piezo2b Regulates Vertebrate Light Touch Response

Adèle Faucherre,^{1,2,3} Joël Nargeot,^{1,2,3} Matteo E. Mangoni,^{1,2,3} and Chris Jopling^{1,2,3}¹CNRS, UMR-5203, Institut de Génétique Fonctionnelle, Département de Physiologie, Labex Ion Channel Science and Therapeutics, F-34094 Montpellier, France, ²INSERM, U661, F-34094 Montpellier, France, and ³Universités de Montpellier 1 and 2, UMR-5203, F-34094 Montpellier, France

The sense of touch allows an organism to detect and respond to physical environmental stimuli. Mechanosensitive proteins play a crucial role in this process by converting the mechanical cue into a biological response. Recently, the Piezo family of stretch-activated ion channels has been identified as genuine mechanosensitive proteins. We set out to determine whether any of these genes are involved in touch response during zebrafish development. *In situ* hybridization indicates that *piezo2b* is specifically expressed in a subset of neurons (Rohon–Beard cells) responsible for detecting light touch. Using morpholino-mediated knockdown, we specifically targeted *piezo2b* and determined that it is involved in mediating touch-evoked response.

Introduction

Touch is a fundamental ability that allows organisms to sense external environmental forces and elicit an appropriate response. Touch can generate a diverse range of behavioral responses depending on the initial stimuli; for example, touch can induce avoidance from a potential hazard or elicit social interactions (Lumpkin et al., 2010). To detect and ultimately produce a specific response, a multitude of specialized cell types have arisen, possessing specific mechanosensory molecules dedicated toward determining particular environmental forces (Lumpkin et al., 2010). Mechanosensitive ion channels, present in the cell membrane, represent a class of such molecules that can detect mechanical stimuli and convert them into a biological response (Xiao and Xu, 2010). Although mechanosensitive ion channels have been detected in virtually all organisms via electrophysiological recordings, the difficulties associated with expressing putative channels in systems that would allow for a detailed analysis have meant that their identities remain largely elusive (Hamill and Martinac, 2001; Xiao and Xu, 2010). Recently, the family of PIEZO proteins have been identified as genuine mechanosensitive ion channels (Coste et al., 2010). These stretch-activated channels are able to detect changes in cell membrane tension resulting in the opening of the channel and an influx of cations (Coste et al., 2010; Gottlieb and Sachs, 2012). Homologs of the *piezo* genes have been identified in a variety of species ranging from protozoa to vertebrates (Coste et al., 2010). Mammals have two *piezo* genes, *piezo1* (*fam38A*) and *piezo2* (*fam38B*). These genes are expressed in a number of mechanosensitive tissues such as

kidney, lung, and colon. In particular, *piezo1* is highly expressed in the skin, while *piezo2* shows an elevated expression pattern in dorsal root ganglion cells (Coste et al., 2010).

Determining the biological processes regulated by the *piezo* genes is currently the subject of intense investigation (Nilius and Honoré, 2012). Recently, the *piezo* genes have been linked to mechanical nociception. The fruit fly *Drosophila melanogaster* has a single homolog *Dmpiezo*. Consequently, *Dmpiezo* knock-out larvae fail to respond to noxious mechanical stimuli yet retain normal responsiveness to touch and other noxious stimuli (Kim et al., 2012).

We sought to determine whether any of the *piezo* genes were involved in regulating touch response in vertebrates. Zebrafish acquire the ability to respond to light touch between 24 and 27 hours post-fertilization (hpf), as evidenced by the writhing behavioral response exhibited by embryos following tactile stimulation (Pietri et al., 2009). The mechanosensitive Rohon–Beard cells innervating the skin mediate touch responsiveness in zebrafish embryos via their free nerve endings (Metcalf et al., 1990; Nakano et al., 2010). Here we report that *piezo2b* is expressed in Rohon–Beard cells of developing zebrafish embryos and is essential for mediating touch response.

Materials and Methods

Zebrafish strains and husbandry. Zebrafish were maintained under standardized conditions, and experiments were conducted in accordance with the European Communities council directive of November 1986. Embryos were staged according to Kimmel et al. (1995); however, it is not possible to determine the sex at these stages of development.

Morpholinos and injections. Morpholino oligonucleotides (MOs) were obtained from Gene Tools and were injected into one-cell stage embryos. The *Pz2b MO1* morpholino targets the start codon of *piezo2b*. The *Pz2b MO2* morpholino targets the 5′ untranslated region (UTR) of *piezo2b* and does not overlap with the *Pz2b MO1* morpholino. The *Pz2b MO3* morpholino targets the exon1/intron1 splice site of *piezo2b*. The *trpa1b MO* targets the start codon of *trpa1b*. The sequences of the injected MOs are as follows: *Pz2b MO1* (ATG), 5′-GCGCAGATGTAGCTCTGCTTCATGA-3′; *Pz2b MO2* (5′ UTR), 5′-ACTTGAACACTTTGACGAGAGCTGC-3′; *Pz2b MO3* (splice), 5′-GTCTATATTATACCATCATAGCGAT-3′; and *Trpa1b MO* (ATG), 5′-TCACTAACTCCTTTCCAACTGCAT-3′.

In situ hybridization. *In situ* hybridization were performed as described previously (Jopling and den Hertog, 2005). The *piezo* probes were gen-

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Correspondence should be addressed to Dr. Chris Jopling, Institut de Génétique Fonctionnelle, 141 Rue de la Cardonille, 34094 Montpellier Cedex 05, France. E-mail: chris.jopling@igf.cnrs.fr.

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erated by cloning an ~500 bp fragment of each gene from embryonic cDNA. The *trpa1b* probe was a gift from Alex Schier (Harvard University, Cambridge, MA; Prober et al., 2008). Fragments were ligated into the pGEM-T EASY vector and subsequently linearized. Antisense probes were synthesized as described previously (Jopling and den Hertog, 2005). Two-color and high-resolution fluorescent, double-whole-mount *in situ* hybridizations were performed as described previously (Jowett, 2001; Brend and Holley, 2009).

Immunohistochemistry. For immunohistochemistry, embryos were fixed overnight at 4°C in a solution of 4% paraformaldehyde in PBS. After fixation, samples were washed in PBS with 0.1% Tween-20 (PBST) and blocked at room temperature with 10% bovine serum albumin in PBST. Primary antibody incubation was conducted overnight at 4°C in blocking buffer with the zn-12 antibody (ZIRC, University of Oregon, Eugene, OR) at a 1:100 dilution. Secondary-antibody incubation was conducted overnight at 4°C in PBST with an Alexa Fluor-594 anti-mouse antibody (Jackson ImmunoResearch) at a 1:1000 dilution.

Light touch response. Touch-evoked behaviors were elicited by touching an embryo/larvae up to three times with a pair of No. 5 forceps between 24 and 27 hpf, as previously described (Low et al., 2010). A response was deemed positive when writhing behavior was observed.

Tail pinch assay. Tail pinch-evoked behaviors were elicited by pinching the tail of an embryo/larvae with a pair of No. 5 forceps between 24 and 27 hpf, as previously described (Low et al., 2010). A response was deemed positive when writhing behavior was observed.

Mustard oil assay. Chemosensory-evoked behavior was elicited by placing embryos in a 500 μ M mustard oil solution diluted in embryo media (06019–10M, Fluka), as previously described (Low et al., 2010). A response was deemed positive when writhing behavior was observed. For more detailed analysis, time-lapse images were recorded. Single writhes were counted when the tail was coiled either to the left or right of the embryo.

Mosaic Rohon–Beard cell labeling and counting. A *HuC:mem-TdTomato* construct was used to mosaically label Rohon–Beard cells, as described previously (Zhao et al., 2006; Faucherre et al., 2009). For counting Rohon–Beard cells, embryos were first labeled with the zn-12 antibody. Subsequently, a bright-field image was made to identify the somite boundaries, then the Rohon–Beard soma in the closest focal plane were counted.

Imaging. Behaviors and still images of fixed embryos were recorded using a CoolSNAP Color cf camera (Photometrics) mounted on a stereomicroscope (Zeiss). Images were acquired with MetaMorph software. Fluorescent *in situ* hybridization and immunohistochemistry images were acquired with a Leica TCS SP8 inverted confocal laser scanning microscope with a 20 $\times$, 40 $\times$, or 63 $\times$ oil-immersion objective. z-stacks were acquired at 0.5, 2, or 3 μ m intervals, using Alexa Fluor-594 for imaging (561 nm excitation, 585–610 nm emission). Maximal projections and analysis were performed with ImageJ software.

Results

The zebrafish homologs of *piezo1* and *piezo2a* have been previously identified (GenBank accession No. XP_696355.4 and XP_002666625, respectively; Coste et al., 2010; Faucherre et al., 2013). However, *in silico* analysis using the Ensembl database indicates a third homolog, *piezo2b* (GenBank accession No. XP_003198010), is also present in zebrafish. Gene duplication is not uncommon in zebrafish and is most likely caused by a whole-genome duplication event during zebrafish evolution (Hultman et al., 2007). Protein sequence analysis indicates that the zebrafish PIEZOS show a similar homology to their mammalian counterparts: human PIEZO1 versus zebrafish PIEZO1, 59.2%; human PIEZO2 versus zebrafish PIEZO2A, 66.9%; and human PIEZO2 versus zebrafish PIEZO2B, 62.6%. To determine the expression of the *piezo* genes during early development, we designed antisense RNA probes covering the initial 500 bp of each gene. *In situ* hybridization analysis reveals that only *piezo2b* is expressed in the Rohon–Beard cells throughout the tail and in the trigeminal gan-

Figure 1. The expression pattern of *piezo2b* during zebrafish development. **A–C**, *In situ* hybridization analysis using an antisense *piezo2b* probe at 24 hpf. **A**, *Piezo2b* expression can be observed in the trigeminal ganglion (arrowhead) and Rohon–Beard cells (box). **B**, Dorsal view showing *piezo2b* expression in the trigeminal ganglion (arrowhead). **C**, Higher magnification of the boxed region in **A**. *Piezo2b* expression can be detected in the Rohon–Beard cells (arrowheads). **E**, To ensure that these observations were specific, we performed an identical assay using a sense *piezo2b* probe, which did not label anything. **D**, *In situ* hybridization analysis using an antisense *trpa1b* probe indicates that this gene is also expressed in Rohon–Beard neurons (arrowheads). **F**, **G**, The other two zebrafish *piezo* homologs, *piezo1* and *piezo2a*, do not appear to be expressed in Rohon–Beard cells.

glion in the head, similar to the chemosensory *trpa1b* ion channel (Prober et al., 2008; Fig. 1A–G). Consequently, we focused our attention on *piezo2b* to determine whether it played a role in the touch response elicited by this set of neurons.

To target *piezo2b* during zebrafish development, we used non-overlapping antisense morpholino oligonucleotides targeting either the start codon or the 5' UTR (Pz2b-MO1 and Pz2b-MO2). Morpholinos were titrated to concentrations that did not cause any gross developmental defects/necrosis. To determine whether *piezo2b* genes have a role in touch response, we performed a previously described light touch assay (Low et al., 2010). Control embryos responded normally to light touch stimulation exhibiting a writhing behavioral response (Fig. 2A,C). Conversely, *piezo2b* morphants showed a complete lack of touch response to tactile stimulation (Fig. 2A,C).

To ensure that the defect we observed in *piezo2b* morphants was specific for the light touch response, and was not due to a global neural defect, we performed a number of other behavioral assays. Tail pinching also elicits a similar writhing behavior; however, in mutant zebrafish lacking a light touch response, this assay still produces an effect, indicating that the sensory systems used to detect either stimulus are distinct (Low et al., 2010). We found that *piezo2b* morphants respond normally to the tail pinch assay compared with control embryos, indicating that the mechanical nociceptive system used to detect these stimuli is unaffected by *piezo2b* knockdown (Fig. 2D). As an additional control, we de-

Figure 2. *Piezo2b* morphants are unresponsive to light touch but respond normally to mechanical and chemical stimuli. Touch response stimulation was performed on 24–27 hpf wild-type and *piezo2b* morphant embryos. **A**, wt panel, Time-lapse images of a wild-type embryo's response to touch-evoked stimuli. *Piezo2b* morphant embryos fail to respond to touch response stimulation (Pz2b MO panel). *Trpa1b* morphants respond normally to light touch (Trpa1b MO panel). **B**, RT-PCR analysis of Pz2b-MO3 morphant embryos. The Pz2b-MO3 targets the exon1/intron1 splice site. Primers designed to the start of exon1 and the end of exon2 (arrows) are able to amplify the correct 270 bp fragment (top band) from wild-type embryo cDNA (lane 1). With morphant embryo cDNA, this band disappears, indicating that the correct splicing of *piezo2b* has been disrupted (excess primers form the lower band in all lanes). **C**, Quantitative analysis of wild-type, *piezo2b*, and *trpa1b* morphants subjected to touch response stimulation. *Piezo2b* morphants generated with morpholinos targeting the start codon (Pz2b-MO1), a nonoverlapping morpholino directed toward the 5' UTR (Pz2b-MO2), or a splice-blocking morpholino targeting the exon1/intron1 boundary (Pz2b-MO3) fail to respond to tactile stimulation. The effect is enhanced slightly by increasing the concentration of morpholinos. *Trpa1b* morphants (Trpa1b-MO) respond normally in this assay (n = cumulative number of embryos from three independent assays). **D**, A tail pinch assay was performed on the same wild-type, *piezo2b*, and *trpa1b* morphants used in the previous light touch assay. The response of *piezo2b* and *trpa1b* morphants was indistinguishable from that of wild-type control embryos (n = cumulative number of embryos from three independent assays). **E**, *Piezo2b* morphants respond the same as control embryos when challenged with mustard oil. The assay was performed on the same wild-type and *piezo2b* morphants used in the previous light touch assay. However, mustard oil fails to elicit a response from *trpa1b* morphants (Trpa1b-MO; n = cumulative number of embryos from three independent assays). To analyze this phenotype in more detail, time-lapse images were recorded allowing us to count the number of writhings in response to mustard oil stimulation, an example of a wild-type embryo response is shown in **F** (black arrow indicates when a single writhing has been initiated). **G**, *Piezo2b* morphants respond to mustard oil with a similar number of writhings to that observed in wild-type embryos, while *trpa1b* morphants barely elicit a response to mustard oil (n = 10 embryos analyzed for each condition). Error bars indicate $\pm$ SEM. $*p < 0.001$, two-tailed Student's t test.

signed a third *piezo2b* morpholino (Pz2b-MO3) targeting the exon1/intron1 splice site (Fig. 2B). RT-PCR analysis of Pz2b-MO3 morphant embryos confirmed that normal splicing of the target mRNA had been disrupted (Fig. 2B). We subsequently

found that knockdown of *piezo2b* using the Pz2b-MO3 morpholino also resulted in a loss of light touch response without affecting the embryos' ability to react to tail pinch stimuli (Fig. 2C,D). Zebrafish embryos have previously been shown to respond to

Figure 3. Rohon–Beard cells develop normally in *piezo2b* morphants. **A–D**, Rohon–Beard cells are clearly visible in wild-type embryos mosaically expressing mem-TdTomato under the control of the *HuC* promoter. **A**, Bright-field image of a wild-type embryo at 24 hpf mosaically expressing *HuC*:mem-TdTomato. **B**, The same embryo viewed with RFP-filtered UV light. The Rohon–Beard cells are indicated with arrowheads. **C, D**, The same embryo at higher magnification. The Rohon–Beard cells are indicated with arrowheads. **E**, Bright-field image of a *piezo2b* morphant embryo at 24 hpf mosaically expressing *HuC*:mem-TdTomato. **F**, The same embryo viewed with RFP-filtered UV light. The Rohon–Beard cells are indicated with arrowheads. **G, H**, The same embryo at higher magnification. The Rohon–Beard cells are indicated with arrowheads. Note: because the *HuC* promoter-driven mem-TdTomato expression is mosaic, this is not a quantitative assay and only illustrates that Rohon–Beard cells are present in *piezo2b* morphants. **I–K**, The average number of Rohon–Beard cells does not change following *piezo2b* knockdown. **I**, Bright-field image of a 24 hpf embryo immunolabeled with the zn-12 antibody; the yellow box delineates the somite boundaries. **J**, Fluorescent image of the same embryo. Three Rohon–Beard cell soma are clearly visible within the somite boundary (yellow box). **K**, Graph indicating the average number of Rohon–Beard cells (RB)/somite in wild-type (wt) embryos, and in two different *piezo2b* morphant groups ($n = 6$ embryos/condition, 5 somites/embryo). Error bar indicates $\pm$ SEM. Somite data were pooled, and no statistical difference was observed between these groups. **L–Q**, The gross morphology of Rohon–Beard soma and neurites is unaffected following *piezo2b* knockdown. **L**, Fluorescent image of a wild-type embryo labeled with the zn-12 antibody; peripheral neurites can be seen extending from individual Rohon–Beard soma to innervate the skin. Scale bar, 25 μ m. **M**, The same image at a higher magnification. Scale bar, 25 μ m. **N–Q**, Similar images taken from two different *piezo2b* morphant groups; the gross morphology of the Rohon–Beard soma and their neurites appears normal when compared with that of the wild-type controls ($n = 6$ embryos analyzed/condition).

noxious chemical stimuli such as mustard oil with increased locomotive activity (Prober et al., 2008). The ion channel *trpa1b*, which is also expressed in Rohon–Beard neurons (Fig. 1D), is essential for this chemosensation. Consequently, *trpa1b* mutant embryos do not respond to mustard oil; however, they do retain touch responsiveness (Prober et al., 2008). These observations suggest that Rohon–Beard neurons are able to detect diverse sensory stimuli. To further investigate these intriguing observations we also designed a morpholino to *trpa1b* (*trpa1b*-MO). We found that knockdown of *trpa1b* did not affect either the light touch or mechanical nociceptive response of zebrafish embryos (Fig. 2A, C, D). However, *trpa1b* morphants did display a severely reduced chemosensory response to mustard oil (Fig. 2E–G). Conversely, we were unable to detect any observable difference in the response of *piezo2b* morphants to mustard oil when compared with control embryos (Fig. 2E–G). Together, these results

indicate that the loss of light touch response observed in *piezo2b* morphants is not due to a global neural defect.

Next we assessed whether *piezo2b* knockdown affected Rohon–Beard cell development/homeostasis. One possible explanation for the light touch response defect we observed in *piezo2b* morphants is that knockdown of this gene results in the loss of or disrupts the function of Rohon–Beard cells. To analyze this, we used a previously reported *HuC*:mem-TdTomato construct, which, when injected into zebrafish embryos, mosaically labels a subset of neurons including Rohon–Beard cells (Zhao et al., 2006; Faucherre et al., 2009; Fig. 3A–H). *piezo2b* morphants were first selected by performing the light touch stimulation to ensure that they did not respond to this assay. In this manner, we were able to clearly detect Rohon–Beard cells in *piezo2b* morphants, indicating that these neurons are still present (Fig. 3A–H). To further ensure that Rohon–Beard cell numbers did not differ between

Figure 4. A subset of *piezo2b*-expressing Rohon–Beard cells also express *trpa1b*. **A–D**, Two-color *in situ* hybridization with *piezo2b* labeled in red (**A**, **B**) and *trpa1b* in blue (**C**, **D**). Individual embryos were initially imaged to detect the red stain. Subsequently, following the blue stain, the same individual embryos were imaged again, allowing us to determine coexpression. All of the *trpa1b*-positive cells are also positive for *piezo2b* (**B**, **D**; black arrowheads); however, it is also possible to detect *piezo2b*-positive/*trpa1b*-negative Rohon–Beard cells (**D**; white arrowhead). **E–H**, A similar *in situ* hybridization assay performed with *trpa1b* in red (**E**, **F**) and *piezo2b* in blue (**G**, **H**). All of the *trpa1b*-positive cells are also positive for *piezo2b* (**F**, **H**; black arrowheads); however, it is also possible to detect *piezo2b*-positive/*trpa1b*-negative Rohon–Beard cells (**H**; white arrowheads). **I**, **J**, Double-fluorescent *in situ* hybridization for *piezo2b* (**I**; green) and *trpa1b* (**J**; red). **K**, All *trpa1b*-positive cells also express *piezo2b*.

wild-type and morphant embryos, we performed immunohistochemistry using the zn-12 antibody, which effectively labels Rohon–Beard cells (Metcalf et al., 1990). In this manner, we were able to determine that the average number of Rohon–Beard cells/somites did not differ significantly between wild-type and morphant embryos (Fig. 3I–K). More detailed analysis revealed that the gross morphology of neurites extending from Rohon–Beard soma appears to be unaffected in *piezo2b* morphants (Fig. 3L–Q). Together, these results indicate that knockdown of *piezo2b* does not affect Rohon–Beard cell development or homeostasis as these neurons are present and appear normal in *piezo2b* morphant embryos.

Because *piezo2b* and *trpa1b* are both expressed in Rohon–Beard neurons, this raises two possible scenarios regarding the differences in the behavioral responses we have observed between these respective morphant embryos. First, *piezo2b* and *trpa1b* could be expressed in distinct subsets of Rohon–Beard neurons; consequently, the detection of different sensory stimuli could be restricted to defined populations of these cells. The second possibility is that both of these genes are expressed in the same neurons that subsequently perform a multisensory role. To ascertain which of these hypotheses is correct, we performed a variety of double *in situ* hybridization assays on zebrafish embryos. Using a two-color approach, we found that 100% of *trpa1b*-positive Rohon–Beard neurons also express *piezo2b* (Fig. 4A–H); however, it was also possible to detect numerous *piezo2b*-positive/*trpa1b*-negative Rohon–Beard cells ($n = 34$ embryos analyzed; Fig. 4A–H). This indicates that *trpa1b* is expressed in a subset of *piezo2b*-positive Rohon–Beard neurons. To further substantiate these findings, we performed double-fluorescent *in situ* hybridization assays for both *piezo2b* and *trpa1b*. Consequently, we found that 100% of *trpa1b*-positive Rohon–Beard cells were also positive for *piezo2b*, and, as we had observed in the previous assays, we were also able to detect *piezo2b*-positive/*trpa1b*-negative Rohon–Beard neurons ($n = 16$ embryos analyzed; Fig. 4I–K). Because all *trpa1b*-positive Rohon–Beard neurons are also positive for *piezo2b*, we determined that knockdown of *piezo2b* does not disrupt the overall functionality of these cells. Despite being unable to sense light touch due to the loss of *piezo2b*, zebrafish are still perfectly capable of responding to chemosensory stimuli. To-

gether, this leads us to conclude that *piezo2b* is essential for sensing light touch.

Discussion

Of the three zebrafish *piezo* homologs, only *piezo2b* showed a specific neural expression pattern, appearing in the trigeminal and Rohon–Beard neurons that innervate the skin and are involved in sensing external stimuli (Prober et al., 2008). In particular, Rohon–Beard cells are specified very early during zebrafish embryogenesis and provide initial mechanosensation, typified by the light touch response (Reyes et al., 2004; Rossi et al., 2009). Similarly, *piezo2* is expressed in mammals in dorsal root ganglion neurons, which relay sensory information, such as touch, to the CNS (Coste et al., 2010). Because *piezo2b* morphants lack a light touch response, it has been suggested that this gene is involved in the mechanosensory function of this subset of neurons. A recent study in *D. melanogaster* has indicated that the fly homolog *Dmpiezo* is involved in mechanical nociception. Mutant larvae that lack *Dmpiezo* do not respond to noxious mechanical stimulation but still retain a light touch response (Kim et al., 2012). However, we found that *piezo2b* morphants responded normally in a mechanical nociception assay (tail pinch), similar to a previously reported zebrafish mutant that also lacks a light touch response (Low et al., 2010). This suggests that there is an evolutionary divergence in the sensory role played by *piezo* between vertebrates and nonvertebrates. It is unlikely that the mechanosensory function of the PIEZO protein itself differs drastically between these two species and is most likely due to a difference in sensory neural expression of *piezo* between *Drosophila* larvae and zebrafish embryos.

Another possible explanation for the lack of touch response observed in *piezo2b* morphants is that neural function in general has been perturbed in some manner. However, the fact that we could not observe any noticeable differences between *piezo2b* morphants and wild-type control embryos when we performed chemosensory or mechanical nociceptive assays indicates that this is unlikely. Furthermore, analysis of Rohon–Beard cells and neurites indicates that these appear to be unaffected by *piezo2b* knockdown, indicating that the function, rather than the development of these neurons has been perturbed in *piezo2b* mor-

phants. Interestingly, another ion channel, *trpa1b*, which plays a chemosensory role, is also expressed in Rohon–Beard neurons (Prober et al., 2008). We found that *trpa1b* morphants do not react to mustard oil stimulation but still retain a light touch response. In *piezo2b* morphants, the reverse is true: light touch sensation is lost, while chemosensitivity is retained. Because all of the *trpa1b*-positive Rohon–Beard neurons also express *piezo2b*, we concluded that knockdown of *piezo2b* does not affect the differentiation/homeostasis of the particular neurons it is expressed in or indeed the ability of these cells to sense noxious chemical stimuli. Rather, it points to a direct role for *piezo2b* in the mechanosensation of light touch. In agreement with our findings, a recent study used a combination of transgenic reporter lines to show that different subtypes of Rohon–Beard cells are present in developing zebrafish embryos (Palanca et al., 2013). Our results indicate that the chemosensory *trpa1b*-positive Rohon–Beard neurons are a subset of the light touch-sensing *piezo2b* cells.

At present, little is known about the mechanisms involved in touch mechanosensation. However, it has been suggested that stretch can evoke calcium influx in neurons (Bhattacharya et al., 2008), a feature that could potentially be regulated by stretch-induced ion channels such as *piezo2b*. Furthermore, a recent study into the neuropathy mechanical allodynia found that knockdown of *piezo2* could attenuate the light touch-evoked pain associated with this condition (Eijkelkamp et al., 2013). Consequently, identification of a specific *piezo2* blocker will have an enormous impact on the lives of patients suffering from this condition.

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