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The mouse *slalom* mutant demonstrates a role for Jagged1 in neuroepithelial patterning in the organ of Corti

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The Notch signalling pathway has recently been implicated in the development and patterning of the sensory epithelium in the cochlea, the organ of Corti. As part of an ongoing large-scale mutagenesis programme to identify new deaf or vestibular mouse mutants, we have identified a novel mouse mutant, *slalom*, which shows abnormalities in the patterning of hair cells in the organ of Corti and missing ampullae, structures that house the sensory epithelia of the semicircular canals. We show that the *slalom* mutant carries a mutation in the *Jagged1* gene, implicating a new ligand in the signalling processes that pattern the inner ear neuro-epithelium.

INTRODUCTION

Lateral inhibition has been implicated as the proposed mechanism by which decisions are made regarding sensory hair cell versus supporting cell in the sensory patches of the developing inner ear (1,2). Mouse mutations have enabled the roles of the various players in this pathway to be determined. Mice homozygous for a targeted null mutation of *Jagged2* (*Jag2*) develop supernumerary hair cells (3), whereas a mutation in *lunatic fringe* (*Lfng*) partially suppresses the effects of the *Jag2* mutation (4). Moreover, the expression patterns of *Notch1* and ligands, *Jag2* and *Delta1* (*Dll1*), are consistent with them playing a role in lateral inhibition that may determine the ordered array of sensory hair cells and supporting cells that is the organ of Corti responsible for auditory transduction (1,3,5). In addition, Delta–Notch signalling has been implicated in lateral inhibition in sensory cell differentiation from studies in zebrafish (6). Misregulation of delta genes and a Serrate homologue have been observed in the zebrafish *mind bomb* mutant where the ear sensory patches consist solely of hair cells. There is also a deltaA zebrafish mutant which has increased numbers of hair cells (7). Whereas the downstream pathways of Notch signalling within the sensory epithelium in the cochlea are less well characterized, they may include proneural genes, such as *Math1*, and the *HES* family of bHLH proteins (8). Mouse mutants carrying a deletion of *Math1* fail to develop hair cells (9).

Two large-scale ENU mouse mutagenesis programmes have recently been described whose purpose has been to undertake a systematic and genome-wide effort to recover a large number of novel mutations affecting a diverse array of phenotypic areas involved with genetic disease (10,11). One feature of these programmes has been the recovery of a large number of dominant phenotypes showing deafness and/or vestibular dysfunction as models of human genetic deafness. We have characterized from this screen a new dominant phenotype, *slalom*, and identified the underlying gene. *slalom* is encoded by the *Jag1* gene implicating a new signalling molecule in the pathways that determine the patterning of the neuro-epithelium in the inner ear.

RESULTS

Identification and characterization of the *slalom* mutation

The ENU mutagenesis programme carried out within the UK incorporated a comprehensive phenotype assessment tool, SHIRPA, involving a battery of up to 40 simple tests, used to detect novel phenotypes in the areas of lower motoneuron/muscle function, spinocerebellar function, sensory function, neuropsychiatric function and autonomic function (12,13). As part of the panel of tests to determine sensory function we included a click-box test to assess deafness along with a number of tests to investigate vestibular function (see http://www.mgu.har.mrc.ac.uk/mutabase/). Over 60 dominant phenotypes showing deafness and/or vestibular dysfunction have been identified to date and many inheritance tested, including the mutant *slalom*.

The *slalom* mutant showed a positive Preyer response to the click-box test, indicating that it was not deaf. However, *slalom* did show subtle headweaving/shaking and poor negative geotaxis indicative of a vestibular defect. Detailed structural and ultrastructural studies (Fig. 1) demonstrated a variety of

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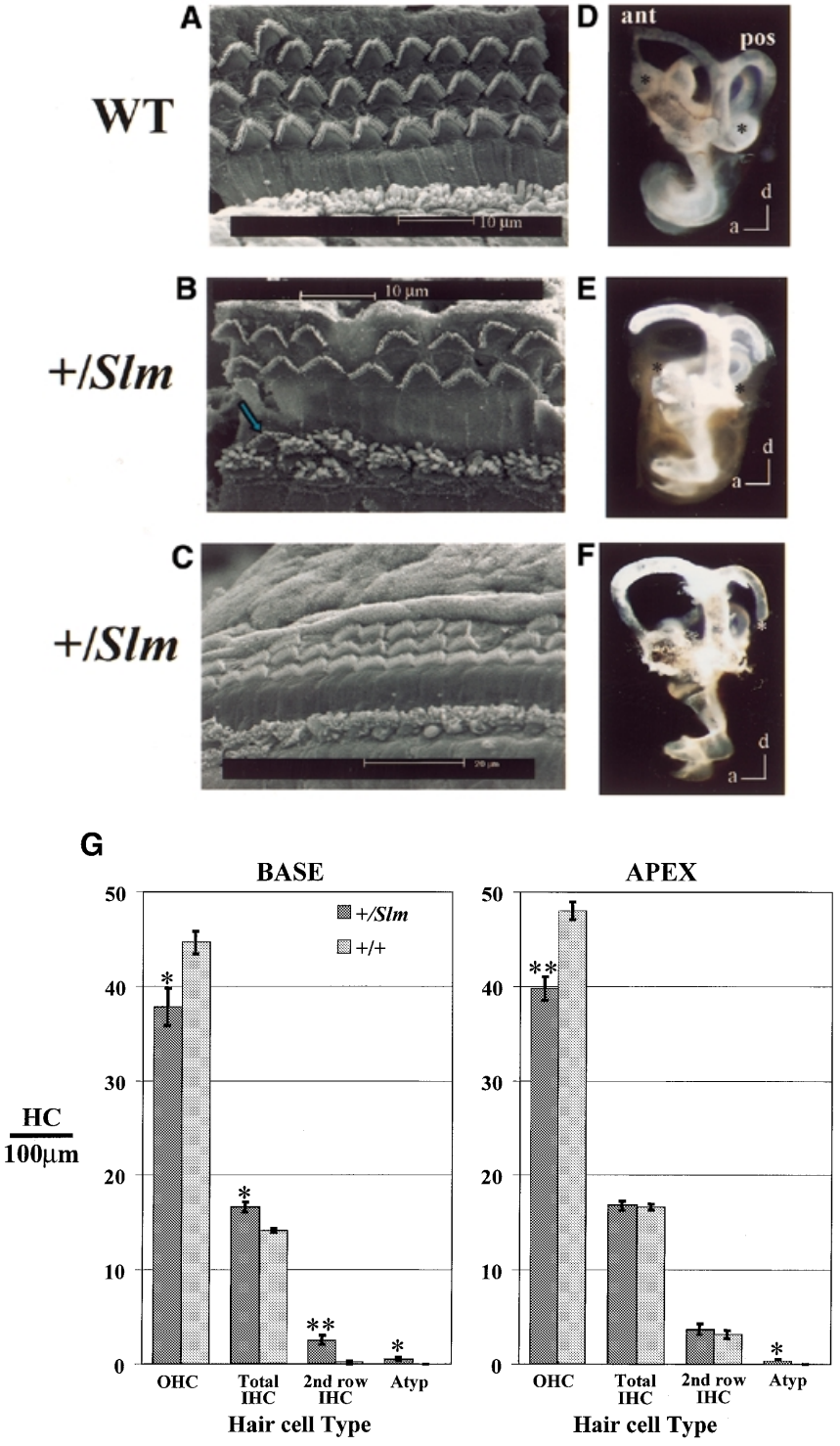

Figure 1. (**A**–**C**) Scanning electron micrographs of the organ of Corti from wild-type (WT) and *slalom (Slm)* heterozygote mice. Note in *+/Slm* mouse in some regions of the organ of Corti only two rows of outer hair cells are observed whereas in others there are three. There are also extra inner hair cells, as well as the presence of atypical inner hair cells that have outer hair cell morphology (arrow). (**D**–**F**) Paintfills of the inner ear from wild-type and *+/Slm* mice. The *+/Slm* mouse in (E) reveals truncation of both the anterior (ant) and posterior (pos) semicircular canals and the absence of both the posterior and anterior ampullae (*). The *+/Slm* mouse in (F) reveals truncation of only the posterior canal (*). The posterior ampullae is also missing. a, anterior; d, dorsal. (**G**) Hair cell counts from *slalom (+/Slm)* and wild-type cochlea in basal and apical turns. Base: +/+, $n = 6$; *+/Slm*, $n = 7$. Apex: +/+, $n = 5$; *+/Slm*, $n = 9$. OHC, outer hair cells, IHC, inner

inner ear anomalies in both the vestibular system and the organ of Corti. Firstly, *slalom* shows truncation of either the posterior or anterior semi-circular canals (Fig. 1F). Occasionally, truncation of both canals is also observed (Fig. 1E). When either or both canals are truncated, the corresponding posterior and/or anterior ampullae are absent. These vestibular defects presumably contribute to the headweaving/shaking behaviour of the *slalom* mutant. We also used scanning electron microscopy (SEM) to examine the organ of Corti of *slalom* mutants. *slalom* shows reduced numbers of outer hair cells. In some areas, the organ of Corti completely lacks one row of outer hair cells (Fig. 1B); in other regions, isolated outer hair cells are missing from one of the three rows, row 3 (Fig. 1C). In addition, occasional supernumary and atypical hair cells with outer hair cell morphology are seen within the inner hair cell row (Fig. 1B). Outer hair cell counts (Fig. 1G) reveal a significant decrease (15%) in the basal turns and 17% decrease in the apical turns of *slalom* mice. Inner hair cell counts reveal a significant increase in the number of inner hair cells in the base of the *slalom* cochlea, largely due to additional inner hair cells which appear to form a second row, although this increase was not observed in the apical regions of the cochlea (Fig. 1G). Moreover, the atypical hair cells within the inner hair cell row of both the base and apex of *slalom* cochlea are not seen in the controls.

Genetic mapping and positional candidate cloning of *slalom*

We undertook the genetic mapping of the *slalom* mutant using a speed backcross approach (10) which takes advantage of the fact that founder mice from the mutagenesis programme are BALB/c/C3H hybrids. Backcross progeny are generated using sperm from the founder or their male progeny (if the founder is not male) to fertilize C3H eggs. We employed a rapid genotyping approach involving pooling of affected backcross progeny DNAs and fluorescent genotyping using a panel of 100 microsatellite markers (14). Initial linkage to chromosome 2 was confirmed by genotyping of individual DNAs and the *slalom* mutation was shown to lie between *D2Mit272* and a cluster of markers—*D2Mit106*/*D2Mit34*/*D2Mit133*—in the region of the *Jagged1* (*Jag1*) gene (data not shown). Given this map location, there were several reasons to consider *Jag1* a strong positional candidate for *slalom*. Firstly, Alagille's syndrome patients are heterozygous for mutations in the *JAG1* gene and, amongst a variety of clinical features, they demonstrate partial or missing posterior canals (15,16). Occasionally, the anterior canal is also affected. Secondly, the *coloboma* mouse mutation is caused by a deletion on mouse chromosome 2 which encompasses *Jag1* (17). *coloboma* shows head-shaking behaviour. Finally, *Jag1* is known to be expressed during early development of the organ of Corti throughout the developing sensory patch, later localizing to the supporting cells (3). Although, a *Jag1* knock-out has been constructed ($Jag1^{dDSL}$) neither behavioural anomalies indicative of vestibular dysfunction nor detailed ultrastructural analyses of the inner ear were reported (17). However, examination of homozygous embryos ($Jag1^{dDSL}/Jag1^{dDSL}$) indicated defects in embryonic and yolk-sac vasculature (see below).

We carried out mutation analysis of the *Jag1* gene by RT–PCR of kidney RNA from both wild-type (BALB/c and BALB/c/C3H genomes) and *slalom* mutants (Fig. 2). RT–PCR products covering the entire coding sequence were cloned and sequenced. A C→T missense mutation was identified that causes a proline to serine change at codon 269 in the second epidermal growth factor (EGF)-like repeat. This change was not identified in the parental genomes. This proline residue is conserved in all Jagged/Serrate homologues characterized to date across a wide variety of species (Fig. 3D). Both the first and second EGF-like repeats appear to play a role in modulating the affinity of interaction between *Jagged1* and *Notch* receptors (18).

Vascular defects and neural tube anomalies in *slalom* homozygotes

Identification of the *slalom* mutation allowed us to unambiguously type embryonic homozygotes and to compare their phenotype with $Jag1^{dDSL}/Jag1^{dDSL}$ embryos and with wild-type (Fig. 3). Whereas $Jag1^{dDSL}/Jag1^{dDSL}$ embryos die at E11.5 (17), *slalom* homozygotes survive to E12.5. *slalom* homozygote embryos did not demonstrate the craniofacial haemorrhaging observed in $Jag1^{dDSL}/Jag1^{dDSL}$ embryos (17), but there appeared to be reduced large vasculature in the yolk-sac similar to the $Jag1^{dDSL}/Jag1^{dDSL}$ phenotype in all 11 homozygotes identified (Fig. 3B and D). In addition, some *slalom* E12.5 homozygotes (3/11 from a total of 39 embryos dissected at E11.5 and E12.5), appear to show neural tube defects, similar to *Notch1* mutant homozygotes (19) (Fig. 3E). Also two homozygote embryos were found that were retarded in development, lagging at least two days behind their siblings.

DISCUSSION

We have demonstrated that a mutation in the *Jag1* gene leads to disturbances of the patterning of the organ of Corti in the inner ear, characterized by reduced numbers of outer hair cells, particularly in the third row, and loss of sensory structures in the vestibular system. A similar phenotype has also been seen in the *Headturner* mutant, isolated in the German mutagenesis screen, which also carries a mutation in *Jag1* (11; A.E. Kiernan *et al*., submitted). This reduction in the numbers of outer hair cells we see in *slalom* is in contrast to the increase to four rows observed in the *Jag2* mutant (3). In the *slalom* mutant we see a significant increase of inner hair cells in the base (~15%), but not as many as in the *Jag2* mutant, where there is an increase of ~50% of the inner hair cell number (3). For outer hair cells, it would appear that *Jag1* and *Jag2* may play reciprocal roles in signalling from the supporting and hair cells, respectively.

Both *Notch1* and *Jag1* are expressed primarily in the supporting cells of the developing organ of Corti at E18 (4,20). At the same time, *Jag2* expression is detectable in both developing inner and outer hair cells. A model of lateral inhibition mediated by Notch signalling has been proposed based on the pattern of cell types in the epithelium, in which every hair cell is surrounded by supporting cells (1–3,21). The model assumes that the hair cell phenotype is the default fate in the developing cells in the organ of Corti. *Jag2* is expressed in a subset of cells activating Notch in neighbouring cells and preventing their development as hair cells. Inactivation of *Jag2* leads to the development of additional hair cells, as the lateral inhibition model would predict. Equally, mice heterozygous for a Notch mutation also show an increase in hair cells consistent with

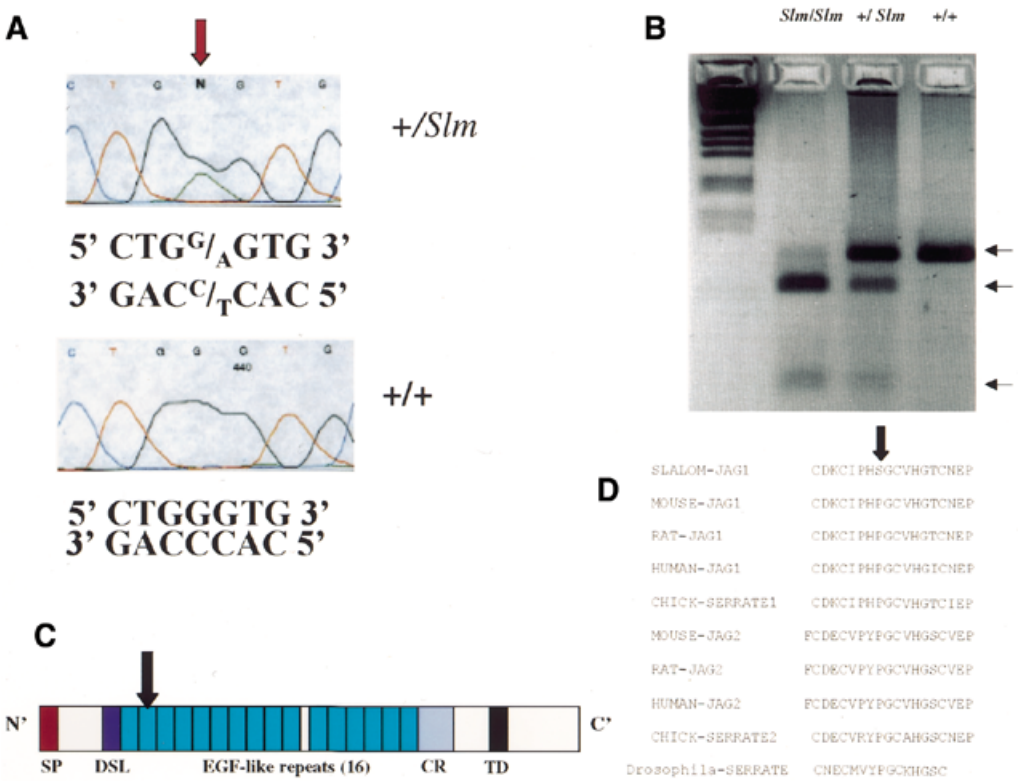

Figure 2. *Slalom* is mutated in the *Jagged1* gene. (**A**) Slalom is a C→T missense mutation that causes a proline to serine change. Traces show direct sequencing of PCR products (reverse strand) from heterozygote and wild-type mice. (**B**) The mutation introduces a *Dde*1 restriction site. Using primers flanking the mutation-containing exon, wild-type mice reveal a single 107 bp fragment following digestion whereas mice homozygous for this mutation have both 27 and 80 bp fragments. Heterozygous mice show all three fragments (indicated by arrows). (**C**) Schematic diagram of mouse *Jagged1* protein structure. The mutation in *Slalom* lies in the second EGF domain as indicated by the arrow. SP, signal peptide; DSL, conserved domain shared by *Delta, Serrate* and *Lag2;* CR, cysteine-rich region; TD, transmembrane domain. (**D**) Amino acid alignments of the second EGF-like repeats of the *Serrate/Jagged* family of molecules from the *Slalom* mutant, mouse, rat, human, chick and *Drosophila*. Note the proline is conserved in all the wild-type sequences (arrow).

Jag2 signalling playing a role in maintaining supporting cell fate (4). It is now clear from the analysis of the *slalom* mutant that we need to consider the role of *Jag1* in the signalling pathways in the developing organ of Corti.

On the basis of the phenotypic effects of mutations in the *Jag1* and *Jag2* genes and taking into account their expression patterns, we have developed one possible model for *Jag1* and *Jag2* signalling in the patterning of the organ of Corti (Fig. 4). We propose that *Jag1* may play a role in determining supporting cell fate, but also may signal (via an unknown receptor) to adjacent developing sensory cells to inhibit *Jag1* expression in these cells. Signalling of *Jag2* from developing sensory cells via Notch may reinforce expression of *Jag1* in supporting cells, while down-regulating *Jag2,* thus providing both a positive and negative feedback loop to establish the differentiation of supporting and sensory cells. In *Jag2* mutants, *Jag2* signalling to adjacent cells is lost and as a consequence *Jag1* expression in supporting cells may not be maintained and hair cells develop. We hypothesize that *slalom* is a hypomorphic mutant leading to reduced *Jag1* levels in supporting cells. Indeed, the proline to serine change observed in the *slalom* mutant seems likely to cause loss of function by affecting the role of the second EGF repeat in binding to *Notch* receptors. Under our proposed model, a reduction in *Jag1* signalling from supporting cells would lead to raised levels of expression of *Jag1* in developing hair cells and as a consequence, some may fail to differentiate properly as observed in the *slalom* mutant.

It has been reported recently that down-regulation of *Jag1* in antisense experiments, with cultures of the developing neuroepithelium, leads to an increase in numbers of both inner and outer hair cells (20), and in contrast leads to the reduction in outer hair cells observed in the *slalom* mutant. However, these differences would be explicable if *slalom* is, as we suggest, a loss of function, semi-dominant mutation. The *slalom* hair cell phenotype is observed in heterozygotes, presumably with levels of *Jag1* activity ~50% of wild-type. If *Jag1* is indeed important for determining the fate of the supporting cells, as well as signalling to neighbouring sensory cells, a reduction in the level of *Jag1* could reduce the number of cells developing into hair cells, whereas the complete loss of *Jag1* could lead to an increase in hair cell number (Fig. 4). Our proposed model assumes that neither *Jag1* nor *Jag2* are completely responsible for determining cell fate in the developing organ of Corti (if they were, there would be either no hair cells or no supporting cells in *Jag1* and *Jag2* mutants, respectively), but instead act in a combinatorial manner along with other signalling molecules to tilt the balance towards a hair cell or supporting cell fate. Furthermore, counting supporting cells is difficult, so we have no clear evidence whether there are additional supporting cells in the *Jag1* mutants or reduced numbers of supporting cells in *Jag2* mutants. Scanning electron microscopy of the *slalom*

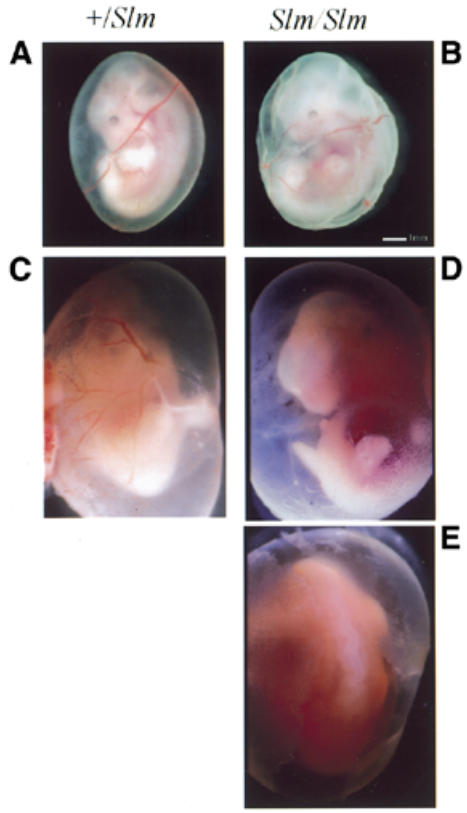

Figure 3. E12.5 embryos from heterozygote intercrosses. (**A** and **C**) Heterozygote embryos that have normal vasculature in the yolk sac. (**B** and **D**) Depleted vasculature in the yolk sacs of *Slm/Slm* embryos. (**E**) Neural tube of a *Slm/Slm* embryo revealing neural tube kinks.

mutant does not reveal any disturbances in the relationship between the outer hair cells and the supporting cells around them. It is clear that further studies are needed to determine why the extra inner hair cells are present, as our proposed model does not fully explain all of these observations.

In conclusion, the identification of the *slalom* mutant demonstrates that *Jag1* signalling has a pivotal role in the normal development of the organ of Corti and specification of some of the vestibular sensory epithelia. It will now be important to investigate further its relationship to other members of the Notch signalling pathway in the inner ear and their roles in the development of both the auditory and balance system of mammals.

MATERIALS AND METHODS

Mapping and mutation screening

We generated backcross progeny for mapping by mating the founder F1 *Slalom* mutant to C3H, this cross also serving as an inheritance test. We phenotyped all progeny for headweaving behaviour. Thirty DNAs were pooled from affected individuals and then genome scanned as described (10,14). Once a region of linkage was identified, we confirmed the map position by genotyping and haplotype analysis of individual affected backcross progeny. We carried out mutation analysis of the *Jag1* gene by RT–PCR of kidney RNA, followed by cloning of RT–PCR products and sequencing. Results were confirmed by direct sequencing of PCR products.

Genotyping of embryos

Embryos were aged based on the day of the vaginal plug being E0.5. We genotyped embryos using the *Dde*I restriction site

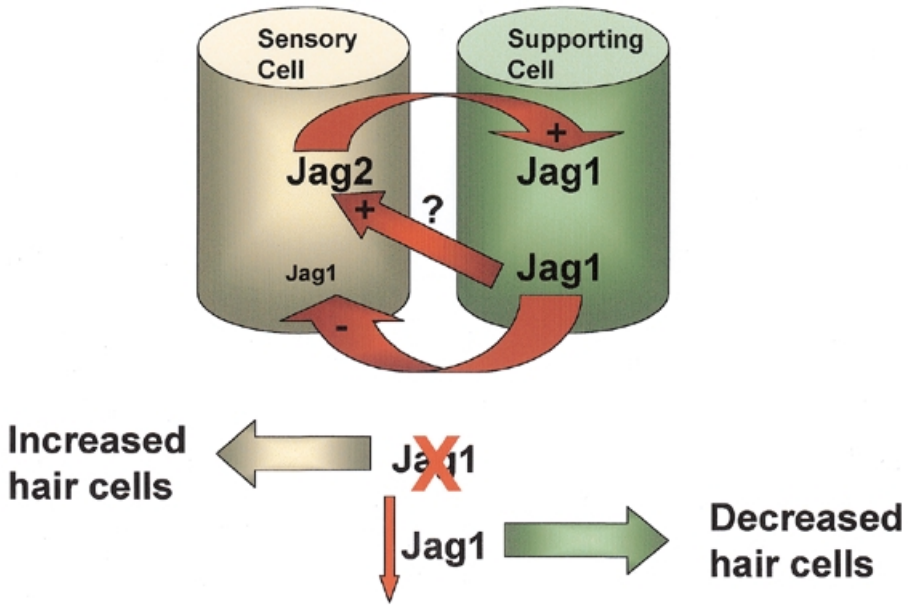

Figure 4. A model for *Jag2* and *Jag1* interactions between sensory and supporting cells in the organ of Corti in the inner ear. *Jag2*, expressed in sensory hair cells, maintains expression of *Jag1* in neighbouring supporting cells. *Jag1* plays a role in determining supporting cells where it is expressed, and acts on sensory cells to inhibit *Jag1* expression. *Jag1* could also reinforce expression of *Jag2* in sensory cells. Complete ablation of *Jag1* expression by antisense (18) would lead to increased numbers of hair cells. A hypomorphic mutant, as hypothesized for *slalom*, with lowered levels of *Jag1* acitivity would lead to decreased numbers of sensory hair cells.

introduced by the mutation (Fig. 3B). Embryo DNA was amplified using the primers: 5′-CTGTACTGCGACAAGTG-CATC-3′ and 5′-TTGTCACAGAGCTGTCCACC-3′ and PCR products digested with *Dde*I prior to gel electrophoresis.

Paintfilling

We carried out paintfills essentially as described (22). We decapitated both wild-type and +/*Slm* mice and then fixed the heads in Bodians fixative (75% ethanol, 5% formalin, 5% glacial acetic acid) overnight followed by washing (minimum 2 h for each wash) twice in each of the following solutions: 75, 95 and 100% ethanol. We then bisected heads and cleared them overnight in methyl salicylate. We injected the endolymphatic compartment of the inner ears via either the common crus or cochlea employing a pulled glass capillary pipette (20–40 μm diameter) filled with 1% gloss paint in methyl salicylate. Finally, we dissected the ears free of the skull and photographed them in methyl salicylate using an Olympus DP10 digital camera.

SEM

We prepared samples for SEM using the osmium tetroxide-thiocarbohydrazide (OTOTO) method as described previously (23,24). Briefly, we dissected inner ears in fixative (2.5% glutaraldehyde in 0.1M phosphate buffer pH 7.2), piercing the windows and apex of the cochlea and samples were allowed to fix for 4 to 5 h rotating at 4°C. We then washed ears overnight in 0.1 M phosphate buffer. For preparation of the organ of Corti, we removed the outer bony shell and stria vascularis. After OTOTO, we dried specimens at critical point, sputter coated and then examined them under a Phillips XL30 scanning electron microscope.

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REFERENCES

1. Lewis, J. (1991) Rules for the production of sensory hair cells. *Ciba Found. Symp.*, **160**, 25–39.
2. Corwin, J.T., Jones, J.E., Katayama, A., Kelley, M.W. and Warchol, M.E. (1991) Hair cell regeneration: The identities of progenitor cells, potential triggers and instructive cues. *Ciba Found. Symp.*, **160**, 103–130.
3. Lanford, P.J., Lan, Y., Jiang, R., Lindsell, C., Weinmaster, G., Gridley, T. and Kelley, M.W. (1999) Notch signaling pathway mediates hair cell development in mammalian cochlea. *Nature Genet.*, **21**, 289–292.
4. Zhang, N., Martin, G.V., Kelley, M.W. and Gridley, T. (2000) A mutation in the lunatic fringe gene supresses the effects of a Jagged2 mutation on inner hair cell development in the cochlea. *Curr. Biol.*, **10**, 659–622.
5. Morrison, A., Hodgetts, C., Gossler, A., Hrabe de Angelis, M. and Lewis, J. (1999) Expression of Delta1 and Serrate1 (Jagged1) in the mouse inner ear. *Mech. Dev.*, **84**, 169–172.
6. Haddon, C., Jiang, Y.-J., Smithers, L. and Lewis, J. (1998) Delta-Notch signalling and the patterning of sensory cell differentiation in the zebrafish ear: evidence from the mind bomb mutant. *Development*, **125**, 4637–4644.
7. Riley, B.B., Chiang, M.-Y., Farmer, L. and Heck, R. (1999) The *deltaA* gene of zebrafish mediates lateral inhibition of hair cells in the inner ear and is regulated by *pax2.1*. *Development*, **126**, 5669–5678.
8. Lanford, P., Shailam, R., Norton, C.R., Gridley, T. and Kelley, M.W. (2000) Expression of Math1 and HES5 in the cochlea of wild-type and Jag2 mutant mice. *J. Assoc. Res. Otolaryngol.*, **1**, 161–171.
9. Bermingham, N.A., Hassan, B.A., Price, S.D., Vollrath, M.A., Ben-Arie, N., Eatock, R.A., Bellen, H.J., Lysakowski, A. and Zoghbi, H.Y. (1999) Math1: An essential gene for the generation of inner ear hair cells. *Science*, **284**, 169–172.
10. Nolan, P.M, Peters, J., Strivens, M., Rogers, D., Hagan, J., Spurr N., Gray, I.C., Vizor, L., Brooker, D. and Whitehill, E. *et al.* (2000) A systematic, genome-wide, phenotype-driven mutagenesis programme for gene function studies in the mouse. *Nature Genet.*, **25**, 440–443.
11. Hrabe de Angelis, M., Flaswinkel, H., Fuchs, H., Rathkolb, B., Soewarto, D., Marschall, S., Heffner, S., Pargent, W., Wuensch, K. and Jung, M. (2000) Genome-wide, large-scale production of mutant mice by ENU mutagenesis. *Nature Genet.*, **25**, 444–447.
12. Nolan, P.M., Peters, J., Vizor, L., Strivens, M., Washbourne, R., Hough, T., Wells, C., Glenister, P., Thornton, C., Martin, J. *et al.* (2000) Implementation of a large-scale ENU mutagenesis program: towards increasing the mouse mutant resource. *Mamm. Genome*, **11**, 500–506.
13. Rogers, D.C., Fisher, E.M., Brown, S.D., Peters, J., Hunter, A.J. and Martin, J.E. (1997) Behavioural and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. *Mamm. Genome*, **8**, 711–713.
14. Isaacs, A.M., Davies, K.E., Hunter, A.J., Nolan, P.M., Vizor, L., Peters, J., Gale, D.G., Kelsell, D.P., Latham, I.D., Chase, J.M. *et al.* (2000) Identification of two new *Pmp22* mouse mutants using large-scale mutagenesis and a novel rapid mapping strategy. *Hum. Mol. Genet.*, **9**, 1865–1871.
15. Oda, T., Elkahloun, A.G., Pike, B.L., Okajima, K., Krantz, I.D., Genin, A., Piccoli, D.A., Meltzer, P.S., Spinner, N.B., Collins, F.S. and Chandrasekharappa, S.C. (1997) Mutations in the human Jagged1 gene are responsible for Alagille's syndrome. *Nature Genet.*, **16**, 235–242.
16. Okuno, T., Takahashi, H., Shibahara, Y., Hashida, Y. and Sando, I. (1990) Temporal bone histopathological findings in Alagille's syndrome. *Arch. Otolaryngol. Head Neck Surg.*, **116**, 217–220.
17. Xue, Y., Gao, X., Lindsell, C.E., Norton, C.R., Chang, B., Hicks, C., Gendron-Maguire, M., Rand, E.B., Weinmaster, G. and Gridley, T. (1999). Embryonic lethality and vascular defects in mice lacking the Notch ligand Jagged1. *Hum. Mol. Genet.*, **8**, 723–730.
18. Shimizu, K., Chiba, S., Kumano, K., Hosoya, N., Takahashi, T., Kanda, Y., Hamada, Y., Yazaki, Y. and Hirai, H. (1999) Mouse Jagged1 physically interacts with Notch2 and other Notch receptors. *J. Biol. Chem.*, **274**, 32961–32969.
19. Huppert, S.S., Le, A., Schroeter, E.H., Mumm, J.S., Saxena, M.T., Milner, L.A. and Kopan, R. (2000) Embryonic lethality in mice homozygous for a processing-deficient-allele of Notch 1. *Nature*, **405**, 966–970.
20. Zine, A., Van de Water, T.R. and de Ribaupierre, F. (2000) Notch signaling regulates the pattern of auditory hair cell differentiation in mammals. *Development*, **127**, 3373–3383.
21. Eddison, M., Le Roux, I. and Lewis, J. (2000). Notch signaling in the development of the inner ear: Lessons from *Drosophila*. *Proc. Natl Acad. Sci. USA*, **97**, 11692–11699.
22. Martin, P. and Swanson, G.J. (1993) Descriptive and experimental analysis of the epithelial remodellings that control semicircular canal formation in the developing mouse inner ear. *Dev. Biol.*, **159**, 549–558.
23. Hunter-Duvar, I.M. (1978) A technique for preparation of cochlear specimens for assessment with the scanning electron microsope. *Acta Otolaryngol. (Stockholm)*, **351** (suppl.), 3–23.
24. Self, T., Mahony, M., Fleming, J., Walsh, J., Brown, S.D.M. and Steel, K.P. (1998) Shaker1 mutations reveal roles for myosin VIIA in both development and function of cochlear hair cells. *Development*, **125**, 557–566.