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Sensory transduction, the gateway to perception: mechanisms and pathology

Stefan G. Lechner & Jan Siemens

The 102nd biannual Boehringer Ingelheim Fonds International Titisee Conference took place in October 2010. In the welcoming atmosphere of the small lakeside resort in the Black Forest, southern Germany, scientists from around the world gathered to discuss current topics and challenges in the area of sensory biology. The research presented covered all of the classical Aristotelian senses (and beyond) and provided a glimpse at recent progress and recurring themes in the sensory systems.

The ability of any living organism to probe and sense stimuli emanating from the surrounding environment (exteroception), as well as to monitor bodily parameters (interoception), is of fundamental importance for its survival. Highly specialized primary sensory cells have evolved to be tuned exquisitely to their respective sensory modality: photoreceptors can detect single photons, chemoreceptors respond to single molecules and mechanoreceptors sense mechanical deflections on the nanometre scale.

Enormous progress has been made in elucidating the molecular machineries that enable transduction, transmission and processing of these diverse physical and chemical stimuli. Given the diversity of the stimuli detected, it is not surprising that the cellular architecture and molecular make-up of the primary sensory cells is similarly diverse. However, common themes among the sensory systems have also emerged.

TRPs: integrators of diverse stimuli

The founding member of the transient receptor potential (TRP) ion-channel family was identified in *Drosophila melanogaster,* where it is involved in transducing a light stimulus into an electric signal in the photoreceptor cells. The TRP super-family of cation channels now includes more than 30 related molecules that respond to a remarkable variety of chemical and physical stimuli, making them suitable candidates for cellular sensors. The physiological roles for most TRP channels remain unknown, but a subset has been implicated in the detection and/or transmission of osmotic, thermal, chemosensory and mechanical stimuli. Although the discovery of *Drosophila* TRP dates back more than 20 years, the way in which TRP channels are activated and regulated *in vivo* is still largely unknown. In *Drosophila*, phototransduction is mediated by a G-protein-coupled phospholipase C (PLC) cascade culminating in the activation of TRP and TRP-like (TRPL) channels. The secondary messengers that gate these channels—or the closely related vertebrate TRPC channels—are unknown, and this represents one of the most enduring mysteries in sensory transduction. It is seldom recognized, however, that the hydrolysis of PIP_2 by PLC not only generates the second messengers $InsP_3$ and DAG, but also releases a proton. At the Titisee conference, Roger Hardie (U. Cambridge, UK) presented data to confirm that light induces a substantial PLC-dependent acidification in *Drosophila* photoreceptors. Moreover, he found that TRP and TRPL channels could be rapidly and reversibly activated by protonophores in cells that had been depleted of PIP_2 by a variety of manipulations. Hardie's results suggest that the combination of PIP_2 depletion and locally released protons might contribute to the activation of TRP channels in photoreceptors (Huang *et al*, 2010).

As well as acting as important receptors in fly photoreceptors, a subset of TRPs have been implicated in temperature detection. It has been suggested that one or more 'thermoTRPs' are broadly tuned to a particular temperature range and, in concert, cover the entire physiologically relevant temperature scale. Although data from cultured cells is in agreement with such a model, the situation seems to be more complex at the level of the organism. In particular, in the warm to hot temperature range, it is unclear to what extent TRPs have a role in temperature detection under physiological conditions, and how much other molecular mechanisms contribute. For the detection of cool to cold temperatures, TRPM8 has gained prominent recognition and three independently generated TRPM8-knockout mouse lines have severe cold phenotypes at the cellular and behavioural level.

The role of TRPA1 in cold perception is among the more-controversial research areas discussed in the TRP arena. Felix Viana (UMH Institute of Neurosciences, Alicante, Spain) provided an interesting twist to this controversy by suggesting that there are organ-specific differences in TRP-mediated cold detection. The somatosensory system recruits TRPM8 for this function, whereas the vagal system—innervating the viscera—might use TRPA1 to report cold temperatures (Fajardo *et al*, 2008). Future studies will tell whether cold is a physiologically relevant stimulus that activates vagal TRPA1, both in reduced preparations and the intact animal. Viana also emphasized a formerly unrecognized role of TRPM8 in cold-mediated tear-flow, which is important for maintaining ocular-surface wetness (Parra *et al*, 2010).

A role for TRPA1 in the detection of environmental irritants and endogenously generated inflammatory agents is well documented and less controversial. Sven-Eric

Jordt (Yale U., USA) discussed the possible role of TRPA1 in the aetiology of asthma and the interesting potential of TRPA1 as a drug target for treating inflammatory airway diseases (Caceres *et al*, 2009).

Asymmetry of auditory transduction

TRP channels have also been proposed to function as mechanoelectrical transducer (MET) channels in the auditory hair-cells of the inner ear that convert auditory stimuli into electrical signals. Speed is an outstanding property of the MET channel; it achieves kinetics fast enough to encode frequencies of up to 100 kHz in species such as bats. Although the TRPP subfamily of ion channels—a TRP subgroup, also known as PKD or polycystin channels—are candidate MET channels, it is also possible that a yet unrecognized ion channel (or ion-channel family) is recruited for this function.

Although the MET channel is unknown, enormous progress has been made recently towards uncovering the molecular mechanism of auditory transduction. To a large extent, our current knowledge is based on genetic studies and the identification of genes that cause hearing impairment. Christine Petit (Insitut Pasteur, Paris, France) pointed out that there are a total of approximately 150 non-syndromic deafness-gene loci, of which 55 deafness-causing genes are known. Several of these deafness-gene loci have been identified and the functions of the proteins that they encode have been characterized by Petit and her co-workers. Interestingly, most of these genes have a function in the primary sensory hair-cell. Hair cells are beautiful, highly specialized structures that are optimized for their mechanosensitive function. An array of stereocilia emanates from the apical surface of the cell. Stereocilia are actin-filled protrusions that are interconnected by protein linkers and their tips are believed to contain the molecular mechanotransduction machinery (Fig 1). Given this specialized (actin-rich) architecture, it might, retrospectively, seem unsurprising that myosin motor proteins are involved in the establishment, maintenance and mechanosensitive functioning of stereocilia.

Additionally, Petit and her co-workers have been at the forefront of identifying and characterizing proteins encoded by genes that are defective in Usher syndrome, a disorder that gives rise to both hearing loss and blindness. Her work has established Usher-syndrome proteins as important constituents of molecular complexes that are crucial for stereocilia bundle development and mechanotransduction (Caberlotto *et al*, 2011).

Interestingly, several Usher-syndrome genes encode adhesion receptors that have been found to be components of the linkers that connect individual stereocilia to a bundle. The linker molecules are crucial for the development and function of this mechanosensitive organelle: auditory stimulation ultimately leads to a deflection of the stereocilia bundle, thereby pulling linker molecules at the stereocilia tip (the so-called tip links). Force imparted by the tip link is believed to be the decisive event in the opening of the enigmatic MET channel that affects hair-cell depolarization (Fig 1).

In addition to their structural role in stereocilia bundle formation and maintenance, two adhesion receptors, cadherin 23 (CDH23) and protocadherin 15 (PCDH15), have a special role in the mechanical-transduction process of hair cells. Ulrich Müller (The Scripps Research Institute, USA) and colleagues found that the two cadherins form the stereocilia tip-link, which is believed to gate the MET channel on auditory stimulation. In the absence of the MET channel, we can only speculate about the way in which such a gating mechanism could work. However, it is interesting to note that the asymmetrical distribution of molecular components of the transduction machinery could be relevant: careful immunohistochemical analysis and binding assays by Müller and co-workers reveal that CDH23 and PCDH15 form heteromers, interacting with their respective amino-termini, whereby the upper part of the tip link is formed by CDH23 and the lower part—which inserts into the tip of the adjacent, smaller stereocilium—is formed by PCDH15 (Fig 1; Kazmierczak *et al*, 2007).

This asymmetry is also reflected in the distribution of other components that are important for stereocilia function, such as myosins and their binding partners. Moreover, the site of the MET channel, indirectly inferred by high-speed calcium imaging, is also localized asymmetrically, as Robert Fettiplace (University of Wisconsin, Madison, USA) explained. He and his colleagues Maryline Beurg and Toni Ricci found that stimulation did not trigger calcium influx in the first row of stereocilia, but that influx occurred in the rows containing smaller stereocilia (Beurg *et al*, 2009). This observation implies that the calcium-permeable MET channels are only located at the bottom-end of each tip link, possibly in the vicinity of PCDH15 (Fig 1). These exciting new findings raise several questions about how asymmetry is achieved and how adaptation processes can be accommodated in such a scenario. However, the most-pressing question remains: what is the molecular identity of the transducer channel?

Somatosensory mechanotransduction

Similarly to auditory hair-cells, sensory neurons that innervate the skin convert mechanical energy into electrical signals that can be processed by the nervous system. Most of our knowledge about how somatosensory mechanotransduction might work at the molecular level has emerged from the seminal work of Martin Chalfie (Columbia University, New York, USA) on touch receptors in the nematode *Caenorhabditis elegans*.

In *C. elegans*, as Chalfie summarized in the keynote lecture, a multi-protein complex is required to transduce mechanical stimuli. The pore of this mechanotransduction complex is formed by the channel proteins MEC-4 and MEC-10, which require the auxiliary subunits MEC-2 and MEC-6 for normal function (Fig 1). On the extracellular side, MEC-4 and MEC-10 are thought to be tethered to the matrix proteins, MEC-1, -5 and -9, which might relay forces exerted on the extracellular environment to the channel proteins. In addition, the tubulins MEC-7 and MEC-12 were shown to be important for the function of *C. elegans* touch-receptor neurons, implicating the cytoskeleton in mechanosensation. Similarly to bacterial mechanotransduction channels, Chalfie suspects that the *C. elegans* touch complex might be activated by force changes in the plane of the plasma membrane, a hypothesis that needs further investigation.

Despite extensive efforts to identify mechanotransduction genes among mammalian *mec*-homologues, only one protein—the stomatin-like protein-3 (SLP-3), a homologue of MEC-2—has been shown to be essential for touch sensation in mice (Fig 1). Gary Lewin (Max-Delbrück-Center for Molecular Medicine, Germany), who characterized SLP-3 function in sensory neurons, presented data that showed mechanotransduction in mice might require a tether protein that connects the transduction channel to the extracellular matrix. By using transmission electron microscopy he could visualize a protein filament with a length of

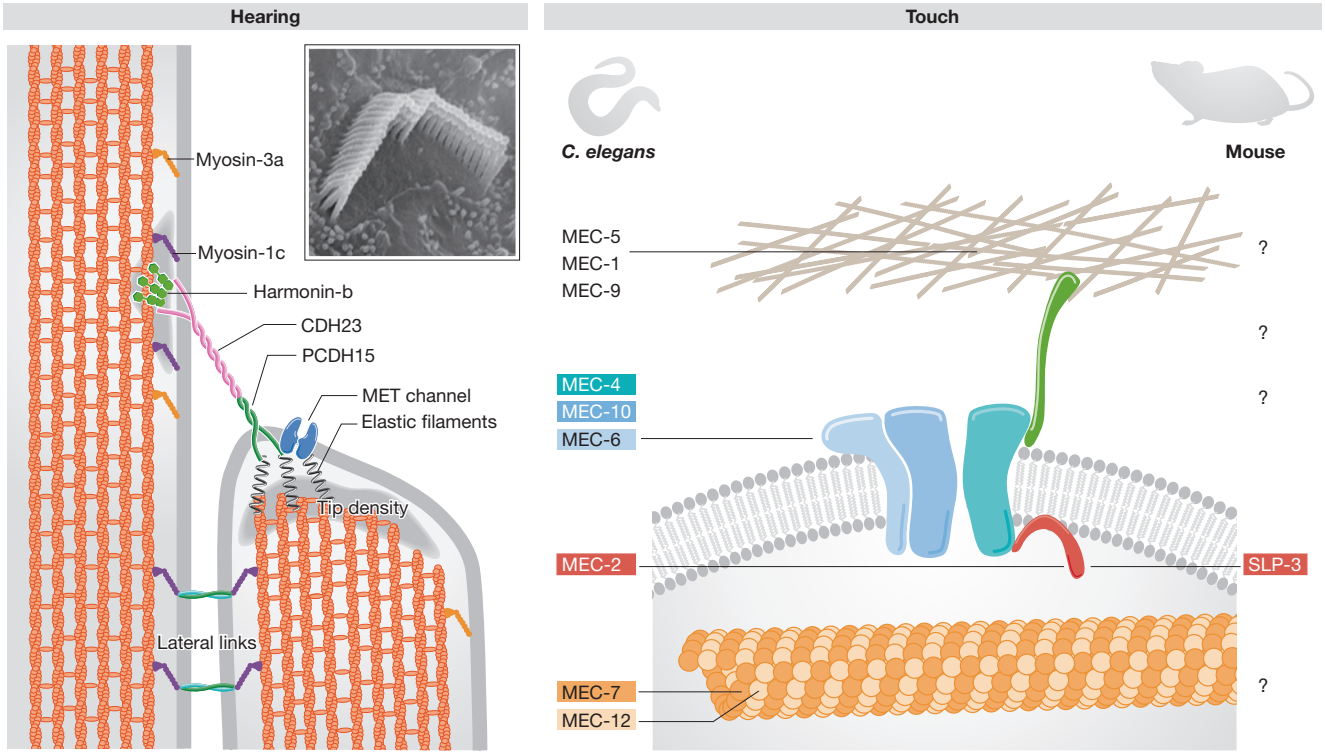

Fig 1 | Mechanotransduction. Left panel (hearing): schematic representation of the asymmetrical molecular transduction machinery spanning two adjacent hair-cell stereocilia. The inset shows an electron micrograph of a stereocilia bundle. Modified from © Schwander *et al*, 2010. Originally published in *J Cell Biol* doi:10.1083/jcb.201001138. Right panel (touch): cartoon depicting the *C. elegans* touch-receptor complex. MEC, mechanosensory abnormality; MET, mechanoelectrical transducer; SLP-3, stomatin-like protein-3.

approximately 100 nm, which is sensitive to cleavage by the site-specific proteases subtilisin and blisterase. When the tether was disrupted by brief protease treatment, mechanotransduction currents recorded from dissociated DRG neurons, as well as mechanically induced action potentials recorded using an *ex-vivo* skin–nerve preparation, were abolished (Hu *et al*, 2010). The molecular identity of the tether protein, however, remains elusive. As the tether was reminiscent of the tip link that connects the stereocilia of auditory hair-cells, Lewin hypothesized that common genes might be required for hearing and touch. Indeed, he showed that hearing-impaired patients with mutations in a known Usher-syndrome gene also exhibit reduced touch sensitivity.

The hypothesis that common genes might be involved in hearing and touch was further supported by data presented by Thomas Jentsch (FMP, Berlin, Germany). Together with Lewin, he found that a voltage-gated potassium channel with a crucial function in auditory hair-cells is also expressed at the mechanosensitive nerve endings of a subset of low-threshold mechanoreceptor afferents. By using mouse models and electrophysiological methods, Jentsch demonstrated that this potassium channel is important for tuning these receptors to low frequencies.

Although the sessions on hearing and touch provided exciting new insights into the molecular mechanisms underlying mechanotransduction, they also reminded us that the key players in this process—the mechanotransduction channels—remain elusive. Or do they? The possible role of Piezos—recently identified mechanically gated ion channels (Piezo-1 and Piezo-2; Coste *et al*, 2010)—in hearing and touch generated lively discussions behind the scenes. It is clear that Piezos will soon be put to the test, and that the mechanotransduction field is entering an exciting period if Piezos turn out to be primary transducers of mechanical stimuli.

Olfaction

The olfactory system can discriminate between a potentially unlimited number of odorants with only a limited repertoire (approximately 400 in humans) of G-protein-coupled odorant receptors. Activation of odorant receptors initiates a signalling cascade that leads to an elevation of cAMP levels, followed by Ca^{2+} influx through cyclic-nucleotide-gated ion channels and the subsequent activation of a Ca^{2+}-activated chloride channel, which mediates a depolarizing efflux of chloride. This efflux of chloride is thought to be crucial for the detection of weak olfactory stimuli, as it amplifies the relatively small Ca^{2+}-mediated depolarization.

Proteins that maintain the high intracellular chloride levels required for this amplification were the subject of a talk by Stefan Frings (U. Heidelberg, Germany). Frings found that the $Na^+/K^+/2Cl^-$ co-transporter NKCC1 is expressed in the sensory cilia of olfactory sensory neurons (OSNs), in which it mediates chloride accumulation in the ciliary lumen. He also found prominent expression of the Cl^-/HCO_3^- exchanger SLC4A1 in sensory cilia, which might provide an additional mechanism for

chloride homeostasis (Hengl *et al*, 2010). Anna Menini (SISSA, Trieste, Italy) discussed the possible role of the recently identified Ca^{2+}-activated chloride channel TMEM16B in OSNs. She showed expression of TMEM16B in the ciliary layer of the mouse olfactory epithelium and presented an extensive functional comparison of currents recorded in the ciliary region of isolated mouse OSNs and in HEK293 cells transfected with TMEM16B, by using both inside-out and whole-cell voltage-clamp techniques. Her findings suggest that TMEM16B is a promising candidate for mediating chloride-based amplification in olfactory transduction (Sagheddu *et al*, 2010).

Another interesting feature of the olfactory system is that a given OSN expresses only one type of olfactory receptor from a genomic repertoire that can be as large as 1,500 genes. The question of how the choice of receptor gene is controlled has puzzled researchers for many years. Peter Mombaerts (MPI for Biophysics, Frankfurt, Germany) presented an elegant approach that might help to gain more insight into this enigmatic process. In order to define the minimal promoters that control the expression of olfactory receptors, Mombaerts has developed a transgenic reporter assay in which promoter transgenes drive the expression of the axonal marker tau-β-galactosidase. He could identify a 13-bp sequence containing a homeodomain binding site that is shared by several promoter elements. When this sequence was multimerized nine times and inserted upstream to a MOR23 minigene, the number of OSNs that express the transgene was dramatically increased. Mombaerts speculated that these homeodomain binding sites modulate the probability of olfactory receptor gene choice, that is, the frequency with which a given olfactory receptor gene is chosen for expression by an OSN (Vassalli *et al*, 2011).

It is unusual to attend a meeting covering such an exciting mix of sensory systems. On behalf of the participants, we thank Thomas Jentsch, Gary Lewin and Christine Petit for putting together such a great meeting with the support of Boehringer Ingelheim Fonds. The range of excellent presentations—far more than can be covered in this review—provided the framework for abundant sensory stimulation, which was enhanced by the superb catering and beautiful surroundings of Lake Titisee.

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Stefan G. Lechner and Jan Siemens are at the Max-Delbrück-Centre for Molecular Medicine (MDC), Berlin, Germany.
E-mail: jan.siemens@mdc-berlin.de

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