Coupling adhesion to actin bundles in the inner ear

Novel functions for novel cadherins

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Genotype and phenotype define two poles of modern biology. The great challenge is to understand the connections that translate gene products into tissue architecture and function. Two recent studies (Boeda *et al.*, 2002; Siemens *et al.*, 2002) exemplify how these connections can be identified during the development of the inner ear.

The sensory hair cells of the inner ear detect sound and postural change by sensing movement in the endolymph fluid of the cochlea and the semicircular canals, respectively. At the subcellular level, mechano-sensing is mediated by organelles called stereocilia (SC)—fine, finger-like protrusions that project from the apices of the hair cells. The morphology and organization of SC are strikingly precise: in mature hair cells, SC increase regularly in height from one side of the cell to the other and are attached to one another by several lateral connections, thereby integrating individual SC into functional units. Developmental mechanisms must therefore exist to coordinate the growth and lateral interactions between SC.

The importance of SC morphogenesis becomes apparent in Usher syndrome type 1. Mouse models of this disease show deafness and vestibular dysfunction and have SC that grow haphazardly and lose lateral interactions with one another. Seven genetic loci are independently able to cause this syndrome when mutated (reviewed in Petit, 2001). The corresponding genes for four of these loci encode the unconventional motor protein myosin VIIa, the cytoplasmic protein harmonin, and two transmembrane proteins of the cadherin superfamily, cadherin 23 (CDH23) and protocadherin 15. How these apparently diverse gene products generate a common phenotype was unclear. However, the two new studies highlighted here have provided a significant advance by demonstrating biochemical and morphological interactions that involve the first three of these proteins.

The cadherins are a superfamily of cell-surface glycoproteins that mediate cell–cell adhesion (Yagi & Takeichi, 2000). CDH23 (Di Palma *et al.*, 2001) is a novel cadherin that is distinguished by a very large extracellular domain that most resembles the *Drosophila* Fat protein and by a cytoplasmic domain that lacks any identifiable

features except for two putative PDZ-binding interfaces (PBIs). In this regard, CDH23 is distinct from the better-characterized classical cadherins (such as E- and N-cadherin), the conserved cytoplasmic tails of which interact with catenin proteins, and thus link them to the actin cytoskeleton. Notably, harmonin proteins contain several PDZ domains and, in the recent papers, both research groups use yeast two-hybrid assays and purified proteins to show that CDH23 and harmonin interact directly (Boeda *et al.*, 2002; Siemens *et al.*, 2002). This interaction seems to involve the PDZ2 domain of harmonin and the carboxy-terminal PBI of CDH23 (Siemens *et al.*, 2002), thus defining a new mode of cytoplasmic interaction for cadherins.

Several harmonin transcripts exist in the inner ear, of which the harmonin b transcript seems to be specific for this organ. Importantly, Boeda *et al.* (2002) further identify a close spatio-temporal relationship between CDH23 and harmonin b in developing hair cells. Both are first detectable in the earliest apical protrusions that extend to become SC. These proteins completely co-localize—initially being found throughout the nascent SC, but becoming progressively restricted to the apices of the hair cells as the SC elongate. They eventually disappear in mature hair cells. Boeda *et al.* (2002) also show that harmonin PDZ1 interacts with myosin VIIa. Strikingly, in mice

Fig. 1 | HeLa cell expressing harmonin b. Six hours after transfection, harmonin b (blue) is enriched at the extremities of actin stress fibres (red), close to the focal adhesion plaques visualized by anti-vinculin staining (green). The right-hand panels are higher magnification views of the boxed area. The figure was kindly provided by B. Boeda and A. El-Amraoui (Institut Pasteur, France).

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Fig. 2 | Schematic model for the biochemical and functional interactions between cadherin 23 (CDH23), harmonin b and myosin VIIa. Binding of CDH23 to harmonin b couples adhesive interactions between growing stereocilia (SC) to the core actin bundles that are necessary for stereociliar growth. This would allow growth of neighbouring SC to be precisely coordinated. An association between harmonin b and myosin VIIa may transport harmonin b to the cadherin, as suggested by Boeda *et al.* (2002). Alternatively, a ternary complex of myosin VIIa, CDH23 and harmonin b, might participate in the cadherin–actin interaction supported by harmonin b.

that are homozygous for a myosin VIIa mutation, harmonin b failed to co-accumulate with CDH23. This caused the SC to grow abnormally—varying irregularly in height and splaying out from one another—as if their lateral interactions were lost. Together, these data strongly suggest that functional as well as biochemical interactions involving myosin VIIa, CDH23 and harmonin b are somehow necessary for proper SC morphogenesis. Note, however, that although mapping of individual binding interactions suggests that a ternary complex of CDH23, harmonin b and myosin VIIa is possible, this has yet to be tested experimentally.

How, then, might CDH23 and harmonin b support SC development? An exciting clue is the demonstration that harmonin b can bundle actin filaments, both *in vitro* and when overexpressed in fibroblasts (Boeda *et al.*, 2002; Fig. 1). It is important to remember that SC are tightly packed with actin filaments that are linked laterally into bundles. Similar to other actin-rich cell-surface protrusions, SC are believed to grow both by polymerization of individual actin filaments and by the incorporation of these filaments into bundles (DeRosier & Tilney, 2000). Filament bundling by harmonin b would thus be predicted to promote SC growth during morphogenesis. Moreover, adhesion mediated by CDH23 could couple nascent SC to one another, ensuring lateral association between SC as they grow. Consistent with this, CDH23 could be localized to lateral tip-to-tip contacts in nascent SC by immuno-electron microscopy (Boeda *et al.*, 2002). Thus, a membrane-based complex of CDH23 and harmonin b provides a mechanism both to promote actin filament bundling (individual SC growth) and to initiate the lateral associations between SC that

couple them into functional units as they grow (Fig. 2). Notably, in this model, CDH23 would mediate *cis*-interactions between membrane projections of the same cells, a novel effect that contrasts with the *trans*-interactions mediated by classical cadherins.

Of course, these data open avenues for further experiments. The ability of cadherin superfamily proteins to mediate adhesion has been proven principally for the classical cadherins, but CDH23 differs markedly from these proteins in primary sequence. Therefore, first it will be important to test directly whether CDH23 is a true adhesion molecule, and if so, whether harmonin b can itself affect the adhesive activity of CDH23. Interestingly, harmonins can oligomerize (Siemens *et al.*, 2002); could this support lateral clustering of CDH23 to modulate adhesion as occurs with classical cadherins (Yap *et al.*, 1998)? Second, the precise role of myosin VIIa remains to be elucidated. Boeda *et al.* (2002) suggest that this motor protein may transport harmonin b in SC; it will be interesting to determine if myosin VIIa can influence the CDH23–harmonin complex itself. Additionally, if myosin VIIa could form a ternary complex with CDH23 and harmonin b, it could potentially stabilize or exert force on the membrane–actin interaction mediated by the latter proteins. Also, does the CDH23–harmonin b complex exert a morphogenetic effect on actin dynamics in SC? Is the complex passively anchored on actin bundles, or can it determine the sites where bundling occurs in developing SC? If so, this would add to the increasing evidence that cadherins can exert active, instructive effects on the actin cytoskeleton (Kovacs *et al.*, 2002). Although there is much to be learned, these recent papers provide important insights into the interactions between gene products that generate a functional organelle.

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