Mechanosensory transduction underlies a wide range of senses, including proprioception, touch, balance, and hearing. The pivotal element of these senses is a mechanically gated ion channel that transduces sound, pressure, or movement into changes in excitability of specialized sensory cells. Despite the prevalence of mechanosensory systems, little is known about the molecular nature of the transduction channels. To identify such a channel, we analyzed Drosophila melanogaster mechanoreceptor defects for mutants in mechanosensory physiology. Loss-of-function mutations in the no mechanoreceptor potential C (nompC) gene virtually abolished mechanosensory signaling. nompC encodes a new ion channel that is essential for mechanosensory transduction.

As expected for a transduction channel, D. melanogaster NOMPC and a Caenorhabditis elegans homolog were selectively expressed in mechanosensory organs.

Our capacity to hear a whisper across a crowded room, detect our position in space, and coordinate our limbs during a stroll through the park is conferred by the mechanical senses. Mechansensory transduction is the process that converts mechanical forces into electrical signals. When mechanoreceptors are stimulated, mechanically sensitive cation channels open and produce an inward transduction current that depolarizes the cell. The opening of mechanosensory transduction channels in vertebrate hair cells takes place within a few microseconds after the onset of a stimulus, too quickly for the generation of second messengers (1). Mechanical stimuli are therefore hypothesized to directly gate these channels. This mode of activation is in sharp contrast to other sensory modalities, such as vision, olfaction, and taste, which use stereotypical G protein–coupled cascades to modulate transduction channels.

Most models of mechanosensory signaling propose that transduction channels be anchored on both sides of the membrane, so that relative movements between the extracellular matrix and the cytoskeleton produce the mechanical tension that gates these channels. In the gating-spring model of mechanosensory transduction in vertebrate hair cells (2, 3), deflection of the mechanically sensitive hair bundle produces shear between adjacent stereocilia that stretches the gating springs. This increase in tension “pulls” the transduction channels open, depolarizes the cell, and triggers neurotransmitter release. Although biophysical data support this model for transduction in hair cells, the molecular identity of the mechanically gated ion channel remains unknown. This is largely due to the paucity of sensory tissue and the small number of transduction channels in each hair cell (4).

Genetic approaches are ideally suited for identifying rare molecules involved in mechanosensory transduction (5–10). The isolation of genetic mutations does not depend on any assumptions about the nature or abundance of the target molecules, other than loss of their function results in a recognizable phenotype. The most extensive genetic dissection of mechanosensory behavior was based on screens for Caenorhabditis elegans touch-insensitive mutants. These studies identified genes involved in the development, survival, function, and regulation of touch receptor neurons (11). Of particular interest were those that likely function in the mechanoelectrical transduction process. This group included degenerins, collagen, stomatin, and tubulins, a finding consistent with the expectation that mechanosensory signaling involves finely orchestrated interactions between ion channels, extracellular matrix, and cytoskeletal components (12).

Degenerins (MEC-4, MEC-10, DEG-1, UNC-8, and UNC-105) are a family of C. elegans ion channels related to vertebrate epithelial sodium channels (13). Because of their critical role in the touch receptor neurons, degenerins have been proposed to function as mechanosensory transduction channels (13). More recently, a C. elegans transient receptor potential (TRP) family member, OSM-9, was shown to be involved in mechanotransduction because it is expressed in sensory dendrites of a subset of ciliated sensory neurons and is required for osmosensation and nose touch (14). Although these genetic studies demonstrated the requirement for degenerins and OSM-9 in mechanoreception, there are no electrophysiological data supporting a role for these channels in the actual transduction process. Drosophila is an attractive model to dissect mechanosensation because it is possible to combine genetic manipulations with electrophysiological recordings from mechanoreceptor neurons (7). The fly’s mechanosensory repertoire includes touch, proprioception, and hearing, mediated by the complement of sensory bristles, campaniform sensilla, chordotonal organs, and type II mechanoreceptors (15). Of these, sensory bristles are particularly amenable to physiological manipulation in the intact animal. Each mechanosensory bristle organ is composed of a hollow hair shaft whose base impinges on the dendritic tip of a bipolar sensory neuron (Fig. 1A). The shaft thus acts as a tiny lever arm in which deflections of the external bristle compress the neuron’s dendritic tip and gate the transduction channels (16). The mechanosensory dendrite is bathed in an unusual high-K\(^{+}\), low-Ca\(^{2+}\) fluid (17), which provides a large positive driving force into the neuron; opening of transduction channels depolarizes the cell and promotes neurotransmitter release.

To identify components of the mechanotransduction machinery, we screened Drosophila touch-insensitive and proprioceptive mutants (7) for defects in the physiology of mechanosensory responses. Those mutants that most likely defined transduction molecules were then characterized.

Wild-type mechanosensory response. To gain electrical access to the sensory neuron,
we removed the tip of the hollow sensory bristle, placed a recording/stimulation pipette over its end, and delivered calibrated mechanical stimuli while recording transduction currents with a voltage-clamp apparatus (17, 18). We analyzed responses from wild-type Drosophila, focusing on electrophysiological features that characterize vertebrate mechanosensory transduction systems: directional sensitivity, steep displacement-response relations, submillisecond latencies between stimulus and response, and sensitivity to displacements of only a few angstroms (3, 4).

Asymmetries in the ultrastructure and transduction machinery of vertebrate mechanosensory organs endow them with directional sensitivity. We reasoned that similar asymmetries may confer directional selectivity to fly bristles (19). Mechanoreceptor currents (MRCs) were recorded from macrochaete bristles throughout the thorax, and all displayed strong directional sensitivity. For instance, when an anterior notopleural bristle was deflected toward the surface of the body, it generated a robust response (Fig. 1B). In contrast, stimuli in all other directions elicited minimal transduction currents. Hereafter, stimuli in the excitatory direction will be referred to as “positive,” and those in the opposite direction will be referred to as “negative.”

To characterize the range of responses of a macrochaete, we gave sensory bristles positive and negative step stimuli that ranged between +35 and −17.5 μm (Fig. 2A, lower traces). During positive displacements, we recorded a transient increase in the MRC that peaked at ~210 pA and was followed by a gradual, but incomplete, decline to the resting current level (Fig. 2A, upper traces). During negative displacements, only a small negative MRC was observed (~6 pA). Because the neuron adapted to this new negative position, the return of the bristle to its resting state is sensed as a positive deflection and results in a concomitant 100-pA transient current. A displacement-response curve derived from 20 thoracic bristles was fitted using a three-state model (20); the results showed that the mechanoreceptor neuron is most sensitive to stimuli between 0 and 10 μm and saturates at ~35 μm (Fig. 2B).

Recording of fly mechanoreceptor responses under conditions that allow the detection of microsecond-scale events showed latencies of ~200 μs (Fig. 2C). Because this response time is ~100 times as fast as the fastest known second-messenger cascade, fly mechanosensory transduction is unlikely to rely on second messengers.

Vertebrate hair cells detect mechanical stimuli of atomic dimensions (4). Although we were unable to deliver displacements this small, we elicited small transduction currents by stimuli of only 100 nm (Fig. 2D). Because of the lever action of the bristle shaft, however, a 100-nm stimulus at the end of a cut bristle produces a much smaller displacement at the neuronal dendritic tip. On the basis of the geometry of the fly macrochaete bristles (21), we estimate that the corresponding displacement at the base of the bristle would be ~50-fold less, or 2 nm. This level of sensitivity would allow the neuron to perceive displacements of only one-half the thickness of its plasma membrane.

Adaptation permits mechanoreceptors to continuously adjust their range of responsiveness, thus enabling the cell to detect new displacements in the presence of an existing stimulus. In vertebrate hair cells, the adaptation machinery restores nearly the full dynamic range of response with each maintained displacement (22). To investigate adaptation in fly mechanoreceptors, we measured the response to a series of test stimuli before and during adapting steps that varied between −14 and +14 μm (Fig. 3A). Responses obtained before the adapting steps were then used to produce an A(X) curve that was shifted along the displace-
ment axis to fit the data generated during each adapting stimulus (Fig. 3B) (22). By plotting the size of the shift as a function of the size of the adapting step, we measured how much of the cell’s response is retained at each adapting step. The adaptation process preserved ~85% of the dynamic range (slope = 0.85; Fig. 3C). Incomplete adaptation may allow the cell to continue to “perceive” the sustained stimulus yet remain receptive to new stimuli. This level of adaptation closely resembles that seen in vertebrate hair cells (22); the similarity extended to the time course (time constant = 18 ms) of the adaptation process (Fig. 3D) (22). Together, these results suggest that the core transduction components in fly bristles and vertebrate hair cells are functionally related.

**nompC mechanosensory responses.** To identify components of the transduction machinery, we screened 27 different Drosophila mechanosensory transduction mutants (7) for defects in transduction currents. On the basis of uncoordinated phenotypes, these mutants fell into 20 complementation groups (23). One of these, nompC, was particularly interesting. At a behavioral level, three of the nompC alleles showed severe uncoordination, whereas another (nompC2) showed moderate clumsiness. The three severe mutants (nompC1, nompC2, and nompC4) displayed a dramatic loss of MRC, with transduction currents of ~10% that of control animals (Fig. 4, A and B). In contrast, the nompC4 allele exhibited almost normal MRC amplitudes but displayed severely defective adaptation. The time constant of adaptation in nompC4 was 50 ms, versus 277 ms for control flies (Fig. 4C). Because the MRC and the adaptation process are intimately tied to the function and regulation of the mechanically gated ion channel, we suspected that the nompC gene product was either a component of the adaptation machinery or a transduction channel.

Why are nompC flies behaviorally uncoordinated, given that they have normal response amplitudes? One possibility is that the abnormally fast decay of the MRC would decrease the number of action potentials by limiting the...
time in which the cell is depolarized. To test this postulate, we stimulated control and nompC4 animals with a step stimulus while recording action potentials through the bristle (17). As hypothesized, the number of action potentials in nompC4 was less than half that of control flies (Fig. 4, D and E). These results explain the behavioral phenotype of nompC4 and further support nompC as a critical player in the transduction process.

Mapping, rescue, and cloning of nompC. nompC was mapped to position 25D7 on the left arm of the second chromosome (Fig. 5A). Three overlapping cosmids clones spanning this interval (Fig. 5A) were tested for rescue of the nompC phenotype by P element-mediated germ line transformation (24). Cosmid C fully rescued the physiological and behavioral defects of nompC mutants (Fig. 5B). Sequences from cosmid C were used to screen a Drosophila antennal cDNA library (25), and two 6.1-kb cDNAs were isolated. Sequence analysis of the full 33-kb cosmid and the two cDNA clones showed a single transcriptional unit encoding a predicted polypeptide of 1619 amino acids (Fig. 5C). This gene is split into 13 exons, spanning 18 kb of genomic DNA. Using the polymerase chain reaction (PCR), we isolated this candidate gene from nompC1, nompC2, nompC3, and nompC4 mutants and determined their nucleotide sequences. All four alleles have single base changes that result in either nonsense or missense mutations.

Fig. 5. Identification of the nompC gene. (A) Genetic and molecular characterization of the nompC interval. nompC was localized to the 25D7 region of the second chromosome by deficiency mapping. Df(2L)tkv2 and Df(2L)clh2 deleted or disrupted nompC; Df(2L)clh2−5 and Df(2L)clh2−4 complemented the nompC phenotype; I(2)25Sc failed to complement nompC (deleted segments are indicated by thin lines). A phage clone (l79) from a nearby chromosomal walk (39) was used as a starting point for isolating cosmids A through C (24). Arrows depict the orientation of predicted transcriptional units from cosmid C. (B) Cosmid C rescued the physiological and behavioral defects of all nompC alleles. (C) The diagram shows the structure of the nompC locus. The gene is divided into 13 exons, producing a 6.1-kb transcript. The structure was derived by comparison of genomic and cDNA sequences. ATG and TAA refer to the initiator and terminator codons, respectively. The location and nature of the mutations in the four nompC alleles are shown above the gene map.

Fig. 6. nompC encodes a new ion channel. (A) NOMPC is a 1619- amino acid protein (26) with 29 ANK repeats (blue boxes) and six predicted transmembrane domains (black boxes); P refers to putative pore region. The four nompC mutations are indicated above the protein feature map. (B) Alignment of D. melanogaster and C. elegans NOMPC proteins. The two sequences display 41% identity (black shading) and 58% similarity (gray shading). The 29 ANK repeats are indicated by blue boxes; the six predicted transmembrane domains (S1 through S6) are indicated by black bars above the sequence. On the basis of similarity to other ion channels, a proposed pore region (P) was assigned between S5 and S6.
nompC each have nucleotide changes that introduce premature termination codons; in contrast, nompC has an A → T change at residue 4820 that results in a C → Y change at amino acid residue 1400 (26) (Figs. 5C and 6A).

A search of protein and nucleotide databases revealed that the NOMPC gene encodes a previously unidentified ion channel with an exceptional feature: the 1150 NH₂-terminal amino acid residues consist of 29 ankyrin (ANK) repeats (Fig. 6B). ANK repeats are 33-residue motifs that mediate specific protein-protein interactions with a diverse repertoire of macromolecular targets (28). Although we do not know the function of the ANK repeats in NOMPC, it is notable that ANK repeats are particularly prominent in the assembly of macromolecular complexes between the plasma membrane and the cytoskeletal network (29).

TRPs are a diverse family of cation channels found in both vertebrates and invertebrates and are implicated in calcium signaling (30), pain transduction (31), and chemosensory transduction (14). In all, pairwise comparison between the channel domains of NOMPC and the various TRP family members revealed ~20% identity (~40% similarity), establishing NOMPC as a new distant member of this channel family (27).

NOMPC is expressed in mechanosensory organs. To examine the expression pattern of the nompC transcript, we performed RNA in situ hybridizations to tissue sections of late-stage pupae (25). We found that NOMPC is selectively expressed in ciliated mechanosensory organs, including microchaetes (Fig. 7A), macrochaetes (Fig. 7B), and bristles on the fly’s proboscis (Fig. 7C). Control hybridizations with sense probes produced no specific signals in any of these cells (32). Given the strong uncoordinated phenotype of nompC mutants, we reasoned that nompC should also be required in proprioceptive organs, which include the ciliated chordotonal neurons. Indeed, NOMPC is expressed in chordotonal organs of the halteres (Fig. 7D), as well as in the leg joints and Johnston’s organ (32). The expression profile of nompC in mechanoreceptive bristles and chordotonal organs accords with the physiological (loss of MRC) and behavioral (uncoordinated) phenotypes of nompC mutants and supports NOMPC as a mechanosensory transduction channel.

We wondered why Ce-NOMPC was not isolated in the various screens for C. elegans touch-insensitive mutants. As it turns out, body-touch sensitivity in C. elegans is mediated by nonciliated touch cells. To determine the expression profile of the C. elegans nompC gene, we fused 4.5 kb of upstream sequences and the first four ANK repeats of Ce-NOMPC to a green fluorescent protein (GFP) reporter (24). The construct was injected into worms, and the transformed progeny was inspected for GFP expression. Multiple transformants were examined, and in all cases, fluorescent signals were observed in CEPV, CEPD, and ADE neurons (Fig. 7, E through G). These mechanosensory neurons have ciliated dendrites and may be the functional equivalent of the fly ciliated mechanosensory neurons (33, 34). Notably, the C. elegans NOMPC-GFP fusion localized to the sensory dendrites, the proposed site of mech-
anosensory transduction in these cells (Fig. 7F).

Concluding remarks. Several lines of evidence support NOMPC’s role as a mechanosensory transduction channel. First, at the primary sequence level, NOMPC has similarity to bona fide ion channels. Second, loss-of-function mutations in nompC virtually eliminate mechanotransduction responses, and a single point mutation in the channel alters the behavior of the transduction currents. Third, nompC is selectively expressed in mechanosensory organs in Drosophila. Furthermore, the C. elegans homolog localizes to the presumed site of mechanoelectrical transduction. Last, it is expected that transduction channels are tethered to the cytoskeleton; the 29 ANK repeats of NOMPC are ideally suited to interact with the cytoskeleton and transduction partners. This number of ANK repeats is selectively expressed in mechanosensory organs in Drosophila. Additionally, the C. elegans homolog localizes to the presumed site of mechanoelectrical transduction. Last, it is expected that transduction channels are tethered to the cytoskeleton; the 29 ANK repeats of NOMPC are ideally suited to interact with the cytoskeleton and transduction partners.

References and Notes

18. Unless indicated otherwise, all wild-type recordings were made from the Canton S strain. Other strains and stocks were obtained from either the Bloomington Dro-
ophila Stock Center, J. Sidonya, or L. Lorenzo were generated in our laboratory. Mechanosensory mutants were prepared for recording, and pipette solutions were as described (7). In this preparation, we clamped the transmembrane potential (TEP) at +40 mV, the average TEP for wild-type bristles. The recording configuration consisted of two recording reference electrodes placed in the thorax of the fly and a recording/stimu-
lation electrode slipped over the end of a cut bristle, thus making a circuit across the sensory epithelium through the recording electrode. Voltage and current re-
sponses were measured through a modified headstage with a voltage-clamp amplifier (AxoPatch 1-D, Axon Instruments, Foster City, CA) in either current- or volt-
age-clamp mode to minimize capacitive and compensation. Responses were low-pass filtered at half-power frequencies of 100 to 10,000 Hz before sampling at intervals of 6 to 2000 μs. After subtraction of 4-mV liquid junction potential, the resting potential across the mechanosensory epithelium was, on average, 41 mV. Movements of the recording electrode were driven by a piezoelectrical stage (PZS-100 HS, Burleigh Instruments, Fishers, NY). To eliminate mechanical res-
olution of the pipette, input signals driving the piezo-
electrical device were low-pass filtered at a half-power frequency of 100 Hz (10 kHz for Fig. 2C, 1 kHz for action potentials) with an eight-pole Bessel filter (Med-
el 3282, Krohn-Hite, Avon, MA). In Figs. 1 through 7, the stimulus trace represents the driving voltage to the piezoelectric device. Displacements of the stimulus probe were calibrated with an etched micrometer grid. Bristles were displaced over a range of 35 μm. The bristle position faithfully followed that of the stimulat-
ing electrode.
24. nompC corresponds to (IQ)25D0c DNA cloning, se-
quencing, characterization of mutant alleles, and Drosophila melanogaster (Dm) transformations were per-
25. nompC cDNAs were identified with a 0.7-kb probe from exon 12 to screen an antennal cDNA library. The nompC gene structure and protein sequence were predicted by the program FGENESH and modified by the deletion of a 70–amino acid sequence (the end of exon 8 and the beginning of exon 9), which introduced a hydrophilic segment in the midst of an ANK repeat that was inconsistent with Dm-NOMPC’s structure. Extension of exon 19 by 54 base pairs added a late amino acid residues with homology to Dm-NOMPC followed by three stop codons. A translational fusion of Ce-
nompC and GFP was constructed with a GFP expres-
sion vector, pD95S81. A 62-kb Ce-nompC sequence was amplified by long-range PCR from genomic DNA (wild-type strain N2) with a primer 4.5 kb upstream of the presumptive initiator methionine and a primer corre-
sponding to the end of the third exon. Clones were sequenced at the site of insertion to ensure proper orientation of the insert within the vector. Germ line transformation was performed as described (35). Worms from six independent transgenic lines were viewed by fluorescence microscopy; cell position and morphology were used to identify neurons.
27. Single-letter abbreviations for the amino acid resi-
dues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
35. E. R. Savin, thesis, Massachusetts Institute of Tech-
nology (1996).
37. Using a variety of screening strategies, we searched genomic and cDNA libraries for homologs of NOMPC but did not identify additional related molecules. Searches of the 90% identity-homolog genomic sequence also failed to identify NOMPC homologs. Additional related channels, however, may reside in the unsequenced gaps, or a different type of chan-
el may mediate the current remaining in nompC mutants.
42. We acknowledge M. Kernan for introducing us to Dro-
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