Chapter 5
Mechanosensory Transduction in the Nematode *Caenorhabditis elegans*

Nikos Kourtis and Nektarios Tavernarakis

Abstract Mechanotransduction, the process of converting a mechanical stimulus into a biological signal, appeared very early in the evolution and underlies a plethora of fundamental biological processes such as osmosensation, touch, hearing, balance and proprioception. Mechanosensory transduction has been studied extensively in simple animal models such as the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*. Genetic and physiological studies have revealed that specialized macromolecular complexes, encompassing mechanically gated ion channels, play a critical role in the conversion of mechanical energy into cellular response. Members of two large ion channel families, the degenerin/epithelial sodium channels (DEG/ENaC) and the transient receptor potential ion channels (TRP), have emerged as candidate mechanosensitive channels. Several auxiliary proteins associate with the core mechanosensitive channels to form the mechanotransducing apparatus in specialized mechanosensory cells. *C. elegans* displays a variety of mechanosensory behaviours. In this chapter, we survey the mechanisms of mechanosensory transduction in *C. elegans*. The exceptional amenability of this simple metazoan to genetic and molecular manipulations has facilitated the dissection of the mechanotransduction process to unprecedented detail.

Key words: Degenerin · Ion channels · Proprioception · Touch receptor neurons · TRP channels

5.1 Introduction

Animals receive and process information about their surroundings through specialized sensory cells. Ubiquitous mechanical stimuli permeate the environment of every living cell and every organism. The process by which cells convert mechanical energy into electrical or chemical signals is called *mechanotransduction*. Because mechanical force is everywhere, mechanosensation probably represents one

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of the oldest sensory transduction pathways that evolved in living organisms, from bacteria to humans (Blount and Moe, 1999; French, 1992; Gillespie and Walker, 2001; Sackin, 1995) The capacity to respond and adjust to mechanical inputs plays a pivotal role in numerous fundamental physiological phenomena such as the perception of sound and gravity, which underlie our senses of hearing and balance (Garcia-Anoveros and Corey, 1997; Hackney and Furness, 1995; Kellenberger and Schild, 2002). Touch sensation and proprioception (the coordinated movement of our body parts) are additional manifestations of responsiveness to mechanical stimulation (Garcia-Anoveros and Corey, 1996; Tavernarakis and Driscoll, 1997; Tavernarakis et al., 1997; Welsh et al., 2002). Moreover, mechanotransduction is equally critical for the stretch-activated reflexes of vascular epithelia and smooth muscle, and in the regulation of systemic fluid homeostasis and blood pressure (baroreception; Garcia-Anoveros and Corey, 1996; Lee and Huang, 2000; Tavernarakis and Driscoll, 2001a; Tavi et al., 2001; Welsh et al., 2002). Mechanotransduction is also important for the prevention of polyspermy during fertilization, cell volume and shape regulation, cell locomotion and tissue development and morphogenesis (Ingber, 1997; Ko and McCulloch, 2001; Rossier et al., 1994). In plants, mechanotransduction is the basis of gravitaxis and turgor control (Lynch et al., 1998; Pickard and Ding, 1993). In protists (*Paramecium*, *Stentor*) mechanotransduction underlies gravikinesis (the swimming against the gravity vector in order to avoid sedimentation; Block et al., 1999; Gebauer et al., 1999; Hemmersbach et al., 2001; Marino et al., 2001).

All living organisms have developed highly specialized structures that are receptive to mechanical forces originating either from the surrounding environment or from within the organism itself. Mechanotransducers are among the most elaborate and efficient, such structures, which are responsible for sensory awareness, for example, those facilitating touch, balance proprioception and hearing (Garcia-Anoveros and Corey, 1997; Gillespie and Walker, 2001; Hackney and Furness, 1995; Tavernarakis and Driscoll, 2001a). The mechanisms underlying the capability of living cells to receive and act in response to mechanical inputs are among the most anciently, implemented during evolution. Proteins with mechanosensitive properties are ubiquitously present in eubacteria, archaea and eukarya, and are postulated to have been an essential part of the physiology of the Last Universal Ancestor (Kloda and Martinac, 2001; Koch, 1994; Koprowski and Kubalski, 2001; Martinac, 2001). The first mechanosensitive processes may have evolved as backup mechanisms for cell protection, e.g. to reduce intracellular pressure and membrane tension during osmotic swelling. Subsequent organismal diversification and specialization resulted in variable requirements for mechanotransduction in different organisms (Norris et al., 1996). Hence, evolutionary pressure has shaped a large repertoire of mechanotransducers, optimized for a great assortment of tasks that range from maintenance of intracellular osmotic balance and pressure, to our impressive ability of hearing and discriminating sounds, and reading Braille code with our fingertips (Gillespie and Walker, 2001; Hamill and Martinac, 2001).

In this chapter, we describe *C. elegans* mechanosensory behaviours and survey the genes implicated in mechanosensory perception. We also discuss the relevant mechanisms underlying mechanotransduction in the nematode.

5.2 *C. elegans*: Background Information

Caenorhabditis elegans is a small (about 1mm length), hermaphroditic, soil-dwelling nematode. The size and simple dietary demands permit easy and cheap cultivation of the animal in the lab. The worm completes a reproductive life cycle in 2.5 days at 25°C progressing from a fertilized embryo through four larva stages, to become an egg-laying adult which lives for about 2–3 weeks (Brenner, 1974). Under non-favourable conditions such as starvation, high temperature or overcrowding, larvae may enter an alternative life stage, called dauer larva, a very resistant larval form that survives for months (Golden and Riddle, 1984). The simple body plan, the transparent egg and cuticle, and the nearly invariant developmental plan of this nematode has facilitated exceptionally detailed developmental and anatomical characterization of the animal (Ward et al., 1975; White et al., 1986; www.wormatlas.org). The complete sequence of somatic cell divisions from the fertilized egg to the 959-cell adult hermaphrodite has been described (Sulston and Horvitz, 1977). The *C. elegans* nervous system consists of 302 neurons of 118 types that interconnect in a stable and reproducible manner to create a variety of neural circuits (White et al., 1976; White et al., 1986). Individual neurons can be ablated through laser microsurgery and genetic manipulation, and neuronal pathways responsible for different behaviours have been characterized.

C. elegans is especially amenable to both forward and reverse genetic analysis. Mutagenized parents segregate homozygous F2 mutant progeny without any requirement for genetic crossing. Self-fertilization of heterozygotes leads to genetically homogeneous populations, while crossing with males that appear at a small percentage in the population facilitates genetic manipulations. Rapid and precise genetic mapping can be achieved, by taking advantage of a dense single nucleotide polymorphism map (Koch et al., 2000; Wicks et al., 2001). A physical map of the *C. elegans* genome, consisting of overlapping cosmid and YAC clones covering most of the six chromosomes, has been constructed to facilitate cloning of genes that have been positioned on the genetic map (Coulson et al., 1988; Waterston and Sulston, 1995). Double-stranded RNA mediated interference (dsRNAi) enables probable loss-of-function phenotypes to be readily evaluated (Fire et al., 1998; Tavernarakis et al., 2000). Transgenic animals carrying the gene of interest can be easily constructed by microinjecting the transgene into the gonad of hermaphrodite animals (Mello and Fire, 1995).

C. elegans displays a variety of behaviours, including mechanosensitive behaviours. The broad range of genetic and molecular tools available in the worm facilitates thorough and multifaceted investigation of the pathways that govern these behaviours.

5.3 *C. elegans* Mechanosensory Behaviours

Many behaviours displayed by *C. elegans* are direct manifestations of mechanosensitivity, making it exceptionally attractive for investigating mechanotransduction (Bargmann and Kaplan, 1998; Baumeister and Ge, 2002; Chalfie, 1993; Chalfie

and Sulston, 1981; Chalfie et al., 1985; Driscoll and Tavernarakis, 1997; Herman, 1996; Kaplan and Horvitz, 1993; Mah and Rankin, 1992; O'Hagan and Chalfie, 2006; Rankin, 1991; Syntichaki and Tavernarakis, 2004; Tavernarakis and Driscoll, 1997; Wicks and Rankin, 1995; Wolinsky and Way, 1990). The best characterized such behaviour is the response to a gentle mechanical stimulus delivered transversely along the body of the animal, typically by means of an eyelash hair attached onto a toothpick (the 'gentle body touch response'; (Chalfie and Sulston, 1981; Chalfie et al., 1985; Herman, 1996; Tavernarakis and Driscoll, 1997)). We discuss studies elucidating the molecular mechanisms of this touch response in the following section. Other mechanosensory responses are the generation and maintenance of the characteristic coordinated sinusoidal pattern of locomotion (analogous to proprioception; (Li et al., 2006; Tavernarakis and Driscoll, 1997; Tavernarakis et al., 1997); see below), and the nose touch response, which can be further categorized into the head-on collision response and the head withdrawal response (Driscoll and Kaplan, 1996; Kaplan and Horvitz, 1993).

When animals collide with an obstacle in a nose-on fashion during the course of normal locomotion they respond by reversing their direction of movement (Bargmann and Kaplan, 1998; Colbert et al., 1997). This response is independent of touch receptor neurons, needed for gentle touch (Chalfie and Sulston, 1981). Three classes of mechanosensory neurons, ASH, FLP, and OLQ, mediate this avoidance response (Bargmann and Kaplan, 1998; Herman, 1996; Kaplan and Horvitz, 1993; Wicks and Rankin, 1995). Each of these sensory neurons accounts for a part of the normal response, which is quantitative with normal animals responding about 90% of the time. Laser ablation and genetic studies have demonstrated that each sensory neuron contributes to the overall responsiveness as follows: ASH, 45%; FLP, 29%; and OLQ, 5%. The remaining 10% of the responses are mediated by the ALM and AVM neurons, which sense anterior body touch (Driscoll and Kaplan, 1996; Kaplan and Horvitz, 1993). It is unclear what distinguishes the function of the three nose touch neurons. One attractive possibility is that these cells differ in their sensitivities and that the intensities of nose touch stimuli vary according to the violence of the collision. If this were the case, it would be expected that the most sensitive neuron (ASH) would account for the majority of responses while less sensitive neurons (FLP and OLQ) would account for the remainder. In addition to their mechanosensory properties, the ASH neurons are part of a chemosensory organ, the amphid sensilla, with their sensory endings exposed to the external environment (Perkins et al., 1986; Ward et al., 1975). The ASH neurons serve chemosensory and osmosensory functions, mediating avoidance of osmotic repellents (Hart et al., 1999; Hart et al., 1995). Several classes of chemosensory neurons respond to multiple chemical stimuli in *C. elegans*. However, ASH is unique among them in responding to such divergent stimuli. In this respect, ASH neurons are similar to vertebrate neurons that sense painful stimuli, which are called nociceptors. For their multi-sensory capabilities, the ASH neurons have been categorized as polymodal sensory neurons (Driscoll and Kaplan, 1996; Kaplan and Horvitz, 1993).

In addition to DEG/ENaC proteins, another major family of channel proteins implicated in sensory mechanotransduction is the transient receptor potential (TRP)

ion channels. TRPs are non-specific cation-permeable channels that are present in diverse species ranging from yeast, flies, and worms to humans (Kahn-Kirby and Bargmann, 2006). All TRP channels appear to form tetrameric assemblies and include six predicted transmembrane domains and a variable number of ankyrin motifs, which are suggested to mediate protein-protein interactions. Members of individual subfamilies may bear several other domains, such as coiled-coil motifs, protein kinase domains, transmembrane segments, and TRP domains (Montell, 2005).

In *C. elegans*, the TRPV (vallinoid TRP) subfamily genes *osm-9* and *ocr-2* are required for the aversive responses of ASH neurons to various noxious stimuli, including high osmomolarity and noxious chemicals (Bargmann et al., 1990; Hilliard et al., 2005; Troemel et al., 1995). The OSM-9 and OCR-2 proteins localize to the sensory cilia of ASH, suggesting a direct role in sensory transduction (Colbert et al., 1997). Additionally, OSM9::GFP is expressed in FLP, OLQ and PVD mechanosensory neurons. Several genetic studies suggest that the function of the putative OSM-9/OCR-2 ion channel is regulated by G protein signalling and specific polyunsaturated fatty acids (PUFAs), which act upstream of OSM-9/OCR-2 to modulate nociceptive responses in ASH neurons, including the mechanosensory nose touch avoidance behaviour (Kahn-Kirby et al., 2004; Roayaie et al., 1998). Expression of a mammalian TRPV4 protein in ASH can rescue defects in osmotic and nose-touch avoidance in *osm-9* mutants (Liedtke et al., 2003).

C. elegans also distinguishes textural differences in its substrate. When worms enter a bacterial lawn they slow their movement, a behaviour known as basal slowing. This response is indeed mechanosensory since worms entering a lawn of Sephadex beads instead of bacteria, slow similarly (Sawin et al., 2000). This behaviour depends on the CEP, ADE and PDE dopaminergic neurons which have ciliated sensory endings, embedded in the cuticle (Perkins et al., 1986; Ward et al., 1975; White et al., 1986). Laser ablation experiments confirmed that these neurons are required for response to small particles (Sawin et al., 2000). In support of this model, dopaminergic neurons transduce an inhibitory signal to the motor circuit (White et al., 1986).

The complicated male-mating behaviour of *C. elegans* is probably based on chemosensory and mechanosensory cues (Liu and Sternberg, 1995). Males have 87 additional neurons, comparing with the hermaphrodite many of which are ciliated and considered to be sensory (Sulston et al., 1980). Two genes, *lov*-1 and *pkd*-2, needed for male mating have been implicated in mechanical signalling. *lov-1* and *pkd-2*. LOV-1 and PDK-2 are the nematode homologs of mammalian PDK-1 and PDK-2 TRPP ion channels respectively (Corey, 2003). Interestingly, mutations in the mammalian PDK-1 and PDK-2 cause autosomal dominant polycystic kidney disease (ADPKD). PDK-1 and PDK-2 form a Ca^{2+}-permeable ion channel which is mechanically activated by fluid flow in certain epithelial cells (Nauli et al., 2003).

An additional mechanosensitive behaviour in *C. elegans* is the tap withdrawal reflex, where animals retreat in response to a tap on the culture plate (Chiba and Rankin, 1990; Rankin et al., 2000; Wicks and Rankin, 1997). Worms respond to a diffuse mechanical stimulus (a tap to the side of the dish they are resting on) by

either accelerating forward movement or by initiating backward movement (Chiba and Rankin, 1990; Rankin et al., 2000). Given that the stimulus is not spatially coherent and that the animal's response is variable, it was proposed that the tap response reflects the simultaneous activation of the anterior and posterior touch cells. The behavioural outcome is likely determined by the integration of these two antagonistic circuits.

Mechanotransduction appears to also play a regulatory role in processes such as egg laying, feeding, defecation, and maintenance of the pseudocoelomic body cavity pressure (Avery, 1993; Du and Chalfie, 2001; Liu and Thomas, 1994; Liu and Sternberg, 1995; Thomas et al., 1990; Wolinsky and Way, 1990). These behaviours add to the large repertoire of mechanosensitive phenomena, amenable to genetic and molecular dissection in the nematode (Bargmann and Kaplan, 1998; Syntichaki and Tavernarakis, 2004; Tavernarakis and Driscoll, 2001b).

5.4 The Gentle Body Touch Transduction System

In its natural habitat, the soil, *C. elegans* encounters a large number of mechanical stimuli. While crawling on surfaces of soil particles, the worm receives external forces generated by bumping on soil materials and other animals. The laboratory assay for the gentle body touch response involves a mild stroke of the animal with an eyelash hair attached to a toothpick, transversely to the anterior-posterior body axis (Chalfie and Sulston, 1981; Chalfie et al., 1985; Syntichaki and Tavernarakis, 2004). When no response is observed, animals are prodded with a thin platinum wire to confirm that they are touch insensitive rather than paralyzed (gentle-touch insensitive animals typically still respond to a strong stimulus-the harsh touch response) (Chalfie et al., 1985; Chalfie and Wolinsky, 1990; Driscoll and Kaplan, 1996; Wolinsky and Way, 1990). Depending on the part of the body touched, animals will either accelerate or initiate forward movement (when stimulated at the posterior or the tail), or reverse and move backwards (when stimulated at the anterior part of the body). Hermaphrodite, male, juvenile (except L1), and dauer animals respond identically to touch. The response is adaptive: repetitive stimulation leads to short periods of insensitivity (Mah and Rankin, 1992; Rankin et al., 2000; Wicks and Rankin, 1995).

5.4.1 The Sensory Cells

The touch reflex of the mature animal involves six touch receptor neurons, 5 pairs of interneurons and 69 motorneurons (Chalfie, 1993; Chalfie et al., 1985). The six touch receptor neurons share common ultrastructural features and express many of the same genes (Chalfie and Sulston, 1981; Chalfie and Thomson, 1979; Chalfie and Thomson, 1982). The six touch receptor neurons were originally designated as the microtubule cells because of distinctive bundles of 15-protofilament (pf; tubulin

dimmer filaments) microtubules that fill their processes (ALML/R: anterior lateral microtubule cell, left/right; AVM: anterior ventral microtubule cell; PLML/R; posterior lateral microtubule cell, left/right; and PVM: posterior ventral microtubule cell; (Chalfie, 1993; Chalfie et al., 1986; Chalfie et al., 1985; Chalfie and Thomson, 1979; Chalfie and Thomson, 1982)). Two fields of touch sensitivity, anterior and posterior are defined by the arrangement of the six touch receptor neurons along the body axis (Fig. 5.1; Chalfie et al., 1985; Tavernarakis and Driscoll, 1997). All six cells have anteriorly directed processes and, except for PVM, an anterior branch. The processes of touch neurons are localized in the hypodermis, just beneath the cuticle, an ideal position for sensing external stimuli and vibrations. All six cells are dispensable for the viability of the organism. Apart from insensitivity to gentle body touch, laser ablation of all six neurons does not result in any additional adverse effects (Chalfie, 1995; Chalfie et al., 1985; Tavernarakis and Driscoll, 1997).

Laser microsurgery established that PLML and PLMR are required for response to a touch to the tail. If either is present, tail touch sensitivity is observed. When both are ablated, animals are completely insensitive to gentle touch stimuli administered to the posterior (Chalfie et al., 1985; Chalfie et al., 1983; Kitamura et al., 2001). Either ALML or ALMR can mediate a response to a mechanical stimulus delivered to the anterior part of the body. AVM, which is added into the touch circuitry postembryonically, can mediate a weak response to some stimuli but not all. In animals in which both ALM cells are killed, partial touch sensitivity returns 35–40 hours after hatching, which is attributable to AVM being generated. PVM alone does not produce a touch response, but its synaptic

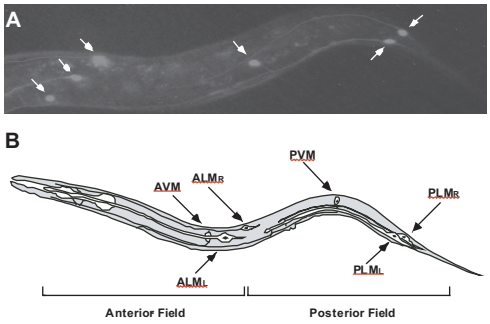

Fig. 5.1 The *C. elegans* touch receptor neurons. (A) Visualization of touch receptors. Worms are expressing the Green Fluorescence Protein (GFP) under the control of the *mec-4* promoter, which is active only in the six-touch receptor neurons. Arrows indicate the cell bodies of the neurons. (B) Schematic diagram , showing the position of the six touch receptor neurons in the body of the adult worm. The two fields of touch sensitivity are defined by the arrangement of touch receptor neurons along the body axis. The ALMs and AVM mediate the response to touch over the anterior field whereas PLMs mediate the response to touch over the posterior field. PVM does not mediate touch response by itself (Chalfie, 1995; Chalfie, 1997; Chalfie et al., 1985; Tavernarakis and Driscoll, 1997). Reproduced from (Voglis and Tavernarakis, 2005) with copyright permission of the Academia Publishing House Ltd

pattern implicates it in the touch behaviour (Chalfie, 1993; Chalfie and Au, 1989; Chalfie et al., 1985).

Bundles of darkly staining large diameter microtubules distinguish the touch receptor neurons (Chalfie and Thomson, 1979; White et al., 1976). Cross bridges between microtubules of a bundle are observed in micrographs obtained with electron microscopy, and may increase the structural integrity of the bundle. These microtubules are unique to the nematode touch receptor neurons and contain 15-protofilament microtubules, a unique feature of these six cells (Chalfie and Thomson, 1982; Tavernarakis and Driscoll, 1997). In most eukaryotic cells, α- and β-tubulin co-assemble into 13-protofilament microtubules, whereas the vast majority of microtubules in *C. elegans* cells have 11-protofilament (Chalfie and Thomson, 1982; White et al., 1986). In normal touch receptors, 11-protofilament microtubules typical of most other cells in this nematode are occasionally observed. If the 15-protofilament microtubules are eliminated by mutation, the number of 11-protofilament microtubules in the touch cell processes increases (Chalfie, 1993; Chalfie et al., 1986; Chalfie and Thomson, 1979; Fukushige et al., 1999; Savage et al., 1989). Individual microtubules are 10–20 μm, but overlap and create bundles, filling in that way the process of the neuron which is 400–500 μm long (Chalfie and Thomson, 1979). The distal ends of the microtubules are apposed to the plasma membrane and are associated with structures of a diameter up to twice that of the microtubules (Chalfie and Thomson, 1979).

Touch receptors also are uniquely surrounded by an osmiophillic extracellular material referred to as the mantle. The amount of mantle varies along the length of the process (Chalfie and Sulston, 1981). The mantle is needed for the attachment of the touch receptor process to the body wall, bringing it to an ideal position for detection of external mechanical stimuli.

5.4.2 The C. elegans Mechanosensory Apparatus

To identify molecules dedicated to touch transduction, Martin Chalfie and colleagues mounted a forward genetics approach to isolate gentle body touch-insensitive nematode mutants (Chalfie and Au, 1989; Chalfie et al., 1986; Chalfie and Sulston, 1981; Du and Chalfie, 2001; Gu et al., 1996; Tavernarakis and Driscoll, 1997). Briefly, populations of wild type, touch sensitive animals were mutagenized and touch insensitive individuals were sought among their descendants by stroking with an eyelash hair and prodding with a platinum wire (Chalfie, 1997). During the course of this very tedious screening process, over 417 mutations in 17 different genes, randomly distributed in all six chromosomes of *C. elegans* were isolated (Driscoll and Kaplan, 1996; Tavernarakis and Driscoll, 1997). By design, the screen yields mutations in genes that are fairly specific for normal gentle body touch perception. For example, gene mutations with pleotropic effects that result in lethality or uncoordinated and paralyzed phenotypes would have been missed. In addition to being touch insensitive *mec* mutants tend to be lethargic when grown normally in the presence of amble food (Driscoll and Kaplan, 1996). Reduced spontaneous movement

is probably due to their inability to sense micro vibrations in their environment, interaction with external objects or stretch produced by the locomotory movements themselves. However, when starved or during mating they move as well as wild type. The 17 genes isolated are designated as the *mec* genes for their '*mec*hanosensory abnormal' phenotype. Corroborating the high specificity of the screen, while most of the alleles generated cause complete touch insensitivity, only few other abnormalities accompany the mutants (Driscoll and Kaplan, 1996). Depending on their role and point of action, *mec* genes can be loosely classified into three main categories. First, the regulatory/specification genes which control the expression touch receptor neuron specific genes or modify the activity of the mechanotransducer complex; second, the *mec* genes encoding core structural components of the mechanosensitive ion channel; and third the genes encoding peripheral, associated proteins.

5.4.2.1 Genes Needed for the Development of Touch Receptor Neurons

The UNC-86 and MEC-3 transcription factors are essential for proper development and differentiation of the six touch receptor neurons (Chalfie and Sulston, 1981; Duggan et al., 1998; Way and Chalfie, 1988; Way and Chalfie, 1989; Xue et al., 1992). UNC-86 is a POU domain protein which is required in several distinct neuroblast lineages for daughter cells to become different from their mothers (Finney and Ruvkun, 1990; Finney et al., 1988). UNC-86 activates the expression of *mec-3* which encodes a LIM-type homeodomain protein needed for the differentiation of the six touch receptor cells (Way and Chalfie, 1989). In mutants lacking *mec-3* activity, the touch receptors express none of their unique differentiated features and appear to be transformed to other types of neurons (Way and Chalfie, 1988). UNC-86 and MEC-3 bind cooperatively as a heterodimer to the *mec-3* promoter as well as to the promoters of other genes required for the function of touch cells (Duggan et al., 1998; Way and Chalfie, 1988; Way and Chalfie, 1989). *unc-86* and *mec-3* genes are also expressed in the PVD and FLP neurons which also function as mechanoreceptors but they do not express the same genes as the touch receptor neurons (Way and Chalfie, 1989).

5.4.2.2 Genes Needed for Function of Touch Receptor Neurons

Four *mec* genes can be classified in the category of core structural components of the putative mechanosensory ion channel in touch receptor neurons, *mec-2*, *mec-4*, *mec-6* and *mec-10*. MEC-4 and MEC-10 form the core ion channel, while MEC-2 and MEC-6 physically interact with the channel subunits to shape and modulate their gating properties. Animals bearing loss-of-function mutations in *mec-4* or *mec-10* are touch-insensitive despite the fact that in these mutant backgrounds the touch receptor neurons develop normally and exhibit no apparent defects in ultra structure (Driscoll and Chalfie, 1991; Huang and Chalfie, 1994; Tavernarakis and Driscoll, 1997). *mec-4* and *mec-10* encode homologous proteins related to

subunits of the multimeric amiloride sensitive Na^+ channel which mediates Na^+ re-absorption in vertebrate kidney, intestine and lung epithelia (the ENaC channel; (Kellenberger and Schild, 2002; Rossier et al., 1994)). In addition to being involved in mechanotransduction, MEC-4, MEC-10 and several other related nematode proteins have a second, unusual property: specific amino acid substitutions result in aberrant channels that induce the swelling and subsequent necrotic death of the cells in which they are expressed (Driscoll, 1996; Driscoll and Chalfie, 1991; Hall et al., 1997; Harbinder et al., 1997). This pathological property is the reason that this family of proteins was originally called degenerins (Chalfie et al., 1993; Chalfie and Wolinsky, 1990; Tavernarakis and Driscoll, 2001a; Tavi et al., 2001). *C. elegans* degenerins, together with their mammalian relatives, the ENaCs, comprise the large DEG/ENaC family of ion channels (Fig. 5.2). The relationship of these channel subunits to subunits of an amiloride-sensitive ENaCs is intriguing because amiloride is a general inhibitor of mechanosensitive ion channels (Alvarez de la Rosa et al., 2000; Hamill et al., 1992; Hamill and McBride, 1996; Hoger et al., 1997; Lane et al., 1991; Rossier et al., 1994; Voilley et al., 1997).

mec-4 is expressed solely in the six touch receptor neurons, while *mec-10* in addition to the six touch receptor neurons, is expressed in two other neuron pairs that may mediate stretch-sensitive responses (FLPs and PVDs; (Driscoll and Chalfie, 1991; Driscoll and Tavernarakis, 1997; Huang and Chalfie, 1994; Tavernarakis and Driscoll, 1997)). Interestingly, a MEC-4::GFP fusion localizes in distinct puncta along the processes of the touch receptor neurons (Fig. 5.3). Such punctuate localization may reflect the distribution of mechanotransducing complexes on the axon of the touch receptor neuron. MEC-4 and MEC-10 co-localize exclusively in mechanosensitive neurons, where they may co-assemble into a mechanically-gated ion channel. Optical imaging studies using genetically encoded Ca^{2+} indicators (cameleons), which monitor intracellular Ca^{2+} changes in response to gentle body touch support this hypothesis (Suzuki et al., 2003). More definitive proof is provided by recent electrophysiological, whole-cell voltage-clamp recordings of mechanoreceptor currents (MRCs) from the PLM touch receptors *in vivo* (O'Hagan et al., 2005). The mechanoreceptor currents are extremely rapid; they turned on within a millisecond of force application and quickly decreased in amplitude, characteristic of adaptation. MRCs recorded from PLM neurons are probably representing transduction by direct activation of the channel and not through second messengers. MRCs are absent in MEC-4, MEC-2 and MEC-6 null mutants, whereas mechanoreceptor current was only partially decreased when testing mutations in non-conserved regions of the proteins (O'Hagan et al., 2005). In addition, MRCs are reduced but not eliminated in worms that carry a null mutation in *mec-7* and *mec-12* genes, affecting α and β-tubulin which forms the 15-protofilament microtubule bundles. These results question the absolute requirement of microtubules for touch transduction.

MEC-2 is an additional component of the channel complex. *mec-2* encodes a 481-amino acid protein and is expressed in the touch receptor neurons and in a few additional neurons in the nerve ring region (Du and Chalfie, 2001; Gu et al., 1996; Huang et al., 1995). MEC-2 features three candidate protein-protein interaction domains (Fig. 5.4). First, part of the amino-terminal domain (situated in part between AA 42–118) is needed for the proper localization of a *mec-2/lacZ* fusion

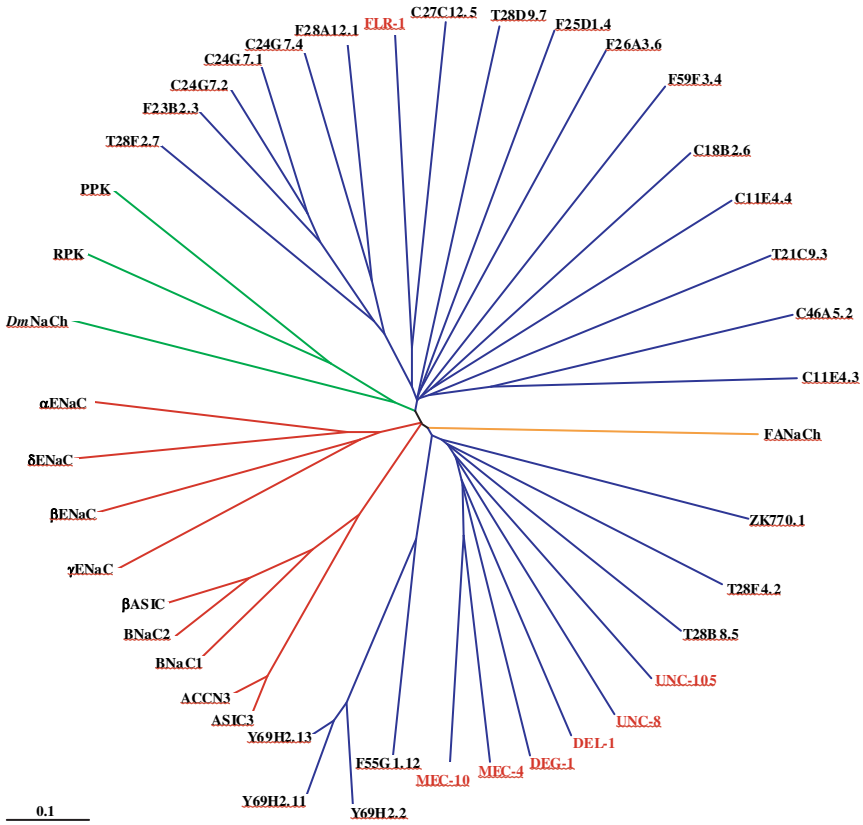

Fig. 5.2 Phylogenetic relationships among DEG/ENaC proteins. Nematode degenerins are shown with blue lines. The current degenerin content of the complete nematode genome is included. The seven genetically characterized (DEG-1, DEL-1, FLR-1, MEC-4, MEC-10, UNC-8 and UNC-105) are shown in red. Representative DEG/ENaC proteins from a variety of organisms, ranging from snails to humans, are also included (mammalian: red lines; fly: green lines; snail: orange line). The scale bar denotes relative evolutionary distance equal to 0.1 nucleotide substitutions per site (Sadoshima et al., 1992). Reproduced from (Voglis and Tavernarakis, 2005) with copyright permission of the Academia Publishing House Ltd

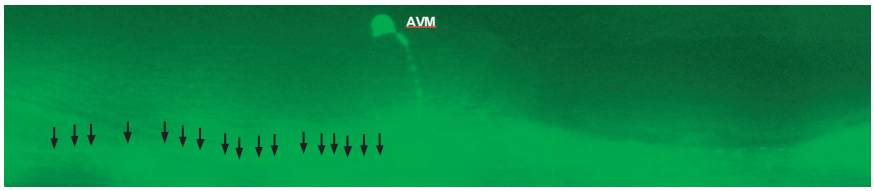

Fig. 5.3 Punctuate localization of a putative mechanosensitive ion channel subunit. Image of an AVM touch receptor neuron expressing a GFP-tagged MEC-4 protein. Fluorescence is unevenly distributed along the process of the neuron in distinct puncta, which may represent the location of the mechanotransducing apparatus. Reproduced from (Voglis and Tavernarakis, 2005) with copyright permission of the Academia Publishing House Ltd

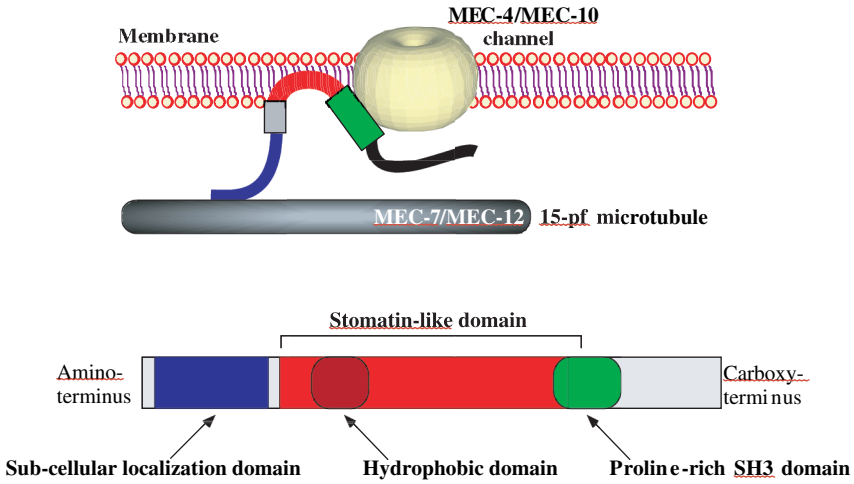

Fig. 5.4 Schematic representation and topology of the MEC-2 protein. Conserved domains as well as hydrophobic regions are highlighted. Putative interactions with the degenerin channel and the cytoskeleton are indicated (Goodman et al., 2002). Reproduced from (Voglis and Tavernarakis, 2005) with copyright permission of the Academia Publishing House Ltd

protein to the touch receptor process. Second, the carboxy-terminal domain includes a proline-rich region that is similar to SH3-binding domains. Third, the central region (AA 114–363) encompasses an SPFH domain with a membrane-associated hydrophobic part (AA 114–141) and a cytoplasmic hydrophilic part that together exhibit 65% identity to the human red blood cell protein stomatin (Huang et al., 1995; Tavernarakis et al., 1999). The SPFH domain is the common denominator of stomatins, prohibitins, flotilins and bacterial *Hfl*K/C proteins, all of which are membrane associated regulators (Fig. 5.5; Tavernarakis et al., 1999). Stomatin, also known as band 7.2b protein, is a membrane-associated protein originally identified as a component of human red cells (Delaunay et al., 1999; Sedensky et al., 2001; Snyers et al., 1998; Stewart, 1997; Stewart et al., 1993). In humans, stomatin is missing from erythrocyte membranes in autosomal dominant hemolytic disease overhydrated hereditary stomatocytosis, despite an apparent normal stomatin gene. Many of the 54 mutant *mec-2* alleles have dominant effects and exhibit a complex pattern of inter-allelic complementation (Chalfie and Sulston, 1981; Gu et al., 1996), indicating that MEC-2 protein molecules form higher order complexes. However, there is also genetic data suggesting that MEC-2 interacts with the specialized touch cell microtubules encoded by *mec-7* and *mec-12* (α-tubulin and β-tubulin respectively; (Gu et al., 1996; Huang et al., 1995)). Normally, a *mec-2/lacZ* fusion protein is distributed along the touch receptor axon (Huang et al., 1995). The axonal distribution of a MEC-2::lacZ fusion protein is mildly disrupted in a *mec-7* null or *mec-12* strong loss-of-function background, implying that the 15-protofilament microtubules are not essential for the localization of MEC-2 to the neuronal process. However, two specific *mec-12* missense alleles interfere dramatically with localization of MEC-2 fusion proteins, restricting the fusion proteins to the cell body (Huang et al., 1995).

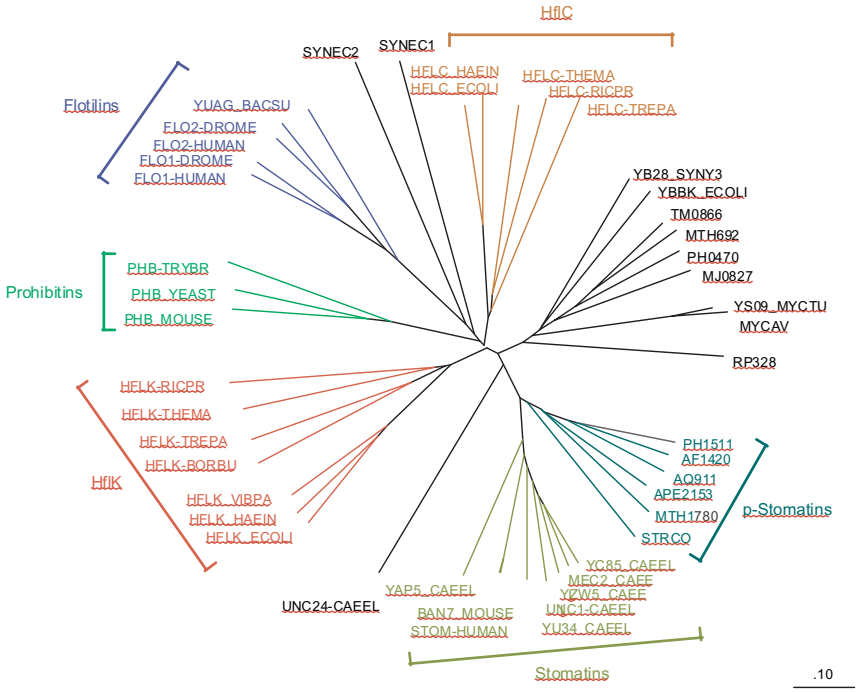

Fig. 5.5 Phylogenetic relations among SPFH domain proteins. A dendrogram showing distance relationships among most of the stomatin protein super-family members (the complete ClustalW generated alignment on which the dendrogram was based is available at http://www.imbb.forth.gr/worms/worms/alignment.gif). The dendrogram was constucted with the neighbor-joining method based on pairwise distance estimates of the expected number of amino acid replacements per site (0.10 in the scale bar), and visualized by TreeTool. Protein sub-families are denoted in different colours (Tavernarakis et al., 1999)

MEC-2 colocalizes with MEC-4 in the six touch receptor neurons and is distributed along neuronal processes in punctuate pattern (Zhang et al., 2004). This is consistent with the co-immunoprecipitation of the two proteins in Xenopus oocytes (Goodman et al., 2002). The stomatin-like domain of MEC-2 interacts specifically with the N-terminus cytoplasmic region of MEC-4 (Zhang et al., 2004). Punctuate expression of MEC-2 is disrupted in the *mec-4(u253)*, *mec-6(u450)* and *mec-10(u20)* loss-of-function mutants indicating that the MEC-2 subcellular localization depends on the other partners of the mechanosensory complex (Zhang et al., 2004). These genetic studies, which do not by themselves prove a direct interaction, have recently been complemented by elegant heterologous expression experiments in *Xenopus* oocytes that support physical interaction between MEC-2 and the channel subunits MEC-4 and MEC-10 (Goodman et al., 2002). Reconstitution of channel activity in *Xenopus* oocytes revealed that MEC-2 regulates the activity of the MEC-4/MEC-10 channel, providing the first direct support for the hypothesis that stomatin-like proteins interact with and regulate ion channels (Goodman et al., 2002; Stewart et al., 1993). This interaction appears to dramatically potentiate the conductivity of the channel in

oocytes. Co-expression of MEC-2 with the hyperactive MEC-4(d) and MEC-10(d) derivatives in *Xenopus* oocytes resulted in about 40-fold increase in the amplitude of amiloride-sensitive ionic currents, and this amplification allowed currents to be detected even with wild-type MEC-4 and MEC-10 proteins (Goodman et al., 2002; Stewart et al., 1993). Visualization of tagged MEC-4(d) and MEC-10(d) in live oocytes demonstrated that MEC-2 does not increase the number of MEC-4(d)/MEC-10(d) channels that reach the plasma membrane, and probably acts by regulating their activity. In *mec-2(u37)* loss-of-function mutants the touch-evoked currents are abolished confirming that MEC-2 is one of the major components needed for the proper function of the MEC-4/MEC-10 ion channel (O'Hagan et al., 2005).

A second stomatin-like protein, UNC-24 appears to be part of the channel complex. In addition to the stomatin domain, UNC-24 has a lipid transfer domain (Barnes et al., 1996; Sedensky et al., 2001), probably important for the membrane localization of the membrane channel complex. The *unc-24* gene is expressed in the touch neurons, while the UNC-24 protein appears in puncta that colocalize with MEC-4 and MEC-2. Mutation of *unc-24* enhances the Mec phenotype caused by *mec-4* and *mec-6* temperature sensitive alleles (Zhang et al., 2004). UNC-24 appears to interact with MEC-2 and MEC-4 through its stomatin-like domain.

mec-6 encodes a 377-amino acid protein and is expressed in muscle cells, neurons and other tissues (Chelur et al., 2002). Recessive *mec-6* mutations disrupt touch sensitivity but do not cause detectable changes in touch cell ultrastructure (Chalfie and Sulston, 1981; Tavernarakis and Driscoll, 1997). *mec-6* alleles have the interesting property that they completely block *mec-4(d)* and *mec-4*(A673V)-induced touch cell degeneration (Harbinder et al., 1997; Huang and Chalfie, 1994; Tavernarakis and Driscoll, 1997). MEC-6 encodes a protein with limited similarity to Paraoxonases/Arylesterases that physically interacts with MEC-4 and MEC-10 (Chelur et al., 2002). MEC-6 has a short cytoplasmic N-terminus, a single transmembrane domain, and a large extracellular C-terminus. How exactly MEC-6 acts to influence MEC-4/MEC-10 channel activity is unknown. Nevertheless, it appears that *mec-6* mutations do not affect *mec-4* transcription, although they do cause full-length MEC-4::LacZ or MEC-4::GFP reporter fusion chimeras to be rapidly degraded (N. T. unpublished observations; (Chelur et al., 2002)). Thus, working hypotheses concerning the function of MEC-6 focus on two possibilities. First, MEC-6 is another subunit needed for channel function or assembly, or second, it mediates localization or post-translational modification essential for MEC-4 and MEC-10 activity/stability. It should be noted that MEC-6 function is not exclusively related to the MEC-4/MEC-10 touch receptor channel. *mec-6* mutations also suppress the deleterious consequences of neurodegeneration-inducing mutations in other *C. elegans* degenerins including *deg-1*, *unc-8* and partly *unc-105* ((Chalfie and Wolinsky, 1990; Liu et al., 1996; Shreffler et al., 1995; Tavernarakis et al., 1997); N.T unpublished observations). *mec-6* loss-of-function mutations affect localization of the MEC-4 channel and disrupt touch evoked membrane currents (Chelur et al., 2002).

Although the exact stoichiometry of the components of the mechanotransducer channel complex is not known, genetic data suggest that several proteins are

present in multiple copies and in various combinations. Two subgroups of *mec* genes encoding peripheral components required for mechanotransduction in the touch receptor neurons can be defined, those encoding intracellular (*mec-7*, *mec-12*) and those encoding extracellular (*mec-1*, *mec-5*, *mec-9*) proteins (Driscoll and Kaplan, 1996; Driscoll and Tavernarakis, 1997; Tavernarakis and Driscoll, 1997). As described previously, the touch receptor processes are filled with bundled 15-protofilament microtubules. Mutations in two genes, *mec-7* and *mec-12*, disrupt the formation of these microtubules (Chalfie and Au, 1989; Chalfie and Sulston, 1981; Fukushige et al., 1999; Hamelin et al., 1992; Savage et al., 1989; Savage et al., 1994). *mec-7* encodes a β-tubulin expressed at high levels in the touch receptor neurons (Hamelin et al., 1992; Savage et al., 1989; Savage et al., 1994). MEC-7 is highly conserved–apart from the carboxy-terminal domain that is characteristically highly variable; only 7 amino acids differ from other β-tubulins. *mec-12* encodes an α-tubulin expressed at high levels in the touch receptor neurons but also expressed in several other neurons that do not assemble 15-protofilament microtubules (Fukushige et al., 1999). Thus, the presence of the MEC-12 tubulin is not sufficient to nucleate assembly of the touch-cell specific microtubules. As is the case for *mec-7*, many *mec-12* mutations are semi-dominant or dominant and are likely to disrupt subunit interactions or protofilament assembly (Gu et al., 1996). Recent data suggest that tethering of the channel complex to the microtubules is not essential for transduction, as mechanoreceptor currents are reduced but not eliminated in *mec-7* β-tubulin mutants (O'Hagan et al., 2005) and *mec-7* and *mec-12* null mutations do not prevent the formation of channel puncta (Emtage et al., 2004; Zhang et al., 2004).

In *mec-1* mutants, touch cells generally lack the mantle and associated periodic specializations of the overlying cuticle (Chalfie and Sulston, 1981; Gu et al., 1996; Savage et al., 1994). *mec-1* is expressed in touch receptor neurons, other lateral neurons and intestinal muscles. It encodes a likely secreted protein with multiple Kunitz-type serine protease inhibitor and EGF domains. The Kunitz and EGF domains are likely to be protein interaction domains. The C terminus of MEC-1 is needed for touch sensitivity, while the N terminus mediates the attachment of the touch neuron processes to the hypodermis (Emtage et al., 2004). MEC-1 is localized along the touch receptor processes in a punctuate manner and colocalizes with MEC-5 and the MEC-4/MEC-10 mechanosensory channel complex (Emtage et al., 2004).

mec-5 mutations disrupt the extracellular matrix in a subtle manner; the mantle in a wild-type animal can be stained with peanut lectin, whereas the mantle in *mec-5* mutants cannot (Chalfie and Sulston, 1981; Du et al., 1996; Gillespie and Walker, 2001). *mec-5* encodes a novel collagen type that is secreted by hypodermal cells (Du et al., 1996). The central portion of the *mec-5* protein is made up of Pro-rich Gly-X-Y repeats. *mec-5* mutations, many of which are temperature-sensitive, cluster toward the carboxy terminus of the protein and affect these repeats. Genetic interactions suggest that *mec-5* influences MEC-4/MEC-10 channel function (for example, *mec-4* and *mec-10* mutations can enhance the *mec-5(ts)* mutant phenotype; (Gu et al., 1996)). Thus, a specialized collagen could interact with the touch receptor channel, perhaps acting to provide gating tension.

mec-9 mutations do not alter mantle ultrastructure in a detectable manner, despite the fact that *mec-9* encodes a protein that appears to be secreted from the touch receptor neurons (Chalfie and Sulston, 1981; Du et al., 1996). The *mec-9* gene generates two transcripts, the larger of which encodes an 834 amino acid protein (MEC-9L) that is expressed only by the touch receptors (Chalfie and Sulston, 1981; Du et al., 1996). Akin to MEC-1, the predicted MEC-9L protein contains several domains related to the Kunitz-type serine protease inhibitor domain, the Ca^{2+}-binding EGF repeat, the non-Ca^{2+}-binding EGF repeat and a glutamic acid-rich domain (Chalfie and Sulston, 1981; Du et al., 1996). Single amino acid substitutions that disrupt MEC-9 function affect the two Ca^{2+}-binding EGF repeats, the sixth EGF repeat and the third Kunitz-type domain, thus implicating these regions as important in MEC-9 function (Chalfie and Sulston, 1981; Du et al., 1996). How MEC-9 is needed for touch cell activity is not clear, but it is interesting that MEC-9 appears specialized for protein interactions and that agrin, a protein that acts to localize acetylcholine receptors, has a domain structure that appears similarly specialized (agrin features multiple EGF and Kazal-type serine protease inhibitor repeats; (Rupp et al., 1992a; Rupp et al., 1992b; Rupp et al., 1991)). *mec-9* mutations are dominant enhancers of a *mec-5(ts)* allele, suggesting that these proteins might interact in the unique mantle extracellular matrix outside the touch receptor neuron (Du et al., 1996; Gu et al., 1996).

5.5 Proprioception

C. elegans senses forces arising within the body itself during movement, a phenomenon called proprioception. Animal locomotion results from alternate contraction and relaxation of dorsal and ventral body wall muscles, which generates a canonical sinusoidal pattern of movement (White et al., 1986; Wolinsky and Way, 1990). The arrangement of the body wall muscles and their synaptic inputs restricts locomotion to dorsal and ventral turns of the body. The body wall muscles are organized into two dorsal and two ventral rows. Each row consists of 23 or 24 diploid mononucleate muscle cells arranged in an interleaved pattern (Francis and Waterston, 1991; Moerman et al., 1996; Waterston et al., 1980; Williams and Waterston, 1994). Distinct classes of motorneurons control dorsal and ventral body muscles. To generate the sinusoidal pattern of movement, the contraction of the dorsal and ventral body muscles must be out of phase. For example, to turn the body dorsally, the dorsal muscles contract, while the opposing ventral muscles relax. Interactions between excitatory and inhibitory motorneurons produce a pattern of alternating dorsal and ventral contractions (Francis and Waterston, 1991; Hresko et al., 1994; Tavernarakis and Driscoll, 1997). Relatively little is known about how the sinusoidal wave is propagated along the body axis. Adjacent muscle cells are electrically coupled via gap junctions, which could couple excitation of adjacent body muscles. Alternatively, ventral cord motorneurons could promote wave propagation since gap junctions connect adjacent motorneurons of a given class (Chalfie et al., 1985; White et al., 1976; White et al., 1986). A third possibility is that motorneurons could themselves act as stretch receptors so that contraction of

body muscles could regulate adjacent motorneuron activities, thereby propagating the wave (Tavernarakis and Driscoll, 1997; Tavernarakis and Driscoll, 2001b; Tavernarakis et al., 1997). Numerous mutations disrupt normal sinusoidal locomotion in *C. elegans*, resulting in animals with movement defects ranging from total paralysis, to severe uncoordination, to subtle and almost imperceptible irregularities in movement (Tavernarakis and Driscoll, 1997; Tavernarakis et al., 1997). Unusual, semi-dominant (sd), gain-of-function mutations in the gene *unc-8* induce transient neuronal swelling of embryonically derived motorneurons as well as some neurons in the head and tail ganglia, and severe uncoordination (Park and Horvitz, 1986b; Shreffler et al., 1995; Tavernarakis et al., 1997). Swelling is absent at hatching and peaks in severity late in L1 and L2. unc-8 encodes a degenerin expressed in several motor neuron classes, in some interneurons and in nose touch sensory neurons. Interestingly, semi-dominant unc-8 alleles alter an amino acid in the region hypothesized to be an extracellular channel-closing domain defined in studies of deg-1 and *mec-4* degenerins. The genetics of unc-8 are further similar to those of *mec-4* and *mec-10*; specific *unc-8* alleles can suppress or enhance *unc-8(sd)* mutations in trans, suggesting that UNC-8::UNC-8 interactions occur (Shreffler et al., 1995; Tavernarakis et al., 1997). Another degenerin family member, *del-1* (degenerin-like) is co-expressed in a subset of neurons that express *unc-8* (the VA and VB motor neurons) and is likely to assemble into a channel complex with UNC-8 in these cells (Tavernarakis et al., 1997). The UNC-8 and DEL-1 proteins include all domains characteristic of degenerin family members and are likely to adopt similar transmembrane topologies (amino and carboxy termini situated inside the cell and a large extracellular domain that includes three cysteine-rich regions). Neither degenerin has any primary sequence features that are markedly different from other *C. elegans* family members although one somewhat atypical feature of UNC-8 is that it has a relatively long C-terminal domain that shares some primary sequence homology with the extended C-terminus of another degenerin implicated in locomotion, UNC-105 (Liu et al., 1996; Park and Horvitz, 1986a).

The exact function of the UNC-8 degenerin channel in motorneurons was elucidated through genetic approaches. *unc-8* null mutants have a subtle locomotion defect; they inscribe a path in an E. coli lawn that is markedly reduced in both wavelength and amplitude as compared to wild type (Tavernarakis et al., 1997). This phenotype indicates that the UNC-8 degenerin channel functions to modulate the locomotory trajectory of the animal. How does the UNC-8 motor neuron channel influence locomotion? As mentioned earlier, one highly interesting morphological feature of some motorneurons (in particular, the VA and VB motorneurons that co-express unc-8 and del-1) is that their processes include extended regions that do not participate in neuromuscular junctions or neuronal synapses. These "undifferentiated" process regions have been hypothesized to be stretch-sensitive (White et al., 1986). Given the morphological features of certain motor neurons and the sequence similarity of UNC-8 and DEL-1 to the candidate mechanically-gated channels MEC-4 and MEC-10, we have proposed that these subunits co-assemble into a stretch-sensitive channel that might be localized to the undifferentiated regions of the motor neuron process (Tavernarakis et al., 1997). When activated by the localized body stretch that occurs during locomotion, this motor neuron channel

potentiates signaling at the neuromuscular junction, which is situated at a distance from the site of stretch stimulus (Fig. 5.6). The stretch signal enhances motorneuron excitation of muscle, increasing the strength and duration of the pending muscle contraction and directing a full size body turn. In the absence of the stretch activation, the body wave and locomotion still occur, but with significantly reduced amplitude because the potentiating stretch signal is not transmitted. This model bears similarity to the chain reflex mechanism of movement pattern generation. However it does not exclude a central oscillator that would be responsible for the rhythmic locomotion. Instead, we suggest that the output of such an oscillator is further enhanced and modulated by stretch sensitive motorneurons (Tavernarakis and Driscoll, 1997; Tavernarakis et al., 1997). One important corollary of the *unc-8* mutant studies is that the UNC-8 channel does not appear to be essential for motor neuron function. If this were the case, animals lacking the *unc-8* gene would be severely paralyzed. This observation strengthens the argument that degenerin channels function directly in mechanotransduction rather than merely serving to maintain the osmotic environment so that other channels can function.

Muscle cells may also play part in the coordination of locomotion by sensing their own extent of stretch. Mutations in the muscle degenerin *unc-105* cause muscle hypercontraction (Garcia-Anoveros et al., 1998). The muscle hyper-contraction phenotype caused by dominant *unc-105* mutations can be suppressed by mutations near the carboxy-terminus of *let-2*, a gene that encodes the α2 chain of type-IV collagen found in the basement membrane between muscle cells and the hypodermis (Liu et al., 1996). It is tempting to speculate that LET-2 normally carries gating tension to the UNC-105 channel, when the muscle is stretched, thus providing regulatory feedback for muscle contraction. However, results from *in vivo* electrophysiogy suggest that UNC-105 is not involved in the formation of a muscle stretch receptor complex (Jospin et al., 2004).

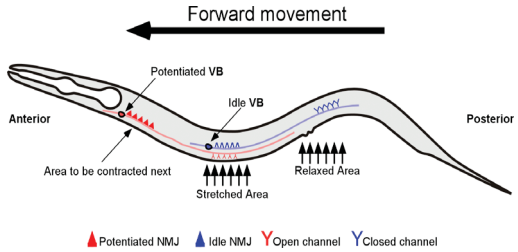

Fig. 5.6 A model for UNC-8 involvement in stretch-regulated control of locomotion. Schematic diagram of potentiated and inactive VB class motor neurons. Neuro-muscular junctions (signified by triangles) are made near the cell body (Tavernarakis et al., 1997; White et al., 1986). Mechanically-activated channels postulated to include UNC-8 (and, possibly in VB motor neurons, DEL-1) subunits (signified by Y figures) are hypothesized to be concentrated at the synapse-free, undifferentiated ends of the VB neuron. Mechanically-gated channels could potentiate local excitation of muscle. Body stretch is postulated to activate mechanically-gated channels which potentiate the motor neuron signal that excites a specific muscle field. Sequential activation of motor neurons that are distributed along the ventral nerve cord and signal non-overlapping groups of muscles, amplifies and propagates the sinusoidal body wave (NMJ: neuromuscular junction). Reproduced from (Voglis and Tavernarakis, 2005) with copyright permission of the Academia Publishing House Ltd

In addition to DEG/ENaC ion channels, a transient receptor potential (TRP) channel has recently been found to be critical for proprioception in *C. elegans*. TRP cation channels are present in all eukaryotes, from yeast to mammals and can be divided into seven subfamilies based on sequence similarities (Montell, 2005). TRP channels are linked to many physiological processes ranging from temperature sensation to mechanosensation and osmosensation (Caterina et al., 1999; Caterina et al., 1997; Colbert et al., 1997; Peier et al., 2002; Tobin et al., 2002). The *trp-4* gene is orthologous to the *Drosophila* nompC channel which is critical for hair cell mechanotransduction (Walker et al., 2000) and to the zebrafish nompC which is required for the function of auditory hair cells (Sidi et al., 2003). *C. elegans trp-4* mutants generate abnormal locomotion waves, with exaggerated body bands and larger than normal wave amplitudes (Li et al., 2006). TRP-4 acts in a single interneuron, called DVA and TRP-4 channels may act as stretch receptors in these neurons to provide sensory feedback to the locomotor control circuit. Thus, DVA appears to be a primary proprioreceptor neuron which modulates the shape of the locomotion sinusoidal wave.

5.6 Mechanotransduction in Other Organisms

Genetic studies of sensory mechanotransduction, which were initiated in *C. elegans* and are now also being carried out in *Drosophila* and in mammals, have converged to reveal a limited set of underlying mechanisms (Eberl, 1999; Gillespie and Walker, 2001; Harteneck et al., 2000; Kellenberger and Schild, 2002). For example, the model proposed for mechanotransduction in the touch receptor neurons (Fig. 5.7) and motorneurons of *C. elegans* shares the same underlying principle and features of the proposed gating mechanism of mechanosensory ion channels in Drosophila sensory bristles and the channels that respond to auditory stimuli in the hair cells of the vertebrate inner ear (Fettiplace and Fuchs, 1999; Gillespie and Walker, 2001; Hamill and McBride, 1996; Hudspeth, 1989; Hudspeth et al., 2000; Jaramillo and Hudspeth, 1991; Pickles and Corey, 1992; Pickles et al., 1991; Tavernarakis and Driscoll, 1997; Weinbaum et al., 2001). Hair cells have bundles of a few hundred stereocilia on their apical surface, which mediate sensory transduction Stereocilia are connected at their distal ends to neighboring stereocilia by filaments called tip links. The integrity of the tip links is essential for channel opening and the mechanosensitive channels appear to be situated at the ends of the stereocilia, near the connecting tip links. Directional deflection of the stereocilia relative to each other introduces tension on the tip links, which is proposed to open the mechanosensitive hair cell channels directly. This remarkable convergence of independent studies in distant species, strongly suggests that different mechanotransducers in different systems have evolved to strictly adhere to the same set of principles. Besides the DEG/ENaC family of ion channels, members of the TRP family are involved in mechanosensation in different organisms. (Alvarez de la Rosa et al., 2000; Duggan et al., 1998; Minke and Cook, 2002; Montell, 2001; Tavernarakis and Driscoll, 2001a; Welsh et al., 2002). Experiments in *Drosophila* revealed the involvement of TRP-like channel genes in the function of

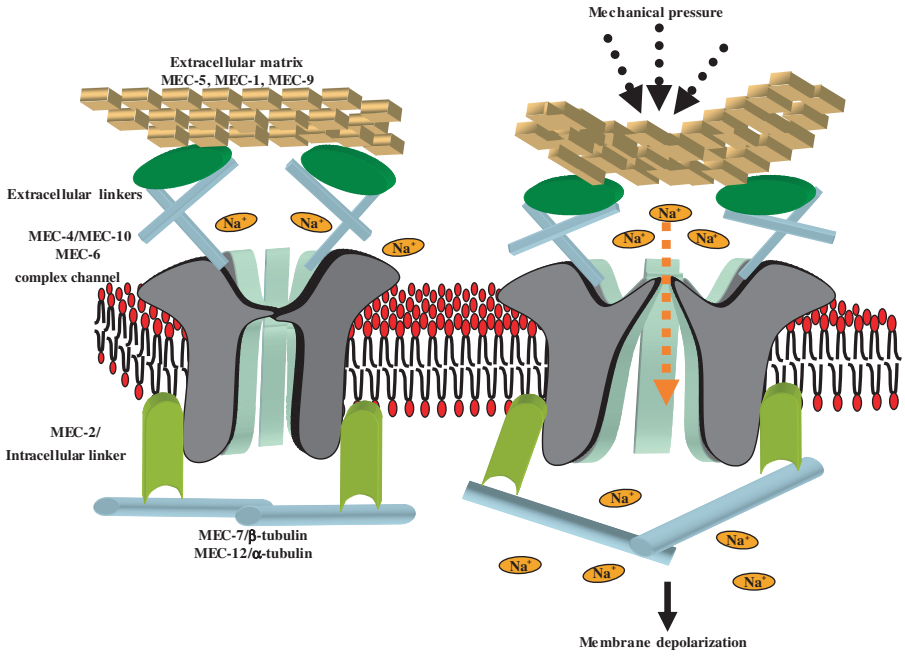

Fig. 5.7 A model for a mechanotransducing complex in *C. elegans* touch receptor neurons. The extracellular matrix contains MEC-5, MEC-9 and MEC-1; the sensory transduction channel is formed by MEC-4, MEC-10 and possibly MEC-6; MEC-2 enhances channel activity and tethers the channel to specialized microtubules containing MEC-7/β-tubulin and MEC-12/α-tubulin. Recent findings suggest that mechanotransduction can occur even when the 15-protofilament microtubules are missing in *mec-7* mutants, questioning in that way the necessity of tethering of the complex to microtubules (O'Hagan et al., 2005). In the absence of mechanical stimulation, the channel is closed and the sensory neuron is idle. Application of mechanical force to the body of the animal results in distortion of a network of interacting molecules that opens the degenerins channel. Na^+influx depolarizes the neuron, initiating a cascade that leads to the integration of the stimulus

mechanosensoty bristles (*nompC*; (Walker et al., 2000)), auditory receptors (*iav* and *nan*; (Gong et al., 2004)), and nociceptors (*painless*; (Tracey et al., 2003)). Another member of the TRP protein family, the TRPA1 channel has been identified as a candidate mechanotransducing channel in the mouse (Corey et al., 2004). *In situ* hybridization revealed that the TRPA1 channel is expressed in the cochlea organ of Corti, which contains the auditory hair cells. Additional colocalization experiments link TRPA1 to mechanosensation: TRPA1 is expressed together with two accessory proteins of the mechanosensory apparatus, myosin 1c and cadherin 23, at the tips of stereocilia throughout the kinocilium and in the pericuticular zone. Whole-cell patch clamp recording of inner hair cells in mice show that the transduction current produced is significantly reduced in the absence of TRPA1, indicating that this channel is a component of the mechanosensitive transduction channel of vertebrate hair cells (Corey et al., 2004). Additionally, two TRP proteins, a NompC-like protein and TRPA1 are required for hair cell function in zebrafish (Corey et al., 2004; Sidi et al., 2003).

Despite enormous progress on the illumination of vertebrate mechanosensory cell biology achieved in recent years, there is still a striking gap between the biophysical information that has accumulated and our understanding of the molecular aspects of mechanosensation. Sophisticated experiments in mice and humans revealed many genes involved in the development and function of the mammalian cochlea and have cumulated in the formulation of the gating-spring model for hair cell mechanotransduction (Gillespie, 1995; Gillespie and Walker, 2001). However, many pieces of the mechanotransducing apparatus puzzle are still missing. Work in lower vertebrates such as birds, amphibians and fish has also contributed significantly in complementing and extending the studies with mammals. In these animals mechanosensory structures are often much easier to access, follow and monitor providing large potential for investigating the molecular basis of auditory transduction (Ashmore, 1998; Smotherman and Narins, 2000). An increasing amount of evidence suggests that some mammalian DEG/ENaC proteins may play a role in mechanosensation similarly to their nematode counterparts. In mammals, there are strong indications that ENaC subunits may be components of the baroreceptor mechanotransducer, one of the most potent regulators of anterial pressure and neurohumoral control of the circulation (Drummond et al., 1998; Drummond et al., 2001). Members of the ASIC (acid sensing ion channel) subgroup of the DEG/ENaC family have been implicated in mechanotransduction in mammals. BNC1 (brain Na^+ channel; also known as MDEG, BNaC1, ASIC2; (Garcia-Anoveros and Corey, 1997; Price et al., 1996; Waldmann et al., 1996; Waldmann and Lazdunski, 1998)) has emerged as promising candidate for a mechanosensitive channel. In BNC1 null mice touch receptor neurons of the skin produce fewer action potentials than in wild type animals over a comparable range of stimuli (Price et al., 2000).

5.7 Conclusions

Studies in *C. elegans* have contributed critical insights into the cellular and molecular mechanisms of mechanotransduction (Fig. 5.7; Chalfie, 1997; Syntichaki and Tavernarakis, 2004; Tavernarakis and Driscoll, 1997; Tavernarakis and Driscoll, 2001b). Recently developed powerful methodologies, such as direct electrophysiological recordings from neurons and imaging of genetically encoded calcium sensors provide the unique opportunity to investigate the properties of the mechanotransduction apparatus in the context of live, behaving animals.

Although our understanding of metazoan mechanosensation has been advanced significantly, open questions still remain. While specific DEG/ENaC and TRP ion channels have been directly implicated in the process of mechanotransduction the *C. elegans* genome encodes many more members of these ion channel proteins. The role of these proteins in mechanotransduction remains to be elucidated.

An additional major question that remains to be addressed is whether the mammalian counterparts of the *C. elegans* degenerins play specialized roles in mechanical signalling in humans. A significant step toward addressing this ques-

tion has been accomplished with the demonstration that BNC1 is involved in mechanosensory signalling in the skin as we have described above. Even though the candidacy of BNC1 for being in the core of a mechanotransducing complex was greatly boosted by these results, a demanding critic would argue that albeit very strong, it still remains just a candidacy. The potential role of BNC1 as part of the core mechanotransducing channel can still only be inferred from these experiments and is not directly proven. It is still possible that BNC1 forms or participates in an auxiliary channel that facilitates the function of the actual mechanotransducing channel. A BNC1 knockout does not completely eliminate the responses to mechanical stimuli (Price et al., 2000). The incomplete nature of the BNC1 deficiency effects indicates that even if BNC1 is indeed part of the core mechanosensory channel, it most likely is not the only critical one. Alternatively, there might be more than one, different mechanotransducing complexes within one neuron, with different properties and composition. The above arguments however, are by no means confined to BNC1. On the same basis, MEC-4/MEC-10 and UNC-8/DEL-1 in *C. elegans* as well as PPK in *Drosophila* might not be parts of the real mechanotransducer but only auxiliary ion channels. The recent identification of another strong candidate mechanosensory channel, the *Drosophila* NompC, adds to the list of candidate mechanosensitive ion channels (Walker et al., 2000). Evidence implicating NompC in mechanotransduction is especially convincing given the supporting electrophysiological analysis that is feasible in this system, and the availability of mutants with altered properties and intermediate effects (Walker et al., 2000). Therefore, NompC homologues in other organisms, including humans, emerge putative mechanosensitive ion channels. Even in this case however, there are caveats; the absence of NompC does not completely eliminate mechanosensitive currents in *Drosophila* hair bristles. Furthermore, the identities and properties of force-generating tethers of NompC in mechanotransducing complexes will need to be determined. Another issue that needs to be addressed is the potential interplay between DEG/ENaC and NompC channels in mechanosensory cells before a clear understanding of mechanotransduction can be achieved.

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References

Alvarez de la Rosa, D., C. M. Canessa, G. K. Fyfe, and P. Zhang. 2000. Structure and regulation of amiloride-sensitive sodium channels. Annu Rev Physiol 62:573–94.

Ashmore, J. 1998. Mechanosensation: swimming round in circles. Curr Biol 8:425–7.

Avery, L. 1993. The genetics of feeding in Caenorhabditis elegans. Genetics 133:897–917.

Bargmann, C. I., and J. M. Kaplan. 1998. Signal transduction in the Caenorhabditis elegans nervous system. Annu Rev Neurosci 21:279–308.

Bargmann, C. I., J. H. Thomas, and H. R. Horvitz. 1990. Chemosensory cell function in the behavior and development of Caenorhabditis elegans. Cold Spring Harb Symp Quant Biol 55:529–38.

Barnes, T. M., Y. Jin, H. R. Horvitz, G. Ruvkun, and S. Hekimi. 1996. The Caenorhabditis elegans behavioral gene unc-24 encodes a novel bipartite protein similar to both erythrocyte band 7.2 (stomatin) and nonspecific lipid transfer protein. J Neurochem 67:46–57.

Baumeister, R., and L. Ge. 2002. The worm in us - Caenorhabditis elegans as a model of human disease. Trends Biotechnol 20:147–8.

Block, I., N. Freiberger, O. Gavrilova, and R. Hemmersbach. 1999. Putative graviperception mechanisms of protists. Adv Space Res 24:877–82.

Blount, P., and P. C. Moe. 1999. Bacterial mechanosensitive channels: integrating physiology, structure and function. Trends Microbiol 7:420–4.

Brenner, S. 1974. The genetics of Caenorhabditis elegans. Genetics 77:71–94.

Caterina, M. J., T. A. Rosen, M. Tominaga, A. J. Brake, and D. Julius. 1999. A capsaicin-receptor homologue with a high threshold for noxious heat. Nature 398:436–41.

Caterina, M. J., M. A. Schumacher, M. Tominaga, T. A. Rosen, J. D. Levine, and D. Julius. 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–24.

Chalfie, M. 1995. The differentiation and function of the touch receptor neurons of Caenorhabditis elegans. Prog Brain Res 105:179–82.

Chalfie, M. 1997. A molecular model for mechanosensation in Caenorhabditis elegans. Biol Bull 192:125.

Chalfie, M. 1993. Touch receptor development and function in Caenorhabditis elegans. J Neurobiol 24:1433–41.

Chalfie, M., and M. Au. 1989. Genetic control of differentiation of the Caenorhabditis elegans touch receptor neurons. Science 243:1027–33.

Chalfie, M., E. Dean, E. Reilly, K. Buck, and J. N. Thomson. 1986. Mutations affecting microtubule structure in Caenorhabditis elegans. J Cell Sci Suppl 5:257–71.

Chalfie, M., M. Driscoll, and M. Huang. 1993. Degenerin similarities. Nature 361:504.

Chalfie, M., and J. Sulston. 1981. Developmental genetics of the mechanosensory neurons of Caenorhabditis elegans. Dev Biol 82:358–70.

Chalfie, M., J. E. Sulston, J. G. White, E. Southgate, J. N. Thomson, and S. Brenner. 1985. The neural circuit for touch sensitivity in Caenorhabditis elegans. J Neurosci 5:956–64.

Chalfie, M., and J. N. Thomson. 1979. Organization of neuronal microtubules in the nematode Caenorhabditis elegans. J Cell Biol 82:278–89.

Chalfie, M., and J. N. Thomson. 1982. Structural and functional diversity in the neuronal microtubules of Caenorhabditis elegans. J Cell Biol 93:15–23.

Chalfie, M., J. N. Thomson, and J. E. Sulston. 1983. Induction of neuronal branching in Caenorhabditis elegans. Science 221:61–3.

Chalfie, M., and E. Wolinsky. 1990. The identification and suppression of inherited neurodegeneration in Caenorhabditis elegans. Nature 345:410–6.

Chelur, D. S., G. G. Ernstrom, M. B. Goodman, C. A. Yao, A. F. Chen, R. O'Hagan, and M. Chalfie. 2002. The mechanosensory protein MEC-6 is a subunit of the C. elegans touch-cell degenerin channel. Nature 420:669–73.

Chiba, C. M., and C. H. Rankin. 1990. A developmental analysis of spontaneous and reflexive reversals in the nematode Caenorhabditis elegans. J Neurobiol 21:543–54.

Colbert, H. A., T. L. Smith, and C. I. Bargmann. 1997. OSM-9, a novel protein with structural similarity to channels, is required for olfaction, mechanosensation, and olfactory adaptation in Caenorhabditis elegans. J Neurosci 17:8259–69.

Corey, D. P. 2003. New TRP channels in hearing and mechanosensation. Neuron 39:585–8.

Corey, D. P., J. Garcia-Anoveros, J. R. Holt, K. Y. Kwan, S. Y. Lin, M. A. Vollrath, A. Amalfitano, E. L. Cheung, B. H. Derfler, A. Duggan, G. S. Geleoc, P. A. Gray, M. P. Hoffman, H. L. Rehm, Tamasauskas, D., and D. S. Zhang. 2004. TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. Nature 432:723–30.

Coulson, A., R. Waterston, J. Kiff, J. Sulston, and Y. Kohara. 1988. Genome linking with yeast artificial chromosomes. Nature 335:184–6.

Delaunay, J., G. Stewart, and A. Iolascon. 1999. Hereditary dehydrated and overhydrated stomatocytosis: recent advances. Curr Opin Hematol 6:110–4.

Driscoll, M. 1996. Cell death in C. elegans: molecular insights into mechanisms conserved between nematodes and mammals. Brain Pathol 6:411–25.

Driscoll, M., and M. Chalfie. 1991. The mec-4 gene is a member of a family of Caenorhabditis elegans genes that can mutate to induce neuronal degeneration. Nature 349:588–93.

Driscoll, M., and J. M. Kaplan. 1996. Mechanotransduction, p. 645–677. *In* D. L. Riddle, T. Blumenthal, B. J. Meyer, and J. R. Pries (ed.), The Nematode C. elegans, II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Driscoll, M., and N. Tavernarakis. 1997. Molecules that mediate touch transduction in the nematode Caenorhabditis elegans. Gravit Space Biol Bull 10:33–42.

Drummond, H. A., M. P. Price, M. J. Welsh, and F. M. Abboud. 1998. A molecular component of the arterial baroreceptor mechanotransducer. Neuron 21:1435–41.

Drummond, H. A., M. J. Welsh, and F. M. Abboud. 2001. ENaC subunits are molecular components of the arterial baroreceptor complex. Ann N Y Acad Sci 940:42–7.

Du, H., and M. Chalfie. 2001. Genes regulating touch cell development in Caenorhabditis elegans. Genetics 158:197–207.

Du, H., G. Gu, C. M. William, and M. Chalfie. 1996. Extracellular proteins needed for C. elegans mechanosensation. Neuron 16:183–94.

Duggan, A., C. Ma, and M. Chalfie. 1998. Regulation of touch receptor differentiation by the Caenorhabditis elegans mec-3 and unc-86 genes. Development 125:4107–19.

Eberl, D. F. 1999. Feeling the vibes: chordotonal mechanisms in insect hearing. Curr Opin Neurobiol 9:389–93.

Emtage, L., G. Gu, E. Hartwieg, and M. Chalfie. 2004. Extracellular proteins organize the mechanosensory channel complex in C. elegans touch receptor neurons. Neuron 44:795–807.

Fettiplace, R., and P. A. Fuchs. 1999. Mechanisms of hair cell tuning. Annu Rev Physiol 61:809–34.

Finney, M., and G. Ruvkun. 1990. The unc-86 gene product couples cell lineage and cell identity in C. elegans. Cell 63:895–905.

Finney, M., G. Ruvkun, and H. R. Horvitz. 1988. The C. elegans cell lineage and differentiation gene unc-86 encodes a protein with a homeodomain and extended similarity to transcription factors. Cell 55:757–69.

Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, and C. C. Mello. 1998. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391:806–11.

Francis, R., and R. H. Waterston. 1991. Muscle cell attachment in Caenorhabditis elegans. J Cell Biol 114:465–79.

French, A. S. 1992. Mechanotransduction. Annu Rev Physiol 54:135–52.

Fukushige, T., Z. K. Siddiqui, M. Chou, J. G. Culotti, C. B. Gogonea, S. S. Siddiqui, and M. Hamelin. 1999. MEC-12, an alpha-tubulin required for touch sensitivity in C. elegans. J Cell Sci 112:395–403.

Garcia-Anoveros, J., and D. P. Corey. 1997. The molecules of mechanosensation. Annu Rev Neurosci 20:567–94.

Garcia-Anoveros, J., and D. P. Corey. 1996. Touch at the molecular level. Mechanosensation. Curr Biol 6:541–3.

Garcia-Anoveros, J., J. A. Garcia, J. D. Liu, and D. P. Corey. 1998. The nematode degenerin UNC-105 forms ion channels that are activated by degeneration- or hypercontraction-causing mutations. Neuron 20:1231–41.

Gebauer, M., D. Watzke, and H. Machemer. 1999. The gravikinetic response of Paramecium is based on orientation-dependent mechanotransduction. Naturwissenschaften 86:352–6.

Gillespie, P. G. 1995. Molecular machinery of auditory and vestibular transduction. Curr Opin Neurobiol 5:449–55.

Gillespie, P. G., and R. G. Walker. 2001. Molecular basis of mechanosensory transduction. Nature 413:194–202.

Golden, J. W., and D. L. Riddle. 1984. The Caenorhabditis elegans dauer larva: developmental effects of pheromone, food, and temperature. Dev Biol 102:368–78.

Gong, Z., W. Son, Y. D. Chung, J. Kim, D. W. Shin, C. A. McClung, Y. Lee, H. W. Lee, D. J. Chang, B. K. Kaang, H. Cho, U. Oh, J. Hirsh, M. J. Kernan, and C. Kim. 2004. Two interdependent TRPV channel subunits, inactive and Nanchung, mediate hearing in Drosophila. J Neurosci 24:9059–66.

Goodman, M. B., G. G. Ernstrom, D. S. Chelur, R. O'Hagan, C. A. Yao, and M. Chalfie. 2002. MEC-2 regulates C. elegans DEG/ENaC channels needed for mechanosensation. Nature 415:1039–42.

Gu, G., G. Caldwell, and M. Chalfie. 1996. Genetic interactions affecting touch sensitivity in Caenorhabditis elegans. Proc Natl Acad Sci U S A 93:6577 - 82.

Hackney, C. M., and D. N. Furness. 1995. Mechanotransduction in vertebrate hair cells: structure and function of the stereociliary bundle. Am J Physiol 268:C1–13.

Hall, D. H., G. Gu, J. Garcia-Anoveros, L. Gong, M. Chalfie, and M. Driscoll. 1997. Neuropathology of degenerative cell death in Caenorhabditis elegans. J Neurosci 17:1033–45.

Hamelin, M., I. M. Scott, J. C. Way, and J. G. Culotti. 1992. The mec-7 beta-tubulin gene of Caenorhabditis elegans is expressed primarily in the touch receptor neurons. Embo J 11:2885–93.

Hamill, O. P., J. W. Lane, and D. W. McBride, Jr. 1992. Amiloride: a molecular probe for mechanosensitive channels. Trends Pharmacol Sci 13:373–6.

Hamill, O. P., and B. Martinac. 2001. Molecular basis of mechanotransduction in living cells. Physiol Rev 81:685–740.

Hamill, O. P., and D. W. McBride, Jr. 1996. The pharmacology of mechanogated membrane ion channels. Pharmacol Rev 48:231–52.

Harbinder, S., N. Tavernarakis, L. A. Herndon, M. Kinnell, S. Q. Xu, A. Fire, and M. Driscoll. 1997. Genetically targeted cell disruption in Caenorhabditis elegans. Proc Natl Acad Sci U S A 94:13128–33.

Hart, A. C., J. Kass, J. E. Shapiro, and J. M. Kaplan. 1999. Distinct signaling pathways mediate touch and osmosensory responses in a polymodal sensory neuron. J Neurosci 19:1952–8.

Hart, A. C., S. Sims, and J. M. Kaplan. 1995. Synaptic code for sensory modalities revealed by C. elegans GLR-1 glutamate receptor. Nature 378:82–5.

Harteneck, C., T. D. Plant, and G. Schultz. 2000. From worm to man: three subfamilies of TRP channels. Trends Neurosci 23:159–66.

Hemmersbach, R., B. Bromeis, I. Block, R. Braucker, M. Krause, N. Freiberger, C. Stieber, and M. Wilczek. 2001. Paramecium–a model system for studying cellular graviperception. Adv Space Res 27:893–8.

Herman, R. K. 1996. Touch sensation in Caenorhabditis elegans. Bioessays 18:199–206.

Hilliard, M. A., A. J. Apicella, R. Kerr, H. Suzuki, P. Bazzicalupo, and W. R. Schafer. 2005. In vivo imaging of C. elegans ASH neurons: cellular response and adaptation to chemical repellents. Embo J 24:63–72.

Hoger, U., P. H. Torkkeli, E. A. Seyfarth, and A. S. French. 1997. Ionic selectivity of mechanically activated channels in spider mechanoreceptor neurons. J Neurophysiol 78:2079–85.

Hresko, M. C., B. D. Williams, and R. H. Waterston. 1994. Assembly of body wall muscle and muscle cell attachment structures in Caenorhabditis elegans. J Cell Biol 124: 491–506.

Huang, M., and M. Chalfie. 1994. Gene interactions affecting mechanosensory transduction in Caenorhabditis elegans. Nature 367:467–70.

Huang, M., G. Gu, E. L. Ferguson, and M. Chalfie. 1995. A stomatin-like protein necessary for mechanosensation in C. elegans. Nature 378:292–5.

Hudspeth, A. J. 1989. How the ear's works work. Nature 341:397–404.

Hudspeth, A. J., Y. Choe, A. D. Mehta, and P. Martin. 2000. Putting ion channels to work: mechanoelectrical transduction, adaptation, and amplification by hair cells. Proc Natl Acad Sci U S A 97:11765–72.

Ingber, D. E. 1997. Tensegrity: the architectural basis of cellular mechanotransduction. Annu Rev Physiol 59:575–99.

Jaramillo, F., and A. J. Hudspeth. 1991. Localization of the hair cell's transduction channels at the hair bundle's top by iontophoretic application of a channel blocker. Neuron 7:409–20.

Jospin, M., M. C. Mariol, L. Segalat, and B. Allard. 2004. Patch clamp study of the UNC-105 degenerin and its interaction with the LET-2 collagen in Caenorhabditis elegans muscle. J Physiol 557:379–88.

Kahn-Kirby, A. H., and C. I. Bargmann. 2006. TRP channels in C. elegans. Annu Rev Physiol 68:719–36.

Kahn-Kirby, A. H., J. L. Dantzker, A. J. Apicella, W. R. Schafer, J. Browse, C. I. Bargmann, and J. L. Watts. 2004. Specific polyunsaturated fatty acids drive TRPV-dependent sensory signaling in vivo. Cell 119:889–900.

Kaplan, J. M., and H. R. Horvitz. 1993. A dual mechanosensory and chemosensory neuron in Caenorhabditis elegans. Proc Natl Acad Sci U S A 90:2227–31.

Kellenberger, S., and L. Schild. 2002. Epithelial sodium channel/degenerin family of ion channels: a variety of functions for a shared structure. Physiol Rev 82:735–67.

Kitamura, K. I., S. Amano, and R. Hosono. 2001. Contribution of neurons to habituation to mechanical stimulation in Caenorhabditis elegans. J Neurobiol 46:29–40.

Kloda, A., and B. Martinac. 2001. Molecular identification of a mechanosensitive channel in archaea. Biophys J 80:229–40.

Ko, K. S., and C. A. McCulloch. 2001. Intercellular mechanotransduction: cellular circuits that coordinate tissue responses to mechanical loading. Biochem Biophys Res Commun 285:1077–83.

Koch, A. L. 1994. Development and diversification of the Last Universal Ancestor. J Theor Biol 168:269–80.

Koch, R., H. G. van Luenen, M. van der Horst, K. L. Thijssen, and R. H. Plasterk. 2000. Single nucleotide polymorphisms in wild isolates of Caenorhabditis elegans. Genome Res 10: 1690–6.

Koprowski, P., and A. Kubalski. 2001. Bacterial ion channels and their eukaryotic homologues. Bioessays 23:1148–58.

Lane, J. W., D. W. McBride, Jr., and O. P. Hamill. 1991. Amiloride block of the mechanosensitive cation channel in Xenopus oocytes. J Physiol 441:347–66.

Lee, R. T., and H. Huang. 2000. Mechanotransduction and arterial smooth muscle cells: new insight into hypertension and atherosclerosis. Ann Med 32:233–5.

Li, W., Z. Feng, P. W. Sternberg, and X. Z. Xu. 2006. A C. elegans stretch receptor neuron revealed by a mechanosensitive TRP channel homologue. Nature 440:684–7.

Liedtke, W., D. M. Tobin, C. I. Bargmann, and J. M. Friedman. 2003. Mammalian TRPV4 (VR-OAC) directs behavioral responses to osmotic and mechanical stimuli in Caenorhabditis elegans. Proc Natl Acad Sci U S A 100 Suppl 2:14531–6.

Liu, D. W., and J. H. Thomas. 1994. Regulation of a periodic motor program in C. elegans. J Neurosci 14:1953–62.

Liu, J., B. Schrank, and R. H. Waterston. 1996. Interaction between a putative mechanosensory membrane channel and a collagen. Science 273:361–4.

Liu, K. S., and P. W. Sternberg. 1995. Sensory regulation of male mating behavior in Caenorhabditis elegans. Neuron 14:79–89.

Lynch, T. M., P. M. Lintilhac, and D. Domozych. 1998. Mechanotransduction molecules in the plant gravisensory response: amyloplast/statolith membranes contain a beta 1 integrin-like protein. Protoplasma 201:92–100.

Mah, K. B., and C. H. Rankin. 1992. An analysis of behavioral plasticity in male Caenorhabditis elegans. Behav Neural Biol 58:211–21.

Marino, M. J., T. G. Sherman, and D. C. Wood. 2001. Partial cloning of putative G-proteins modulating mechanotransduction in the ciliate stentor. J Eukaryot Microbiol 48:527–36.

Martinac, B. 2001. Mechanosensitive channels in prokaryotes. Cell Physiol Biochem 11:61–76.

Mello, C., and A. Fire. 1995. DNA transformation. Methods Cell Biol 48:451–82.

Minke, B., and B. Cook. 2002. TRP channel proteins and signal transduction. Physiol Rev 82: 429–72.

Moerman, D. G., H. Hutter, G. P. Mullen, and R. Schnabel. 1996. Cell autonomous expression of perlecan and plasticity of cell shape in embryonic muscle of Caenorhabditis elegans. Dev Biol 173:228–42.

Montell, C. 2001. Physiology, phylogeny, and functions of the TRP superfamily of cation channels. Sci STKE 2001.

Montell, C. 2005. The TRP superfamily of cation channels. Sci STKE 2005.

Nauli, S. M., F. J. Alenghat, Y. Luo, E. Williams, P. Vassilev, X. Li, A. E. Elia, W. Lu, E. M. Brown, S. J. Quinn, D. E. Ingber, and J. Zhou. 2003. Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. Nat Genet 33:129–37.

Norris, V., M. S. Madsen, and P. Freestone. 1996. Elements of a unifying theory of biology. Acta Biotheor 44:209–18.

O'Hagan, R., and M. Chalfie. 2006. Mechanosensation in Caenorhabditis elegans. Int Rev Neurobiol 69:169–203.

O'Hagan, R., M. Chalfie, and M. B. Goodman. 2005. The MEC-4 DEG/ENaC channel of Caenorhabditis elegans touch receptor neurons transduces mechanical signals. Nat Neurosci 8:43–50.

Park, E. C., and H. R. Horvitz. 1986. C. elegans unc-105 mutations affect muscle and are suppressed by other mutations that affect muscle. Genetics 113:853–67.

Park, E. C., and H. R. Horvitz. 1986. Mutations with dominant effects on the behavior and morphology of the nematode Caenorhabditis elegans. Genetics 113:821–52.

Peier, A. M., A. J. Reeve, D. A. Andersson, A. Moqrich, T. J. Earley, A. C. Hergarden, G. M. Story, S. Colley, J. B. Hogenesch, P. McIntyre, S. Bevan, and A. Patapoutian. 2002. A heat-sensitive TRP channel expressed in keratinocytes. Science 296:2046–9.

Perkins, L. A., E. M. Hedgecock, J. N. Thomson, and J. G. Culotti. 1986. Mutant sensory cilia in the nematode Caenorhabditis elegans. Dev Biol 117:456–87.

Pickard, B. G., and J. P. Ding. 1993. The mechanosensory calcium-selective ion channel: key component of a plasmalemmal control centre? Aust J Plant Physiol 20:439–59.

Pickles, J. O., and D. P. Corey. 1992. Mechanoelectrical transduction by hair cells. Trends Neurosci 15:254–9.

Pickles, J. O., G. W. Rouse, and M. von Perger. 1991. Morphological correlates of mechanotransduction in acousticolateral hair cells. Scanning Microsc 5:1115–24.

Price, M. P., G. R. Lewin, S. L. McIlwrath, C. Cheng, J. Xie, P. A. Heppenstall, C. L. Stucky, A. G. Mannsfeldt, T. J. Brennan, H. A. Drummond, J. Qiao, C. J. Benson, D. E. Tarr, R. F. Hrstka, B. Yang, R. A. Williamson, and M. J. Welsh. 2000. The mammalian sodium channel BNC1 is required for normal touch sensation. Nature 407:1007–11.

Price, M. P., P. M. Snyder, and M. J. Welsh. 1996. Cloning and expression of a novel human brain Na+ channel. J Biol Chem 271:7879–82.

Rankin, C. H. 1991. Interactions between two antagonistic reflexes in the nematode Caenorhabditis elegans. J Comp Physiol [A] 169:59–67.

Rankin, C. H., T. Gannon, and S. R. Wicks. 2000. Developmental analysis of habituation in the Nematode C. elegans. Dev Psychobiol 36:261–70.

Roayaie, K., J. G. Crump, A. Sagasti, and C. I. Bargmann. 1998. The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in C. elegans olfactory neurons. Neuron 20:55–67.

Rossier, B. C., C. M. Canessa, L. Schild, and J. D. Horisberger. 1994. Epithelial sodium channels. Curr Opin Nephrol Hypertens 3:487–96.

Rupp, F., W. Hoch, J. T. Campanelli, T. Kreiner, and R. H. Scheller. 1992. Agrin and the organization of the neuromuscular junction. Curr Opin Neurobiol 2:88–93.

Rupp, F., T. Ozcelik, M. Linial, K. Peterson, U. Francke, and R. Scheller. 1992. Structure and chromosomal localization of the mammalian agrin gene. J Neurosci 12:3535–44.

Rupp, F., D. G. Payan, C. Magill-Solc, D. M. Cowan, and R. H. Scheller. 1991. Structure and expression of a rat agrin. Neuron 6:811–23.

Sackin, H. 1995. Mechanosensitive channels. Annu Rev Physiol 57:333–53.

Sadoshima, J., T. Takahashi, L. Jahn, and S. Izumo. 1992. Roles of mechano-sensitive ion channels, cytoskeleton, and contractile activity in stretch-induced immediate-early gene expression and hypertrophy of cardiac myocytes. Proc Natl Acad Sci USA 89:9905–9.

Savage, C., M. Hamelin, J. G. Culotti, A. Coulson, D. G. Albertson, and M. Chalfie. 1989. mec-7 is a beta-tubulin gene required for the production of 15-protofilament microtubules in Caenorhabditis elegans. Genes Dev 3:870–81.

Savage, C., Y. Xue, S. Mitani, D. Hall, R. Zakhary, and M. Chalfie. 1994. Mutations in the Caenorhabditis elegans beta-tubulin gene mec-7: effects on microtubule assembly and stability and on tubulin autoregulation. J Cell Sci 107:2165–75.

Sawin, E. R., R. Ranganathan, and H. R. Horvitz. 2000. C. elegans locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. Neuron 26:619–31.

Sedensky, M. M., J. M. Siefker, and P. G. Morgan. 2001. Model organisms: new insights into ion channel and transporter function. Stomatin homologues interact in Caenorhabditis elegans. Am J Physiol Cell Physiol 280:1340–8.

Shreffler, W., T. Magardino, K. Shekdar, and E. Wolinsky. 1995. The unc-8 and sup-40 genes regulate ion channel function in Caenorhabditis elegans motorneurons. Genetics 139:1261–72.

Sidi, S., R. W. Friedrich, and T. Nicolson. 2003. NompC TRP channel required for vertebrate sensory hair cell mechanotransduction. Science 301:96–9.

Smotherman, M. S., and P. M. Narins. 2000. Hair cells, hearing and hopping: a field guide to hair cell physiology in the frog. J Exp Biol 203:2237–46.

Snyers, L., E. Umlauf, and R. Prohaska. 1998. Oligomeric nature of the integral membrane protein stomatin. J Biol Chem 273:17221–6.

Stewart, G. W. 1997. Stomatin. Int J Biochem Cell Biol 29:271–4.

Stewart, G. W., A. C. Argent, and B. C. Dash. 1993. Stomatin: a putative cation transport regulator in the red cell membrane. Biochim Biophys Acta 1225:15–25.

Sulston, J. E., D. G. Albertson, and J. N. Thomson. 1980. The Caenorhabditis elegans male: postembryonic development of nongonadal structures. Dev Biol 78:542–76.

Sulston, J. E., and H. R. Horvitz. 1977. Post embriyonic cell lineages of the nematode *Caenorhabditis elegans*. Dev Biol 56:110–156.

Suzuki, H., R. Kerr, L. Bianchi, C. Frokjaer-Jensen, D. Slone, J. Xue, B. Gerstbrein, M. Driscoll, and W. R. Schafer. 2003. In vivo imaging of C. elegans mechanosensory neurons demonstrates a specific role for the MEC-4 channel in the process of gentle touch sensation. Neuron 39:1005–17.

Syntichaki, P., and N. Tavernarakis. 2004. Genetic models of mechanotransduction: the nematode Caenorhabditis elegans. Physiol Rev 84:1097–153.

Tavernarakis, N., and M. Driscoll. 2001. Degenerins. At the core of the metazoan mechanotransducer? Ann N Y Acad Sci 940:28–41.

Tavernarakis, N., and M. Driscoll. 2001. Mechanotransduction in Caenorhabditis elegans: the role of DEG/ENaC ion channels. Cell Biochem Biophys 35:1–18.

Tavernarakis, N., and M. Driscoll. 1997. Molecular modeling of mechanotransduction in the nematode Caenorhabditis elegans. Annu Rev Physiol 59:659–89.

Tavernarakis, N., M. Driscoll, and N. C. Kyrpides. 1999. The SPFH domain: implicated in regulating targeted protein turnover in stomatins and other membrane-associated proteins. Trends Biochem Sci 24:425–7.

Tavernarakis, N., W. Shreffler, S. Wang, and M. Driscoll. 1997. unc-8, a DEG/ENaC family member, encodes a subunit of a candidate mechanically gated channel that modulates C. elegans locomotion. Neuron 18:107–19.

Tavernarakis, N., S. L. Wang, M. Dorovkov, A. Ryazanov, and M. Driscoll. 2000. Heritable and inducible genetic interference by double-stranded RNA encoded by transgenes. Nat Genet 24:180–3.

Tavi, P., M. Laine, M. Weckstrom, and H. Ruskoaho. 2001. Cardiac mechanotransduction: from sensing to disease and treatment. Trends Pharmacol Sci 22:254–60.

Thomas, J. H., M. J. Stern, and H. R. Horvitz. 1990. Cell interactions coordinate the development of the C. elegans egg-laying system. Cell 62:1041–52.

Tobin, D., D. Madsen, A. Kahn-Kirby, E. Peckol, G. Moulder, R. Barstead, A. Maricq, and C. Bargmann. 2002. Combinatorial expression of TRPV channel proteins defines their sensory functions and subcellular localization in C. elegans neurons. Neuron 35:307–18.

Tracey, W. D., Jr., R. I. Wilson, G. Laurent, and S. Benzer. 2003. painless, a Drosophila gene essential for nociception. Cell 113:261–73.

Troemel, E. R., J. H. Chou, N. D. Dwyer, H. A. Colbert, and C. I. Bargmann. 1995. Divergent seven transmembrane receptors are candidate chemosensory receptors in C. elegans. Cell 83: 207–18.

Voglis and Tavernarakis. 2005. Transduction in the nematode *Caenorhabditis elegans.* In: Mechanosensitivity in Cells and Tissues. Ed. Kamkin, Andre and Kiseleva, Irina. Academia Publishing House Ltd, Moscow, p. 23–56.

Voilley, N., A. Galibert, F. Bassilana, S. Renard, E. Lingueglia, S. Coscoy, G. Champigny, P. Hofman, M. Lazdunski, and P. Barbry. 1997. The amiloride-sensitive Na+ channel: from primary structure to function. Comp Biochem Physiol A Physiol 118:193–200.

Waldmann, R., G. Champigny, N. Voilley, I. Lauritzen, and M. Lazdunski. 1996. The mammalian degenerin MDEG, an amiloride-sensitive cation channel activated by mutations causing neurodegeneration in Caenorhabditis elegans. J Biol Chem 271:10433–6.

Waldmann, R., and M. Lazdunski. 1998. H(+)-gated cation channels: neuronal acid sensors in the NaC/DEG family of ion channels. Curr Opin Neurobiol 8:418–24.

Walker, R. G., A. T. Willingham, and C. S. Zuker. 2000. A Drosophila mechanosensory transduction channel. Science 287:2229–34.

Ward, S., N. Thomson, J. G. White, and S. Brenner. 1975. Electron microscopical reconstruction of the anterior sensory anatomy of the nematode Caenorhabditis elegans. J Comp Neurol 160:313–37.

Waterston, R., and J. Sulston. 1995. The genome of *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA 92:10836–10840.

Waterston, R. H., J. N. Thomson, and S. Brenner. 1980. Mutants with altered muscle structure of Caenorhabditis elegans. Dev Biol 77:271–302.

Way, J. C., and M. Chalfie. 1989. The mec-3 gene of Caenorhabditis elegans requires its own product for maintained expression and is expressed in three neuronal cell types. Genes Dev 3:1823–33.

Way, J. C., and M. Chalfie. 1988. mec-3, a homeobox-containing gene that specifies differentiation of the touch receptor neurons in C. elegans. Cell 54:5–16.

Weinbaum, S., P. Guo, and L. You. 2001. A new view of mechanotransduction and strain amplification in cells with microvilli and cell processes. Biorheology 38:119–42.

Welsh, M. J., M. P. Price, and J. Xie. 2002. Biochemical basis of touch perception: mechanosensory function of degenerin/epithelial Na+ channels. J Biol Chem 277:2369–72.

White, J. G., E. Southgate, J. N. Thomson, and S. Brenner. 1986. The structure of the nervous system of Caenorhabditis elegans. Philos Trans R Soc Lond B Biol Sci 314:1–340.

White, J. G., E. Southgate, J. N. Thomson, and S. Brenner. 1976. The structure of the ventral nerve cord of Caenorhabditis elegans. Philos Trans R Soc Lond B Biol Sci 275:327–48.

Wicks, S. R., and C. H. Rankin. 1997. Effects of tap withdrawal response habituation on other withdrawal behaviors: the localization of habituation in the nematode Caenorhabditis elegans. Behav Neurosci 111:342–53.

Wicks, S. R., and C. H. Rankin. 1995. Integration of mechanosensory stimuli in Caenorhabditis elegans. J Neurosci 15:2434–44.

Wicks, S. R., R. T. Yeh, W. R. Gish, R. H. Waterston, and R. H. Plasterk. 2001. Rapid gene mapping in Caenorhabditis elegans using a high density polymorphism map. Nat Genet 28:160–4.

Williams, B. D., and R. H. Waterston. 1994. Genes critical for muscle development and function in Caenorhabditis elegans identified through lethal mutations. J Cell Biol 124:475–90.

Wolinsky, E., and J. Way. 1990. The behavioral genetics of Caenorhabditis elegans. Behav Genet 20:169–89.

Xue, D., M. Finney, G. Ruvkun, and M. Chalfie. 1992. Regulation of the mec-3 gene by the C.elegans homeoproteins UNC-86 and MEC-3. Embo J 11:4969–79.

Zhang, S., J. Arnadottir, C. Keller, G. A. Caldwell, C. A. Yao, and M. Chalfie. 2004. MEC-2 is recruited to the putative mechanosensory complex in C. elegans touch receptor neurons through its stomatin-like domain. Curr Biol 14:1888–96.