc (1)</td>
<td>Small otoliths</td>
<td>Motility, tectum necrosis</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td>weiss, wEI (1)</td>
<td>Retarded ear, small otoliths</td>
<td>General retardation</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td>sahne, sah (1)</td>
<td>Small otoliths</td>
<td>Motility</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td>colourless, cls (3)</td>
<td>Small ear and otoliths</td>
<td></td>
<td>d</td>
</tr>
<tr>
<td></td>
<td>puzzle, puc (1)</td>
<td>Small ear and otoliths</td>
<td>Jaw</td>
<td>d</td>
</tr>
<tr>
<td>Collapsed brain ventricles</td>
<td>clorix, clx (1)</td>
<td>Small otoliths</td>
<td>Motility</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>non blond, nob (1)</td>
<td>Small otoliths</td>
<td></td>
<td>e</td>
</tr>
<tr>
<td>No rhombic isthmus</td>
<td>snakehead, snk (6)</td>
<td>Small ear, no otoliths</td>
<td></td>
<td>f</td>
</tr>
<tr>
<td></td>
<td>fullbrain, ful (1)</td>
<td>Small ear</td>
<td>Eyes, pigment, skin</td>
<td>f, d</td>
</tr>
<tr>
<td></td>
<td>otter, ott (1)</td>
<td>Small ear</td>
<td></td>
<td>f</td>
</tr>
<tr>
<td>Immotile</td>
<td>acerebellar, ace (1)</td>
<td>Small ear, often with only one otolith</td>
<td></td>
<td>g</td>
</tr>
<tr>
<td></td>
<td>heart attack, hat (1)</td>
<td>Small ear</td>
<td>Circulation</td>
<td>h</td>
</tr>
<tr>
<td>Eyes, lens, pectoral fins</td>
<td>dreumes, dre (1)</td>
<td>Abnormal ear structure (dorsolateral septum missing)</td>
<td>Homozygous adults are dwarf and lack anal fin</td>
<td>i, c</td>
</tr>
<tr>
<td></td>
<td>leprechaun, lep (2)</td>
<td>Ear structuring retarded</td>
<td></td>
<td>As for dreumes</td>
</tr>
<tr>
<td></td>
<td>ukkie, uki (1)</td>
<td>Ear structuring retarded</td>
<td>Homozygous adults are dwarf and lack anal fin</td>
<td>As for dreumes</td>
</tr>
</tbody>
</table>

Full descriptions of the mutants can be found in the references indicated: a, Schilling et al. (1996); b, Trowe et al. (1996); c, van Eeden et al. (1996b); d, Kelsh et al. (1996); e, Odenhal et al. (1996); f, Jiang et al. (1996); g, Brand et al. (1996); h, Granato et al. (1996); i, Heisenberg et al. (1996).
rise to more severe patterning defects in the jaw than in dog: van gogh (vgo) (Table 2), boxer and dackel (box and dak) (Table 3). In vgo, the ear phenotype is visible at 24 hours, when the otic vesicle is significantly smaller than wild type (Fig. 2L). By 48 hours (Fig. 2M) the vesicle is still relatively small, and most strikingly, no projections are seen from the walls of the vesicle to form semicircular canals. At 120 hours, the ear is minute compared to wild type, and remains a simple undivided cavity (Fig. 2N). Nevertheless, staining for actin at day 5 reveals a single sensory patch within the tiny vesicle (Fig. 3I). Despite the small size of the otic vesicle, and single sensory patch, two fairly normal otoliths develop that do not fuse together (Fig. 2L-N). The jaw phenotype of vgo is striking, showing both anterior and posterior arch defects (Piotrowski et al., 1996). By 120 hours, homozygotes show general oedema; the mutation is lethal. vgo mutants lack expression of both mshC (48 hours; Fig. 4K) and otxl (24 hours, 48 hours; Fig. 5A) in the otic vesicle; the absence of mshC expression supports the view that cristae are absent. Expression of these genes in other regions of the body appears to be normal (see, for example, the expression of otxl in the head at 24 hours; Fig. 5A).

Mutations affecting pigment cells with an ear phenotype

Many of the mutations affecting pigment cells also show otolith, and sometimes other ear defects (Table 3). Mutations in the gene colourless (cls) give rise to a phenotype in which all three pigment cell types - melanophores, xanthophores and iridophores - are greatly reduced or lacking (Kelsh et al., 1996). Homozygous cls embryos have a tiny ear, similar in appearance to that of vgo, containing two small otoliths (Fig. 2O) and a single sensory patch at 120 hours (data not shown). Unlike vgo, however, cls has relatively normal levels, although abnormal patterning, of otxl and mshC expression in the ear. In cls, mshC is expressed in two patches, rather than three in the wild type (Fig. 4L), and otxl is present in a smaller domain than normal (Fig. 5B). Ear defects in the other mutants listed in Table 3 have not been characterised in detail.

Ear size

Expanded ears

In big ears (bge), headphones (hph), lauscher (lau) and one unresolved mutation (tp219e), the ears are grossly distended at 120 hours; in lau and tp219e, the epithelial projections spanning the ear may fail to fuse (data not shown). In bge and hph at least, the sensory patches, as assessed by actin staining, are normal, but appear slightly displaced from one another due to expansion of the ear (Fig. 3J). Otoliths are always normal, and adult homozygotes of bge and hph appear to swim and balance normally under normal lighting conditions. In hph, expression of mshC (48 hours), otxl (24 hours, 48 hours) and paxb (24 hours) in the ear has been analysed, and in all cases is indistinguishable from wild type (data not shown). Development of lateral line neuromasts is unaffected in these mutants (data not shown). It is likely that the genes defined by this set of mutants have non-essential roles in maintaining fluid composition or pressure within the ear.

Small ears

The small ear class includes the gene little ears (lte; Fig. 2P), where, as in bge, patterning of sensory patches appears normal, but all five patches are found very close together inside the abnormally small vesicle (Fig. 3K). This gene may also function to regulate fluid composition within the ear, as no patterning defect is obvious at early stages. For one allele tested, lte abo27, expression of the genes mshC, otxl and paxb appears to be normal in the ear (data not shown). Lateral line neuromasts in lte are normal (data not shown). lte mutants have abnormal balance, swimming and resting on their sides or upside down. Mutations in the gene micrototic (mtc) also give rise to a small ear phenotype, but here the jaw is also affected (data not shown). As in lte, the sensory patches for this mutant appear normal, despite being found close together in the small ear cavity (Fig. 3L).

Mutations affecting the lateral line

Although not systematically screened for, two mutations have been found that affect development of the lateral line system; dog, described above, which has fewer neuromasts than normal, and hypersensitive (hps; see note below Table 2), which displays extra neuromasts along the rostral half of the posterior lateral line at 120 hours (Fig. 6C). Patterning of neuromasts elsewhere at this stage appears normal. The single allele is homozygous viable; hps embryos have not yet been analysed in detail at later stages, but at 1 month of age, they show a pigmentation defect also affecting the trunk region (data not shown).

Mutations affecting balance

Simple behavioural screening for motility identified 17 mutants with abnormal balance, but with no apparent structural ear defect. Their phenotypes vary, but many are similar to those affecting otoliths: the larvae swim or rest on their sides or upside down. Some fish swim along a corkscrew path or show circling behaviour (Granato et al., 1996). These are exactly the defects expected of animals with defective vestibular function, as found in several of the otolith and ear mutations described here, and in many mouse mutations affecting function of the inner ear (see Discussion). Detailed analysis of the inner ear has not yet been done for these mutations. Some mutants with expanded melanophores also show circling behaviour (Kelsh et al., 1996); zwart (zwa), submarine (sum) and dropje (dro) mutants were stained for actin, but these showed normal hair cell patterning (data not shown). However, it is possible that in several of these mutations, the defect, if a vestibular one, is in the VIIIth ganglion or in vestibular pathways of the brain. In two cases, circling mutants show a retinotectal projection abnormality, which is likely to be the cause of the behavioural phenotype (Granato et al., 1996).

In one unresolved mutation, ty85e, in which larvae are less motile than their siblings at 120 hours, hair cell patterning in all five sensory patches is defective, with decreased numbers of hair cells in each patch, and an abnormal appearance of the hair bundles themselves (Fig. 3M,N). No gross morphological ear defect was obvious for this mutation. Because of the motility phenotype, it has not yet been possible to characterise whether this mutant also has defective balance.

DISCUSSION

The ear mutants described here exhibit a range of phenotypes,
from those specifically affecting otoliths, to those where morphology of the ear epithelium is grossly abnormal, and others where additional structures in the embryo are also affected. Although analysis of the phenotypes is at a preliminary stage, simple conclusions can already be drawn about the functions of some of the genes involved. In some cases, the mutant phenotypes also provide interesting tests of causal relationships that have been predicted to exist between different features, both structural and molecular, of the developing ear.

Conclusions from the ear phenotypes

Macular hair cells can differentiate in the absence of otoliths

Otoliths arise by precipitation of calcium carbonate within a matrix thought to be secreted by supporting cells of the sensory maculae (e.g. Hertwig and Schneider, 1986). One might expect, therefore, that the absence of an otolith would sometimes reflect a gross abnormality in, or absence of, its corresponding macula. However, by staining for hair cells with fluorescence-phalloidin, no mutants with defective otoliths have yet been found to have disrupted maculae. For example, in *eis*, one otolith, and in *kei* and *bks*, both otoliths are missing, but in all three mutants, both sensory maculae are present and appear normal. It is possible that supporting cells are absent in these mutants, but the normal spacing of hair cells indicates that this is unlikely. A more plausible interpretation is that there is an abnormality in the organic matrix secreted by the supporting cells, leading to a failure of calcium carbonate precipitation.

Maculae can develop independently of cristae

In some fish species, it is observed that cristae and maculae arise from the same sensory primordia in the ear (Becerra and Anadón, 1993; Iwasaki, 1937; Thomot and Bauchot, 1987). However, each type of sensory patch later shows distinct anatomical and biochemical specialisation. For example, the gene *mshC* shows specific expression in three discrete patches of the wild-type fish ear (Ekker et al., 1992), which are almost certainly the developing cristae; it is not expressed in maculae (this paper; T. T. W., unpublished data). Although no mutants have been found that lack maculae but not cristae, mutant *dog* and *vgo* embryos appear to lack cristae (and *mshC* expression in the ear) but do show some macular development. These phenotypes suggest that the two patch types develop (at least in later stages) according to somewhat different genetic programs, and that the *dog* and *vgo* genes may have specific roles in the development of cristae. Alternatively, it is possible that formation of cristae hair cells is more sensitive to the lack of a particular component in a developmental pathway common to cristae and maculae.

While *mshC* expression normally marks cristae, the *kei* phenotype suggests that its normal pattern and level of expression may not be necessary for their development. In four alleles tested, *kei* shows weak and de-localised *mshC* expression in the ear at 48 hours, with less marked localisation to developing cristae than in the wild type; however, by 120 hours, all these alleles have normal cristae, as judged by actin staining. However, no mutant has been found that lacks *mshC* expression in the ear altogether and yet forms normal cristae, and so a role for *mshC* in crista development remains likely.

Macular hair cell differentiation and otolith formation do not depend on *mshC* or *otx1* expression in the ear

*vgo* mutants, which fail to express either *mshC* or *otx1* in the ear, develop a single sensory patch with normal-looking hair cells, and two relatively normal otoliths, indicating that these homeobox genes are not required in the ear for hair cell differentiation or otolith formation per se. Because *mshC* very likely marks crista primordia in the wild type, it is probable that the single sensory patch in the *vgo* ear is macular in character. In the small ear of the *cls* mutant, however, which also has a single patch at 120 hours, but where *mshC* is expressed earlier, it is possible that the patch consists of fused cristae and maculae.

Sensory differentiation and otolith formation can proceed in the absence of ear morphogenesis

The fact that some hair cell and otolith development takes place even within a grossly malformed ear, such as that in *vgo* or *cls* mutants, is striking; sensory development (of maculae at least) appears to proceed relatively normally, despite semicircular canals being rudimentary or absent. Similar results have been obtained from in vitro studies, where amphibian, avian or mammalian ear epithelium has been cultured in the absence of its surrounding periotic mesenchyme, or implanted into ectopic locations in the embryo (Ginsburg, 1995; Swanson et al., 1990; reviewed in Van De Water, 1983). However, in several mutants where semicircular canal development is disrupted (e.g. *dog*, *vgo*, *cls*), while macular hair cells develop, cristae appear to be abnormal (see above), suggesting that there may be a link between development of semicircular canals and cristae. In the mutants *lte*, *bge*, *lau* and *lph*, where early morphogenesis of the ear is normal, but where ear size is disrupted at later stages, sensory patches (including cristae) develop normally. Such mutants may have abnormal fluid pressure in the ear rather than displaying a patterning defect, and where tested, this is supported by the normal expression of marker genes in the early otic vesicle.

Most mutations affecting ear morphogenesis do not affect the lateral line

In addition to development of the sensory patches, development of lateral line neuromasts also appears to be normal in most of the mutants with abnormal ear morphogenesis. The developmental and evolutionary relationship between the lateral line and the inner ear is an old problem (see Jørgensen, 1989). The original acousticolateralis hypothesis proposed that the lateral line was the more ancient system, and that the inner ear was derived from it (Ayers, 1892). The evolutionary sequence remains uncertain, but there are clear anatomical and functional similarities between lateral line neuromasts and sensory patches of the inner ear (Platt et al., 1989). However, only one mutant, *dog*, appears to have defects in both systems; it lacks a subset of sensory patches in the inner ear (the cristae) and a subset of posterior lateral line neuromasts. Clearly, a direct screen for neuromast and inner ear hair cell defects would be required to test whether the cell types and the mechanisms that produce them are similar, in genetic terms, for the two systems.

The *hps* mutation does not appear to affect ear development. It is possible that this mutation primarily affects tissues in the trunk and not the lateral line primordium itself, as pigmenta-
tion defects are found in the same region as the extra neuro-

masts at later stages. Other mutations affecting structures along

the pathway of migration of the primordium may also be of

interest regarding lateral line development. For example, in the

mutants you too and sonic you (van Eeden et al., 1996a), which

lack a myoseptum, posterior lateral line organs are displaced

ventrally, although the normal number are present (data not

shown). Study of such mutants may lead to the characterisa-

tion of guidance cues that the posterior lateral line primordium

uses as it migrates.

Neural crest abnormalities are often correlated with

inner ear defects

Mutations in 14 genes affect development of both the ear or

otoliths and at least one neural crest derivative (pigment cells

and/or cartilaginous elements of the jaw; vgo, dog, mtc and

mutations listed in Table 3; Kelsh et al., 1996; Odenthal et al.,

1996; Piotrowski et al., 1996; Schilling et al., 1996). Such phe-

notypes may provide evidence for interactions between neural

crest mesenchyme and the otic epithelium, or may reveal genes

with a common role in the development of both these ecto-

dermal neurogenic populations. The neural crest is known,

from quail and chick chimera studies, to contribute to the car-

tilaginous otic capsule and to non-neuronal cells of the VIIIth

ganglion (Noden, 1988). In vitro experiments have revealed

reciprocal interactions between otic epithelium and periotic

mesenchyme, where presence of mesenchyme is required for

organised morphogenesis of the epithelium, and an inductive

influence of the epithelium is required for chondrogenesis of

its surrounding otic capsule (Frenz and Van De Water, 1991;

Van De Water, 1983). The relative roles of neural crest- and

mesodermal-derived mesenchyme in these interactions are not

fully understood; the mutants may help to address this

problem. Several mutations are known to affect development

of both neural crest-derived pigment cells (melanocytes) and

the inner ear in the mouse. For example, those at the W and St

loci affect survival of melanocytes, including those that

populate the stria vascularis of the cochlea. The stria vascularis

normally maintains the ionic composition of the endolymph

within the cochlea; in the absence of melanocytes, it fails to

function correctly, and the mutant mice are deaf (Steel and

Brown, 1994, and references within). However, it is unlikely

that the fish mutants include exactly equivalent phenotypes, as

a direct counterpart of the stria vascularis is not found in the

fish ear.

Despite the fact that several mutants have abnormalities in

both neural crest derivatives and the ear, mutations in only four

genres, snakehead, otter, fullbrain and acerebellar, give rise to

obvious morphological defects in both the brain and the ear

(Table 3). Several studies have suggested that inductive signals

from the hindbrain play a role in development of the inner ear

(reviewed in Van De Water, 1983), and many mouse mutants

that affect development of the hindbrain also show inner ear

phenotypes (reviewed in Deol, 1983; Lyon and Searle, 1989;

Steel and Brown, 1994). It is likely, therefore, that some of the

zebrafish ear mutants have neural tube defects that were not

detectable in the morphological screen. However, expression of

krox20, which marks rhombomeres 3 and 5 in the early

hindbrain, appears to be normal in vgo (data not shown) and

cls (R.N.K., unpublished data), both of which have a small otic

vesicle from early stages. Clearly, analysis with a range of

molecular markers in addition to krox20 will be necessary to
determine whether these mutants have a brain defect; note that

snk, ott and ace mutants, all of which have morphological
defects of the brain and an early (small) ear phenotype, also
show normal krox20 expression (Brand et al., 1996; Jiang et al.,
1996).

The mouse and fish inner ear mutant collections are

complementary

Mutations in nearly 50 genes are already known that affect

morphogenesis and functioning of the inner ear in the mouse

(Lyon and Searle, 1989). Many of these mutants are viable,
and display behavioural phenotypes, involving deafness and/or
balance defects; hence the gene names circler, kreisler, waltzer, shaker, rotating, etc. (reviewed by Deol, 1983; Steel and Brown, 1994). These have primarily been characterised at a behavioural level in adult mice, but are now beginning to be analysed in the embryo in molecular terms (e.g. McKay et al., 1994). In addition, several mutations generated by transgenic methods give rise to phenotypes affecting the inner ear (Chisaka et al., 1992; Ernfors et al., 1995; Lufkin et al., 1991; Mansour et al., 1993; Mark et al., 1993). The zebrafish mutants described here form a useful complement to the collection of mouse mutants, as the fish has a number of advantages for the study of early stages of

ear development. These include the transparency of the fish

embryo, its development outside the mother, and its potential

for early behavioural and electrophysiological tests. Most

importantly, the fish screen has uncovered many ear mutants

with early lethal phenotypes, which are less well represented

in the mouse collection.

Phenotypes not found in the screen

In addition to a discussion of the phenotypes described here, it is informative to mention those phenotypes that might have been predicted, but that were not found.

No mutants lack ears

It is remarkable that no mutants lacking ears were found. Rather, all mutants that survive to 24 hours make otic vesicles in roughly the appropriate location, and with relatively normal initial form. In almost all cases, the ear phenotypes described here are not easily visible under the dissecting microscope until 48 hours, unless an otolith phenotype is evident. Under the compound microscope, a few ear phenotypes are apparent earlier, such as the small ears of vgo and ace (Brand et al., 1996). Lack of ears would be a very obvious phenotype and is unlikely to have been missed in the screen. This argues against the existence of genes with a ‘master’ role in ear development, in contrast to the role of the pax-6 gene in eye development (Quiring et al., 1994). It seems likely, therefore, either that there is redundancy in the functions of genes that govern otic vesicle formation, or that these genes also have functions earlier in development, and that mutations in them would affect other systems so drastically that an ear phenotype would be masked by earlier lethality or gross abnormality. Alternatively, gene products with a role in early ear development may have a substantial maternal component that can compensate for a lack of zygotic expression in the mutant. This latter possibility seems unlikely, however, as formation of the ear occurs relatively late, after the main body plan has been established,
and well after activation of the zygotic genome in the embryo (Kane and Kimmel, 1993).

No mutants lack hair cells, or have too many hair cells. Of 48 different mutant lines studied, none have so far been found to lack hair cells altogether. Although hair cells were not directly screened for, and so any mutation solely affecting their differentiation would have been missed, absence of a macula would almost certainly be concomitant with an otolith defect. Since the screen detected many mutants with otolith abnormalities, a subset of these might be expected to show abnormal hair cell patterning. However, all the otolith mutants tested have normal maculae (see above), and hair cells only appear to be disrupted (but never absent) in a few of the mutants affecting epithelial morphology of the ear. No mutants have yet been found to have extra hair cells within a sensory patch, another phenotype that on its own would have been missed in the screen, but which also might have led to an otolith defect. Because of the regularly spaced array of hair cells in the sensory patches, and the fact that in some species, damaged hair cells are regenerated by division of supporting cells following acoustic trauma, it has been proposed that patterning of inner ear hair cells may take place via a lateral inhibition mechanism (Corwin et al., 1991; Lewis, 1991). Homologues of genes involved in lateral inhibition pathways in Drosophila have recently been identified in vertebrates (see references within Calof, 1995; Simpson, 1995), and some are indeed expressed in the developing inner ear (Myat et al., 1996 and J. Adam, D. Henrique and A. Myat, unpublished data). In this respect, it will be interesting to see whether the mutant white tail, which has a neurogenic-like phenotype, displaying extra Mauthner cells and other neurons (Jiang et al., 1996), also has extra hair cells within the ear or neurons in the VIIIth ganglion.

Conclusions

The ear mutants will be of great value to a wide range of different studies, for example the mechanisms of otolith formation, epithelial morphogenesis and differentiation, and perhaps the development of behavioural characteristics such as the startle response and the vestibulococulomotor reflex. Screening for balance phenotypes is a potentially powerful method for revealing genes with roles in the mechanosensory transduction pathway of sensory hair cells. Due to the very small number of transduction channel molecules believed to be present on each hair cell, their identification by biochemical means presents technical difficulties (Gillespie and Hudspeth, 1991), which a genetic approach could overcome; those mutants with balance phenotypes will be of particular interest in this respect. While the viable mouse mutants, with their behavioural traits, will undoubtedly remain of most clinical relevance as models for human deafness, the zebrafish mutants will be invaluable for developmental studies of the inner ear.

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REFERENCES

Ayers, H. (1892). Vertebrate cephalogenesis. II. A contribution to the morphology of the vertebrate ear, with a reconsideration of its functions. J. Morph. 6, 1-360.


