***P*-Element Insertion Alleles of Essential Genes on the Third Chromosome of *Drosophila melanogaster*: Mutations Affecting Embryonic PNS Development**

**Adi Salzberg,^{*,1} Sergei N. Prokopenko,^{*,†} Yuchun He,^{*} Peter Tsai,^{*} Margit Pál,[‡]
Péter Maróy,[‡] David M. Glover,[§] Péter Deák,^{§,***} and Hugo J. Bellen^{*,†}**

^{*}Howard Hughes Medical Institute, Department of Molecular and Human Genetics and [†]Developmental Biology Program, Baylor College of Medicine, Houston, Texas 77030, [‡]Department of Genetics, University of Szeged, Szeged, H-6701, Hungary, [§]CRC Cell Cycle Genetics Group, Department of Anatomy and Physiology, University of Dundee, Dundee DD1 4HN, United Kingdom and ^{***}Institute of Biochemistry, Biological Research Center, Szeged, H-6726, Hungary

Manuscript received March 12, 1997

Accepted for publication July 29, 1997

ABSTRACT

To identify novel genes and to isolate tagged mutations in known genes that are required for the development of the peripheral nervous system (PNS), we have screened a novel collection of 2460 strains carrying lethal or semilethal *P*-element insertions on the third chromosome. Monoclonal antibody 22C10 was used as a marker to visualize the embryonic PNS. We identified 109 mutant strains that exhibited reproducible phenotypes in the PNS. Cytological and genetic analyses of these strains indicated that 87 mutations affect previously identified genes: *tramtrack* ($n = 18$ alleles), *string* ($n = 15$), *cyclin A* ($n = 13$), *single-minded* ($n = 13$), *Delta* ($n = 9$), *neuralized* ($n = 4$), *pointed* ($n = 4$), *extra macrochaetae* ($n = 4$), *prospero* ($n = 3$), *tartan* ($n = 2$), and *pebble* ($n = 2$). In addition, 13 mutations affect genes that we identified recently in a chemical mutagenesis screen designed to isolate similar mutants: *hearty* ($n = 3$), *dorsototals* ($n = 2$), *pavarotti* ($n = 2$), *sanpodo* ($n = 2$), *dalmatian* ($n = 1$), *missensed* ($n = 1$), *senseless* ($n = 1$), and *sticky ch1* ($n = 1$). The remaining nine mutations define seven novel complementation groups. The data presented here demonstrate that this collection of *P*-elements will be useful for the identification and cloning of novel genes on the third chromosome, since >70% of mutations identified in the screen are caused by the insertion of a *P*-element. A comparison between this screen and a chemical mutagenesis screen undertaken earlier highlights the complementarity of the two types of genetic screens.

A POWERFUL approach to isolate genes involved in a specific developmental process is to screen systematically for mutations that disrupt normal development. This approach has been used successfully in the fruit fly to identify genes that play a role in diverse developmental processes such as general body patterning (NÜSSELEIN-VOLHARD and WIESCHAUS 1980) and different aspects of axonal growth (SEEGER *et al.* 1993; VAN VACTOR *et al.* 1993; KOŁODZIEJ *et al.* 1995; MARTIN *et al.* 1995). More recently, this methodology has been employed in zebrafish to identify genes that affect body pattern formation, morphogenesis and behavior (for review see EISEN 1996).

We have used this approach to identify genes that are required for normal pattern formation in the embryonic peripheral nervous system (PNS) (SALZBERG *et al.* 1994; KANIA *et al.* 1995). The embryonic PNS of *Drosophila* has proven to be an excellent model system to study neurogenesis, since it is relatively simple and since some of the molecular pathways underlying PNS development in the fly seem to be conserved

across animal phyla (reviewed in SALZBERG and BELLEN 1996).

The anatomy of the embryonic PNS has been well studied (DAMBLY-CHAUDIERE and GHYSEN 1986; GHYSEN *et al.* 1986; BODMER and JAN 1987; HARTENSTEIN 1988). It consists of ~650 neurons and 1200 associated non-neuronal cells. There are two major types of sensory organs: external sensory (ES) organs that function as chemoreceptors and mechanoreceptors, and chordotonal (Ch) organs that function as stretch or proprioceptors. In addition, the PNS contains multiple dendritic (MD) neurons that are not associated with specific accessory cells and are thought to function in touch perception.

Genes participating in PNS development in the fly were discovered in various ways. Many genes were identified on the basis of altered bristle patterns in adult flies (*e.g.*, *achaete*, *scute*, *Hairless*, and *extra macrochaetae*). Bristles are secreted by hair cells and constitute the external portion of ES organs in adults. Although the adult PNS is more elaborate than the embryonic PNS, the molecular mechanisms that govern neurogenesis seem to be similar in both systems. Indeed, genes that were initially identified due to their effect on adult neurogenesis were later found to play a role in embryonic neurogenesis as well. Another important group of

Corresponding author: Hugo J. Bellen, Howard Hughes Medical Institute, Room T630, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. E-mail: hbellen@bcm.tmc.edu

¹Present address: Department of Genetics, Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 31096, Israel.

TABLE 1
Deficiencies and mutations used in this study

Deficiency	Cytology	Reference
<i>Df(3L)W5.4</i>	65A; 65E1	ANDERSON <i>et al.</i> (1995)
<i>Df(3L)XDI98</i>	65A2; 65E1	W. A. JOHNSON
<i>Df(3L)pbl-X1</i>	65F3; 66B9	HIME and SAINT (1992)
<i>Df(3L)AC1</i>	67A2; 67D11-13	LINDSLEY and ZIMM (1992)
<i>Df(3L)W4</i>	75B8-11; 75C5-7	LINDSLEY and ZIMM (1992)
<i>Df(3L)Cat</i>	75C1-2; 75F1	LINDSLEY and ZIMM (1992)
<i>Df(3R)Antp17</i>	84A6; 84D13-14	LINDSLEY and ZIMM (1992)
<i>Df(2R)dsx2M</i>	84C1-3; 84E1	LINDSLEY and ZIMM (1992)
<i>Df(3R)by10</i>	85D8-12; 85E7-F1	LINDSLEY and ZIMM (1992)
<i>Df(3R)GB104</i>	85D11-13; 85E10	LINDSLEY and ZIMM (1992)
<i>Df(3R)by62</i>	85D11-14; 85F16	LINDSLEY and ZIMM (1992)
<i>Df(3R)cu</i>	86C1-2; 86D8	LINDSLEY and ZIMM (1992)
<i>Df(3R)kar-Sz28</i>	87C7-8; 87E9-10	LINDSLEY and ZIMM (1992)
<i>Df(3R)sbd45</i>	89B4; 89B10	LINDSLEY and ZIMM (1992)
<i>Df(3R)Dt-BX12</i>	91F1-2; 92D3-6	LINDSLEY and ZIMM (1992)
<i>Df(3R)3450</i>	98E3; 99A6-8	S. HAYASHI
<i>Df(3R)awd-KRB</i>	100C; 100D	BIGGS <i>et al.</i> (1988)

Mutation	Cytological location	Reference
<i>emc</i> ^{O4322}	61D1-2	KARPEN and SPRADLING (1992)
<i>par</i> ^{B200}	64B2-14	SALZBERG <i>et al.</i> (1994)
<i>ut</i> ^{H599} , <i>ut</i> ^{M638}	65A2; 65E1	SALZBERG <i>et al.</i> (1994)
<i>pbl</i> ⁵ (<i>pbl</i> ^{11D})	66B1-2	JÜRGENS <i>et al.</i> (1984)
<i>cycA</i> ^{H170}	68E1-2	SALZBERG <i>et al.</i> (1994)
<i>trn</i> ^{28.4}	70A1-2	CHANG <i>et al.</i> (1993)
<i>misn</i> ^{E95}	84D8-12	SALZBERG <i>et al.</i> (1994)
<i>hrt</i> ^{H246}	84F1-2	SALZBERG <i>et al.</i> (1994)
<i>ded</i> ^{G371}	85A1-7	SALZBERG <i>et al.</i> (1994)
<i>neur</i> ^{F180}	85C4-5	SALZBERG <i>et al.</i> (1994)
<i>hth</i> ^{5E04}	85E-86B	JÜRGENS <i>et al.</i> (1984)
<i>dml</i> ^{I184} , <i>dml</i> ^{I265} , <i>dml</i> ^{M572}	85E5; 85F11	SALZBERG <i>et al.</i> (1994)
<i>hyd</i> ^{I4}	85E6-10	MANSFIELD <i>et al.</i> (1994)
<i>knk</i> ^I	85E6-10	JÜRGENS <i>et al.</i> (1984)
<i>dtl</i> ^{H321} , <i>dtl</i> ^{I186}	86C1-4	SALZBERG <i>et al.</i> (1994)
<i>pros</i> ^{D135}	86E3-20	SALZBERG <i>et al.</i> (1994)
<i>sim</i> ²	87E1	HILLIKER <i>et al.</i> (1980)
<i>Dt</i> ^{F362}	92A2	SALZBERG <i>et al.</i> (1994)
<i>pnt</i> ² (<i>pnt</i> ^{9f})	94F1-2	JÜRGENS <i>et al.</i> (1984)
<i>stg</i> ⁴ (<i>stg</i> ^{2M53})	99A5-6	JÜRGENS <i>et al.</i> (1984)
<i>stg</i> ^{D1235}		KARPEN and SPRADLING (1992)
<i>spdo</i> ^{C55}	99F	SALZBERG <i>et al.</i> (1994)
<i>ttk</i> ^{B330}	100D3	SALZBERG <i>et al.</i> (1994)
<i>sens</i> ^{I235} , <i>sens</i> ^{M256} , <i>sens</i> ^{M608}	3L	SALZBERG <i>et al.</i> (1994)
<i>jay</i> ^{F261} , <i>jay</i> ^{I68}	3R	SALZBERG <i>et al.</i> (1994)
<i>stich</i> ^I ^{D233}	Unknown	SALZBERG <i>et al.</i> (1994)
<i>stich</i> ² ^{D337}	Unknown	SALZBERG <i>et al.</i> (1994)
<i>wan</i> ^{I368} , <i>wan</i> ^{K835}	Unknown	SALZBERG <i>et al.</i> (1994)

genes, the neurogenic genes, was identified because mutations in these genes lead to the formation of supernumerary neurons at the expense of epidermal cells (LEHMANN *et al.* 1983). More recently, novel genes involved in PNS development have been identified in "enhancer detector" screens (BELLEN *et al.* 1989; BIER *et al.* 1989; UEMURA *et al.* 1989; VAESSIN *et al.* 1991). Finally, many new genes affecting PNS development were identified in systematic mutagenesis screens that

capitalized on the availability of excellent cell-specific monoclonal antibodies (SALZBERG *et al.* 1994; KANIA *et al.* 1995; KOŁODZIEJ *et al.* 1995). Here we report the results of a genetic screen designed to identify *Pe*-element insertions on the third chromosome that affect PNS development. Our results suggest that this novel collection of *Pe*-elements is very valuable for the identification and cloning of novel genes and for the isolation of molecularly tagged mutations in known genes on the

TABLE 2
Summary of the third chromosome *P*-element screen

	No. of strains	No. of complementation groups	Percentage of saved strains
Lethal strains screened	2460		
Saved strains	109	26	100
Revertible <i>P</i> elements	66		65 ^a
Mutations allelic to previously identified genes	100	19	92
Novel mutations	9	7	8
PNS phenotypes observed with MAb 22C10 ^b			
Gain of neurons	43 (40) ^c	7	40
Loss of neurons	38 (35)	11	35
Abnormal positioning of neurons	8 (8)	3	7
Defects in axonal pathways	21 (18)	6	19
Neuronal morphology	6 (4)	4	6

^a Based on 101 strains tested for reversion.

^b Note that some strains (83/20, 238/3, 708/6, 1143/7, 1351/8, 1437/2, and 1441/14) exhibit a complex phenotype and are included in more than one phenotypic class.

^c Numbers in parentheses indicate the number of alleles of previously identified genes.

third chromosome. This collection is similar in size and quality to the second chromosome *P*-element collection established by I. KISS and his colleagues (TÖRÖK *et al.* 1993; KANIA *et al.* 1995).

MATERIALS AND METHODS

Stocks: All stocks were maintained on standard *Drosophila* medium at room temperature (ASHBURNER 1989). For genetic nomenclature, cytology, and description of mutations and chromosomes refer to LINDSLEY and ZIMM (1992) and FLYBASE (1997). The construction of mutant stocks is described in the accompanying paper (DEÁK *et al.* 1997). The mutant stocks are of the *y w*; *P[lacZ, w⁺]/TM3, Sb Ser* genotype. The deficiencies and mutations used in this study are listed in Table 1. Most of the deficiency chromosomes and mutant alleles were obtained from the Bloomington Stock Center (Bloomington, IN) and from the Umeå Stock Center (Umeå, Sweden). Deficiencies *Df(3L)W5.4* and *Df(3L)XDI98* were obtained from Dr. W. A. JOHNSON, the *hth*^{SE04} allele was obtained from Dr. C. NÜSSEIN-VOLHARD, the *hyd*¹⁴ allele was obtained from Dr. A. SHEARN, the *sim*² allele was obtained from Dr. S. T. CREWS, and the *trn*^{28.4} allele was obtained from Dr. A. LAUGHON.

Immunocytochemistry: Embryos from each mutant strain were collected overnight and processed for whole mount antibody staining according to standard protocols (BIER *et al.* 1989; BELLEN *et al.* 1992). The primary antibodies, monoclonal antibody (MAb) 22C10 (FUJITA *et al.* 1982; GOODMAN *et al.* 1984; ZIPURSKY *et al.* 1984) or anti- β -galactosidase (Promega) were adsorbed at 4° overnight at 1:50 and 1:1000 dilutions, respectively. The secondary antibody was a biotinylated horse anti-mouse IgG (Vector). Signal detection was done using the Vectastain ABC-HRP kit (Vector) using diaminobenzidine (Sigma) as a substrate. Stained embryos were mounted in 70% glycerol and examined with light microscopy (Zeiss Axioptot).

In situ hybridization to polytene chromosomes: Males from balanced mutant strains were outcrossed to wild-type (*Canton S*) or *y w* females. Salivary glands of the progeny larvae were dissected in Ringer's insect saline and fixed with acetate. The pretreatment and hybridization to the chromosomes were essentially as outlined by LANGER-SAFER *et al.* (1982). Digoxigenin-labeled DNA probes were made from the *P[hwB]* plasmid (WILSON *et al.* 1989) using the Genius kit (Boehringer Mannheim). Following hybridization, the probe was detected using an anti-digoxigenin antibody conjugated to alkaline phosphatase (Boehringer Mannheim) and nitro blue tetrazo-

FIGURE 1.—Cytological location of 116 *P*-element insertions from 104 strains on the third chromosome (12 strains carry more than one insertion). Each insertion is indicated by a circle or a square. ●, *P*-element insertions that are responsible for the observed phenotype in the PNS; ■ insertions that do not cause or may not cause the lethality and PNS phenotype observed.

TABLE 3
Mutations causing gain of neurons or ectopic 22C10 expression

Gene	Cytology	Strain no.	Cytology of P elements	Reversion ^a	Lethal phase ^b	Complementation data	Phenotype	lacZ expression ^c
I. Neurogenic mutations								
neuralized (neur)	85C4-5	328/12	85C9-10	Y	E	neur ^{F180} —	Collapsed CNS, normal number of PNS neurons	Early mesoderm, PNS, CNS, MG
		971/15	85C9-15, 86E		E		Hypertrophic CNS and PNS	CNS, PNS, Ect
		1293/9	85C	Y	E		As above	No staining
		1315/5	85C	Y/N?	E		Hyperplasia in CNS but not PNS	Complex
Delta (Dl)	92A2	495/20	92A	Y	E	Dl ^{F362} —	Hypertrophy of PNS and CNS	Ect, heart, trachea, midline
		926/11	88E, 92A	Y	E		As above	All over
		1053/14	92A	Y	E		As above	Ect, heart, trachea, PNS, all over
		1119/9	92A	Y	E		As above	Ect, pMG, PNS
		1304/3	92A	Y	E		As above	Ect, heart, trachea, PNS, CNS, all over
		1423/11	92A	Y	E		As above	Dark all over
		1440/11	92A	N	E		As above	All over
		1480/11		Y	E		As above	H, CNS, all over
		1485/4	92A	Y	E		As above	Dark all over
		II. Mutations affecting neuronal lineage						
sanpodo (spdo)	99F	970/2	91D	N	E	spdo ^{C55} —	Supernumerary neurons, abnormal neuronal morphology, pathway defects in CNS	No staining
		1309/10	93B1-4	N	E		As above	Amnioserosa, heart, muscles
tramtrack (ttk)	100D3	37/17	100D	N	E-L1	ttk ^{B330} —	Severe ttk phenotype	All over
		250/25	100D	Y	E			As above
		438/31	100D	N	E			Ect, amnioserosa, all over
		702/7	100D	Y	E		22C10 expression in the ectoderm, heart, visceral and somatic mesoderm. No extra neurons in the PNS	All over
		1049/7	100D	Y	E			As above
		1119/4	100D	Y	E			As above
		1184/16	100D	N	E			H, CNS
		1209/5	100D	Y	E			All over
		1209/10	100D	Y	E		Severe ttk phenotype	As above
		1260/10	100D	Y	E			As above
		1281/4	100D	N	E		Severe ttk phenotype	As above
		1325/15	100D	Y	E			Dark all over
		1372/8	73F, 100D	N	E		Severe ttk phenotype	All over
		1396/14	97D	N	E			No staining
		1418/6	87B, 100D	Y	E			Dark all over
		1418/11	100D	Y	E			As above
1424/2		N	E			As above		
1448/2	90C?, 100D	Y	E			No staining		

TABLE 3

Continued

Gene	Cytology	Strain no.	Cytology of <i>P</i> elements	Reversion ^a	Lethal phase ^b	Complementation data	Phenotype	<i>lacZ</i> expression ^c
III. Mutations causing ectopic 22C10 expression								
<i>extra macrochaetae</i> (<i>emc</i>)	61D1-2	94/26	61D	Y	E	<i>emc</i> ^{04322-d}	22C10 expression in the gonads	All over
		203/10	61D		E	<i>emc</i> ^{04322-d}	As above	Early, mesoderm; later, all over
		587/1	61D	Y	E	<i>emc</i> ^{04322-d}	As above	All over
<i>bonus</i> (<i>bon</i>)	92E8-14	977/9	61D, 90D	Y	E	<i>emc</i> ^{04322-d}	As above	As above
		241/8	92E8-14	Y	pA		Two extra neurons anteriorly to each dorsal cluster. One or two very large and round cells darkly stained with the MAb 22C10 are found posterior to the v' cluster	Segmentally repeated pattern
		249/12	92E8-14	Y	pA		As above	Faint all over
		487/6	92E distal	Y	pA		As above	AS, Ph

^a Revertability: Y, revertible; N, nonrevertible.^b Lethal phase: A, adult; E, embryonic; L1, L2, and L3, larval; P, pupal, pA, pharate adult.^c Abbreviations are as follows: AS, anterior spiracles; CNS, central nervous system; Ect, ectoderm; H, head; MG, midgut; Ph, pharynx; pMG, posterior midgut; PNS, peripheral nervous system. Complex pattern refers to *lacZ* expression in many tissues.^d Adult escapers are viable and fertile but exhibit supernumerary macrochaetae.

lium chloride/5-bromo-4-chloro-3-indolyl-phosphate (Boehringer Mannheim). The chromosomes were counterstained with Giemsa (Sigma) and mounted in Permount (Fisher).

Isolation of revertants: To isolate revertants, we used the following procedure: males from each insertional strain (*P*[*lacZ*, *w*⁺]/*TM3*, *Sb* *Ser*) were crossed to *Ly*/*TM3* *ry e Sb* *P*(Δ 2-3, *ry*⁺) females and male progeny that carry both the *P*-element insertion and the transposase gene (*P*[*lacZ*, *w*⁺]/*TM3* *ry e Sb* *P*(Δ 2-3, *ry*⁺)) were isolated and crossed back to virgins of the original insertional strain. The progeny of each cross (at least 500 flies) were scored for non-stubble flies.

Lethal phase analysis: The analysis of lethal phase of *P*-element insertions is described elsewhere (DEAK *et al.* 1997).

RESULTS

Genetic screen: We screened a collection of 2460 strains carrying lethal or semilethal *P*-element insertions on the third chromosome. Embryos were collected from each mutant strain and immunocytochemically stained with MAb 22C10. Stained embryos were mounted and examined under a light microscope for altered patterns of 22C10 staining in the PNS. Mutant phenotypes were confirmed after outcrossing strains that were identified in the primary screen and restaining with MAb 22C10. All strains displaying reproducible phenotypes in the secondary screen were retained for further analysis.

To assign mutations to complementation groups, we crossed mutant strains that exhibited similar phenotypes to each other. In addition, strains exhibiting phenotypes similar to those caused by previously described

mutations were tested for complementation with relevant mutant strains. In summary, we identified 109 mutations that define 19 previously identified and seven novel complementation groups (Table 2).

Cytological mapping and reversion assays: The *P*-element insertions present in 104 of the saved strains were mapped by *in situ* hybridization to polytene chromosomes using *P*(*hwB*) DNA as a probe. To determine the quality of this novel collection of *P* elements, we mapped most of the *P* elements that fail to complement previously isolated genes such as *Delta*, *tramtrack*, *string*, and *cyclin A*. The positions of all mapped *P*-element insertions are shown diagrammatically in Figure 1 and are listed in Tables 3–7. To confirm the mapping positions of *P* elements inserted in novel genes (*e.g.*, *astray*), we carried out complementation tests with deficiencies available in corresponding chromosomal regions. Results of the deficiency mapping are shown in Tables 3–7.

To establish whether the *P*-element insertions are responsible for the mutant phenotypes, we excised the *P* elements and screened for revertants. As summarized in Table 2, the lethality associated with 65% of the *P*-element insertions is revertible (66 out of 101 strains tested). However, this percentage does not reflect accurately the percentage of *P* elements that are responsible for the observed phenotypes. In another 13% of the mutant strains (13/101), the *P* elements are very likely to cause the mutant phenotype as they map to the same

TABLE 4
Continued

Gene	Cytology	Strain no.	Cytology of P elements	Reversion ^a	Lethal phase ^b	Complementation data	Phenotype	<i>lacZ</i> expression ^c
<i>potpourri</i> (<i>potp</i>)	85E	1143/7	85E	Y		<i>dmt</i> ^{M572} +	Variable loss of neurons, decreased staining with 22C10 especially in the CNS, disorganization of the PNS, pathfinding defects	All over
<i>dalmatian</i> (<i>dmt</i>)	85E; 85F1	481/3	85E5-11	Y	pA	<i>dmt</i> ^{I184} — <i>hth</i> ^{5E04} + ^e <i>hyd</i> ^{I4} + ^e <i>knk</i> ^I + ^e	Very mild <i>dmt</i> allele. Occasional loss of neurons, pathway defects and appearance of dark rounded cells	CNS, early PNS
<i>senseless</i> (<i>sens</i>)	3L	1228/4	85D12-20	N		<i>sens</i> ^{I235} — <i>sens</i> ^{M256} — <i>sens</i> ^{M608} + <i>Df(3R)by10</i> + <i>Df(3R)by62</i> + <i>Df(3R)GB104</i> +	Severe loss of neurons	Heart, midline, peripheral glia
1368.02	ND	1368/2	75B11; 75C4	N	E	<i>ded</i> ^{C371} + <i>dmt</i> ^{I184} + <i>misr</i> ⁸¹⁶⁰³ + <i>sens</i> ^{I235} + <i>sens</i> ^{M608} + <i>Df(3L)Cat</i> + <i>Df(3L)W4</i> +	Very low expression of the 22C10 antigen	Midline, gut

^{a-c} See Table 3. Additional abbreviations are as follows: ND, not determined; pPNS, part of the PNS; SG, salivary glands; VNC, ventral nerve cord. Mitotic pattern is characterized by *lacZ* expression in cells within mitotic domains.

^d Adult escapers lack scutellars and many other bristles, have rough eyes, and occasionally lack a haltere or have notched wings.

^e Complementation tests with *dmt*^{I184} and *dmt*^{I265}.

cytological band as other insertions that are revertible (e.g., *pointed*, *pavarotti*, *dorsotonals*). The inability to recover revertants of those insertional mutations may be due to another lethal mutation on the same chromo-

some or a damaged *P* element. Thus, based on the results of reversion assays, mapping of *P*-element insertions, and complementation tests, we estimate that ~78% of the mutations are caused by *P* elements. The

TABLE 5
Mutations causing aberrant positioning of neurons

Gene	Cytology	Strain no.	Cytology of P elements	Reversion ^a	Lethal phase ^b	Complementation data	Phenotype	<i>lacZ</i> expression ^c
<i>tartan</i> (<i>trn</i>)	70A1-2	24/5	70A, 94B	Y	A	<i>trn</i> ^{28.4} —	Mild dorsalization of LCh5	pPNS, gut
		641/17	70A	Y	E	<i>trn</i> ^{28.4} —	Occasional dorsal localization of LCh5	<i>tartan</i> -like expression
<i>dorsotonals</i> (<i>dtl</i>)	86C1-4	1323/7	86C1-6	N	E	<i>dtl</i> ^{H321} —		No staining
<i>pointed</i> (<i>pnt</i>)	94F1-2	1422/4	86C1-4	Y	E-L1			Ect, CNS
		123/9	94F1-3	Y	E-L1	<i>pnt</i> ² —	Dorsal localization of LCh5 in 1-2 segments per embryo	No staining
		608/7	70C4-8, 94F1-3	Y	L3			Very complex
		998/12	94F1-3	N	P			Heart, pMG, unidentified cells
		1183/6			E			No staining

^{a-c} See Table 3. pPNS, part of the PNS.

TABLE 6
Mutations causing aberrant neuronal connections

Gene	Cytology	Strain no.	Cytology of <i>P</i> elements	Reversion ^a	Lethal phase ^b	Complementation data	Phenotype	<i>lacZ</i> expression ^c
<i>pebble (pbl)</i>	66B1-2	83/20	66AB	Y	E	<i>pbl</i> ⁵ – <i>Df(3L)pbl-X1</i> –	Severe fasciculation defects, loss of neurons, remaining cells are enlarged	No staining
		542/3	66AB	Y	P	<i>pbl</i> ⁵ – <i>Df(3L)pbl-X1</i> –	10% of embryos exhibit mild fasciculation defects	MT, faint CNS
<i>astray (aay)</i>	67B1-10	423/14	67B1-10	Y	pA	<i>jay</i> ^{F261} + <i>jay</i> ^{J68} + <i>wan</i> ^{J368} + <i>wan</i> ^{K835} + <i>Df(3L)AC1</i> – <i>pros</i> ^{D135} –	Axonal misrouting, ISN crosses segment boundaries	No staining
<i>prospero (pros)</i>	86E3-20	451/9	84E3-9		E			No staining
		1135/9	76B	N	E			As above
		1167/13	92C	N	E	<i>Df(3R)Dl-BX12</i> + <i>sim</i> ² –		As above
<i>single-minded (sim)</i>	87E1	483/9	64B9-15	N	E	<i>Df(3R)kar-Sz28</i> –		Early mesoderm, muscles
		716/8	75C?	N	E			No staining
		899/14	99A1-4	N	E			H, pPNS, pCNS
		953/8	61C5-9	N	E			Ect, all over
		1002/10	66F		E			Anal pads, HG
		1034/2	85F13-16	N	E			No staining
		1034/3	68E?	N	E			As above
		1034/7		Y	E			
		1050/13	70D	N	E			No staining
		1110/4	88F?	Y?	E			As above
		1111/10	91F; 92A4		E			As above
		1330/8	85F	N	E			As above
		1479/12		Y	Viable			All over
529.08	ND	529/8	83A	N	L2	<i>aay</i> ⁴²³¹⁴ + <i>jay</i> ^{J68} + <i>pros</i> ¹¹⁶⁷¹³ + <i>wan</i> ^{J368} +	Misrouting of axons, mainly in dorsal region. Most frequent in A4 and A5.	No staining

^{a-c} See Table 3. Additional abbreviations are as follows: HG, hindgut; MT, malpighian tubules; ND, not determined; pCNS, part of the CNS; pPNS, part of the PNS.

remaining 22% of mutations fall into two categories: insertional mutations that clearly do not cause the observed phenotype (*e.g.*, *missensed*, several *single-minded* and *string* alleles) and those for which we have no conclusive data (*sticky ch1*, 529.08, 573.02, 1368.02).

Novel genes, that were identified by reversible *P* elements, were named on the basis of their PNS phenotype. We did not name a number of genes identified by a single mutation, since we do not know where they map and/or whether the *P* element is responsible for the observed phenotype. These mutants are designated according to the original number of the insertional strain.

Mutant phenotypes: The 26 complementation groups identified in the screen were classified on the basis of their phenotype into five phenotypic classes: gain of neurons, loss of neurons, abnormal positioning of neurons, defects in axonal pathways and abnormal neuronal morphology (Table 2). Since the classification of mutant phenotypes is based solely on MAb 22C10 staining, the terms "loss of neurons" or "gain of neurons" refer to a loss or gain of MAb 22C10 immunoreac-

tivity and not necessarily to a genuine change in neuronal cell numbers or cell identity. Most mutations that perturb the overall structure of the embryo, such as mutations that affect segmentation, were discarded.

Mutations that cause gain of neurons or ectopic 22C10 expression: We have identified 40 mutations in six complementation groups that cause a gain of neurons (Table 3). Four mutations were found to be allelic to the neurogenic gene *neuralized (neur)* and nine to the neurogenic gene *Delta (Dl)*. All of the mutations in *neur* and *Dl*, with the exception of one *neur* allele, 328/12, cause a typical hyperplasia of the nervous system. Embryos from the 328/12 strain did not exhibit a neurogenic phenotype, but exhibited a phenotype that is very similar to mutations in the *single-minded (sim)* gene, namely a collapsed central nervous system (CNS). However, 328/12 complements *sim* alleles and fails to complement *neur* alleles. In addition, Southern analysis revealed that *P{328/12}* is inserted in the 5' end of the *neur* gene (S. CREWS, personal communication). Hence, we propose that the 328/12 mutation may affect tran-

TABLE 7
Mutations causing abnormal morphology of neurons

Gene	Cytology	Strain no.	Cytology of <i>P</i> elements	Reversion ^a	Lethal phase ^b	Complementation data	Phenotype	<i>lacZ</i> expression ^c
<i>melted</i> (<i>melt</i>)	65E	1441/14	65E	Y	E	<i>ut</i> ^{H599} + <i>ut</i> ^{M638} +	Abnormal morphology and mild loss of neurons	No staining
<i>hearty</i> (<i>hrt</i>)	84F1-2	238/3	84F	Y	E	<i>hrt</i> ^{H246} —	22C10 expression in the heart. Defects in dorsal closure.	Faint PNS, dorsal midline
		708/6 1351/8	84A, 86A 84F	Y?	E E		As above As above	No staining PNS, dorsal midline, heart
<i>sticky ch1</i> (<i>stich1</i>)	ND	1437/2	86A	N		<i>stich1</i> ^{D233} — <i>hth</i> ^{5E04} + ^d	Loss of neurons in v and v' clusters. LCh5 stain very faintly with Mab 22C10 or do not stain at all.	No staining
573.02	ND	573/2	100C	N	E	<i>hrt</i> ^{H246} + <i>melt</i> ^{H4114} + <i>stich1</i> ^{D233} + <i>stich2</i> ^{D337} + <i>Df(3R)awd-KRB</i> +	PNS neurons stain faintly with 22C10 and appear smaller than normal. CNS does not retract fully and display defects in axonal scaffold.	No staining

^{a-c} See Table 3. ND, not determined.

^d Complementation test with *stich1*^{D233}.

scription of *neur* in a subset of cells in the nervous system.

The *sanpodo* (*spdo*) gene was previously identified as being required for proper cell fate specification in the PNS lineages (SALZBERG *et al.* 1994). The gene was mapped by meiotic recombination and deficiency mapping to cytological region 99F. Loss-of-function mutations in the *spdo* gene cause an increase in the number of neurons at the expense of lineage-related cells in the PNS (SALZBERG *et al.* 1994). Two alleles of *spdo* were identified in the present screen, 970/2 and 1309/10. However, in both strains the *P* elements are not inserted in the *spdo* locus at 99F. Interestingly, genomic Southern analysis revealed that both mutations caused rearrangements of the *spdo* locus (C. DYE and H. J. BELLEN, unpublished results). This provides further evidence that exposure of *P* elements to $\Delta 2-3$ transposase can cause chromosomal defects (KANIA *et al.* 1995), making these mutations a valuable tool to clone genes, even when the *P* element does not map to the cytological band of interest.

Eighteen mutations were identified in the *tramtrack* (*tth*) locus (HARRISON and TRAVERS 1990), an insertional hotspot. All of the *tth* alleles cause extra 22C10 immunoreactivity. Some of the alleles (for example, 438/31) cause an increase in the number of neurons in the PNS in addition to 22C10 immunoreactivity in mesodermal and ectodermal cells. Other alleles, such as 702/7, cause a gain of 22C10 immunoreactivity in the ectoderm and mesoderm, but do not alter the number of neurons in the PNS (data not shown). We did

not identify any *tth* alleles that affect specifically the PNS, but not cell identity in other tissues.

Several novel hypomorphic alleles of *extra macrochaetae* (*emc*) were identified in the screen. *emc* was discovered as a gene required for the patterning of sensory organs in the adult fly (BOTAS *et al.* 1982). It is related to a family of helix-loop-helix (HLH) proteins, but lacks a basic DNA binding domain (ELLIS *et al.* 1990; GARRELL and MODOLELL 1990). It was implicated in the spatial patterning of sensory organs by antagonizing the DNA binding activity of basic HLH proteins encoded by proneural genes of the *achaete-scute* complex and *daughterless*.

Embryos homozygous for any of the four *emc* alleles identified in the present screen (94/26, 203/10, 587/1, and 977/9) exhibit a similar phenotype, *i.e.*, 22C10 staining in a group of ~12 cells in the dorsal region of abdominal segments A5–A6 (Figure 2, C and E). These cells are located within the embryonic gonads and are likely to be pole cells. Staining with the anti-VASA antibody that recognizes a protein component of polar granules (HAY *et al.* 1988) shows that VASA is not expressed in these gonadal cells, whereas heterozygous embryos abundantly express VASA in these cells (data not shown). Interestingly, pole cells that do not enter the gonad do express VASA and fail to express the 22C10 antigen, suggesting that the gonadal environment is key to the expression of the 22C10 antigen and the absence of VASA staining. Unlike normal germ cells, the 22C10 expressing cells in these embryos do not form a tight cluster in the mesodermal envelope of

FIGURE 2.—Mutations causing gain of neurons. (A) Lateral view of three abdominal segments of a wild-type stage 16 embryo stained with MAb 22C10. d, dorsal cluster; l, lateral cluster; v and v', ventral clusters. (B) *bonus* (allele 241/8). Note ectopic neurons anterior to the dorsal cluster and posterior to the v' cluster (arrows). (C and E) *extra macrochaetae* (allele 94/26). Ectopic 22C10 expression is evident in the pole cells within the embryonic gonads (arrows). Compare an embryo from the 94/26 stock (E) with a similar view of a wild-type embryo (D). Notice that only the pole cells are affected.

the gonads. Finally, in contrast to the rounded morphology normally displayed by germ cells, these cells are less rounded (arrows in Figure 2C) and occasionally display neuronal-like projections. We therefore conclude that the germ cells are at least partially transformed into neuronal-like cells.

All alleles of *emc* identified in this screen are embryonic lethal. Interestingly, flies transheterozygous for any of the *emc* alleles identified in our screen and previously isolated alleles of *emc* are viable and fertile (Table 3 and data not shown). In addition, they display, similarly to transheterozygous combinations of hypomorphic and amorphic alleles of *emc*, supernumerary macrochaetae.

Three revertible allelic insertions that map to cytological position 92E8-14 define a novel gene that we named *bonus* (*bon*). All *bon* mutations cause a similar phenotype: four extra neurons in each abdominal hemisegment (Figure 2B). Two ectopic neurons are located beneath the ectodermal layer anterior to each dorsal PNS cluster in abdominal segments. The other two ectopic neurons are located just posterior to the v' cluster and appear as large rounded cells that stain darkly with MAb 22C10. The number of neurons in the dorsal and v' clusters themselves is not altered in embryos homozygous for *bon* mutations.

Mutations that cause loss of neurons: We have identified 35 mutations in eight complementation groups that cause a loss of neurons (Table 4). This group in-

cludes mutations in three genes, *pavarotti* (*pav*), *cyclin A* (*cycA*), and *string* (*stg*), that lead to a reduction of the number of neuronal cells with a concomitant increase in cell size. This phenotype denotes mutations that affect cell cycle or cell division because maternal contributions are exhausted during mitotic cycles 14–16, requiring zygotic transcription in the PNS and CNS precursors and their descendants (SALZBERG *et al.* 1994; BHAT *et al.* 1996).

Two strains, 934/13 and 973/8, were found to harbor an insertion in the *pav* gene. The *pav* gene was previously identified in a chemical mutagenesis screen and was mapped to cytological region 64B (SALZBERG *et al.* 1994). Phenotypic analysis of *pav* mutant embryos suggested that in the absence of *pav* cell division in the PNS is impaired and cells that fail to divide grow in size. Further analysis indicated that mutations in the *pav* gene affect cell division (R. R. ADAMS and D. M. GLOVER, personal communication).

Thirteen alleles of *cycA* were identified in the present screen. All of the *cycA* mutant strains carry a revertible *P*-element insertion in the *cycA* locus at cytological position 68E.

In contrast, only six out of 15 alleles of *stg* identified in the screen map to the proper cytological position, 99A. These data suggest that the *stg* locus is an insertional hotspot from which *P* elements excise rapidly and reintegrate at other positions (KANIA *et al.* 1995).

The 816/3 chromosome harbors a lethal mutation in the previously identified *missensed (misen)* gene (SALZBERG *et al.* 1994). Mutations in *misen* lead to a variable loss of neurons and some disorganization of the PNS (Figure 3A). The *misen* gene was mapped by meiotic recombination and deficiency analysis to the 84D8; 84D12 cytological interval (SALZBERG *et al.* 1994). The *P* element inserted in the 816/3 chromosome is probably not related to the *misen* gene as it maps to 70A.

The novel complementation group we called *pot-pourri (potp)* is defined by a single *P*-element insertion allele, 1143/7. Embryos homozygous for this insertion display a variety of defects in the PNS: variable loss of neurons, decreased staining with MAb 22C10 (most prominent in the CNS), general disorganization in the PNS, pathfinding and subtle morphological defects (data not shown). This insertion is revertible and maps at 85E.

The 481/3 strain carries a lethal *P* insertion in the *dalmatian (dmt)* gene. This gene was identified in a previous study and was mapped to chromosomal region 85E; 85F1 (SALZBERG *et al.* 1994). The *P*{481/3} is a revertible insertion that maps at cytological region 85E5-11 and fails to complement the chemically induced *dmt* alleles. Embryos homozygous for the *P*{481/3} insertion display a very mild *dmt* phenotype that consists of infrequent loss of neurons, occasional pathfinding defects and small round cells that stain darkly with MAb 22C10 (Figure 3B). Chemically induced *dmt* alleles cause a much more severe loss of neurons, which is associated with a more general disorganization of the PNS and defects in other tissues (data not shown).

The 1228/4 chromosome carries a lethal mutation in the *senseless (sens)* gene. The *sens* gene was identified in a previous study by three EMS-induced alleles: *sens*^{J235}, *sens*^{M256}, and *sens*^{M608} (SALZBERG *et al.* 1994). Mutations in this gene cause a loss of most chordotonal neurons and many external sensory neurons (Figure 3F). On average, only 15–20 of the 44 peripheral neurons remain in each abdominal hemisegment of embryos homozygous for a mutation in the *sens* gene. The *sens* gene was mapped by meiotic recombination to the left arm of chromosome three (SALZBERG *et al.* 1994). A precise mapping position could not be determined because of chromosomal rearrangements in at least one of the chemically mutagenized chromosomes. The *P*-element insertion in the 1228/4 chromosome was mapped to cytological region 85D12; 85D20. However, attempts to revert the lethality by excising the *P*{1228/4} insertion, or to map the lethal mutation to a deficiency in that region, were unsuccessful (see Table 4). Based on this information we conclude that the *P* insertion in the 1228/4 strain is probably not responsible for the phenotype.

The 1368/2 insertion defines a novel gene required for normal development of the embryonic PNS. Embryos homozygous for the 1368/2 chromosome display

a severe loss of 22C10 immunoreactivity in the PNS and CNS (Figure 3, C and E). All neurons in both the PNS and CNS fail to express normal levels of the 22C10 antigen. The only exceptions are the ventral unpaired midline neurons that exhibit 22C10 expression at levels comparable to wild-type embryos (arrows in Figure 3, C and E). Further phenotypic analysis is required to establish whether the loss of 22C10 immunoreactivity reflects a genuine loss of neuronal cells, a defect in neuronal differentiation, or a disruption of the 22C10-encoding gene itself. The *P*{1368/2} insertion was mapped to cytological position 75B11; 75C4. However, the 1368/2 insertion chromosome complemented deficiencies uncovering the 75B-C region (see Table 4) and could not be reverted to wild type. Therefore, it is likely that a mutation mapping elsewhere is the cause of the observed phenotype.

Mutations that cause abnormal positioning of neurons: The abdominal lateral chordotonal (LCh) neurons originate in a dorsal position and migrate to the lateral region of the embryo later in development (SALZBERG *et al.* 1994). This process is associated with a rotation of the migrating cells. Two genes on the third chromosome were shown to play a role in the process of lateral chordotonal migration and rotation: *u-turn (ut)* and *dorsotonals (dtt)* (SALZBERG *et al.* 1994). Mutations in these genes cause the lateral chordotonal neurons to remain associated with the dorsal PNS cluster or stall along their migratory pathway. Whenever these neurons remain associated with the dorsal cluster, their dendrites point ventrally.

As reported previously, mutations in the *tartan (trn)* gene also cause occasional dorsalization of the LCh neurons (CHANG *et al.* 1993). Embryos homozygous for the 641/17 chromosome exhibit occasional dorsalization of the lateral chordotonal neurons and carry a revertible *P*-element insertion at cytological band 70A. Complementation tests indicated that 641/17 is a novel *trn* allele (Table 5). The 24/5 mutation represents another *trn* allele. It is a revertible insertion that maps at 70A and causes a mild dorsalization of lateral chordotonal neurons. This mutation was found to be semilethal over the 641/17 chromosome and lethal over the *trn*^{28.4} allele.

Two insertional strains, 1323/7 and 1422/4, exhibit a severe “dorsal chordotonals” phenotype (Figure 4B). Embryos homozygous for the 1422/4 insertion display abnormal positioning of the lateral chordotonals in >60% of the abdominal segments. The 1323/7 mutation causes a more severe phenotype and leads to an almost complete dorsalization and occasional loss of these neurons. Both strains carry a single *P*-element insertion at cytological region 86C1-4. The *P*{1323/7} and *P*{1422/4} mutations failed to complement the chemically induced *dtt* alleles, *dtt*^{H321} and *dtt*^{I86}. Based on these data we conclude that 1323/7 and 1422/4 are novel *dtt* alleles and that the *dtt* gene maps at cytological

FIGURE 3.—Mutations causing loss of neurons. (A) *missense* (allele 816/3). Note a variable loss of neurons and some disorganization in the PNS. (B) *dalmatian* (allele 481/3). Note the small round cells that stain darkly with MAb 22C10 (arrows). Other less prominent features of the *dmt* phenotype are occasional loss of neurons and pathfinding defects. (C and E) *1368.02* mutant phenotype. Note a severe reduction of the 22C10 immunoreactivity in both PNS and CNS. Only ventral unpaired midline neurons in the CNS (arrows) exhibit near normal levels of the 22C10 antigen. Compare *1368.02* embryo (E) with a similar view of a wild-type embryo (D). (F) *senseless* (allele 1228/4). A severe loss of most chordotonal neurons and many external sensory neurons in all PNS clusters is prominent.

interval 86C1-4. To confirm this mapping position, we crossed various *dtl* alleles to *Df(3R)cu*, which uncovers chromosomal interval 86C1-86D8. The 1323/7 and

1422/4 mutations complemented this deficiency, whereas *dtl*^{H321} was semilethal and *dtl*^{I186} was lethal over this deficiency. These data may suggest that the EMS-

FIGURE 4.—Mutations causing abnormal positioning of neurons and defects in axonal pathways. (A and B) Dorsal (d) and lateral (l) clusters of two abdominal segments of wild-type (A) and *dorsotonal*s (allele 1323/7) (B) embryos. Mutations in *dtt* lead to the “dorsalization” of the lateral chordotonal neurons. They remain associated with the dorsal cluster (arrow) and their rotation and migration to the lateral position is blocked. (C) *pebble* (allele 83/20). A severe disorganization of the axonal connections is evident. Axons grow along aberrant pathways often crossing segment boundaries (arrow). Notice that there is a loss of neurons and some of the remaining neurons grow abnormally large (arrowheads). (D) *astray* (allele 423/14). The intersegmental nerve crosses segment boundaries (arrow) and makes aberrant connection with neurons in a neighboring segment. (E) 529.08 mutant phenotype. Note a severe misrouting of axons in the dorsal and lateral regions and abnormal connections between dorsal clusters of two adjacent segments.

induced *dtt* alleles are associated with chromosomal rearrangements that affect additional genes, and that the *dtt* gene maps close to the proximal breakpoint of *Df(3R)cu*.

Four strains exhibiting a mild “dorsal chordotonal” phenotype were found to be allelic to *pointed* (*pnt*) (KLAMBT 1993). Embryos homozygous for any of these alleles (123/9, 608/7, 998/12, and 1183/6) display a dorsal localization of the lateral chordotonal neurons in one or two abdominal segments per embryo. The affected neurons remain associated with the dorsal cluster, and their orientation is reversed.

Mutations that cause defects in axonal pathways: We identified 20 mutations that define five complementation groups that affect axonal pathways in the PNS and/or CNS (Table 6).

Two strains, 83/20 and 542/3, harbor revertible *P*-element insertions in the *pebble* (*pbl*) gene. Originally *pbl* was identified in a screen for mutations affecting the pattern of the embryonic cuticle (JÜRGENS *et al.*

1984). Mutations in *pbl* lead to a complete block of cytokinesis in somatic cells in the first postblastoderm mitosis (mitosis 14) (HIME and SAINT 1992; LEHNER 1992). Interestingly, all other aspects of mitosis (nuclear envelope breakdown, chromosome condensation and segregation, spindle assembly) are not affected in *pbl* embryos.

Embryos homozygous for the 83/20 insertion display gross defects in the axonal trajectories in the PNS. The intersegmental nerve grows from the dorsal cluster along aberrant pathways and often fails to connect to the lateral PNS cluster. Axons also cross segment boundaries in the dorsal, lateral and ventro-lateral region (Figure 4C). In addition, these embryos display a loss of neurons with a concomitant increase in the size of the remaining neurons (arrowheads in Figure 4C), a phenotype typical for mutations in genes required for cell cycle or cell division. The 542/3 mutation causes a mild phenotype that is not fully penetrant: ~10% of embryos collected from this strain exhibit mild fascicu-

lation defects. The 542/3 chromosome carries a *P*-element insertion at cytological position 66AB and fails to complement the 83/20 mutation. This complementation data indicate that 83/20 and 542/3 chromosomes carry allelic mutations. In addition, both insertions failed to complement *Df(3L)pbl-X1* and a chemically induced allele of *pbl* (see Table 6). This suggested that 83/20 and 542/3 are novel hypomorphic alleles of *pbl*.

A single insertional strain, 423/14, defines a novel complementation group named *astray* (*aay*). Homozygous 423/14 embryos exhibit gross defects in the axonal trajectories of the PNS, mainly the intersegmental nerve that often crosses segment boundaries and extends to neighboring segments (arrow in Figure 4D). The phenotype observed in the PNS of 423/14 embryos is very similar to the previously described phenotype of mutations in *jaywalker* (*jay*) (SALZBERG *et al.* 1994). However, *P{423/14}* is probably not allelic to *jay* as it complements the available chemically induced *jay* alleles. *P{423/14}* is a revertible insertion that maps at 67B1; 67B10. This mutation failed to complement *Df(3L)AC1*, which uncovers the 67A2; 67D7-13 interval.

Three mutant chromosomes, 451/9, 1135/9, and 1167/13, harbor a mutation in the *prospero* (*pros*) gene and cause a typical *pros* phenotype (DOE *et al.* 1991; VAESSIN *et al.* 1991). However, the *P* elements in these three strains are not inserted in the *pros* locus at cytological position 86E. Similarly, 11 out of 13 strains that were found to carry a mutation in *single-minded* (*sim*) do not harbor a *P* element insertion in the *sim* locus.

Embryos homozygous for the 529/8 mutation display severe misrouting of axons in the dorsal and lateral region (Figure 4E). The intersegmental nerve often crosses segment boundaries and extends to adjacent segments. This phenotype is most frequently (but not only) seen in abdominal segments A4 and A5. In addition, dorsal clusters of neighboring segments occasionally connect to each other and some loss of dorsal PNS neurons occurs. The 529/8 strain harbors a *P*-element insertion at cytological position 83A. The 529/8 chromosome could not be reverted to wild type. It is likely that the 529/8 mutation defines a novel gene, since it complemented other mutations from the same phenotypic class (see Table 6).

Mutations that cause abnormal neuronal morphology: This phenotypic class includes mutations in four complementation groups that alter the morphology of neuronal cells and axonal bundles, or alter the appearance of the PNS clusters when visualized with MAb 22C10 (Table 7).

The 1441/14 mutation defines a novel gene named *melted* (*melt*) because it causes a phenotype similar to the *fondue* mutation (KANIA *et al.* 1995). Embryos homozygous for *melt* display abnormal morphology of all PNS neurons, which is combined with a mild loss of neurons mainly in the ventral region. Neuronal cell bodies appear somewhat larger and more elongated than normal

and the cells seem to melt together in the cluster (arrow in Figure 5A). The 1441/14 chromosome carries a revertible insertion at cytological band 65E.

The 238/3, 708/6 and 1351/8 chromosomes harbor mutations in the *hearty* (*hrt*) gene. This gene was identified previously by a single EMS-induced mutation, *hrt*^{H246} (SALZBERG *et al.* 1994). The axonal bundles in the PNS of embryos homozygous for mutations in the *hrt* gene appear defasciculated and thicker than those of normal embryos (compare Figure 5, C and D). All *hrt* alleles lead to ectopic 22C10 immunoreactivity in the heart (Figure 5E), which is similar to the pattern of 22C10 staining in the heart of embryos homozygous for mutations in the *ttk* gene (data not shown). In addition, heart morphogenesis is not completed properly in embryos homozygous for *hrt* mutations, leaving the posterior region of the dorsal vessel open (arrow in Figure 5E).

The 1437/2 insertional chromosome carries a mutation allelic to *sticky ch1* (*stich1*) identified earlier (SALZBERG *et al.* 1994). Embryos homozygous for the 1437/2 mutation exhibit a loss of neurons and a change in the morphology of the remaining neurons. The loss of neurons is evident mainly in the ventral and lateral PNS clusters. The lateral chordotonal neurons stain very faintly with MAb 22C10 or do not stain at all (Figure 5B). In addition, these neurons display abnormal cell shape and appear somewhat larger. The 1437/2 insertion was mapped to cytological band 86A. This mutation could not be reverted to wild type and no deficiencies are available in the 86A region. Since we did not succeed in mapping of *stich1* by meiotic recombination (SALZBERG *et al.* 1994), we cannot conclude whether the *P{1437/2}* is responsible for the observed phenotype.

The 573/2 insertional chromosome carries a mutation that causes defects in both PNS and CNS (data not shown). In 573/2 mutant embryos, PNS neurons stain faintly with 22C10 when compared to wild type and they appear smaller than normal. The CNS does not retract fully and the axonal scaffold displays a less regular structure than normal. The 573/2 chromosome carries a single nonrevertible insertion that was mapped by *in situ* hybridization to cytological region 100C. Since there are no deficiencies available uncovering the entire 100C region, we could not conclude whether this insertion is responsible for the phenotype. However, results of complementation tests (see Table 7) demonstrate that the 573/2 mutation is not allelic to any of the mutations from the same phenotypic class. This may indicate that the 573/2 mutation defines a novel gene required for proper PNS development.

Analysis of *lacZ* expression: Embryos from each insertional strain were stained with antibodies against β -galactosidase (β -gal) to reveal the β -gal expression pattern caused by the *P*-element insertions. The observed expression patterns are listed in Tables 3–7. More than 20% of strains (26 out of 108 strains examined) exhib-

FIGURE 5.—Mutations affecting neuronal morphology. (A) *melted* (allele 1441/14). The cell bodies of the lateral chordotonal neurons are somewhat larger and more elongated than normal and seem to be associated tightly in the cluster (arrow). (B) *stich1* (allele 1437/2). Note the loss of neurons and morphological abnormalities of the remaining neurons. The lateral chordotonal neurons (arrow) have abnormal cell shape and stain very faintly with MAb 22C10. (C and D). Five abdominal segments of a wild-type (C) and *hearty* (allele 708/6) (D) embryos. Note that axonal bundles in *hrt* embryos appear thicker (arrows). (E) Ectopic 22C10 immunoreactivity in the dorsal vessel of the *hrt* embryo (allele 708/6). Note that the heart morphogenesis is not complete and the posterior region of the heart remains open (arrow).

ited no detectable β -gal expression in the embryo. Another 31% of the examined strains (34/108) expressed the β -gal marker ubiquitously in the embryo. Thus, only ~45% of strains exhibited a tissue-specific expression of the β -gal marker.

Can the phenotypes associated with insertional mutations in the newly identified genes be correlated with their β -gal expression patterns? Our data suggest that such correlation is not obvious in most cases. For example, all *P* elements inserted in the *emc* gene confer ubiquitous *lacZ* expression, whereas *P* elements inserted in *aay* and *stich1* do not confer a visible *lacZ* expression at all. However, insertions in three complementation groups induce β -gal expression patterns that can be correlated with the observed phenotypes. Their patterns are briefly described below.

One of the insertions in *bon*, *P*{241/8}, confers β -gal expression to a subset of CNS neurons and a small subset of PNS cells. In addition, 241/8 embryos display a segmentally repeated staining pattern in unidentified cells some of which are located approximately where the ectopic neurons appear. However, double labeling experiments are required to establish whether the β -gal-expressing cells are the ones transformed into neurons. The other two *bon* insertional alleles, 249/12 and 487/6, display a faint generalized ("all over") staining and staining in the amnioserosa, respectively.

Two insertions in the *hrt* gene, 238/3 and 1351/8, lead to the anti- β -gal staining pattern that correlates nicely with the *hrt* phenotype. These strains express β -gal in the PNS and more strongly in two rows of ectodermal cells along the dorsal midline that stretch from

head to tail. In homozygous 238/3 or 1351/8 embryos anti- β -gal staining is observed also in the heart.

Another mutant which expresses β -gal in the PNS is the *dmt* allele 481/3. This strain exhibits a faint β -gal staining in the CNS and subsets of cells in the PNS, including an early staining (stage 11) of some sensory organ precursor cells.

DISCUSSION

A novel collection of *P* elements on the third chromosome: In this work we describe the isolation of mutations affecting the morphology of the PNS from a novel collection of 2460 *P*-element enhancer detectors that map to the third chromosome (DEÁK *et al.* 1997). Since we carried out a near saturation chemical mutagenesis screen on the same chromosome using the same molecular marker (MAb 22C10) (SALZBERG *et al.* 1994), it is possible to compare the number and types of mutations that were isolated in these two studies (Table 8). The following observations demonstrate that this novel collection of *P* elements is quite valuable.

First, we have isolated mutations in 19 previously identified genes. These include 10 previously cloned genes (*Dl*, *neur*, *pros*, *etc.*) and nine genes that were previously identified in the chemical mutagenesis screens but have not yet been cloned. Five of those (*hrt*, *dmt*, *dtt*, *pav*, and *pbl*) can now be cloned, since the *P*-element insertions map to the right cytological band, are revertible, fail to complement the EMS-induced alleles, and fail to complement deficiencies when available. In addition, novel mutations in four other genes (*misn*, *sens*, *spdo*, and *stich1*) may prove useful to clone the genes as they are likely to be associated with small chromosomal rearrangements in the corresponding loci, as demonstrated for *spdo*.

Second, we estimate that in 78% of the saved strains (79 out of 101 tested for reversion) the *P*-element insertions cause the mutant PNS phenotype, even though some strains (15 out of 101 tested for reversion) carry two insertions or carry single insertions that are not revertible. These estimates are very similar to those found for the collection of lethal *P* elements on the second chromosome (TÖRÖK *et al.* 1993; KANIA *et al.* 1995). This suggests that this collection of *P* elements on the third chromosome is a valuable source to screen for lethal mutations affecting other developmental and morphogenetic processes in the embryo.

Mutations isolated in the screen: This screen allowed us to identify mutations in 26 genes, including seven novel genes. On the basis of few assumptions (randomness of hits and allele frequency), we estimated (SALZBERG *et al.* 1994) that there are ~22 genes required for PNS development on the third chromosome. However, the results of this screen demonstrate that at least 32 genes on the third chromosome are required for PNS development and can be identified by screen-

ing with MAb 22C10 (see Table 8). This discrepancy stems probably from the different nature of the two screens and different types of mutations isolated in them. When screening the *P*-element collection, it became immediately obvious that the phenotypic background (due to second-site mutations) is much lower in this screen than in the chemical mutagenesis screen. We found many fewer morphologically defective embryos in the insertional mutant strains than in those generated by a chemical mutagen. The low frequency of morphological defects among embryos collected from the insertional mutant strains greatly facilitates the identification of mutant embryos with subtle phenotypes in the PNS. This allowed the isolation of genes, such as *trn*, that were not isolated in the previous EMS screen. In addition, we were able to identify novel hypomorphic alleles of known genes (for example, *emc*, *pbl*, and *neur*). The subtle tissue-specific phenotypes associated with these novel alleles have not been described before and, therefore, could be a valuable source of information about the function and/or tissue-specific transcriptional regulation of the corresponding genes. Finally, it is possible that mutations in several genes that are associated with easily detectable phenotypes (for example, *bon*) were not identified in a chemical mutagenesis screen because these loci may be less susceptible to EMS-induced point mutations.

Despite the low genetic load, the ease of carrying out this type of screen compared to an EMS screen, the ability to map mutations cytogenetically, and the observation that >70% of the mutations underlie the observed defects, *P* element screens only complement and, certainly, do not replace chemical mutagenesis screens. Mutations identifying many essential genes could be missed by focusing only on *P*-element screens. The percentage of genes that are not identified in *P*-element screens may be quite significant as demonstrated in Table 8 [32% (6/19) of the genes identified in the EMS screen were not identified in the present *P*-element screen]. In addition, *P*-element mutations do not replace point mutations that may allow isolation of many different types of alleles (MULLER 1932), often permitting a structure-function analysis. Finally, *P* elements are usually inserted in the 5' regulatory regions of the genes resulting in partial loss of function mutations. Therefore, other types of alleles (amorphs, neomorphs, antimorphs, temperature-sensitive mutations) that could be more informative about the functions of a gene are more easily isolated in a chemical mutagenesis screen.

Lethal enhancer detectors are poor reporters of tissue-specific patterns of gene expression: KANIA *et al.* (1995) observed previously in a similar screen on the second chromosome, that *lacZ* expression is not a good indicator to identify genes that affect the development of the PNS. We found that only ~21% (23/108) of the strains saved in the present screen exhibited *lacZ*

TABLE 8

Mutations affecting PNS development identified in the present screen and in a previous EMS screen

Complementation group (mapping position)	No. of alleles in present screen	No. of alleles in EMS screen	References
<i>extra macrochaetae</i> (61D1-2)	4	—	BOTAS <i>et al.</i> (1982) GARRELL and MODOLELL (1990)
<i>veinlet (rhomboid)</i> (62A)	—	1	ROBERTS and EVANS-ROBERTS (1979) JÜRGENS <i>et al.</i> (1984) BIER <i>et al.</i> (1990)
<i>pavarotti</i> (64B2-14))	2	6	SALZBERG <i>et al.</i> (1994)
<i>u-turn</i> (65A2; 65E1)	—	3	SALZBERG <i>et al.</i> (1994)
<i>melted</i> (65E)	1	—	This study
<i>pebble</i> (66B1-2)	2	—	JÜRGENS <i>et al.</i> (1984) HIME and SAINT (1992) LEHNER (1992)
<i>astray</i> (67B-10)	1	—	This study
<i>cyclin A</i> (68E1-2)	13	2	LEHNER and O'FARRELL (1989)
<i>tartan</i> (70A1-2)	2	—	CHANG <i>et al.</i> (1993)
<i>missensed</i> (84D8-12)	1	5	SALZBERG <i>et al.</i> (1994)
<i>hearty</i> (84F1-2)	3	1	SALZBERG <i>et al.</i> (1994)
<i>depleted</i> (85A1-7)	—	2	SALZBERG <i>et al.</i> (1994)
<i>neuralized</i> (85C4-5)	4	4	BOULIANNE <i>et al.</i> (1991)
<i>potpourri</i> (85E)	1	—	This study
<i>dalmatian</i> (85E5; 85F1)	1	4	SALZBERG <i>et al.</i> (1994)
<i>dorsototals</i> (86C1-4)	2	2	SALZBERG <i>et al.</i> (1994)
<i>prospero</i> (86E3-20)	3	6	DOE <i>et al.</i> (1991) VAESSIN <i>et al.</i> (1991)
<i>single-minded</i> (87E1)	13	—	HILLIKER <i>et al.</i> (1980) THOMAS <i>et al.</i> (1988)
<i>Delta</i> (92A2)	9	4	ALTON <i>et al.</i> (1988)
<i>bonus</i> (92E8-14)	3	—	This study
<i>pointed</i> (94F1-2)	4	—	JÜRGENS <i>et al.</i> (1984) KLAMBT (1993)
<i>string</i> (99A5-6)	15	—	JÜRGENS <i>et al.</i> (1984) EDGAR and O'FARRELL (1989)
<i>sanpodo</i> (99F)	2	2	SALZBERG <i>et al.</i> (1994)
<i>tramtrack</i> (100D3)	18	2	HARRISON and TRAVERS (1990)
<i>senseless</i> (3L)	1	3	SALZBERG <i>et al.</i> (1994)
<i>jaywalker</i> (3R)	—	2	SALZBERG <i>et al.</i> (1994)
<i>sticky ch1</i> (?)	1	1	SALZBERG <i>et al.</i> (1994)
<i>sticky ch2</i> (?)	—	1	SALZBERG <i>et al.</i> (1994)
<i>wanderer</i> (?)	—	2	SALZBERG <i>et al.</i> (1994)
529.08 (?)	1	—	This study
573.02 (?)	1	—	This study
1368.02 (?)	1	—	This study

expression in the PNS. Since it was previously reported that ~20% of all enhancer detectors express *lacZ* in the PNS (BELLEN *et al.* 1989; BIER *et al.* 1989), we conclude that lethal insertional mutations selected on the basis of phenotype in the PNS are not enriched for enhancer detectors that report expression in the PNS.

Genetic dissection of PNS development: In summary, we present evidence that there are at least 32 genes on the third chromosome required for the development of the embryonic PNS. This includes genes that were identified in a previous chemical mutagenesis screen (SALZBERG *et al.* 1994) and genes identified in this study. The results of this screen together with our second chromosome *P*-element screen (KANIA *et al.*

1995) bring the total number of genes, identified as being essential and required for PNS development, to 66. This includes 17 previously characterized genes and 49 novel genes. It is likely that the majority of these loci on the second and third chromosomes (which correspond to ~80% of the *Drosophila* genome) are now identified. This provides an excellent starting point for the molecular dissection of genetic pathways operating during PNS development. Cloning of these genes, their functional analyses, and a detailed phenotypic characterization of the mutant phenotypes will be required to get insights into their molecular nature and their developmental roles.

We thank COREY S. GOODMAN for generous supply of MA6 22C10.

We thank KATHY A. MATTHEWS and the Bloomington Stock Center, the Umeå Stock Center, STEVE CREWS, WAYNE A. JOHNSON, ALLEN S. LAUGHON, CHRISTIANE NÜSSLEIN-VOLHARD, and ALLEN SHEARN for sending us fly strains, and BASSEM HASSAN, MARK WU, and GRAEME MARDON for comments on the manuscript. We would like also to thank STEVE CREWS for sharing with us unpublished data. We acknowledge the following support: Medical Research Council (MRC) grant to D.M.G., R. SAUNDERS, and M. ASHBURNER, European Union Cooperation with Central and Eastern Europe Countries (PECO) and Biotechnology grants coordinated by D.M.G., and Hungarian Scientific Research Fund (OTKA) grant from the Hungarian Academy of Sciences to P.M. and P.D. A.S. was a postdoctoral researcher of the Howard Hughes Medical Institute. A portion of this work was done in Israel and was supported by a grant from the Technion Faculty of Medicine Foundation to A.S. H.J.B. is an Associate Investigator of the Howard Hughes Medical Institute.

LITERATURE CITED

- ALTON, A. K., K. FECHTEL, A. L. TERRY, S. B. MEIKLE and M. A. MUSKAVITCH, 1988 Cytogenetic definition and morphogenetic analysis of *Delta*, a gene affecting neurogenesis in *Drosophila melanogaster*. *Genetics* 118: 235–245.
- ANDERSON, M. G., G. L. PERKINS, P. CHITTICK, R. J. SHRIGLEY and W. A. JOHNSON, 1995 *drifter*, a *Drosophila* POU-domain transcription factor, is required for correct differentiation and migration of tracheal cells and midline glia. *Genes Dev.* 9: 123–137.
- ASHBURNER, M., 1989 *Drosophila. A Laboratory Handbook*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- BELLEN, H. J., S. KOOYER, D. D'EVELYN and J. PEARLMAN, 1992 The *Drosophila* Couch potato protein is expressed in nuclei of peripheral neuronal precursors and shows homology to RNA-binding proteins. *Genes Dev.* 6: 2125–2136.
- BELLEN, H. J., C. J. O'KANE, C. WILSON, U. GROSSNIKLUS, R. K. PEARSON *et al.*, 1989 *Pelement*-mediated enhancer detection: a versatile method to study development in *Drosophila*. *Genes Dev.* 3: 1288–1300.
- BHAT, M., A. V. PHILP, D. M. GLOVER and H. J. BELLEN, 1996 Chromatid segregation at anaphase requires the *barren* product, a novel chromosome-associated protein that interacts with topoisomerase II. *Cell* 87: 1103–1114.
- BIER, E., L. Y. JAN and Y. N. JAN, 1990 *rhomboid*, a gene required for dorsoventral axis establishment and peripheral nervous system development in *Drosophila melanogaster*. *Genes Dev.* 4: 190–203.
- BIER, E., H. VAESSIN, S. SHEPHERD, K. LEE, K. MCCALL *et al.*, 1989 Searching for pattern and mutation in the *Drosophila* genome with a P-lacZ vector. *Genes Dev.* : 1273–1287.
- BIGGS, J., N. TRIPOULAS, E. HERSPERGER, C. DEAROLF and A. SHEARN, 1988 Analysis of the lethal interaction between the *prune* and *Killer of prune* mutations of *Drosophila*. *Genes Dev.* 2: 1333–1343.
- BODMER, R., and Y. N. JAN, 1987 Morphological differentiation of the embryonic peripheral neurons in *Drosophila*. *Roux's Arch. Dev. Biol.* 196: 69–77.
- BOTAS, J., J. M. DEL PRADO and A. GARCIA-BELLIDO, 1982 Gene-dose titration analysis in the search of trans-regulatory genes in *Drosophila*. *EMBO J.* 1: 307–310.
- BOULIANNE, G. L., A. DE LA CONCHA, J. A. CAMPOS-ORTEGA, L. Y. JAN and Y. N. JAN, 1991 The *Drosophila* neurogenic gene *neuralized* encodes a novel protein and is expressed in precursors of larval and adult neurons. *EMBO J.* 10: 2975–2983.
- CHANG, Z., B. D. PRICE, S. BOCKHEIM, M. J. BOEDIGHEIMER, R. SMITH *et al.*, 1993 Molecular and genetic characterization of the *Drosophila* *tartan* gene. *Dev. Biol.* 160: 315–332.
- DAMBLY-CHAUDIERE, C., and A. GHYSEN, 1986 The sense organs in the *Drosophila* larva and their relation to the embryonic pattern of sensory neurons. *Roux's Arch. Dev. Biol.* 195: 222–228.
- DEAK, P., M. M. OMAR, R. D. C. SAUNDERS, M. PÁL, O. KOMONYI *et al.*, 1997 *Pelement* insertion alleles of essential genes on the third chromosome of *Drosophila melanogaster*: Correlation of physical and cytogenetic maps in chromosomal region 86E–87F. *Genetics* 147: 1697–1722.
- DOE, C. Q., Q. CHU-LAGRAFF, D. M. WRIGHT and M. P. SCOTT, 1991 The *prospero* gene specifies cell fates in the *Drosophila* central nervous system. *Cell* 65: 451–464.
- EDGAR, B. A., and P. H. O'FARRELL, 1989 Genetic control of cell division patterns in the *Drosophila* embryo. *Cell* 57: 177–187.
- EISEN, J. S., 1996 Zebrafish make a big splash. *Cell* 87: 969–977.
- ELLIS, H. M., D. R. SPANN and J. W. POSAKONY, 1990 *extramacrochaetae*, a negative regulator of sensory organ development in *Drosophila*, defines a new class of helix-loop-helix proteins. *Cell* 61: 27–38.
- FLYBASE, 1997 The *Drosophila* Genome Database. <http://cbbbridges.harvard.edu:7081/>
- FUJITA, S. C., S. L. ZIPURSKY, S. BENZER, A. FERRUS and S. L. SHOTWELL, 1982 Monoclonal antibodies against the *Drosophila* nervous system. *Proc. Natl. Acad. Sci. USA* 79: 7929–7933.
- GARRELL, J., and J. MODOLELL, 1990 The *Drosophila* *extramacrochaetae* locus, an antagonist of proneural genes that, like these genes, encodes a helix-loop-helix protein. *Cell* 61: 39–48.
- GHYSEN, A., C. DAMBLY-CHAUDIERE, E. ACEVES, L. Y. JAN and Y. N. JAN, 1986 Sensory neurons and peripheral pathways in *Drosophila* embryos. *Roux's Arch. Dev. Biol.* 195: 281–289.
- GOODMAN, C. S., M. J. BASTIANI, C. Q. DOE, S. DU LAC, S. L. HELFAND *et al.*, 1984 Cell recognition during neuronal development. *Science* 225: 1271–1279.
- HARRISON, S. D., and A. A. TRAVERS, 1990 The *tramtrack* gene encodes a *Drosophila* finger protein that interacts with the *ftz* transcriptional regulatory region and shows a novel embryonic expression pattern. *EMBO J.* 9: 207–216.
- HARTENSTEIN, V., 1988 Development of *Drosophila* larval sensory organs: spatiotemporal pattern of sensory neurons, peripheral axonal pathways and sensilla differentiation. *Development* 102: 869–886.
- HAY, B. A., L. Y. JAN and Y. N. JAN, 1988 A protein component of *Drosophila* polar granules is encoded by *vasa* and has extensive sequence similarity to ATP-dependent helicases. *Cell* 55: 577–587.
- HILLIKER, A. J., S. H. CLARK, A. CHOVNICK and W. M. GELBART, 1980 Cytogenetic analysis of the chromosomal region immediately adjacent to the *rosy* locus in *Drosophila melanogaster*. *Genetics* 95: 95–110.
- HIME, G., and R. SAINT, 1992 Zygotic expression of the *pebble* locus is required for cytokinesis during the postblastoderm mitoses of *Drosophila*. *Development* 114: 165–171.
- JÜRGENS, G., E. WIESCHAU, C. NÜSSLEIN-VOLHARD and H. KLUDING, 1984 Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. II. Zygotic loci on the third chromosome. *Roux's Arch. Dev. Biol.* 193: 283–295.
- KANIA, A., A. SALZBERG, M. BHAT, D. D'EVELYN, Y. HE *et al.*, 1995 *Pelement* mutations affecting embryonic peripheral nervous system development in *Drosophila melanogaster*. *Genetics* 139: 1663–1678.
- KARPEN, G. H., and A. C. SPRADLING, 1992 Analysis of subtelomeric heterochromatin in the *Drosophila* minichromosome Dp1187 by single *Pelement* insertional mutagenesis. *Genetics* 132: 737–753.
- KLAMBT, C., 1993 The *Drosophila* gene *pointed* encodes two ETS-like proteins which are involved in the development of the midline glial cells. *Development* 117: 163–176.
- KOLODZIEJ, P. A., L. Y. JAN and Y. N. JAN, 1995 Mutations that affect the length, fasciculation, or ventral orientation of specific sensory axons in the *Drosophila* embryo. *Neuron* 15: 273–286.
- LANGER-SAFER, P. R., M. LEVINE and D. C. WARD, 1982 Immunological method for mapping genes on *Drosophila* polytene chromosomes. *Proc. Natl. Acad. Sci. USA* 79: 4381–4385.
- LEHMANN, R., F. JIMENEZ, U. DIETRICH and J. A. CAMPOS-ORTEGA, 1983 On the type and development of mutants of early neurogenesis in *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* 192: 62–74.
- LEHNER, C. F., 1992 The *pebble* gene is required for cytokinesis in *Drosophila*. *J. Cell Sci.* 103: 1021–1030.
- LEHNER, C. F., and P. H. O'FARRELL, 1989 Expression and function of *Drosophila* cyclin A during embryonic cell cycle progression. *Cell* 56: 957–968.
- LINDSLEY, D., and G. ZIMM (Editors), 1992 *The Genome of Drosophila melanogaster*. Academic Press, San Diego.
- MANSFIELD, E. A., E. HERSPERGER, J. BIGGS and A. SHEARN, 1994 Genetic and molecular analysis of *hyperplastic discs*, a gene whose

- product is required for regulation of cell proliferation in *Drosophila melanogaster* imaginal discs and germ cells. *Dev. Biol.* **165**: 507–526.
- MARTIN, K. A., B. POECK, H. ROTH, A. J. EBENS, L. C. BALLARD *et al.*, 1995 Mutations disrupting neuronal connectivity in the *Drosophila* visual system. *Neuron* **14**: 229–240.
- MULLER, H. J., 1932 Further studies on the nature and causes of gene mutations. *Proc. Sixth Int. Congr. Genetics* **1**: 213–255.
- NÜSSLEIN-VOLHARD, C., and E. WIESCHAUS, 1980 Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**: 795–801.
- ROBERTS, D. B., and S. EVANS-ROBERTS, 1979 The genetic and cyto-genetic localization of the three structural genes coding for the major protein of *Drosophila* larval septum. *Genetics* **93**: 663–679.
- SALZBERG, A., and H. J. BELLEN, 1996 Invertebrate versus vertebrate neurogenesis: variations on the same theme? *Dev. Genet.* **18**: 1–10.
- SALZBERG, A., D. D'EVELYN, K. L. SCHULZE, J.-K. LEE, D. STRUMPF *et al.*, 1994 Mutations affecting the pattern of the PNS in *Drosophila* reveal novel aspects of neuronal development. *Neuron* **13**: 269–287.
- SEEGER, M., G. TEAR, D. FERRES-MARCO and C. S. GOODMAN, 1993 Mutations affecting growth cone guidance in *Drosophila*: genes necessary for guidance toward or away from the midline. *Neuron* **10**: 409–426.
- THOMAS, J. B., S. T. CREWS and C. S. GOODMAN, 1988 Molecular genetics of the *single-minded* locus: a gene involved in the development of the *Drosophila* nervous system. *Cell* **52**: 133–141.
- TÖRÖK, T., G. TICK, M. ALVARADO and I. KISS, 1993 P-lacW insertional mutagenesis on the second chromosome of *Drosophila melanogaster*: isolation of lethals with different overgrowth phenotypes. *Genetics* **135**: 71–80.
- UEMURA, T., S. SHEPHERD, L. ACKERMAN, L. Y. JAN and Y. N. JAN, 1989 *numb*, a gene required in determination of cell fate during sensory organ formation in *Drosophila* embryos. *Cell* **5**: 349–360.
- VAESSIN, H., E. GRELL, E. WOLFF, E. BIER, L. Y. JAN *et al.*, 1991 *prospero* is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axonal outgrowth in *Drosophila*. *Cell* **67**: 941–953.
- VAN VACTOR, D., H. SINK, D. FAMBROUGH, R. TSOO and C. S. GOODMAN, 1993 Genes that control neuromuscular specificity in *Drosophila*. *Cell* **73**: 1137–1153.
- WILSON, C., R. K. PEARSON, H. J. BELLEN, C. J. O'KANE, U. GROSS-NIKLAUS *et al.*, 1989 P-element-mediated enhancer detection: an efficient method for isolating and characterizing developmentally regulated genes in *Drosophila*. *Genes Dev.* **3**: 1301–1313.
- ZIPURSKY, S. L., T. R. VENKATESH, D. B. TELOW and S. BENZER, 1984 Neuronal development in the *Drosophila* retina: monoclonal antibodies as molecular probes. *Cell* **36**: 15–26.

Communicating editor: R. S. HAWLEY