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How do we feel?

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Mammalian dorsal root ganglia (DRG) contain a diverse collection of sensory neuronal subtypes specialized to detect different sensory stimuli. Most of these cells respond to mechanical stimulation of some sort, be they proprioceptors detecting body movements, receptors for gentle touch or nociceptors activated by painful levels of pressure, and yet the proteins that detect mechanical stimuli remain unknown. In this issue of *The EMBO Journal*, Gary Lewin and colleagues give insights into the developmental acquisition of mechanosensitivity by different classes of sensory neurons and in doing so, they offer a new approach for determining the molecular basis of this sensory modality.

Until recently, it seemed that unravelling the molecular basis of mammalian touch sensation would be straightforward. Marty Chalfie's group had characterized a multi-protein mechanotransduction complex in *Caenorhabditis elegans* touch-sensing neurons (Chalfie, 2009) and homologues of the crucial players in this ensemble had been found in mammalian sensory neurons. Most compellingly, a group of ion channels related to the channel at the heart of the nematode complex, the acid-sensing ion channels (ASICs), was shown to be richly expressed in the DRG. Two subunits, ASIC3 and ASIC1B, are selectively expressed by sensory neurons. However, despite the early promising results (Price *et al*, 2000), as studies of ASICs and mechanotransduction accumulated, it became apparent that these channels are not essential components of the mammalian mechanosensory apparatus. Subsequently, much attention was paid to the TRP channel family, given their established roles in *Drosophila* mechanotransduction and reports that some mammalian TRP channels are modulated by membrane stretch (Christensen and Corey, 2007). However, a new study by Lechner *et al* (2009) has defined the developmental acquisition of mechanosensitivity by DRG neurons and, by the use of a new mRNA expression screen, concluded that we almost certainly need to move beyond ASICs and TRP channels in our search for the elusive force-transducing ion channels of this system.

Studying somatosensory transduction is confounded by the inaccessibility of the nerve endings, embedded in peripheral tissues, where this process naturally occurs. Consequently, researchers use cultured DRG neurons as a model, in which receptor proteins expressed at peripheral terminals *in vivo* are found on the cell body. Compression of the somatic membrane using a mechanical probe generates a cationic current in sensory neurons (McCarter *et al*, 1999). Different classes of DRG neurons show currents with distinctive physiological and pharmacological properties consistent with the neurons' specific functions; for example, presumptive touch receptors show larger currents with lower activation thresholds than do damage-sensing neurons (nociceptors) (Drew *et al*, 2004, 2007). Supported by studies on neurites *in vitro* (Hu and Lewin, 2006), this model system has become a focus in attempts to define the molecular mechanisms involved in mechanosensation.

Neurogenesis in mouse DRG occurs in two waves; the first wave (at E10–11) gives rise to low-threshold mechanoreceptors, neurons that express TrkB and TrkC neurotrophin receptors, and the second (between E11–13) to a variety of cell types, characterized by TrkA expression, including nociceptors. Developing neurons first undergo a phase of neurite outgrowth, innervating the spinal dorsal horn and the periphery (where they are sensitive to target-derived signalling molecules), before a period of functional maturation. To characterize maturating DRG neurons, Lechner *et al* extracted these cells from developing mice at daily intervals from E11.5 until the day they were born and recorded the currents activated by mechanical stimulation of their somata. These experiments showed that the acquisition of mechanosensitivity occurs in three distinct waves. The first happens at E13.5 in the early-born low-threshold mechanoreceptors (defined by the presence of TrkB and TrkC) when they begin to generate rapidly adapting mechanogated currents. In the second wave, a population of nociceptive neurons begins to express rapidly adapting currents at E15.5, and in the final wave, at birth another population of (possibly overlapping) nociceptors appears, which generates slowly adapting currents in response to mechanical stimulation (see Figure 9 in Lechner *et al* (2009)).

The first two waves occur as the respective peripheral axons of neurons innervate their targets, which led the authors to ask whether a target-released factor induces the expression of mechanically gated channels. Interestingly, acquisition of mechanosensitivity by low-threshold mechanoreceptors is independent of the TrkC ligand, neurotrophin 3, and probably represents a genetically programmed maturational event. Conversely, in nociceptors, nerve growth factor (NGF) exposure, acting through TrkA, is crucial for the appearance of mechanically activated currents. Furthermore, NGF can also prematurely induce such currents in younger nociceptors that are yet to fully extend their processes. Consistent with earlier findings, high levels of NGF in culture increased mechanosensitivity (Di Castro

et al, 2006), shown here to be because of the additional expression of an intermediately adapting mechanically activated current, and NGF sensitivity is maintained in early postnatal neurons. Whether the final wave of persistent current acquisition is because of the ongoing maturation of those neurons or is dependent on a separate secreted factor remains to be determined.

The second part of the paper takes the newly established delineation of mechanosensitivity maturation and uses it to screen for the underlying ion channels. Assuming that the mRNA expression of the transducing channel begins concurrently with the appearance of the functional channel (i.e. translational repression is not occurring at early time points), the authors reason that a screen of mRNAs that are absent (or rare) at E11.5 but abundant at E13.5 and in adult DRG will reveal a shortlist of candidate mechanotransduction genes, whose function could then be assessed.

In this study, the screen was limited to members of the mammalian DEG/ENaC (including the ASICs) and TRP ion-channel families, albeit all 43 members were assessed. The authors found the expression of a small number of these channels was temporally regulated in the anticipated way but, none of them represented outstanding candidates. ASIC2 and ASIC3 subunits were upregulated at the appropriate time but, consistent with results in adult DRG neurons (Drew *et al*, 2004), currents were normal in embryonic neurons lacking these genes. TRPC4 expression emerged along with mechanosensitivity in low-threshold mechanoreceptors, but mice lacking this gene have no striking sensory phenotype (Freichel *et al*, 2005) and its function in DRG might relate to neurite outgrowth (Wu *et al*, 2008). No known stretch-modulated TRP channels were expressed consistently along with the expression of mechanosensation. Overall, these results argue strongly for moving beyond DEG/ENaC and TRP channels in the pursuit of mechanotransduction channels, just as the auditory hair-cell field has done. The screen developed has rich potential if extended to include other ion channels or genome-wide microarray analysis, and similar investigation of gene induction by NGF could show nociceptor-specific mechanosensitive channels.

Our knowledge about mechanosensation in lower organisms has grown rapidly, but many exciting questions remain open for mammalian systems (Chalfie, 2009). In the DRG, does each subtype of sensory neuron express a single transducing channel, deletion of which will result in a strikingly strong phenotype, say a mouse insensitive to mechanical pain or one with entirely uncoordinated movement? Or is there a cache of related channels broadly expressed and capable of partially compensating for one another? Are the DRG sensory channels related to those that function in hearing and balance? And is there a malignant dysregulation of mechanogated ion channels in certain pain conditions? Lechner *et al* have presented an elegant characterization of the somatosensory system in development and have provided a potentially useful new tool in answering these questions.

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