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Review

Transient Receptor Potential Ion Channels and Animal Sensation: Lessons from *Drosophila* Functional Research

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Ion channels of the transient receptor potential (TRP) superfamily are non-selective cationic channels with six transmembrane domains. The TRP channel made its first debut as a light-gated Ca^{2+} channel in *Drosophila*. Recently, research on animal sensation in *Drosophila* disclosed other members of the TRP family that are required for touch sensation and hearing as well as the sensation of painful stimuli.

Keywords: *Drosophila*, Animal, Sensation, TRP, Channel

Functional genomics research (aiming at understanding gene function in an organismal level, which is practically impossible with humans) requires simple genetic model animals. As the gene function and molecular pathways are proven to be conserved across phyla, animal models provide useful information on human genes. The completion of genome sequencing, identification of many cDNA expressed, and systematic disruption of many genes by P-element based technology, all of these make functional genomics research in *Drosophila* much easier than in the past. In *Drosophila* sophisticated molecular genetic tools (such as mosaic analysis and modular expression system) were developed; for example, a single gene can be destroyed in a specific area or cells of organs in an otherwise wild-type animal, allowing for the dissection of the gene function even lethal to animals. Particularly useful to a neurobiologist is the availability of electrophysiological recordings from diverse sensory neurons, including photoreceptors, mechanosensory bristles, and hearing organs. Thus, *Drosophila* affords unparalleled advantages as a model animal for functional research, especially in the area of sensory biology.

Drosophila can see, feel pain and touch, detect temperature, and hear sound so that the molecular components and mechanisms underlying these sensory signalings can be identified and dissected by a functional analysis. Isolation and characterization of components that are involved in the visual transduction pathway in *Drosophila* began 30 years ago. A significant amount of information has been accumulated, allowing us to draw a rather detailed phototransduction cascade (Pak and Leung, 2003). Since *Drosophila* visual transduction employs phosphoinositide signaling, it has provided a model for the lipid signaling pathway *in vivo* (Montell, 1999; Minke and Cook, 2002). However, studies of other sensory modalities, including pain, touch, temperature and hearing, have recently begun in *Drosophila*.

A mutant fly, named *transient receptor potential* (*trp*), was discovered which was defective in phototransduction (Fig. 1) (Cosens and Manning, 1969). The responsible *trp* gene (Montell and Rubin, 1989; Wong *et al.*, 1989) revealed a novel protein with multiple transmembrane domains. A molecular genetic analysis showed that the *trp*-encoding channel was gated by light stimulation in *Drosophila* (Hardie and Minke, 1992). Later, related genes, *trpl* (*trp*-like) and *trpγ*, were identified (Phillips *et al.*, 1992; Xu *et al.*, 2000). The *trp*, *trpl*, and *trpγ*- encoding channels are topologically similar to voltage-gated Na^+ and Ca^{2+} channels, but lack charged amino acids in the voltage sensor (Armstrong and Hille, 1998). This suggests that the *trp*, *trpl* and *trpγ*-encoding channels may not be gated by voltage but by ligands. Indeed, the *trp* and *trpl* channels are not activated by voltage but by down-stream components of PLC-β signaling (Hardie and Minke, 1992). While the diverse sensory modalities apparently look different, it was a surprise that the ion channels that were recently cloned from a touch insensitive mutant *no mechanoreceptor potential* C (*nompC*) and a pain mutant *painless* in *Drosophila* are both distinct members of the TRP superfamily (Kernan *et al.*, 1994; Walker *et al.*, 2000; Tracey *et al.*, 2003). Another *Drosophila* TRP channel belonging to the TRPV family was recently shown to be required for hearing, which was named *nanchung* (Korean meaning deafness) (Kim *et al.*, 2003). Six temperature-gated channels,

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recently identified by whole cell recordings in patch clamp configuration in mammals, are also members of TRP superfamily (Caterina *et al.*, 1997; Caterina *et al.*, 1999; Guler *et al.*, 2002; McKemy *et al.*, 2002; Peier *et al.*, 2002a, 2002b; Smith *et al.*, 2002; Watanabe *et al.*, 2002; Xu *et al.*, 2002; Story *et al.*, 2003; Voets and Nilius, 2003). Altogether, it appears that the TRP channels evolved to carry out sensing of the environment. In this review, I will describe *Drosophila* research on phototransduction, pain and mechanosensation with special focus on TRP channels.

Phototransduction

Most of the components that are involved in phototransduction were discovered from an extensive genetic screening in the late sixties and seventies (Pak, 1995; Pak and Leung, 2003). A functional analysis of these components revealed the pathway of the ubiquitous phosphoinositide signaling *in vivo* and identified the *trp*-encoding protein as a light gated ion channel (Montell, 1999; Minke and Cook, 2002; Pak and Leung, 2003).

Isolation of components Behavioral assays, lacking phototaxis or optomotor responses, led to some defective mutants in phototransduction (Hotta and Benzer, 1969; Pak *et al.*, 1970; Heisenberg, 1971). However, many useful mutants in phototransduction were isolated by inserting electrodes into the eyes of individual mutagenized flies and measuring light-evoked neuronal depolarization, called electroretinogram (ERG), of the photoreceptors (Fig. 1). Ten years of painstaking efforts of ERG-based mutant screening of approximately 10^5 flies led to the identification of some 200 ERG defective mutants (Pak and Leung, 2003). From these mutants, corresponding genes were cloned and their functions were genetically characterized, showing that they play critical roles in phototransduction (Pak 95).

A fly mutant *norpA* (*no receptor potential A*), isolated both by behavioral and ERG-based screenings, exhibits no response to light (Fig. 1) (Hotta and Benzer, 1969; Pak *et al.*, 1970; Heisenberg, 1971). The corresponding *norpA* gene encodes PLC-β (Bloomquist *et al.*, 1988), showing that the PLC-β pathway is involved in visual transduction in *Drosophila*. An important category of defective mutants in phototransduction was isolated from defects in the prolonged depolarizing afterpotential (PDA) (Fig. 1). In wild-type flies, a blue light generates photoactivated metarhodopsin, which is remained stable in the dark and thereby continues activating downstream signaling molecules in the dark, which generates the prolonged depolarizing after-potential (PDA) (Pak, 1995). One class of PDA defective mutants was named *ina* (inactivation but no afterpotential) since they possessed inactivation of photoreceptors but lost the PDA. The other class was named *nina* (neither inactivation nor afterpotential) since they lacked both the PDA and inactivation. Molecular

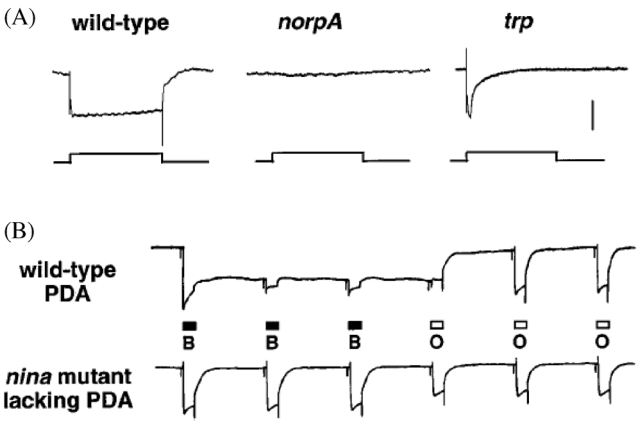

Fig. 1. Electroretinogram recordings (ERG) of wild-type and phototransduction mutants (derived with permission (Montell, 1999)). (A) Five-second pulse of bright white. Wild-type photoreceptors respond to light, while *norpA* and *trp* exhibit no response and transient receptor potential, respectively. (B) Consecutive pulses (4 seconds) of intense blue (B) and orange (O) light. The PDA (prolonged depolarization afterpotential) is formed by the stable blue light-activated form of rhodopsin (metar-rhodopsin). PDA is seen in the wild type, but not in *nina* mutants.

cloning revealed that the *ina* class included eye-specific PKC mutants (*inaC*) and the PDZ-domain containing scaffolding protein INAD (*inaD*). The *nina* class includes rhodopsin (*ninaE*), photoreceptor-specific cyclophilin (*ninaA*), and myosin III (*ninaC*).

The *trp* fly, was first identified from a spontaneous mutant fly (Cosens and Manning, 1969) and later isolated from ERG-based screening (Pak and Leung, 2003). The *trp* photoreceptors exhibit rapid decay of receptor potentials during a prolonged stimulus, thus named *transient receptor potential* (Fig. 1) (Cosens and Manning, 1969; Minke and Cook, 2002). However, double mutants of *trp* and *trpl* lacked complete light response of ion influx (Niemeyer *et al.*, 1996; Reuss *et al.*, 1997; Scott *et al.*, 1997), showing that *trp* and *trpl* fulfill most of light-gated ion channels.

Phototransduction-an overview A cascade of phototransduction, occurring in the rhabdomere (photoreceptor membrane) of *Drosophila* eyes, begins with a photon inducing photoisomerisation of rhodopsin to metarhodopsin (M-Rh, encoded by *ninaE* gene) (Fig. 2). The activated metarhodopsin interacts with a heterotrimeric Gq protein to cause the exchange of GDP for GTP on the Gqα subunit, releasing the Gqα subunit. The Gqα effector is PLC-β; namely, Gqα activates the PLC-β (*norpA* gene), which in turn hydrolyzes a phospholipid PIP_2 (phosphatidyl inositol 4,5, bis-phosphate), generating two potential second messengers, IP_3 (inositol 1,4,5-tris-phosphate) and DAG (diacylglycerol). Three light-sensitive channels (*trp*, *trpl*, *trpγ* genes) are activated by downstream components of PLC-β

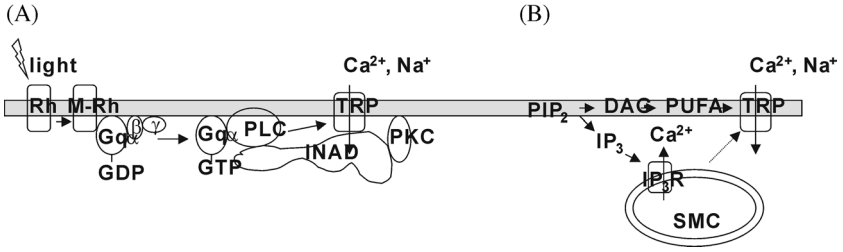

Fig. 2. Elements of the *Drosophila* phototransduction cascade (A) and pathways leading to the activation of TRP ion channels (B). PUFAs (polyunsaturated fatty acids) activate TRP channels while activation of TRP channels by store depletion is questionable (Hardie, 2003).

catalysis. Eye-specific protein kinase C (PKC) (*inaC*), calmodulin and myosin III (*ninaC*) are involved in deactivation and adaptation but not in activation (Smith *et al.*, 1991b; Hardie *et al.*, 1993; Porter and Montell, 1993; Porter *et al.*, 1995). A striking feature of this signaling is that the signaling molecules - rhodopsin, *trp* and *trpl* encoding channels, PKC, calmodulin, myosin III, and PLC-β are all assembled into a signaling complex termed a signalplex (Montell, 1998) or transducisome (Tsunoda *et al.*, 1997) by the scaffolding protein, INAD (Huber *et al.*, 1996; Chevesich *et al.*, 1997; Li and Montell, 2000). The signaling complex may account for the rapid activation and inactivation of phototransduction.

Mechanism of TRP channel activation Hardie and Minke showed that the major *trp* defect was in the Ca^{2+} permeability of the light-sensitive conductance, suggesting that the *trp* gene may encode the light-gated Ca^{2+}-permeable channel (Hardie and Minke, 1992). Indeed, heterologous expressions of both *trp* and *trpl* cDNAs in various cell lines have shown that the *trp* and *trpl*-encoding channels possess cation channel activities (Minke and Selinger, 1996; Montell, 1997; Xu *et al.*, 1997).

How are the *trp* and *trpl* channels activated in phototransduction? Since PLC-β produces second messengers, IP3 and DAG, it is plausible to think that the *trp* and *trpl*-encoding channels could be activated by either IP3 or the DAG dependent pathway. Heterologous expression studies *in vitro*, where the depletion of Ca^{2+} in internal stores upon addition of thapsigargin activates the *trp* channel (Vaca *et al.*, 1994; Xu *et al.*, 1997), support the supposition of the IP3 pathway as an activator of TRP channels. However, many observations challenge this view (Hardie, 2003). First, depleting internal stores using ionomycin or thapsigargin did not activate currents in photoreceptors (Ranganathan *et al.*, 1994). Second, introduction of caged IP3 into the photoreceptors by whole cell recording pipette also failed to activate the channels (Hardie and Raghu, 1998). Most importantly, null mutations in the IP3 receptor gene of *Drosophila* had no effect on phototransduction (Acharya *et al.*, 1997; Raghu *et al.*, 2000). These results suggest that IP(3)R-mediated store depletion is unlikely to underlie the TRP and TRPL activation in *Drosophila* photoreceptors.

Supporting the DAG pathway as an activator of TRP channels, Chyb *et al.* (1999) showed that both the *trp* and *trpl*-encoding channels are activated by polyunsaturated fatty acids (PUFAs), which are likely products of DAG hydrolysis. An analysis of the *rdgA* (*retinal degeneration A*) mutant also supports the DAG pathway. The *Drosophila rdgA* gene encodes the diacylglycerol kinase (DGK) that converts DAG to phophatidic acid (PA) and thus reduces DAG concentration. In *rdgA* flies, in which DAG would be higher, the light-sensitive TRP channel was constitutively active (Raghu *et al.*, 2000). Moreover, *rdgA* flies exhibited early onset retinal degeneration and blindness (Masai *et al.*, 1993). Importantly, the *rdgA;trp* double mutants were rescued, indicating that the degeneration caused by the *rdgA* mutation was by Ca^{2+} influx through the TRP ion channel (Raghu *et al.*, 2000). These observations can best be explained by the DAG pathway activating TRP channels. However, the activation route of PKC by DAG as being an activator of the *trp* and *trpl* is ruled out since eye-specific PKC mutants, *inaC*, display defects in response termination but not in activation (Smith *et al.*, 1991a; Hardie *et al.*, 1993).

Pain Sensation

In animals, painful stimuli are detected by specialized neurons known as nociceptors. A great contribution to pain signaling was made when Caterina *et al.* (1997) cloned an ion channel that was activated by capsaicin (a spicy component of peppers), based on a functional assay *in vitro*, revealing that the channel belongs to the TRP superfamily. In heterologously-expressed cells, the capsaicin receptor (TRPV1) channel is gated by stimuli including noxious heat (>43°C), protons, and capsaicin (Caterina *et al.*, 1997; Tominaga *et al.*, 1998). A related channel, TRPV2, was subsequently cloned by sequence homology and shown to be activated at higher temperatures (>52°C) (Caterina *et al.*, 1999). More recently, channels of the TRPV, TRPM, and TRPN subfamily were identified to be activated by warm (~27-34 °C, ~34-38°C), cool (<~24-28°C) and cold (<17°C) temperatures, respectively. This leads to the suggestion that some ion channels of the TRP family may play a central role in sensing temperature and pain sensation (Montell, 2003;

Voets and Nilius, 2003).

Painless Recently, Tracey *et al.* (2003) described a stereotypical rolling behavior of *Drosophila* larvae in response to noxious heat stimuli >38ºC. Utilizing this pain behavior, they screened mutants with impaired sensitivity to noxious heat and identified 49 such mutants among 1500 EP lines that were screened. One of them, named *painless*[1], exhibits impaired pain sensation while it displays normal mechansensation. However, *painless*[1] elicited a rapid response at >52ºC, indicating that another channel might mediate more intense pain sensation at >52ºC. Notably, the *painless*[1] function is required for mechanical nociception; *painless*[1] larvae respond little to the mechanical forces that are generated by 45 mN Von Frey filaments (0.2 mm diameter), but vigorous rolling was observed in most of the wild-type larvae.

Performing electrophysiological recordings of peripheral sensory neurons from dissected abdominal nerves in third instar larvae, Tracey *et al.* (2003) showed that the mean bulk spiking frequencies of nerves from the wild-type increased 2.6 ± 0.8 times at 38ºC-42ºC when compared to room temperature, but the *painless*[1] did not. This electrophysiological measurement directly demonstrates that *painless* is required for the excitatory response of sensory neurons to noxious heat *in vivo*. However, whether or not the Painless protein directly mediates ionic currents by noxious temperature has not yet been determined.

The *painless* encodes a member of the TRP family and is most closely related to NOMP-C in *Drosophila*. Even though the *painless* is expressed in multidendritic and chordotonal sensory neurons, the multidendritic neurons are probably responsible for pain sensation since chordotonal neuronal signaling is not involved in pain sensation (Kim, 2003). Interestingly, the painless protein was present in the spots that were juxtaposed along the dendritic arbor.

Mechanosensation

Mechanosensation takes on many forms, including proprioception (sensation of body movement), sensation of touch, balance, and hearing. The molecules and mechanisms underlying mechanosensation in vertebrates are largely unknown due to the experimental difficulties that are inherent in the mechano-sensory neuronal system, which is not localized but dispersed throughout the body. A breakthrough in this field was made again by genetic screening using *C.elegans* and *Drosophila*, uncovering a large number of mutants that are defective in touch sensation. Recently, positional cloning of a corresponding gene from a touch insensitive mutant *no mechanoreceptor potential C* (*nompC*) in *Drosophila* revealed that *nompC* encodes a member of the TRP superfamily (Kernan *et al.*, 1994; Walker *et al.*, 2000). On the other hand, Kim *et al.* (2003) demonstrated that an ion channel belonging to TRPV family in *Drosophila* is required for hearing and some proprioception.

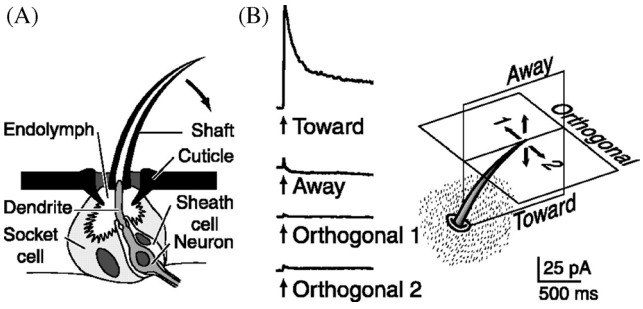

Fig. 3. Diagram of a *Drosophila* bristle sensory organ (A) and directional sensitivity of a bristle to mechanical stimuli (B) (derived with permission (Walker *et al.*, 2000)). (A) The ciliated dendritic tip is bathed in an unusual high K^+ endolymph, which creates a TEP of +40 mV. Displacement of the bristle compresses the sensory cilia and thereby gates mechanosensitive channels, presumably located on the cilia. Clipping the bristle and placing a recording electrode over the tip allows electrical recording. (B) Step stimuli of 20 μm toward the body elicited a robust 100-pA transient current.

Electrophysiology of bristle recording Fruit flies are covered on the skin with a number of bristles, most of which mediate touch sensation. Underneath the hollow bristle (hair shaft) are three cells: the socket cell, the sheath cell, and a ciliated mechanosensory neuron (Fig. 3). Deflection of the external bristle compresses the neuron's cilia and thereby activates a transduction channel to be gated. The mechanosensory cilia is bathed in an unusual high-K^+, low-Ca^{2+} endolymph (Corfas and Dudai, 1990), which is interestingly similar to the endolymph surrounding stereocilia of sensory hair cells in the inner ear of mammals (Anniko and Wroblewski, 1986). The composition of endolymph creates a TEP (transepithelial potential) of +40 mV and a large (~120-mV) driving voltage into the neuron (Walker *et al.*, 2000).

Because of the hollow bristle, electrophysiological measurements are possible with the tip of the bristle clipped and a recording/stimulation pipette placed over its end (Kernan *et al.*, 1994; Walker *et al.*, 2000). Transduction currents are recorded by applying calibrated mechanical stimuli to the bristle in a voltage-clamp set-up. Waker *et al.* (2000) measured wild-type mechanosensory currents with the TEP clamped at +40 mV during each 700-ms stimulus. Displacements toward the body of the fly elicited a robust 100-pA transient current, whereas stimuli away from any other direction produced little response. The latency of the response was measured to be as fast as ~200-μs. The responses of bristle neurons were sensitive to nanometer deflections that were imposed on the sensory cilia. Strikingly, these electrophysiological properties were similar to those of the sensory hair cells in the inner ear of mammals (Strassmaier and Gillespie, 2002).

NOMPC To identify the molecules that are involved in mechanosensory transduction, Kernan *et al.* (1994) initially screened mutagenized fly larvae for defects in withdrawal from a gentle touch. Later, they screened adult mutant flies for uncoordinated behaviors since touch insensitive mutants exhibit uncoordination. This screening led to the identification of 20 complementation groups, including *uncoordinated* (*unc*), *uncoordinated-like* (*uncl*), *no mechanoreceptor potential* (*nomp*), and *reduced mechanoreceptor potential* (*remp*). In the *unc*, *uncl*, and *nomp* mutants, mechanoreceptor potentials of the bristles were reduced or absent (Kernan *et al.*, 1994). The touch insensitivity and uncoordination that was exhibited by these mutants suggest that tactile and proprioceptive sensations are both impaired.

Three of the *nompC* alleles exhibited severe uncoordination, whereas another allele (*nompC*^{4}) showed moderate clumsiness. The three severe mutants displayed a dramatic loss of MRC (mechano-receptor currents) while the *nompC*^{4} allele exhibited almost normal MRC amplitudes, but, interestingly, displayed severely defective adaptation (Walker *et al.*, 2000). Since adaptation is intimately linked to the channel gating, the *nompC*^{4} phenotypes suggest that the *nompC* gene product is a transduction channel.

The *nompC* gene encodes an ion channel of the TRP superfamily with 29 ankyrin (33-residue motifs) repeats (Walker *et al.*, 2000), which implicates in the assembly of macromolecular complexes with the cytoskeletal network (Luna, 1991). A database search in other animals identified homologous molecules in *C. elegans* and fish, but not in other vertebrates. A *C.elegans* NompC-GFP fusion protein is localized to the sensory cilia (Walker *et al.*, 2000), a probable site for mechanotransduction to take place. However, the localization of *Drosophila* NompC has not yet been determined. Recently, zebra fish NOMP-C was shown to be required for hearing (Sidi *et al.*, 2003), but strangely the mammalian counterpart has not yet been found. Whether or not NompC possesses channel activities has not yet been determined.

***Drosophila* hearing** *Drosophila* can hear sound, which plays a critical role during courtship (Hall, 1994). Approaching to a female, an activated male fly extends and vibrates one of its wings, thereby emitting a species-specific song, colloquially known as 'love songs' (Shorey, 1962; Bennet-Clark and Ewing, 1970). Hearing this sound, females become receptive to copulation (Hall, 1994; Greenspan and Ferveur, 2000).

The main component of the 'love songs' is the pulse song with an ~34 ms interval between 5- to 10-ms pulses (Hall, 1994). A second component represents the sine song, a 160-Hz sinusoidal hum, which appears to function as a precourtship stimulatory signal (Von Schilcher, 1976b; Tauber and Eberl, 2001). A third sound is produced by a courted male to give a rejection signal (RS) to a courting male (Paillette, 1991). Right after hearing the RS, the courting male abruptly stops courting behavior.

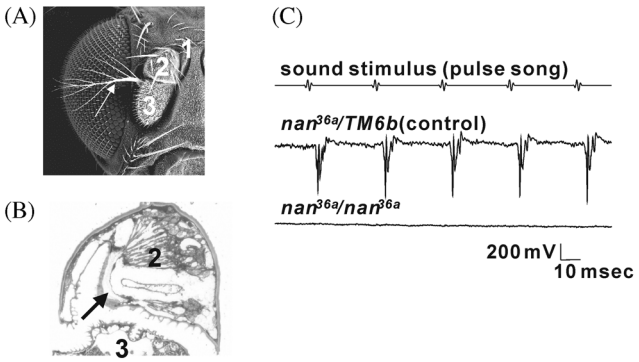

Fig. 4. *Drosophila* hearing organ (A and B). The *nanchung* mutants lack sound-evoked potential (C) (derived with permission (Kim *et al.*, 2003). (A) Sound induces the vibration of arista (arrow)/third segment (labeled 3) of the antenna. (B) Scolopidia in the second segment is connected to the cuticle originating from the third segments at the joints (arrow). (C) Pulse song induces auditory response in wild-type flies, but not in *nan* mutant flies.

Early 1960 and 1970 studies established that the *Drosophila* antennae serve as 'love song' detectors (Ewing, 1978). The antennae are composed of three segments including (from proximal to distal) the first (scape), second (pedicel), and third segments (funiculus) to which arista, an elongated and branched lateral process, is attached (Fig. 4). Ablation of either the third segment (funiculus) or the arista impairs female's receptivity and antennal mutants *aristaless*, in which antenna is transformed into legs, reduce the females receptivity (Manning, 1967). Electrophysiological recordings that demonstrated that Johnston's organ, a mechanosensory chordotonal organ in the second segment (pedicel) of the antenna serves as the auditory sensory organ (Ewing, 1978). Recently, Eberl *et al.* showed that sound-evoked potentials are absent in atonal mutant flies that lack Johnston's organ (Eberl *et al.*, 2000), confirming the notion that Johnston's organ is the *Drosophila* hearing receptor. The arista and third segment are the sound receiver that oscillates in response to the 'love song' (Manning, 1967; Bennet-Clark and Ewing, 1970; Gopfert and Robert, 2002), which causes the sensory cilia of chordotonal neurons in the second segment of the antenna to be mechanically stretched (Caldwell and Eberl, 2002).

Auditory mutants Eberl *et al.* (1997) screened mutagenized flies aiming at isolating components in the auditory transduction pathway by using an auditory behavior assay based on the observation of von Schilcher (Von Schilcher, 1976a), who found that a group of males would vigorously court one another if presented with the pulse song. From 400 homozygous viable lines that were screened, 15 lines were isolated that displayed consistent failure in the auditory behavior response (Eberl *et al.*, 1997). Among the 15 lines, only one line, 5P1, gave no response to the sound and was named *beethoven* (*btv*)

(Eberl *et al.*, 2000). The rest of the courtship-defective lines showed no apparent defects in sound-evoked potentials, implying that the failure to evoke courtship behavior is caused by defects other than auditory transduction. The *btv* mutants showed normal mechanoreceptor potentials in bristles (Eberl *et al.*, 2000), indicating that *btv* is specific to chordotonal mechanosensation. A second gene affecting chordotonal organs but not bristle-mediated mechanosensation is *touch-insensitive larva B* (*tilB*) (Eberl *et al.*, 2000). The *tilB*/*Y* hemizygous males show a complete absence of sound-evoked courtship behavior and no sound-evoked potentials. The *tilB* and *btv* mutants exhibited axonemal defects of the sensory cilia (Eberl *et al.*, 2000), suggesting that axoneme of chordotonal cilia plays a critical role for mechanotransduction. However, the corresponding genes to *btv* and *tilB* have yet to be cloned to molecularly characterize their functions.

Since touch response and hearing are both mechanosensation, Eberl *et al.* (2000) examined whether mutants (*unc*, *nomp*, *remp* class mutants) that eliminated bristle receptor potentials also abolished the sound evoked response. Most of the mutants that were defective in bristle-mediated mechanosensation were also defective in sound-evoked auditory response. This suggests that both the mechanosensation mediating touch and sound share many components for mechanotransduction to be carried out.

Nanchung The nature of transduction channels mediating mechanotransduction in Johnston's organ of the antenna is largely uncharacterized. Even though *nompC* encodes a TRP channel that is required for mechanosensation in *Drosophila* bristle organs, the discovery that sound-evoked potential is unaffected in *nompC* (Eberl *et al.*, 2000) suggests that another channel must constitute the mechanosensor in Johnston's organ of *Drosophila*. Recently, Kim *et al.* (2003) demonstrated that the TRPV family ion channel encoded by *nanchung* is required for hearing transduction. The Nanchung channel is gated in response to membrane swelling induced in a hypotonic solution. The increase of intracellular Ca^{2+} concentration is due to the influx of an extracellular Ca^{2+} since EGTA in extracellular solution abolished Ca^{2+} increase in cells. The Nan channel mediates Na^{+} and K^{+} currents, as shown by whole-cell recordings in patch clamp configuration. Interestingly, the Nan channel is expressed exclusively in chordotonal neurons, suggesting that other mechanosensitive neurons in *Drosophila* use other mechanosensitive channels. Importantly, the Nan protein is specifically localized to the sensory cilia of chordotonal neurons in Johnston's organ. Since the sensory cilia is the site where mechanotransduction takes place, the cilia localization of Nan suggests that Nan may play a role in mechanosensation. Consistent with its expression in the auditory neurons, *nan* auditory nerves do not respond to sound, as shown by sound-evoked potential. An electron microscopic observation showed no ultrastructural defects of the chordotonal neurons, including sensory cilia in the antenna of *nan* flies. Altogether, the Nanchung channel meets most of the criteria that is required for a mechanosensitive channel.

Concluding Remarks

Three decades of functional research in phototransduction at the genomic level in *Drosophila* have led to the identification of most of the components that are required for the phototransduction process. Since these components employ phospho-inositide signaling, *Drosophila* research on phototransduction has provided valuable insight into the lipid signaling *in vivo*. The *trp*, *trpl* and *trpγ* channels were disclosed as light-activated channels in phototransduction and gated downstream of the phosphoinositide signaling. Even though it is still controversial what activates TRP channels, evidence favors DAG and its metabolites rather than store depletion that is induced by IP3 as an activator of TRP channels in phototransduction (Hardie, 2003).

In recent years many members of the TRP family were discovered as required for sensory signaling. These are as diverse as mechanosensation, pain sensation, temperature detection, and hearing. Six mammalian TRP members are required for the sensation of a wide range of temperatures from <as low as 17ºC to as high as> 53ºC. Genetic screening in *Drosophila* disclosed the touch insensitive mutant *nompC* and the painless mutant *painless*, both of these encode distinct TRP members. A functional analysis of the *Drosophila* TRPV channel, encoded by *nanchung*, is required for hearing transduction. *Drosophila* NompC led to the identification of zebrafish NompC that is required for hearing in fish.

Transduction physiology of mechanoreceptor bristles and chordotonal organs that mediate touch and hearing in *Drosophila* is similar to that of vertebrate hair cells, including a high-K^{+}, low-Ca^{2+} receptor endolymph bathing both the mechanosensitive cilia of fly mechanoreceptors and hair bundles of sensory hair cells in mammals. Recently, it was demonstrated that *Drosophila* chordotonal organs and vertebrate auditory hair cells are developmentally related; cell fate specification of *Drosophila* and vertebrate auditory cells is governed by a functionally similar transcription regulator, *atonal* in *Drosophila* (Eberl *et al.*, 2000) and Math1 (atonal orthorlogue of mammals) in mammals (Bermingham *et al.*, 1999). Importantly, Math1 functionally rescues *Drosophila ato* mutants (Ben-Arie *et al.*, 2000) and *ato* rescues Math1 null mice (Wang *et al.*, 2002), suggesting that genes and machinery that are required for building *Drosophila* and mammalian hearing organs are derived from a common ancestor. We anticipate that in the future powerful genetics that are available for the functional analysis of *Drosophila* genome will provide a resource of components and insights into their function and pathway underlying pain and mechanosensation.

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