

Genetic Dissection of Mechanosensory Transduction: Mechanoreception-Defective Mutations of *Drosophila*

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Summary

To identify genes involved in mechanotransduction, *Drosophila* larvae were screened for X-linked mutations affecting a behavioral response to touch. Many mutations that caused nondevelopmental defects were recovered, among them multiple alleles of the genes *uncoordinated* (*unc*) and *uncoordinated-like* (*uncl*). Adult flies mutant in these genes showed reduced viability that was associated with behavioral phenotypes ranging from reduced locomotor activity to total uncoordination. Behavioral analysis of mosaic flies indicated that external sensory bristles are a focus of the *unc* mutant defect. Extracellular recordings from mutant mechanosensory bristles revealed that mechanoreceptor potentials were absent or reduced in both *unc* and *uncl* mutants. A second genetic screen, for *unc*-like uncoordination, yielded mutations in seven genes on the second chromosome; mutations in five of these genes also reduced or eliminated bristle mechanoreceptor potentials. These mutants provide the basis for a genetic, electrophysiological, and molecular dissection of mechanotransduction.

Introduction

Sensitivity to mechanical stimuli is so widespread as to be a defining characteristic of animal life. Many of the senses we trust the most, yet take most for granted (not only touch and hearing, but also the proprioceptive and visceral senses), depend on the conversion of mechanical stimuli to neuronal electrical signals. But despite the ubiquity and importance of mechanotransduction, less is known about its molecular basis than about that of any of the other modes of sensory signal transduction.

Mechanosensitive cells are diverse, ranging from single-celled ciliates to highly specialized sensory cells such as the hair cells of the vertebrate lateral line and inner ear (Ito, 1992). In the absence of a description of the molecular structures involved, it is not known whether similar mechanisms underlie mechanoreception in these different cells. From the very detailed biophysical studies that have been performed on vertebrate hair cells (Hudspeth, 1989), some generalizations may be attempted. First, the rapid onset of a receptor potential, too rapid to involve the intracellular diffusion of second messengers, implies a direct link between the receptor and the gated membrane conductance. This suggests that

mechanosensory receptors may be structurally dissimilar from the G protein-linked receptors that transduce other sensory stimuli, such as vision (Hargrave and McDowell, 1993), taste (Margolskee, 1993), and olfaction (Anholt, 1993), via second messengers. Second, electrophysiological studies of the gated conductances in hair cells suggest that the number of channels and therefore the number of linked receptors may be low, from tens to hundreds per hair cell (Howard and Hudspeth, 1988). Thus, attempts to identify components of the transducing machinery by similarity with previously described receptors, or by direct biochemical characterization, may be misleading or difficult. Instead, we adopted a functional strategy, identifying genes by isolating mutations that affect mechanosensory behavior.

Genetic approaches have already identified some gene products involved in mechanosensation. In the ciliate *Paramecium*, mutations affecting a touch-evoked change in swimming behavior define several loci in a sensorimotor pathway (Saimi and Kung, 1987); however, all affect only steps subsequent to the generation of a receptor potential. A saturating screen of the nematode *Caenorhabditis* (Chalfie and Au, 1989) has identified many touch-insensitive *mec* mutants, some of which are insensitive although the mechanosensory neurons are correctly specified and morphologically normal. Recently, it has been shown that two loci defined by touch-insensitive mutations, *mec-4* and *deg-1*, encode proteins similar in sequence to an amiloride-sensitive epithelial sodium channel from rat colon (Canessa et al., 1993; Lingueglia et al., 1993).

Drosophila provides a model system that is particularly well suited to the study of mechanotransduction. The cuticular mechanosensory organs of the adult fly enable mechanotransduction to be assayed electrophysiologically in a system also tractable to genetic and behavioral analysis. These organs include chordotonal and campaniform sensilla, which respond to deformation of the surrounding cuticle, and bristles that can be deflected by external stimuli. Different subsets of these have tactile, proprioceptive, and auditory functions, and they are involved in a variety of behaviors, including coordinated movement, withdrawal from touch, and grooming (McIver, 1985). The development of mechanosensory organs has been intensively studied in both embryo and pupa, and there exist many mutations affecting their specification and gross external morphology (Lindsley and Zimm, 1992). In addition, there is a large collection of molecular markers for the sensory neuron and its surrounding support cells (Zipursky et al., 1984; Bodmer et al., 1987; Ghysen and O'Kane, 1989; Hartenstein and Jan, 1992). In adult flies, the bristles are abundant and easily accessible. Macrochaete bristles, though large enough to be stimulated by hand, are innervated by single

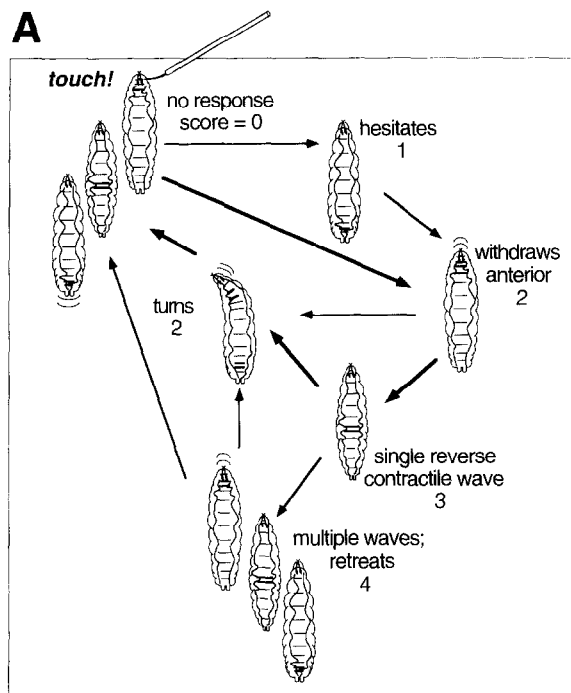
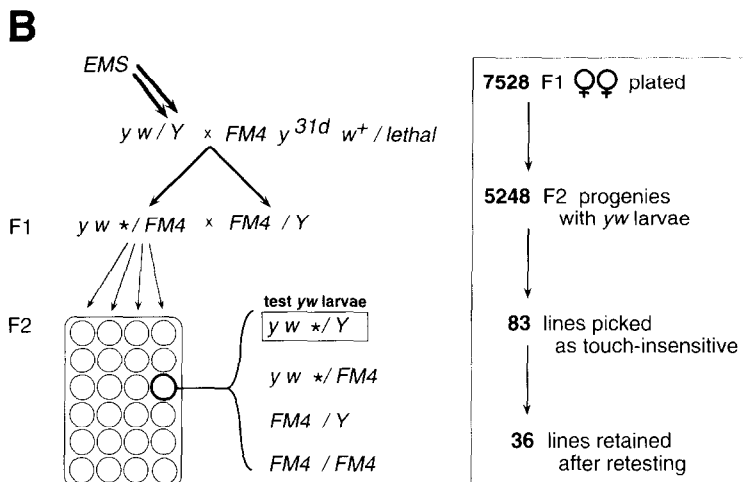


Figure 1. Screening Larvae for Touch Insensitivity

(A) Stereotypical responses of larvae to touch. Thicker arrows indicate the sequences of behavior most often observed. Larvae moving forward were stroked with the tip of an eyelash across one side of the thoracic segments. They typically responded by first contracting their anterior segments to withdraw from a stimulus, then either turning away from the stimulus or completing the wave of reverse contraction before resuming forward movement in a new direction. Occasionally, larvae will also go into full retreat (thinner arrows) by performing several reverse waves of contraction. To quantitate the responsiveness of a larva, scores of 0–4 were assigned to the behaviors indicated.

(B) Mutagenesis and crossing scheme. Mated F1 females, each carrying a different mutagenized *y* (yellow) *w* (white) X chromosome, were placed singly in chambers of a multiwell plate for egg laying. F2 hemizygous *y w* male larvae were distinguished for testing from their sibs by mouthhook color (brown in *y* mutants instead of black in *y*⁺ or *y*^{31d}) and Malpighian tubule color (colorless in *w* mutants). Lines of interest were recovered and their touch response quantitated in subsequent generations. Numbers of lines (i.e., individual mutagenized chromosomes) at each stage of the screen are shown in the box at the right.



sensory neurons; their operation can be assayed behaviorally by monitoring an evoked scratch reflex (Vandervorst and Ghysen, 1980). Most importantly, receptor potentials and action potentials in the single sensory neuron can be directly detected by extracellular recording through the hollow bristle shaft (Hodgson et al., 1955; Wolbarsht and Dethier, 1958).

Mutations affecting grooming behavior have been identified in *Drosophila* (Phillis et al., 1993); some of these may affect the operation of the sensory bristles. However, we suspected that because mechanotransduction is involved in so many different behaviors, global defects in mechanosensation would lead to adult inviability. We therefore screened for touch insensitivity at the larval stage and used anatomical criteria to identify those lines with functional rather than developmental defects. Here, we describe the results

of the larval genetic screen, followed by behavioral and electrophysiological analyses of selected mutants. Mutations in two genes in particular, *uncoordinated* and *uncoordinated-like* (*unc* and *uncl*), were found to eliminate mechanotransduction; their adult phenotypic profiles were then used in a new screen for mutations affecting mechanosensation. The genes thus identified provide the basis for a molecular genetic dissection of mechanosensory signal transduction.

Results

Screening Larvae for Touch Insensitivity

Flies hemizygous for *sc*¹⁰⁻¹, a composite mutation of the *achaete-scute* complex, fail to develop most of the adult external mechanosensory organs (Villares

and Cabrera, 1987). These animals show low viability associated with severe uncoordination: the adult flies are quite unable to walk or stay upright and usually become stuck in the culture medium immediately after eclosion. This observation suggested that mutants with a severe functional defect in external mechanoreception would likely not be recovered as adults. We therefore screened for defects in larval mechanosensory behavior.

Wild-type larvae move and feed on the surface of their culture medium in a series of forward waves of segmental contraction. A stroke across the thoracic segments with the tip of an eyelash evokes a limited set of responses, ranging from withdrawal by contracting only the anterior segments to retreat by performing one or more complete reverse waves of contraction (Figure 1A). Every larval segment bears a variety of external and internal mechanosensory organs, including sensory hairs, campaniform sensilla, chordotonal organs, and multidendritic neurons (Dambly-Chaudière and Ghysen, 1986; Bodmer and Jan, 1987); any or all of these could be involved in both sensing the stimulus and governing the response.

We screened male larvae that were made hemizygous for chemically mutagenized X chromosomes for defects in this withdrawal response. The screen was carried out on over 5200 lines arising from single mated F1 females (Figure 1B). To test a single line, several larvae from each F2 progeny were stimulated, and their response was examined. If two or more larvae failed to respond or showed an abbreviated response, the progeny was retained and a stock was established from the sib classes. The number of lines retained at this stage was 83. In a second round of testing, the touch responsiveness of each line was quantitated. A score was assigned to each stage of the withdrawal sequence (Figure 1A). The highest-scoring behavior observed in response to each stimulus was recorded. Each larva was stimulated four times, and the scores (0–4) were added for a maximum total of 16. This total was averaged over many larvae. The unmutagenized *y w* stock scored 10.3 (SE = 0.24). The 28 lines that scored from 0.9 (representing the lack of almost any response) to less than 8.0 are listed in Table 1. Most of these mutant lines displayed reduced motility or other larval behavioral phenotypes in addition to a reduced response to touch. These phenotypes could indicate general defects that impaired the motor response to touch, but they could also result from altered mechanosensory input and therefore did not alone disqualify a line from further study.

Twenty-six of the 28 lines that showed reduced larval touch response also are lethal or showed greatly reduced viability as adults (Table 1). In each case, this could be due to either the behavioral mutation or to a second lethal mutation being induced on the same chromosome (which is not an unlikely event under the chemical mutagenesis conditions used; see Experimental Procedures). As the behavioral phenotypes could not be scored well enough in single larvae for

recombination mapping, the viability defect was mapped by recombination and by the use of duplicated X chromosome segments to rescue mutant lethality. Mutations covered by the same duplication were then be crossed to each other to test whether they were in the same lethal complementation group.

The *touch-insensitive larva B*, *unc*, and *uncl* Genes

The number found of lethal or semilethal complementation groups with multiple alleles was four (Table 1). The alleles within each group came from separately mutagenized batches of chromosomes and therefore arose independently. The recovery of multiple independent lethal hits at the same locus by a behavioral selection confirms that the lethal and behavioral phenotypes of these alleles are indeed caused by single mutations. In this work, we focus on the three groups that included the least touch-responsive lines. We used chromosomal duplications to map all three to the base of the X chromosome, in the cytogenetic interval 19E–20 (see Experimental Procedures). The group comprising lines 318, 145, 410, and 356 was identified as the *unc* gene, and that comprising lines 394, 365, and 331 was identified as *uncl* (see below). The third group, lines 184 and 349, was named the touch-insensitive larva B (*tilB*) gene. (*tilA* and *tilC* refer to other loci that are represented by single mutations and are not described further.)

Many different kinds of defects could interfere with the behavioral response to touch. Insensitivity could result from a defect in the specification or patterning, rather than the operation, of sensory organs. To check for developmental defects of the nervous system in insensitive lines, the late embryonic CNS and PNS were immunostained with monoclonal antibodies (MAbs) that recognize either the entire nervous system (MAB 22C10; Zipursky et al., 1984; Bodmer et al., 1987) or specific structures in the chordotonal and external sensory organs (MAB 21A6; Pollack, 1985). The nervous system morphologies of *unc*, *uncl*, and *tilB* mutant embryos were indistinguishable from wild type (data not shown).

Adult Behavioral Defects: Identification of *unc* and *uncl* Alleles

The external morphology of the mechanosensory organs is most easily observed in the macrochaete and microchaete bristles of the adult cuticle. However, most of the mutants isolated in our screen are adult lethal. Therefore, to generate mutant adult sensory organs, we made gynandromorph mosaics in which marked patches of mutant tissue occur in an otherwise wild-type animal.

In mosaic patches mutant for either *unc* or *uncl*, bristle morphology and pattern were normal. However, in ventral *unc* mutant patches that included one or more legs, a clear behavioral phenotype was observed: severe uncoordination of the mutant legs (see Figure 3A). In wholly mutant animals, it is this uncoordination that is in fact the cause of lethality. Most

Table 1. Lines Isolated in Larval Touch-Insensitivity Screen

Line Number	Locus, Allele	Cytogenetic Location	Touch-Response Score (SE n)	Larval Motility	Other Larval Phenotypes	Adult Viability	Other Adult or Mosaic Phenotypes
89	<i>tilA</i> ¹ <i>tilA</i> ^{1/+}	12E-13A	0.9 (0.24, 27) 4.5 (0.60, 16)	+ ++	Curling, writhing	- +	<i>tilA</i> ^{1/+} Sedentary
394	<i>uncl</i> ^b		2.4 (0.54, 30)	++	Rearing up	+/-	Sedentary, slight uncoordination.
365	<i>uncl</i> ⁷		3.1 (0.46, 47)	+/+		+/-	Sedentary
331	<i>uncl</i> ⁸	20	3.4 (0.55, 43)	+	Frequent turning	-	Severe uncoordination
318	<i>unc</i> ²⁵		3.2 (0.62, 23)	++		-	
145	<i>unc</i> ²⁶	19E	3.4 (0.45, 43)	++		+/-	Severe uncoordination
410	<i>unc</i> ²⁷	19E	3.4 (0.61, 35)	++		-	Severe uncoordination
356	<i>unc</i> ²⁸		4.0 (0.70, 22)	++		-	
184	<i>tilB</i> ¹	20	3.7 (0.37, 49)	+++		+/-	Sedentary
349	<i>tilB</i> ²	20	5.6 (0.46, 31)	+++		+/-	Sedentary; does not fly.
154	<i>tilC</i> ¹	10A-11A	3.2 (0.53, 25)	+++		+/-	All movements slow.
375			4.2 (1.00, 19)	+	Often found curled.	-	
70			4.4 (0.70, 13)	+	Larval CNS and PNS deranged.	-	
376			4.8 (0.46, 30)	+	Freezes stiff on stimulation.	-	
80	<i>tilD</i> ¹	13F-14A	4.9 (0.63, 13)	+		-	
200			5.2 (0.83, 12)	++		-	Bristle loss or transformation; legs paralyzed.
415	<i>tilD</i> ²		6.1 (0.55, 20)	+		-	
103	<i>tilD</i> ³		8.1 (0.52, 38)	+		-	
75			5.4 (0.52, 31)	+	Flaccid larvae, turn in a V shape.	-	
411			5.6 (0.23, 19)	+		-	
366			6.2 (0.65, 33)	+	Often found on side.	+/-	
147			6.7 (0.45, 32)	++		-	Bristles misshapen or pattern disarranged; legs swollen, kinked.
350			6.9 (0.88, 26)	++	Freeze stiff on stimulation.	-	
344	<i>forked</i>		7.1 (0.71, 23)	+		-	
310			7.4 (0.37, 32)	++	Looping, caterpillar-like movement.	+	
301			7.4 (0.36, 55)		Frequent rearing	+	
317			7.9 (0.37, 26)	+		-	Trichomes and bristles absent in mosaic patch.
78			7.9 (0.30, 46)	+++		-	
Wild type (y w)			10.3 (0.24, 88)	+++		+	

Lines with a touch-response score of less than eight are listed, ranked by score. Lines with allelic mutations are grouped together, at the rank position of the most severely affected allele. Cytogenetic position was determined by rescuing the lethal and behavioral phenotypes of mutant chromosomes with the following duplications. *tilA*: *Dp(1;f)LJ9* but not *Dp(1;f)LJ8*; *uncl*, *unc*; *tilB*: *Dp(1;Y)y⁺ct⁺* and *Dp(1;Y)y⁺fcl⁺*; *tilC*: *Tp(1;2)v^{65b}*; *tilD*: *Dp(1;Y)shi⁺* and *Dp(1;4)r⁺*. Locations of *unc*, *uncl*, and *tilB* were further refined by deficiency mapping (Figure 2). Touch-response score is on a scale of 0 (equals no response) to 16; see text for scoring method. SE, standard error; n, number of larvae scored. Larval motility was scored by inspection; +++ indicates wild type, ++ indicates visibly reduced motility, and + indicates severely reduced motility. Other larval phenotypes are described only if noted in at least two or more independent observations. Adult viability in normal conditions was scored relative to the sib male class *FM4/Y*. Complete lethality is indicated by -; +/- indicates less than 10% viability. Other adult phenotypes include defects in bristle morphology seen in mosaic patches of mutant tissue and behavioral phenotypes seen in survivors from semiviable lines.

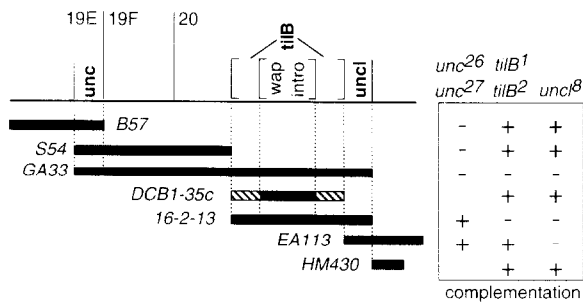


Figure 2. Deficiency Map of *unc*, *uncl*, and *tilB* Alleles

Lines 145, 318, 356, and 410 carry novel *unc* mutations, and lines 331, 365, and 394 carry novel *uncl* mutations. These alleles map to intervals containing only the *unc* or *uncl* genes. *tilB* mutations define a novel locus at the cytogenetic interval uncovered by *Df(1)16-2-13* but not by *Df(1)DCB1-35c*. Hatched bars indicate the two alternative map locations of *tilB* (either proximal or distal of *wings-apart* [*wap*] and *introverted* [*intro*]). Minus signs indicate noncomplementation (i.e., lethality for *unc* and *uncl* or sedentary behavior for *tilB*) of the deficiency/mutant combinations. The deficiency map is derived from Perrimon et al. (1989).

unc and *uncl* hemizygous animals survive through the larval and pupal stages if reared in the absence of competing wild-type sibs. But, immediately upon eclosion, all adult *unc* flies show uncoordination so severe that they can neither stand nor walk, leading to an early death by adhesion, a similar phenotype to that of the bristleless mutant *sc⁷⁰⁻¹*. Adult flies from the *uncl* mutant line 331 show a similar, though somewhat milder, uncoordination: they are barely able to walk. Other *uncl* alleles showed a lesser degree of uncoordination, or sedentary behavior.

unc and *uncl* were previously identified in the course of an intensive mutagenesis and mapping of genes at the base of the X chromosome (Lifschytz and Falk, 1968; Schalet and Lefevre, 1973; Schalet and Lefevre, 1976; Miklos et al., 1987; Perrimon et al., 1989). The adult lethality and uncoordination of the mutations isolated in our screen are similar to the previously described *unc* and *uncl* phenotypes. Furthermore, our touch-insensitive *unc* mutations fail to complement the phenotype of previously isolated *unc* alleles: when lines 145 and 410 were crossed to *unc*²⁴, only a few *unc*²⁴/145 or *unc*²⁴/410 female offspring were recovered, and these showed severe uncoordination. Fine-scale deletion mapping placed the lethal mutations in lines 145 and 410 in a cytogenetic interval occupied only by *unc* (Figure 2). Line 331 similarly failed to complement the viability and behavioral defects of *uncl*⁵ and was mapped to an interval that contains only *uncl*. Therefore, the lines 145 and 410, and by inference lines 318 and 356, carry novel alleles of *unc*, while lines 331, 365, and 394 carry novel alleles of *uncl*. The novel mutations have been named *unc*²⁵-*unc*²⁸ and *uncl*⁶-*uncl*⁸.

The *tilB* locus is identified by two alleles, *tilB*¹ and *tilB*². Adults hemizygous for either allele eclose at a reduced frequency from heterozygote stocks, but

show close to full survival if reared in isolation. External morphology and patterning of the sensory bristles are normal. However, unlike wild-type flies that show continuous activity, *tilB* flies are usually sedentary. Moreover, they are unable to walk upside down or climb the sides of culture vials. Fine-scale deletion mapping of the sedentary phenotype (Figure 2) placed *tilB* in a newly defined interval close to the genes *wings-apart* and *introverted*.

Looking for a Defective Focus: Behavioral Analysis of *unc* Mosaics

Adult uncoordination, like larval insensitivity, could be the result of either CNS or PNS defects; for example, a defect in the motor output of the thoracic ganglia or in sensory input from the affected legs. To test whether *unc* affects mechanosensory bristle function, we turned to the bristle scratch reflex, a behavior that allows the operation of single mechanosensory organs to be tested. Decapitated wild-type flies respond to mechanical stimulation of a single macrochaete bristle by sweeping the stimulated area with one leg. The first and third legs respond to different, well-defined fields of bristles (Vandervorst and Ghysen, 1980). The various thoracic bristles also differ in the frequency with which a response is elicited, the tegular bristles at the base of the wing being the most reliable (Corfas and Dudai, 1990). We found that mechanical stimulation of bristles on the dorsal abdomen also invariably produced a third-leg response.

We analyzed the performance of this reflex in gynandromorph mosaics that carried patches of the male *y unc* mutant cuticle on the dorsal thorax or abdomen of a female *y⁺ unc⁺* fly (Figure 3). Only mosaics in which the legs were coordinated (and were therefore mostly *y⁺ unc⁺*) were used. These gynandromorph mosaics arise by the loss of an unstable X chromosome early in embryonic development (Janning, 1978), long before the pupal cell divisions that give rise to the adult sensory organs (Hartenstein and Posakony, 1989). Therefore, the four component cells of a bristle (sensory neuron, socket, bristle, and sheath cells) should all have the same genotype. Both mutant (*y*) and wild-type (*y⁺*) bristles in each mosaic animal were stimulated, the latter to confirm the ability of a leg to respond to bristles within its field. Control mosaics carrying the parental *y unc⁺* chromosome were also generated and scored. Our results (Figures 3B and 3C) demonstrate a strong correlation between a *y unc* bristle genotype and failure to elicit a scratch reflex. This is most clearly seen for the tegular and abdominal bristles for which responses can reliably be elicited when they are *y⁺ unc⁺* or *y unc⁺*, but which rarely or never give rise to a response when they are *y unc*.

These results indicate that to trigger a scratch reflex, *unc⁺* function is required in the sensory organs or at a closely linked site on the *Drosophila* blastoderm fate map. Although the genotypes of the other reflex pathway elements (the interneurons and motor neurons of the CNS) were not ascertained, their ventral

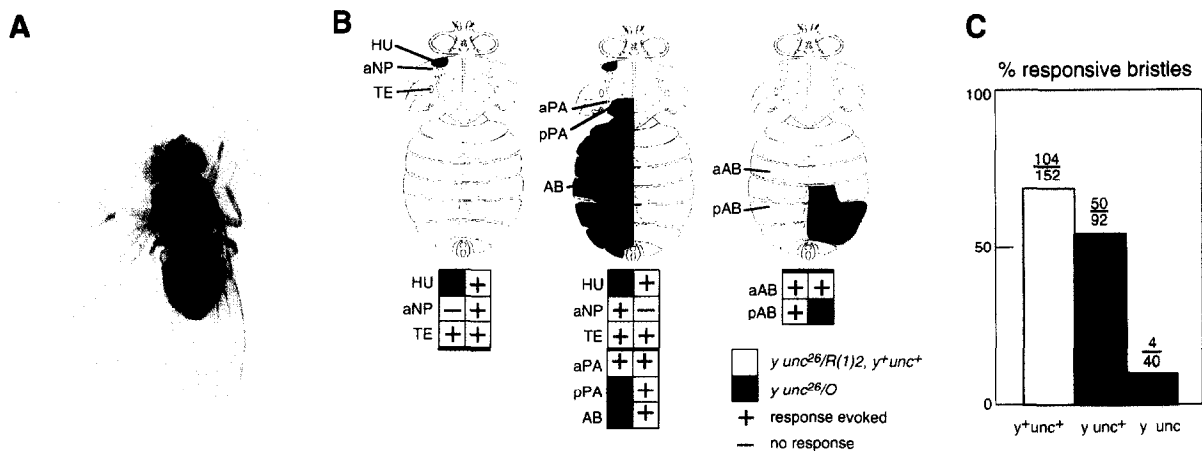


Figure 3. Uncoordination and Loss of Scratch Reflex in *unc* Mosaics

(A) Uncoordination in a gynandromorph mosaic for *unc*²⁶, with the entire left side male and mutant and the right side female and wild type. Note the held-out wing and tangled legs on the mutant side. Mosaics were generated by loss of the unstable *R(1)* (ring X) chromosome from female embryos of the genotype *R(1)2 y⁺w^c unc²⁶/y w unc²⁶* in early cell divisions, giving rise to large adult patches of male XO tissue. These patches are male and hemizygous for *unc*, the cuticular marker *yellow* (*y*), and the eye marker *white* (*w*). (B) Scratch reflex responses in mosaic animals indicate a peripheral focus of the *unc* defect. Shown are projections of the cuticle from three example mosaic animals, with colored areas highlighting *unc*²⁶ mutant patches. Legs and ventral areas were *y⁺unc⁺* in all cases. The results of stimulating specific bristles to evoke a scratch reflex are shown below each mosaic. Plus sign indicates a responsive bristle. A heavy line separates bristles in the responsive fields of the first and third legs. Stimulation (deflection anteriorly) of the humeral (HU), anterior notopleural (aNP), and tegular (TE) bristles evokes a scratch response by the ipsilateral first leg; the third leg responds to deflection of the postalar (PA) and abdominal (AB) bristles. Individual bristles were scored as nonresponsive (minus sign) if no response by the appropriate leg was evoked by any of at least four stimuli. Abbreviations: a-, anterior; p-, posterior. (C) Histogram of the results from mosaics for a control parental *y⁺unc⁺* (closed bar) chromosome and combined results for four *unc* mutations (*unc*²⁵⁻²⁸, colored bar). For each genotype, the number of bristles scored as responsive is shown above the number tested. Abbreviations: *y*, *yellow*; *w*, *white*; a-, anterior; p-, posterior.

position on the *Drosophila* blastoderm fate map (Kankel and Hall, 1976) suggests a closer correlation with the ventral *y⁺unc⁺* cuticle than with the dorsal cuticle. More importantly, the normal response evoked in mosaic animals by stimulating control wild-type bristles demonstrated that the same legs that failed to respond to stimulation of *unc* mutant bristles were in fact capable of a response. Thus, components involved in the motor response, such as interneurons, leg motor neurons, and leg muscles, must be functional.

***unc* and *uncl* Are Defective in Mechanoreceptor Potential Generation**

The loss of mechanosensory bristle function in *unc* mutants could result from a failure of mechanotransduction by the sensory neuron or support cells or from a defect in downstream functions of the sensory neuron; for instance, an error in projection of the sensory axon within the CNS or a defect in synaptic function. To determine whether the defects are at the level of transduction, electrophysiological recordings from wild type and from *unc*, *uncl*, and *tilB* mutant mechanosensory bristles were performed.

We measured mechanically evoked changes in the transepithelial potential (TEP), a voltage difference between the apical and basal sides of the sensory epithelium that is characteristic of insect external sensory organs. The generation and maintenance of the TEP

is a reflection of the anatomy and ion-pumping activities of the sensory support cells. The three support cells surround the neuron concentrically, forming tight junctions among each other and the surrounding epithelium. The apical surfaces of the support cells and the cuticle enclose the dendrite of the sensory neuron in a cavity filled with a specialized receptor lymph. This fluid is unusually rich (for an extracellular fluid) in potassium ions (Grünert and Gnatzy, 1987) and is functionally equivalent to the potassium-rich endolymph of the vertebrate cochlea (Kuijpers and Bonting, 1970). Pumping of potassium ions into the receptor lymph by the bristle and socket cells results in a TEP difference, with the apical side of the epithelium being positively charged. In series with the transmembrane potential of the neuron, the TEP contributes to the electromotive force that drives receptor-gated current into the neuron (Thurm and Koppers, 1980).

With an electrode placed over the cut end of a macrochaete bristle (Figure 4A), the TEP is recorded at rest as a positive potential in the range +10 to +50 mV. Mechanical stimulation of the bristle by controlled movement of the recording electrode generates a mechanoreceptor potential (MRP). This is recorded as a negative deflection of the TEP (Figure 4B; Figure 5A) owing to the flow of cations from the receptor lymph into the neuron. The response is rapid, beginning within 3 ms of the bristle deflection, and is main-

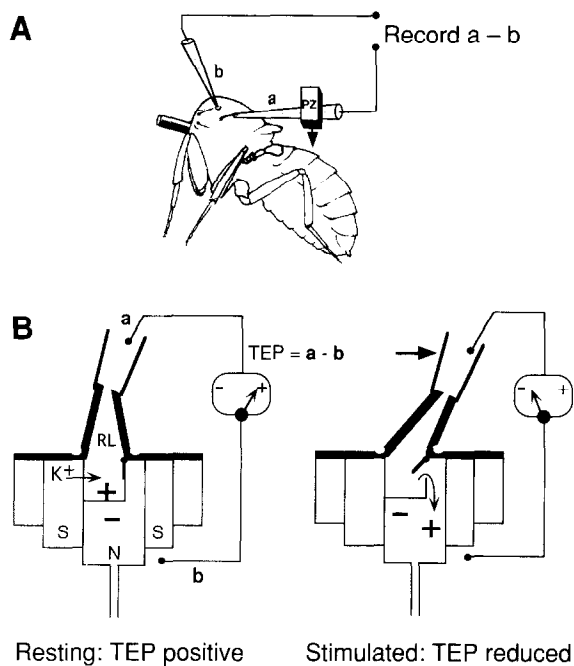


Figure 4. Recording TEP from Bristles

(A) A fly, its head and wings removed, is mounted on a pin. The recording/stimulating electrode (a) is placed over the cut end of a thoracic bristle (humeral or anterior notopleural). A reference electrode (b) pierces the cuticle and contacts the hemolymph; a differential amplifier multiplies the TEP difference (a-b). To stimulate the bristle, the electrode (a) is mounted on a piezoelectric (PZ) stage; voltage commands generate a 1/2 s, ramp-and-hold mechanical stimulus, which deflects the bristle by about 10° in the sagittal plane.

(B) Schematic diagram of electrical events underlying the TEP and MRP. Support cells (S) secrete a potassium-rich (K⁺) receptor lymph (RL) and maintain a positive TEP. Upon mechanical stimulation, a conductance in the sensory neuron (N) is opened, and an inward current flows, thus reducing the TEP. This change is recorded as the MRP. The mechanical gating of the conductance is schematic; its actual structure is unknown.

tained (though it adapts) during the stimulus. Upon relaxation of the bristle, the TEP returns to the resting level.

The MRP response of wild-type bristles was robust and reproducible, correlating in amplitude with the resting TEP value in each bristle type (Figure 5B). In contrast, mechanoreceptor potentials are completely absent in *unc*²⁶ and *unc*²⁷ mutant flies, even at high TEP values (Figure 5C). Moreover, a synthetic deficiency for *unc*, generated by producing flies carrying the deficiencies *Df(1)S54* and *Df(1)B57*, also eliminated the MRP response. In all *unc* genotypes, the range of TEP values did not differ significantly from wild type.

*unc*¹⁸ hemizygotes display mechanoreceptor potentials that are reduced, but not eliminated (Figure 5D). *uncl*⁷, the least severe of the *uncl* mutations in adult behavior, shows near-normal MRP amplitudes. However, a synthetic deficiency for *uncl*, comprising the deficiencies *Df(1)GA33* and *Df(1)EA113*, causes extreme uncoordination and completely eliminates MRPs (Figure 5D, bottom panels). Thus, the *uncl* null

phenotype is similar to that of *unc*, and the *uncl*⁷ and *uncl*¹⁸ alleles represent hypomorphic alleles of this locus. Hemizygotes for *tilB* alleles showed TEP values and MRP amplitudes within wild-type ranges (data not shown).

New Adult Uncoordination Mutants Also Show MRP Loss

The behavioral and electrophysiological phenotypes of *unc* and *uncl* suggest that both larval touch insensitivity and adult uncoordination are consequences of an inability to generate a mechanoreceptor potential. The survival to the pharate adult stage of animals that lack these genes suggested that a screen for very uncoordinated adults might recover further mechanoreception-defective mutants. We therefore screened the second chromosome for mutations causing adult inviability due to severe uncoordination. Lines carrying chemically mutagenized *cn bw* chromosomes were generated, and the culture vials were first pre-screened for the presence of homozygous *cn bw* (white-eyed) pharate adults but the absence of eclosed, motile *cn bw* flies.

In the prescreen, 296 pupal-viable, adult-lethal lines were selected from 2892 F3 lines. Survival of homozygotes in subsequent generations was aided by the use of a dominant temperature-sensitive embryonic lethal mutation to kill the competing sibs by shifting the cultures to 29°C. Homozygous pharate adults were picked from the preselected lines and allowed to eclose in humid chambers, and the behavior of the adult flies was observed. *cn bw* flies from 10 of the preselected lines showed severe uncoordination similar to that seen in *unc* mutants. These fell into seven complementation groups with respect to lethality and uncoordinated behavior. When bristle recordings were performed on mutant flies, mutants from 3 of the 7 groups, including two for which multiple alleles were recovered, showed almost complete loss of MRPs (Figure 6). We have named these mutated loci *no mechanoreceptor potential A*, *B*, and *C* (*nompA*, *nompB*, and *nompC*). A fourth locus, *nompD*, had an intriguing phenotype: elimination of MRPs in all bristles except the dorsal humerals, in which MRPs were invariably wild-type. This finding suggests that different functional components of mechanosensory transduction may be expressed in different bristles. Mutation of a fifth gene, *reduced mechanoreceptor potentials* (*remp*) caused reduction in both MRP amplitudes and TEP values. The lower TEP values alone do not explain the reduced MRPs as large TEPs were recorded from some bristles in which MRP amplitude was still small. Single mutations at each of two loci, *uncoordinated with mechanoreceptor potential* (*ump*) *A* and *B*, had no apparent effect on TEP or MRP values (data not shown).

Discussion

Our knowledge of mechanotransduction is remark-

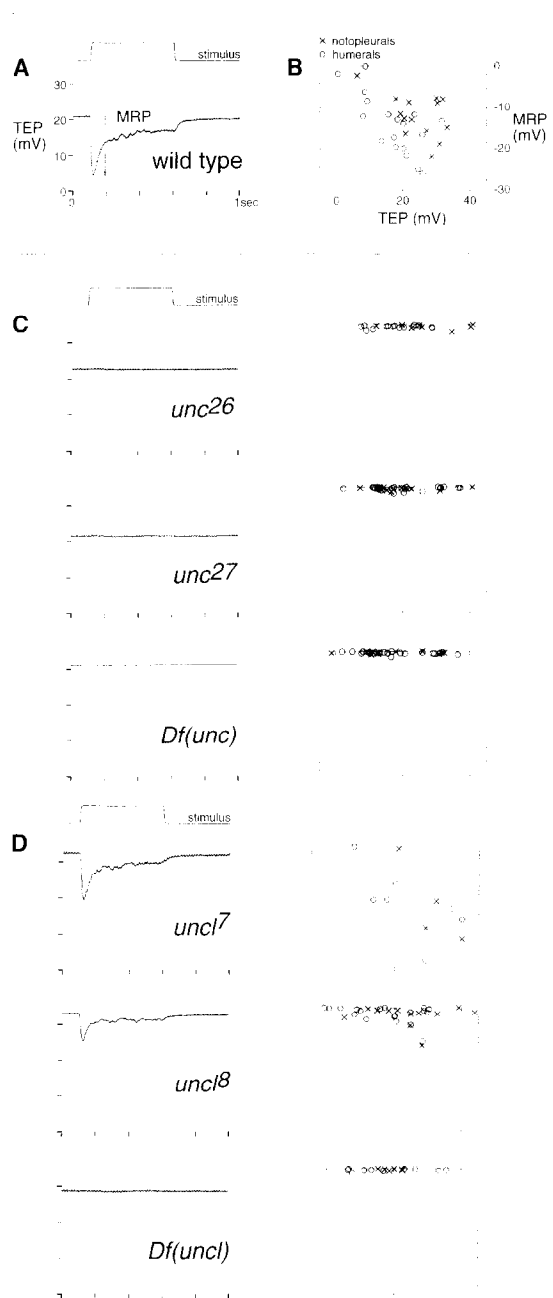


Figure 5. *unc* and *uncl* Mutations Eliminate or Reduce MRPs
TEPs and MRPs were recorded from *unc*, *uncl*, and control wild-type flies that were collected as pupae and used within 1–3 days after eclosion. Measurements were made from the humeral and anterior notopleural bristles.

(A) Single TEP trace recorded from a control wild-type bristle. The stimulus trace indicates linear displacement of the bristle electrode. An identical stimulus command was used in all experiments. However, the angle of bristle deflection depended on the variable length of the cut bristle. MRP amplitude was calculated as the difference between the TEP value immediately prior to the stimulus onset and the minimum TEP recorded during the stimulus.

(B) Plot of the largest MRP recorded from individual bristles (from at least three trials) versus the TEP value immediately prior to stimulus onset. Each data point represents a different bristle. No more than six bristles (two notopleurals and four humerals) from any one fly are plotted.

able for both its detail on the biophysical level and its paucity at a molecular level. For instance, the mechano-electrical events underlying signal transduction in vertebrate hair cells have been analyzed to a degree such that the gating forces and stiffness of single receptor elements can be estimated (Howard and Hudspeth, 1988). However, little is known about the molecular structures underlying these properties or, indeed, about the molecular components of any mechanosensory organ. A molecular description of mechanoreception would allow us to understand how structure dictates the mechanical and transducing properties of these receptors, and it would provide the means to find out whether similar molecules and mechanisms underlie the operation of the wide variety of mechanosensitive cells. Unfortunately, the difficulty of biochemical analyses on the small amount of material in mechanosensory epithelia has inhibited progress in this area. A further difficulty arises from the structural complexity of many mechanosensory organs. Mechanoreceptors usually operate in a context of accessory structures that filter and direct mechanical stimuli, so that in vitro assays for isolated components may not be meaningful.

These problems were circumvented by a genetic approach: genes encoding molecules involved in mechanotransduction were identified by the effect of mutations on mechanosensory behavior. As we did not know a priori precisely what phenotypes would be shown by mechanosensory-defective mutants, we began with a broadly inclusive screen: defects in the larval response to touch. From among a large set of lines with reduced touch responses, we used anatomical, behavioral, and electrophysiological criteria to identify mutations in two genes, *unc* and *uncl*, that are required in sensory bristles for mechanotransduction. These are the first mutations shown to affect mechanoreceptor potentials in any system.

Validity and Scope of the Screens

By the behavioral criteria used in the larval screen, *unc* and *uncl* mutants were among the most severely affected of the selected lines. Mutations in these loci were isolated repeatedly, indicating that this behavioral screen indeed selected, and probably saturated the X chromosome, for mutations eliminating mechanotransduction in sensory bristles.

We then used the adult phenotype of *unc* and *uncl*, i.e., inviability due to severe uncoordination, to screen the second chromosome. This proved to be a

(C) Sample traces and MRP versus TEP plots for three *unc* mutant genotypes. *Df(unc)* refers to the overlap of the deficiencies *Df(1)B57* and *Df(1)S54*, both of which uncover *unc*. Note the complete loss of MRPs at all TEP values.

(D) Sample traces and MRP versus TEP plots for three *uncl* mutant genotypes; *Df(uncl)* indicates an overlap of the deficiencies *Df(1)GA33* and *Df(1)EA113*. Note that MRPs are totally eliminated in the synthetic deficiency.

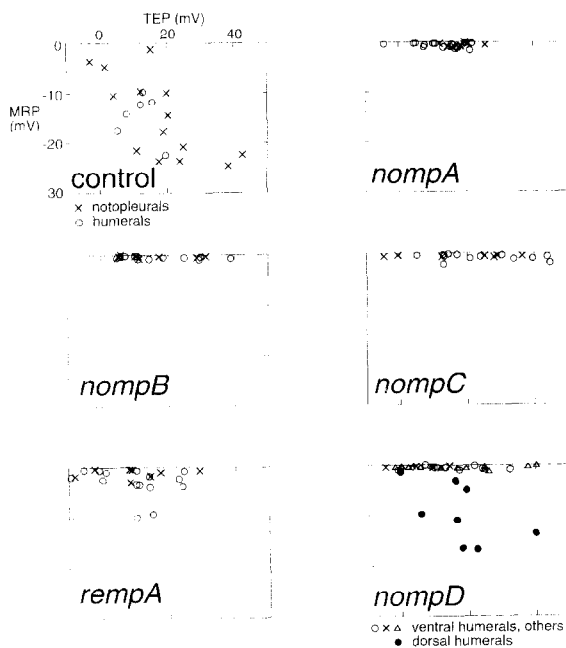


Figure 6. Second Chromosome Uncoordination Mutants with Reduced or Absent MRPs

Shown are plots of MRP amplitudes versus TEP for mutants in each of five complementation groups. *nompA*, *nompB*, and *nompC* alleles show dramatic reduction in MRPs. *nompD* shows normal responses only in dorsal humeral bristles. *rempA* shows reduced MRP amplitudes. For groups with multiple alleles (two in *nompA* and three in *nompB*), the results from different alleles and from noncomplementing combinations are included. Control recordings are from flies carrying *nompA*, *-B*, and *-C* mutations in complementing heterozygous combinations.

more rapid and efficient means of identifying novel *unc*-type mutations: a majority of the novel mutants recovered had severe defects in bristle mechanotransduction. Again, multiple mutations were isolated in two of these *nomp* loci, suggesting a potentially saturable range of targets.

The number of genes that can be mutated so as to completely eliminate mechanotransduction is striking. The second chromosome was not completely saturated and the third, comprising most of the remaining 40% of the euchromatic genome, has yet to be screened. Therefore, many more *nomp* genes probably remain to be found. Such genetic susceptibility is also a feature of vertebrate hair cells: in humans and mice, many different genetic conditions cause sensorineural deafness (Steel, 1991; Reardon, 1992). Thus, mechanotransduction appears to involve either a biochemical pathway with many mutable components acting strictly in series, or more likely, a complex assembly in which loss or malfunction of any single component would eliminate function.

Possible Targets of Mechanotransduction Mutations

What might these components be? In the bristle preparation, while receptor potentials are recorded directly (albeit extracellularly), the stimulus is applied

to the external bristle shaft and not to the sensory dendrite. The mutable targets therefore include extracellular components that conduct the mechanical stimulus to the surface of the dendritic membrane. At the sensory neuron, transduction molecules presumably include a gated ion channel and modulating elements. The short latency of the response (less than 3 ms) argues against a diffusible second messenger pathway linking receptor deformation to channel gating; for instance, the *Drosophila* photoresponse, one of the fastest known second messenger pathways, has a latency of 20–25 ms (Ranganathan et al., 1991). Thus, as in vertebrate hair cells (Hudspeth, 1989), direct mechanical action is more likely. This may require cytoskeletal structures to anchor the transducing elements or to provide a resistance against which a stimulus can act.

Loss of mechanoreceptor potentials could also result from the absence of the transducing cell or its degeneration, as in the *Caenorhabditis elegans* mutants *mec-4* and *deg-1*. The normal morphology of the embryonic PNS argues against a developmental defect being the cause of the touch insensitivity in *unc* and *uncl* larvae. The external structures of the adult sensory organs, the bristle shaft and socket, also appear normal, indicating wild-type development of the support cells that construct them. Moreover, the normal TEP values of *unc* and *uncl* mutants indicate that support cell function and the integrity of the epithelial cell layer are retained. Degeneration of the sensory neuron after embryonic and pupal development has not been excluded, but would have to occur rapidly, as the mutant phenotypes are already evident in both first-instar larvae and newly eclosed adults.

Mutants, such as *tilB*, *tilC*, *umpA*, and *umpB*, that show abnormal mechanosensory behavior but retain bristle mechanoreceptor potentials may be affected in downstream neuronal functions (for instance, neurotransmitter synthesis, axonal pathfinding, or synaptic function). Alternatively, such mutations may affect different mechanosensory organs or other cells that also contribute to the behaviors examined.

Behavioral Phenotypes of Mechanoreception-Deficient Mutants

Can a loss of mechanotransduction in bristles explain the behavioral phenotypes of *unc* and *uncl* mutants? The connection between physiology and behavior is clearest for the scratch reflex: the same sensory organs in which the normal *unc* gene product is required for normal behavior are those shown to be physiologically defective in *unc* mutants. Insensitivity to touch is also likely to be the cause of the reduced touch response in *unc* larvae. This behavior, however, may be mediated by other types of mechanosensors in addition to bristle organs: campaniform sensilla and chordotonal organs are also present on the larval cuticle, and the effect of *unc* and *uncl* on these organs is not yet known.

The degree of uncoordination observed in *unc* and *uncl* adults is remarkable, but it may be explained by considering the proprioceptive as well as the tactile functions of mechanoreceptors. Proprioceptors are mechanosensory organs that are stimulated by changes in attitude or posture. For instance, they may report the static angle of a limb joint or the mechanical load on a leg. In insects, many proprioceptors are external. They include hair plates: groups of bristles, each similar to a tactile bristle, that are positioned at leg joints so as to be deflected by changes in joint angle (Wright, 1976). The sensory feedback provided by such proprioceptors is known to be involved in regulating locomotion. Experiments in which single proprioceptors are ablated show only modest effects on behavior, but more drastic interventions, such as amputation or restraint of the middle legs of an insect, cause major changes in gait (Delcomyn, 1985). A global loss of proprioceptive as well as tactile mechanosensory feedback could therefore lead to severe uncoordination. The phenotypes of *uncl* hypomorphic mutants, in which the degree of uncoordination correlates with the severity of the physiological defect, support this interpretation. The possibility that uncoordination in *unc* and *uncl* is due to an additional defect in the CNS cannot be excluded. However, the association between loss of mechanotransduction and uncoordination was strengthened by the results of screening for autosomal mutations that cause severe uncoordination: of 10 such mutations that define 7 loci, 8 mutations in 5 loci affect mechanotransduction.

Together, the phenotypes of the *unc*, *uncl*, and *nomp* mutations define a syndrome of behavioral phenotypes associated with loss of mechanotransduction. This definition will speed the identification of the remaining genes required for the operation of mechanosensory bristles. Characterization of the corresponding gene products will provide the means for a molecular understanding of how sensory cells transduce mechanical stimuli.

Experimental Procedures

Drosophila Mutagenesis and Chromosomes

Flies were mutagenized by feeding them 18 mM (X chromosome screen) or 25 mM (second chromosome screen) EMS in a sucrose solution (Grigliatti, 1986). Most preexisting mutations, rearrangements, and balancer chromosomes are described elsewhere (Lindsley and Zimm, 1992). The deficiencies used in mapping *unc*, *uncl*, and *tilB* mutations are described elsewhere (Perrimon et al., 1989).

Larval Screen

To grow larvae for screening in multiwell plates, a 10% yeast–10% sucrose–3% agar medium was used. This was supplemented with 0.3 g/l riboflavin to enhance the distinction between wild-type and *white* mutant Malpighian tubule colors (Oster, 1951). Plates were incubated at 25°C for 3 days, and the parents were discarded before screening. Two or more *y w* larvae in each well were stimulated several times, and their response was observed. If no response or an aberrant response was reproducibly evoked from two or more larvae, the line was retained. To recover a line of interest, the food plug from the multiwell plate was transferred to a vial. Quantitation of the touch response was per-

formed twice; in the second instance, the lines were coded so that their identity was not known to the experimenter. Scores from the two rounds were well correlated ($R = 0.74$).

Mapping and Complementation Testing

Mapping adult lethality by recombination: adult-lethal *y w* chromosomes were made heterozygous with chromosomes carrying the markers $y^+ w^+ cv m$ and $y^+ w^+ g f$, and the frequencies of recovery of each combination of markers in hemizygous males were determined. Results were normalized to the frequencies obtained by recombination with the unmutagenized *y w* chromosome. This yielded approximate map locations that guided cytogenetic mapping. In lines 344, 375, and 376, two well-separated lethal loci were mapped. Thus, these lines could not have been rescued by any single duplication.

Cytogenetic mapping: females heterozygous for *y w* (*mutant*) chromosomes were crossed to males carrying one of a set of duplicated (*Dp*) X chromosome segments, which covered in aggregate about 46% of the euchromatic X chromosome. Progenies were examined for survival of the *y w;Dp* class. For complementation testing, mutations covered by the same duplication were crossed inter se, and the viability and behavioral phenotype of the *y w* (*mutant 1*)/*y w* (*mutant 2*) progeny was examined.

All 28 touch-insensitive lines were also tested for complementation of the bang-sensitive mutations *bang-sensitive*, *bang-senseless*, *easily-shocked*, and *technical knockout*, which display paralysis following mechanical stimulation and were considered possible sites of mechanosensory mutations. Heterozygote progeny from all crosses did not show paralysis after vibration on a laboratory vortex mixer, indicating that no mutations of the bang-sensitive loci were among those recovered.

Immunocytochemistry

To stain mutant embryos with MAbs specific for the nervous system, mutations of interest were kept heterozygous with balancer chromosomes marked with a ubiquitously expressed β -galactosidase gene (FM6-12 or FM6-2; S. Shepherd, personal communication). Stage 16 embryos were dechorionated in bleach, fixed, and stained (protocol 95 in Ashburner, 1989). Primary staining was with a mixture of the mouse MAb 22C10 or 21A6 and mouse anti- β -galactosidase. Secondary antibody was goat anti-mouse conjugated with horseradish peroxidase. Hemizygous mutant male embryos were distinguished by the absence of ubiquitous staining. Some larvae were fileted for better visibility of the nervous system.

Generation of Mosaics

Gynandromorph mosaics were generated by making heterozygotes for the unstable ring X chromosome *R(1)2*, $y^+ wvC$ and *y w* mutant chromosomes. Mosaic patches for some mutations were generated with a yeast-derived system of FLP recombinase-mediated mitotic recombination (Golic, 1991). FRT inserts FRT101 (Golic and Lindquist, 1989) or FRT18A (Xu and Rubin, 1993) were recombined onto *y w* mutant chromosomes. Recombination was induced by heat-shock expression of the FLP recombinase in the genotype *y w* (*mutant*) *FRT/y^+ w^+ FRT*; *FLP2B/+* to yield patches of homozygous *y w* (*mutant*) *FRT* tissue.

TEP and MRP Recording

Initial attempts at recording from bristles in y/y^+ mosaics revealed that *y* bristles usually showed very reduced or no TEP and therefore showed reduced or absent MRPs. (In the rare cases where an adequate TEP was obtained, normal MRP responses were observed.) Therefore, mutations of interest were always placed in a y^+ genotype. Mutations at the base of the X chromosome (*unc*, *uncl*, or *tilB*) were recombined with a $y^+ w^+ g$ *bas f* chromosome. Several $y^+ w^+ g^+ f^+$ (*mutant*) recombinants of each mutant allele were tested. Similar recombinants constructed from the parental *y w* chromosome served as wild-type controls.

For recordings, the bristle electrode was filled with a high-potassium, low-calcium saline solution: 121 mM K^+ , 9 mM Na^+ , 0.5 mM Ca^{2+} , 4 mM Mg^{2+} , 35 mM glucose, and 5 mM HEPES (pH 7.1). The reference electrode was filled with a standard low-

calcium saline (128 mM Na⁺, 2 mM K⁺, and other ions as above). When both electrodes were placed in contact with thoracic extracellular space, no potential differences greater than 4 mV were observed; any such cis-epithelial potentials were neglected in our measurements of TEP. The bristle electrode was mounted on a piezoelectric microstage (PZS-100HS, Burleigh Instruments), which followed computer-generated signals to give a ramp-and-hold 20 μm displacement. Potentials were subtracted and amplified (100× gain, no filtering) with a World Precision Instruments DAM-60 or Dagan EX-1 differential amplifier and then were digitized (200 μs sample period), displayed, and analyzed on a Macintosh computer equipped with a MacAdios analog-to-digital converter running Superscope II software (GW Instruments).

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