A Gain-of-Function Screen for Genes That Affect the Development of the Drosophila Adult External Sensory Organ

Salim Abdelilah-Seyfried,* Yee-Ming Chan,* Chaoyang Zeng,† Nicholas J. Justice,* Susan Younger-Shepherd,* Linda E. Sharp,* Sandra Barbel,* Sarah A. Meadows,* Lily Yeh Jan* and Yuh Nung Jan*

**Howard Hughes Medical Institute, Departments of Physiology and Biochemistry, University of California, San Francisco, California 94143-0725 and †Department of Biological Sciences, University of Wisconsin, Milwaukee, Wisconsin 53201*

Manuscript received November 16, 1999
Accepted for publication February 24, 2000

ABSTRACT

The Drosophila adult external sensory organ, comprising a neuron and its support cells, is derived from a single precursor cell via several asymmetric cell divisions. To identify molecules involved in sensory organ development, we conducted a tissue-specific gain-of-function screen. We screened 2293 independent *P*-element lines established by P. Rørth and identified 105 lines, carrying insertions at 78 distinct loci, that produced misexpression phenotypes with changes in number, fate, or morphology of cells of the adult external sensory organ. On the basis of the gain-of-function phenotypes of both internal and external support cells, we subdivided the candidate lines into three classes. The first class (52 lines, 40 loci) exhibits partial or complete loss of adult external sensory organs. The second class (38 lines, 28 loci) is associated with increased numbers of entire adult external sensory organs or subsets of sensory organ cells. The third class (15 lines, 10 loci) results in potential cell fate transformations. Genetic and molecular characterization of these candidate lines reveals that some loci identified in this screen correspond to genes known to function in the formation of the peripheral nervous system, such as *big brain*, *extra macrochaetae*, and *numb*. Also emerging from the screen are a large group of previously uncharacterized genes and several known genes that have not yet been implicated in the development of the peripheral nervous system.

THE development of the Drosophila adult external sensory (es) organ, a mechanosensory bristle, involves lateral inhibition and asymmetric division, two mechanisms that underlie numerous developmental processes (Posakony 1994; Jan and Jan 1995; Campos-Ortega 1996). First, a single sensory organ precursor (SOP) cell is selected from a proneural cluster, a group of cells that are competent to become neuronal precursors, via lateral inhibition. Genes within the *achaete-scute* complex (AS-C) and the *daughterless* (*da*) gene are required to confer neuronal potential to these cells (Ghysen and Dambly-Chaudiere 1989). After the SOP cell is singled out, it divides asymmetrically to produce two different secondary precursor cells, IIA and IIB. IIA gives rise to two external cells: one shaft cell (trichogen) and one socket cell (tormogen). IIB gives rise to the internal cells: one neuron, one sheath cell, and, for at least one class of es organs, an additional glial cell (Hartenstein and Posakony 1989; Gho *et al.* 1999).

The Notch (N) signaling pathway mediates the cell-cell interactions that occur during lateral inhibition. The transmembrane protein Notch is a receptor and its principal ligand during lateral inhibition is Delta (reviewed in Artavanis-Tsakonas *et al.* 1999). Within the proneural cluster, Notch signaling is mediated through the transcription factor Suppressor of Hairless [Su(H)] and results in the activation of target genes at the *Enhancer of split* [*E*(*spl*)] locus (Schweisguth and Posakony 1992; Fortini and Artavanis-Tsakonas 1994; Bailey and Posakony 1995; Jarriault *et al.* 1995; Lecourtois and Schweisguth 1995). Hairless (H) is believed to act as an antagonist of Notch through physical interaction with Su(H) (Brou *et al.* 1994; Bang *et al.* 1995).

Both Notch-mediated cell-cell interactions and asymmetric segregation of the cell-intrinsic determinant Numb operate during divisions of the SOP lineage (Posakony 1994; Rhyu *et al.* 1994). During divisions of the SOP cell and its progeny, Numb protein is unequally segregated to one of the two resulting daughter cells. In that cell, Numb inhibits the activity of N, which receives signals from two redundant ligands, Delta and Serrate (Rhyu *et al.* 1994; Frise *et al.* 1996; Guo *et al.* 1996; Zeng *et al.* 1998a). The pathways downstream of Notch are different for the asymmetric divisions of IIA and IIB cell lineages. Su(H) acts as a transducer of Notch signaling only within IIA and her daughter cells; the

Corresponding author: Yuh Nung Jan, Howard Hughes Medical Institute, Departments of Physiology and Biochemistry, Third and Parnassus Ave., University of California, San Francisco, CA 94143-0725. E-mail: ynjan@itsa.ucsf.edu

downstream molecules that mediate Notch signaling in the IIB cell lineage are unknown (Wang *et al.* 1997). A potential downstream target of Su(H) in IIA is *tramtrack* (*ttk*), a gene that does not appear to have a function during lateral inhibition (Guo *et al.* 1995, 1996). Another gene that affects lineage events and might be a component of the Notch signaling pathway is *sanpodo* (Dye *et al.* 1998; Skeath and Doe 1998).

The Notch signaling cascade in the SOP cell lineage differs from that involved in lateral inhibition. Additional components involved in N signaling during asymmetric divisions of the SOP lineage remain to be identified (*e.g.*, ones that are specific for the IIB cell lineage).

Many genes with a function in lateral inhibition or asymmetric divisions of the adult es organ lineage, such as *N*, *Delta*, *numb*, *prospero* (*pros*), and *ttk*, were initially identified due to embryonic loss-of-function (lof) phenotypes (Lehmann *et al.* 1981, 1983; Uemura *et al.* 1989; Doe *et al.* 1991; Vaessin *et al.* 1991; Xiong and Montell 1991; Salzberg *et al.* 1994). However, pleiotropy or redundancy of gene function may hamper the identification of other genes important for the formation of the adult es organ. One strategy to identify such genes is to look for gain-of-function (gof) phenotypes.

For this purpose, we screened 2293 independent Drosophila lines with the modular *P*-element-based EP (*e*nhancer/*p*romoter) misexpression element devised by P. Rørth (Rørth 1996; Rørth *et al.* 1998). This misexpression element contains upstream activating sequence (UAS) sites that are recognized by the transcriptional activator Gal4 (Brand and Perrimon 1993). Tissue-specific overexpression of genes that lie near the EP element can be achieved by using a line that expresses Gal4 in specific cells. In cells that both express Gal4 and carry the EP element, Gal4 binds to the UAS sites and causes misexpression of the adjacent gene.

On the basis of overexpression studies with genes previously shown to be involved in adult es organ formation, we expected certain phenotypes from such a gof screen. Overexpression of genes such as *numb*, *ttk*, *Su*(*H*), *H*, and *N* give phenotypes opposite to the respective lof phenotypes (Bang and Posakony 1992; Lieber *et al.* 1993; Rhyu *et al.* 1994; Schweisguth and Posakony 1994; Guo *et al.* 1995; Doherty *et al.* 1997; Wang *et al.* 1997). Overexpression of *N* or its transducer *Su*(*H*) during lateral inhibition results in loss of entire es organs due to suppression of SOP formation. At later stages, during asymmetric division, overexpression of these two genes produces up to four external cells, all socket-like, due to IIB-to-IIA cell and/or shaft-to-socket cell transformations (Lieber *et al.* 1993; Schweisguth and Posakony 1994; Wang *et al.* 1997; Doherty *et al.* 1997; Figure 1). Conversely, misexpression of *H*, which antagonizes Notch signaling, results in increased numbers of SOPs, IIA-to-IIB, and socket-to-shaft transformations (Bang and Posakony 1992).

In our screen, we first identified lines that produced visible misexpression phenotypes in the external cells of the es organ, *i.e.*, the daughters of IIA. Next, we analyzed the effect of misexpression on the sheath cell, a daughter of IIB. Finally, we examined the effect of reducing *N* or *H* function on the gof phenotype. These analyses, combined with preliminary molecular characterizations, have led to the identification of genes previously shown to be important for es organ development, as well as other genes that may be involved in this process.

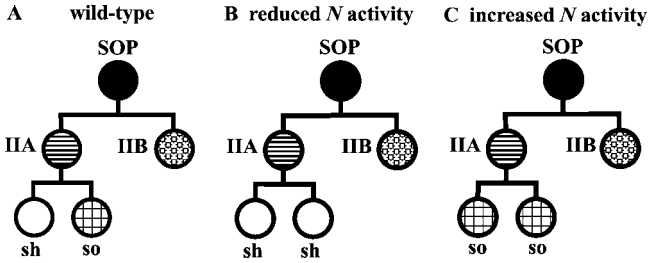

Figure 1.—Potential cell fate transformations in the IIA sublineage. (A) In wild-type, IIA divides asymmetrically to give rise to shaft (sh) and socket (so) cells. (B) Reduction of N signaling results in socket-to-shaft transformations. (C) Conversely, increased N signaling (*e.g.*, in *Hairless* mutants) results in shaft-to-socket transformations. Genetic interactions were assayed on the basis of the effects of the EP misexpression on heterozygous mutant *N* or *H* phenotypes and vice versa.

MATERIALS AND METHODS

Drosophila stocks: The collection of 2293 EP target element lines was a generous gift of P. Rørth through the Berkeley Drosophila Genome Project. For tissue-specific analysis of the misexpression effects, the individual EP lines were crossed to *sca-Gal4*, a *P{Gal4}* line with an insertion at the *scabrous* locus (Nakao and Campos-Ortega 1996). The *sca-Gal4* line expresses Gal4 in SOP and surrounding cells and later in the lineage of the es organ. To test the effects of different levels of expression, parents from initial crosses were serially transferred and progeny from individual crosses were raised at 18, 25, and 29° during larval and pupal stages. The phenotypes at 29° were generally stronger and more penetrant. All subsequent crosses were maintained at 29°.

The *A101* line carries an insertion of $P\{lacZ,ry^{+}\}$ at the *neuralized* locus (Usui and Kimura 1993). It expresses nuclear β-galactosidase in the SOP cell and the es organ lineage. On the notum, lacZ expression is strongest in the nuclei of the two external support cells. The *pros-lacZ* enhancer trap line $P\{lacZ,ry^{+}\}$ expresses β-galactosidase in the sheath cell. We visualized β-galactosidase expression by X-gal staining of pharate adults.

Genetic interactions: To test genetic interactions with *N*, males from individual EP lines were crossed to $w^{a}N^{55E11}/FM6$; *sca-Gal4*/*CyO* females and the phenotypes of $w^{a}N^{55E11}/+$; *sca-Gal4*/+ flies carrying one copy of the EP element were compared to those of *FM6*/+; *sca-Gal4*/+ flies carrying one copy of the EP element and to those of $w^{a}N^{55E11}/+$; *sca-Gal4*/+ flies without the EP element. Most lines that showed a positive interaction were retested using a reciprocal crossing scheme with $w^{a}N^{55E11}/w{\cdot}Y$; *sca-Gal4*/*CyO* males ($w{\cdot}Y$ is a partial duplication of the first chromosome including the *N* locus). Genetic interactions with *H* were tested by crossing males from individual EP lines with *y w*; *sca-Gal4*/*CyO*; $FRT\ H^{E21}/TM3$ females.

Phenotypes of *y w*; *sca-Gal4*/+; *FRT* H^{E21}/+ flies with one copy of the EP element were compared to those of *y w*; *sca-Gal4*/+; *TM3*/+ flies carrying one copy of the EP element and to those of *sca-Gal4*/+; *FRT* H^{E21}/+ flies without the EP element. For most crosses, parents were serially transferred and progeny from individual crosses were maintained at 18, 25, and 29° during larval and pupal stages. This genetic interaction scheme allowed us to evaluate changes of the EP misexpression phenotypes as an enhancement or suppression. In addition, enhancement or suppression of the *H* mutant phenotype was evaluated. Since *N*/+ flies lack a bristle phenotype, only the enhancement of *N* haploinsufficiency could be detected.

Molecular analysis: Genomic sequences flanking the 3′end of the EP misexpression element were isolated by plasmid rescue using *Eco*RI or *Sac*II (Pirotta 1986). Sizes of three independent clones for each plasmid rescue were compared to determine the number of insertions per line. In total, there were 7 lines with two insertions (7/105 = 6.7%). Genomic sequences adjacent to the EP element were sequenced.

Flanking sequences were analyzed by searching the Berkeley *Drosophila* Genome Project (BDGP) and National Center for Biotechnology Information databases. Expressed sequence tags (EST) within a 3-kb distance from EP element insertion sites were tested for sequence similarities using "blastx" searches. Sequenced genomic regions within a 3-kb distance from EP element insertions for which no candidate transcripts had been identified were tested using open reading frame finders. Only significant sequence similarities were reported (see Table 1).

RESULTS

Using the modular misexpression system (Rørth 1996; Rørth *et al.* 1998), we misexpressed genes in the SOP cell and its neighbors and examined the effects on the development of the adult external sensory organ. The *sca-Gal4* line was chosen as driver because it is expressed in clusters of cells surrounding the presumptive macro- and microchaetae on the notum and head (Figure 2). Expression persists in the SOP lineage. All misexpression phenotypes described in this paper are produced by *sca-Gal4* in conjunction with an EP insertion. We then examined the effects of reducing *N* or *H* function on the gof phenotype. The enhancer trap lines *A101* and *pros-lacZ* were used to assist our characterization of misexpression phenotypes. *A101-lacZ* expresses β-galactosidase strongly in the nuclei of the two external support cells, while *pros-lacZ* expresses β-galactosidase specifically in the sheath cell, one of the internal cells.

In total, 4.6% of the lines (105/2293) produced phenotypes affecting the number or fate of outer cells of the es organ. These phenotypes fall into three major classes:

1. class I: loss of external support cells (sockets and shafts)
2. class II: supernumerary es organs or support cells
3. class III: potential cell fate transformations, with increases in one cell type associated with loss of another cell type.

Tables 1 and 2 summarize the molecular, phenotypic, and genetic interaction data presented in this study. Many EP lines resulted in phenotypes with characteristics of more than one class. To simplify the classification, all EP lines with potential lineage transformation phenotypes were grouped into class III independently of other phenotypes. Similarly, among the remaining EP lines, those with phenotypes that include supernumerary es organs or subsets of support cells were grouped into class II independently of other phenotypes. Many lines in all three classes also exhibited an altered morphology of shaft or socket cells.

Loss of external cells: We identified 52 lines representing 40 loci that produced loss of some or all of the external and internal support cells. Loss of both external and internal support cells could arise from loss of the entire es organ. Alternatively, the support cells could have been transformed into neurons. Genes responsible for such phenotypes could interfere with lateral inhibition and function in lineage decisions, prevent cell cycle progression, or result in cell lethality.

This is the largest class of EP lines and includes *P*-element insertions into genes known to have important functions in asymmetric cell division, lateral inhibition, and other aspects of development. For example, misexpression of *extra macrochaetae* (*emc*) by *EP(2)0415* caused a loss of macro- and microchaetae (Figure 3A) that resembles the phenotype of a dominant *emc* mutation (emc^D; Craymer 1980). *emc* acts as a repressor that blocks the activity of *achaete* and *scute* gene function during sensory organ neurogenesis (Ellis *et al.* 1990; Garrell and Modolell 1990; Skeath and Carroll 1991; Van Doren *et al.* 1991) and its misexpression is predicted to block SOP formation.

Another example is the misexpression of *escargot* (*esg*) [by *EP(2)0683*, *EP(2)0684*, *EP(2)2009*, *EP(2)2159*, and *EP(2)2408*], which caused the most severe loss of es organs observed in this screen. In *EP(2)0684* and *EP(2)2009*, there was an almost complete loss of es organs on the notum (Figure 3B). *esg* encodes a zinc finger protein that acts as a repressor of Scute/Daughterless-dependent transcription *in vitro* (Whiteley *et al.* 1992; Fuse *et al.* 1994). It also acts as negative regulator of endoreplication in imaginal tissues (Hayashi *et al.* 1993; Hayashi 1996).

We also identified several genes known to be required for correct cell cycle progression. *dacapo* [*EP(2)2584*] is a cyclin-dependent kinase inhibitor that is required during embryogenesis for a timely exit from the cell cycle (Lane *et al.* 1996; de Nooij *et al.* 1996). Misexpression of *dacapo* produced a loss of external cells of scutellar and dorsocentral macrochaetae (Figure 3C). In some cases, there was a single prospero-positive cell that was no longer accompanied by shaft and socket cells. Another gene, *divisions abnormally delayed* (*dally*), encodes a proteoglycan that is required for normal cell cycle progression (Nakato *et al.* 1995) and might act as coreceptor for Wingless (Lin and Perrimon 1999; Tsuda *et al.* 1999). Misexpression of this gene by *EP(3)3168*

TABLE 1

Summary of phenotypic, molecular, and genetic interaction data

Locus	Map position	EP no.	Insertion site	Misexpression phenotype	Genetic interactions	
					Notch	*Hairless*
				Class I: Loss of external cells		
Elf 1 beta		*EP(2)2298*	Clot 300 −77 bp of LD03616	Loss of single or few scutellar and dorsocentral macrochaetae	—	None
	02B16-18	*EP(X)1232*	Matches AC017852 clot 13174 ∼−3.3 kb of SD09936	Loss of external support cells of few scutellar and dorsocentral macrochaetae without loss of sheath cell, no socket/one shaft	None	ni
	12A8-10	*EP(X)1335*	Matches AC013189	Loss of external support cells of several scutellar and dorsocentral macrochaetae without loss of sheath cell, dot-like shaft morphology	—	—
		EP(X)1508	Matches AC013189		—	None
	13F1-2	*EP(X)1581*	Matches AC019935	Loss of few scutellar macrochaetae, no socket/two shafts	—	None
Drosophila lim-domains only (dlmo)	17C1-2	*EP(X)1306*	−107 bp of HL02308	Loss of several scutellar and dorsocentral macrochaetae	—	—
		EP(X)1383	−143 bp of transcript	Loss of several scutellar and dorsocentral macrochaetae	—	None
		EP(X)1394	−162 bp of HL02308		—	ni
	18F1-2	*EP(X)1344*	Clot 3975 −70 bp of LD37284	Loss or ectopic position of several scutellar and dorsocentral macrochaetae, socket morphology abnormal	ni	ni
Hypothetical protein1	22F1-4	*EP(2)0566a*	Clot 1268	Loss of several scutellar macrochaetae, one socket/no shaft/one sheath cell, dot-like shaft morphology	None	None
	35D1-4	*EP(2)0566b*	−847 bp of SD10914			
	23C4-5	*EP(2)0383*	Matches AC019920	Loss of several scutellar macrochaetae, no socket/one shaft and one socket/no shaft/one sheath cell	Yes	Yes
		EP(2)2122	Matches AC019920		—	—
	26A1-2	*EP(2)2299*	Matches DS06477	Severe loss of microchaetae on abdomen and loss of scutellar and dorsocentral macrochaetae, one socket/no shaft/one sheath cell	None	None
		EP(2)0595a	Matches DS06477			
	66D1-2	*EP(2)0595b*	Clot 6267 −358 bp of GM12356		Yes	—
Krueppel-homolog alpha-isoform	26B7-9	*EP(2)2289*	+562 bp of transcript	Severe loss of microchaetae on abdomen and loss of scutellar and dorsocentral macrochaetae, short and thickened shaft morphology	ni	ni
Heterogeneous nuclear RNA-associated protein (hnRNP27C)	27C4-5	*EP(2)0748*	Clot 1057 +10 bp of LD46853	Loss of scutellar macrochaetae, one socket/no shaft, dot-like shaft morphology	—	None
Homology with CFTR	29A1-C1	*EP(2)2146a*	Clot 6022 −46 bp of GM05532	Loss of external support cells of several scutellar macrochaetae without loss of sheath cell, one socket/two shafts, and no socket/one shaft/one sheath cell	None	—

(*continued*)

TABLE 1

(Continued)

Locus	Map position	EP no.	Insertion site	Misexpression phenotype	Genetic interactions	
					Notch	*Hairless*
Ubiquitin-conjugating enzyme *UbcD2*	32A	*EP(2)2146b*	Antisense orientation clot 4090 +47 bp of LD47532			
	30F5-6	*EP(2)0594*	Clot 381 LD22483 within intron 5′ of CDS	Loss of external support cells of scutellar and dorsocentral macrochaetae without loss of sheath cell, no socket/one shaft and one socket/no shaft, dot-like shaft morphology	—	—
elongation factor 4A (*elF-4A*)	34A5-6	*EP(2)2317*	Clot 8989 −259 bp of GH06371	Massive loss or reduction of shaft cell morphology, socket with shaft-like protrusions	ni	ni
	34C4-5	*EP(2)2599*	Matches AC018307	Loss of shaft, dot-like socket morphology	—	—
escargot (*esg*)	35D1-2	*EP(2)0683*	Antisense orientation +692 bp of transcript (CDS at +1007 bp)		None	ni
		EP(2)0684	+564 bp of transcript	Complete loss of all macrochaetae and microchaetae on notum, no sheath cells	—	—
		EP(2)2009	+673 bp of transcript	Complete loss of all macrochaetae and microchaetae on notum	—	ni
		EP(2)2159	+795 bp of transcript		—	—
		EP(2)2408	+649 bp of transcript			
Gliotactin (*Gli*)	35D7	*EP(2)2306*	−41 bp of transcript	Loss of few scutellar or dorsocentral macrochaetae, no socket/one shaft	Yes	Yes
Myocyte enhancing factor (*Mef2*)	46C1-2	*EP(2)2002a*	−163 bp of transcript	No socket/one shaft/one sheath cell	—	—
	57E1-2	*EP(2)2002b*	Matches DS08012			
dacapo (*dap*)	46B1-2	*EP(2)2584*	+16 bp of transcript	Loss of external support cells of scutellar and dorsocentral macrochaetae without loss of sheath cell, no socket/one shaft and one socket/no shaft, dot-like shaft morphology, socket cell abnormal	None	—
longitudinals lacking (*lola*)	47A13	*EP(2)0343*	−45 bp of transcript	Loss of external support cells of few scutellar and dorsocentral macrochaetae without loss of sheath cell, no socket/one shaft	—	—
	47D5-6	*EP(2)2359*	Clot 13362 −89 bp of SD06302	Loss of a few scutellar macrochaetae	—	ni
	57A5-6	*EP(2)2356*	Matches AC01428 clot 5433 −1989 bp of LP02972	Loss of scutellar and dorsocentral macrochaetae, no socket/one shaft, short and thickened shaft with branching tips	None	None
		EP(2)2586	−1855 bp of LP02972		ni	ni
		EP(2)2587	−2225 bp of LP02972			

(*continued*)

TABLE 1

(Continued)

Locus	Map position	EP no.	Insertion site	Misexpression phenotype	Genetic interactions	
					Notch	*Hairless*
inscuteable (*insc*)	57B1-4	*EP(2)2010*	−184 bp of transcript	Loss of several scutellar macrochaetae, no socket/one shaft, one socket/two shafts, and no socket/no shaft/one sheath cell	Yes	Yes
apontic (*apt*)	59F1-2	*EP(2)2373*	−300 bp of transcript	Loss of scutellar and dorsocentral macrochaetae, several additional subalar macrochaetae, dot-like shaft morphology	—	—
	61C3-4	*EP(3)3104*	Clot 5381 −28 bp of GM08528	Loss of external support cells of a few scutellar and dorsocentral macrochaetae without loss of sheath cell, one socket/no shaft and one socket/two shafts, dot-like socket/shaft morphology	—	—
extra macrochaetae (*emc*)	61D1-2	*EP(3)0415*	−102 bp of transcript	Loss of macrochaetae, no socket/one shaft/one sheath cell, rarely shaft with branching tip	—	Yes
		EP(3)3620	−72 bp of transcript		—	—
Homolog of human *zyg*	62A1-2	*EP(3)3673*	Clot 11184 +36 bp of LD02105	Loss of external support cells of scutellar and dorsocentral macrochaetes, sheath cell present or absent, no socket/one shaft, dot-like shaft morphology	Yes	Yes
pebble (*pbl*)	66A17-18	*EP(3)3415*	−10 bp of transcript	Loss of several scutellar macrochaetae, no socket/one shaft and one socket/two shafts, rarely one socket/one shaft/two sheath cells	None	None
division abnormally delayed(*dally*)	66E1-2	*EP(3)3168*	−39 bp of transcript	Loss of one scutellar or dorsocentral macrochaetae, with patched-Gal4: loss of scutellar macrochaetae	—	—
	77B1-9	*EP(3)3121*	Antisense orientation clot 2764 +75 bp of LD12337	Loss of scutellar and dorsocentral macrochaetae, rarely two shafts/one sockets, no shaft/one socket and one shaft/no socket, thin or stumpy shaft	ni	—
	77C1-2	*EP(3)3519*	Clot 11156 −8 bp of LP01915	Loss of macrochaetae, no socket/one shaft, thickened shaft	Yes	Yes
	89B-C	*EP(3)3463*	Matches AC019746 clot 61 −1986 bp of CK00053	Loss of external support cells of scutellar macrochaetae without loss of sheath cell, no socket/one shaft, dot-like shaft morphology, large flat socket cells	None	None
	92E-F	*EP(3)3073*	Clot 1101 +59 bp of LD05530	Loss of external support cells of most scutellar and dorsocentral macrochaetae without loss of sheath cell, one socket/two shafts and one socket/one shaft/two sheath cells	None	—
rab11	93C1-2	*EP(3)3017*	+222 bp of transcript (CDS at +189 bp)	Loss of scutellar and dorsocentral macrochaetae, no socket/one shaft/one sheath cell	None	—
DNA polymerase alpha 180 kD subunit of *E2F*	93E8-9	*EP(3)3707*	Within intron	Short and thin shaft morphology	—	—
gliolectin	93F6-8	*EP(3)3449*	−41 bp of transcript	With 109-68 Gal4: loss of several scutellar macrochaetae, one socket/two shafts, thin shaft morphology	—	—

(*continued*)

TABLE 1

(Continued)

Locus	Map position	EP no.	Insertion site	Misexpression phenotype	Genetic interactions	
					Notch	*Hairless*
Nucleolytic polyadenylate-binding protein homolog gene	95D5-6	*EP(3)3278*	Antisense orientation clot 2669 +598 bp of LP03871	Loss of scutellar and dorsocentral macrochaetae	None	None
	95F11-12	*EP(3)3716*	Clot 3795 +34 bp of GH27517	With patched-Gal4: loss of several scutellar macrochaetae	None	—
	100C6-7	*EP(3)1076*	Clot 3746 within LD21971	Loss or ectopic positioning of a few scutellar and dorsocentral macrochaetae	—	None
fat facets (*faf*)	100D1-3	*EP(3)0381*	−572 bp of transcript	Loss of external support cells of scutellar and dorsocentral macrochaetae without loss of sheath cell, shaft with branched tip, on abdomen dot-like shaft morphology	None	—
				Class II: Supernumerary es organs or support cells		
	01B13-14	*EP(X)1216a*			None	None
CyclinA/cdk2 assoc. p19 (RNA polymerase II elongation factor-like protein)	13C7-8	*EP(X)1216b*	Matches AC020129 −2.9 kb of transcript	Additional macrochaetae, two sockets/two shafts, no socket/one shaft/one sheath cell, small shaft morphology	None	None
Poly(ADPribose) glyco-hydrolase	3E1-2	*EP(X)0351*	Antisense orientation +103 bp of CDS	Additional scutellar macrochaetae, several dorsocentral macrochaetae replaced by microchaetae	—	ni
Mitochondrial carrier homolog	3E6-7	*EP(X)1408*	Antisense orientation clot 12032 within GH17330	Two sockets/two shafts	Yes	Yes
	7B1-2	*EP(X)1523*	Matches AC013057	One or two additional scutellar macrochaetae	None	None
scalloped (*sd*)	13F1-2	*EP(X)1435*	+384 bp of transcript (5′ of CDS)	Additional macrochaetae, loss of a few microchaetae on notum	—	None
	21B1-2	*EP(2)0456*	Clot 7864 −72 bp of LD26519	Additional scutellar macrochaetae, one socket/two shafts, rarely no socket/two shafts	None	None
split ends (*spen*)	21B4-6	*EP(2)2583*	within transcript	Additional scutellar and dorsocentral macrochaetae, no socket/one shaft/sheath cell	Yes	Yes
	21C4-6	*EP(2)2237*	Clot 13365 −57 bp SD06353	Too many or too few scutellar or dorsocentral macrochaetae, frequently two shafts/one socket of macro- and microchaetae, one shaft/no socket and no shaft/one socket, smaller shaft morphology, ocelles absent or greatly reduced	—	—

(*continued*)

TABLE 1

(Continued)

Locus	Map position	EP no.	Insertion site	Misexpression phenotype	Genetic interactions	
					Notch	*Hairless*
yan	22D1-2	*EP(2)0598*	−98 bp of transcript	Too many or too few scutellar macrochaetae, no socket/one shaft-, one socket/two shaft-, and two socket/one shaft phenotypes, on abdomen short shaft morphology	None	None
		EP(2)2500	−88 bp of transcript		—	—
		EP(2)0787	Matches AC014073	Loss of scutellar macrochaetae, few dot-like sockets	—	—
		EP(2)0816	Matches AC014073		None	—
	27F3-5	*EP(2)1221*	Matches AC014073	Loss of scutellar and dorsocentral macrochaetae, no socket/one shaft	None	None
		EP(2)1121a	Matches AC014073	Too many or too few scutellar macrochaetae, no socket/one shaft/one sheath cell and one socket/two shafts	None	None
		EP(2)1121b			None	None
big brain (*bib*)	30F	*EP(2)2278*	−110 bp of transcript	Too many or too few macrochaetae	—	—
	35B1-2	*EP(2)0965*	Matches AC004118	Additional scutellar and dorsocentral macrochaetae	None	—
		EP(2)2039	Matches AC004118		—	ni
Adh distal factor 1 (*Adf1*)	42C1-2	*EP(2)0815*	+8 bp of transcript	With patchedGal4: additional scutellar and dorsocentral macrochaetae	None	ni
	48B1-2	*EP(2)0622*	Clot 14224 −171 bp of LP12012	Additional scutellar and dorsocentral macrochaetae	None	—
	52B1-3	*EP(2)1229*	Matches AC017160	Too many or too few scutellar and dorsocentral macrochaetae, no socket/one shaft, one socket/two shafts, two sockets/one shaft, and two sockets/two shafts, abnormal shaft morphology	Yes	—
		EP(2)2316	Matches AC017160	Too many or too few scutellar and dorsocentral macrochaetae, no socket/one shaft, abnormal shaft morphology	None	—
	53D1-2	*EP(2)0639*	Antisense orientation clot 13052 +480 bp of SD02913	Two sockets/two shafts/one sheath cell, one socket/two shafts and two sockets/one shaft, loss of macrochaetae, abnormal shaft morphology	—	Yes
		EP(2)2148	Antisense orientation clot 13052 +507 of SD02913	Two sockets/two shafts, one socket/two shafts, and no socket/one shaft, loss of scutellar macrochaetae, dot-like shaft morphology	—	Yes
		EP(2)2402	Antisense orientation clot 13052 +508 bp of SD02913		—	—
		EP(2)2437	Antisense orientation clot 13052 +507 bp of SD02913	Two sockets/two shafts and one socket/two shafts, loss of macrochaetae, dot-like shaft morphology	None	—
BTB domain gene	55B5-10	*EP(2)0647*	Clot 1493 −779 bp of LD08847	Two sockets/two shafts, one socket/no shaft, and no socket/one shaft/one sheath cell, thickened and dot-like shaft morphology	Yes	Yes

(*continued*)

TABLE 1

(Continued)

Locus	Map position	EP no.	Insertion site	Misexpression phenotype	Genetic interactions	
					Notch	*Hairless*
bancal (*bl*)	57A3-4	*EP(2)0954*	Within transcript 3′ of CDS	Additional scutellar macrochaetae, loss of several macrochaetae, one socket/no shaft and one socket/two shafts, shaft with branched tip	Yes	Yes
	60E3-6	*EP(2)2251*	Matches AC018245	With patched-Gal4: one additional scutellar macrochaetae	—	—
	61C7-8	*EP(3)3622*	Matches AC017329 clot 800 −2071 bp of GH02109	Tufting and many ectopic scutellar and dorsocentral macrochaetae, four sockets/no shaft, three sockets/three shafts, two sockets/two shafts, and two sockets/one shaft, dot-like shaft morphology	Yes	Yes
	61C7-8	*EP(3)3208*	Matches AC020437	Additional scutellar macrochaetae, no socket/one shaft	None	None
	62A1-2	*EP(3)2409*	Clot 3540 +7 bp of LD16669	Additional scutellar macrochaetae, two sockets/one shaft	Yes	ni
nuclear fallout (*nuf*)	70D3	*EP(3)3324*	−58 bp of LD08622	Additional scutellar macrochaetae, one socket/two shafts	—	—
		EP(3)3339	−482 bp of transcript	Too many or too few scutellar macrochaetae	—	—
	86F6-7	*EP(3)3015a*	Clot 5714 −66 bp of GH21481	Additional scutellar macrochaetae, no socket/one shaft/one sheath cell, no socket/no shaft/one sheath cell, and one socket/no shaft, short and thickened shaft morphology	ni	—
	93C4-5	*EP(3)3015b*			ni	ni
	94A1-2	*EP(3)3474*	Clot 2649 −55 bp of LD31046	Additional scutellar and dorsocentral macrochaetae; no socket/one shaft/one sheath cell	—	—
hedgehog (hh)	94E2	*EP(3)3521*	+215 bp of transcript (CDS at +639 bp)	Additional scutellar macrochaetae, short and thickened shaft morphology	—	—
	96E5-6	*EP(3)3559*	Clot 2122 −152 bp of LD33329	Too many or too few scutellar macrochaetae, short and thickened shaft morphology	—	—
New regulatory subunit of PP2A	97F1-2	*EP(3)3559*	Clot 2122 −9 bp of LD02456	Two sockets/two shafts, two sockets/one shaft, and one socket/two shafts, dot-like socket morphology	None	None
			Class III: Potential cell fate transformations			
	08F8-10	*EP(X)1149*	Matches AC020080	Potential socket-to-shaft and shaft-to-socket transformations, severe loss of scutellar and dorsocentral macrochaetae, short and thickened shaft morphology, sockets with shaft-like protrusions	—	—
		EP(X)1179	Matches AC020080		—	—
	10D4-6	*EP(X)1503*	Clot 241 −114 bp of LD35241	Potential shaft-to-socket transformations, no socket/one shaft/one sheath cell, additional scutellar macrochaetae, forked-like shaft morphology	Yes	Yes

(*continued*)

TABLE 1

(Continued)

Locus	Map position	EP no.	Insertion site	Misexpression phenotype	Genetic interactions	
					Notch	*Hairless*
numb (*nb*)		*EP(2)2542*	−511 bp of transcript	Potential socket-to shaft transformations, one socket/two shafts, one socket/no shaft, and two sockets/ one shaft, rarely one socket/one shaft/two sheath cells or no socket/two shafts, small shaft and socket cells	ni	None
	30B1-12					
		EP(2)2478	Clot 13259 −43 bp of SD04839	Potential IIA-to-IIB or neuron-to-sheath transformations, no socket/one shaft and one socket/two shafts, on wing margin potential socket-to-shaft transformations	—	Yes
	32D1-2					
twine (*twe*)		*EP(2)0613*	−290 bp of transcript	Potential shaft-to-socket transformations, rarely four sockets/no shaft, additional scutellar and dorsocentral macrochaetae, two sockets/two shafts, one socket/two shafts, no socket/one shaft, short and thickened shaft morphology	None	None
	35F1-2					
grapes (*grp*)	36A10	*EP(2)0587*	Within first intron, −460 bp from CDS	Potential shaft-to-socket transformations on abdomen, one socket/no shaft, no socket/one shaft, loss of external support cells of several scutellar and dorsocentral macrochaetae without loss of sheath cell	None	ni
	54C7-8	*EP(2)0386*	Clot 13804 −570 bp of LP07188	Potential shaft-to-socket transformations on abdomen, no socket/one shaft, rarely no socket/two shafts/two sheath cells, abnormal shaft morphology	Yes	Yes
		EP(2)0988	Clot 13804 −574 bp of LP07188	Potential shaft-to-socket transformations on abdomen, loss of several scutellar and dorsocentral macrochaetae, shaft and socket morphology	ni	None
	97F7-9	*EP(3)0596*	Antisense orientation clot 13184 −25 bp of 3′ end of SD04125	Potential shaft-to-socket transformations, loss of several scutellar and dorsocentral macrochaetae, shaft with branched tips or dot-like morphology	ni	Yes
	98F6-7	*EP(3)3390*	Matches AC017691	Potential IIA-to-IIB or neuron-to-sheath transformations, loss of external support cells of macro- and microchaetae on notum and duplication of sheath cells, shaft with branched tip	None	None
string (*stg*)	99A5-6	*EP(3)1213*	∼−1.5 kb of transcript	Potential shaft-to-socket transformations, two sockets/two shafts, two sockets/one shaft, and one socket/two shafts	None	None
		EP(3)3261	+270 bp of transcript (CDS at +392 bp)	Three sockets/one shaft, two sockets/two shafts, two sockets/one shaft, two sockets/no shaft, tufting with up to four shafts, frequently several sheath cells associated with es organ clusters	—	—
		EP(3)3426	−37 bp of transcript		—	—
		EP(3)3432	Antisense orientation		None	—

Insertion sites, as determined by 3′ flanking sequences, are indicated: For known genes, the position is indicated as either 5′ (−) or 3′ (+) relative to the transcription start site or the coding start site (CDS). For insertions into ESTs, the clot number and relative position to the EST start is indicated. Matches with genomic sequence contigs are listed. Genetic interaction studies with *N* and *H* are summarized: Yes, genetic interaction was found (details provided in Table 2); None, no genetic interaction was found; ni, genetic interaction was tested but was not informative or could not be interpreted; −, not tested.

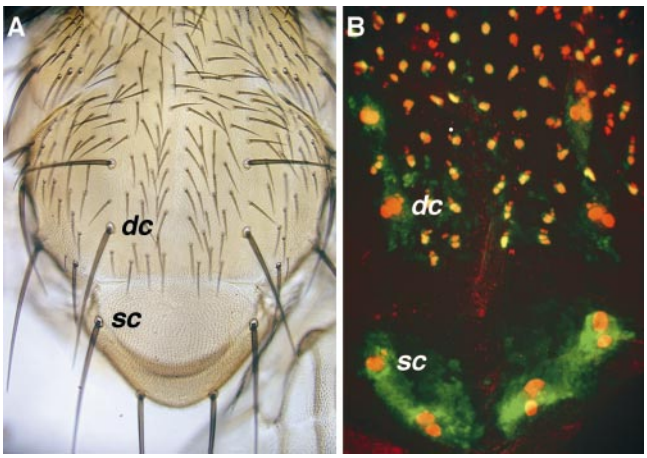

Figure 2.—Macro- and microchaetae are arranged in stereotyped patterns on the notum of Drosophila (for recent review on es organ pattern formation, see Simpson *et al.* 1999). (A) Four dorsocentral (dc) and four scutellar (sc) macrochaetae decorate the adult notum. (B) *sca-Gal4* expresses Gal4 (in green, driving *UAS-GFP*) within the four cut-expressing cells of the es organ (red) and surrounding cells (Blochlinger *et al.* 1993). On the scutellum and between the dorsocentral macrochaetae, *sca-Gal4* is expressed not only in the developing sensory organs but also in surrounding domains.

resulted in the occasional loss of scutellar or dorsocentral macrochaetae. Misexpression of these genes could interfere with SOP lineage events by blocking cell cycle progression (*e.g.*, by forcing the SOP cell to exit mitosis) or, in the case of *dally*, by affecting Wingless signaling, which is involved in the patterning of es organs (Phillips and Whittle 1993).

A large number of *P*-element insertions targeted genes that are known to have essential functions during development but have not previously been implicated in sensory organ development. One line, carrying an insertion at the *inscuteable* (*insc*) locus [*EP(2)2010*], exhibited a loss of external structures of scutellar macrochaetae without a concurrent loss of the prospero-positive sheath cell. Whether this phenotype is entirely due to altered expression of *insc*, which serves an essential function in asymmetric divisions of delaminating neuroblasts and embryonic muscle progenitor cell divisions (Kraut *et al.* 1996; Carmena *et al.* 1998), requires further study. One potential complication is the presence of the gene *skittles*, which encodes the phosphatidylinositol 4-phosphate 5-kinase, in the first intron of *insc*. Misexpression of *skittles* has been shown to generate ectopic es organs (Hassan *et al.* 1998). It is not clear whether misexpression of *insc*, *skittles*, or both is driven by *EP(2)2010*.

Other known developmental regulators found in this screen include *gliotactin* [*EP(2)2306*], which encodes a transmembrane protein that functions in peripheral glia to establish the blood-nerve barrier (Auld *et al.* 1995); *fat facets* [*EP(3)0381*], which encodes a deubiquitination enzyme required for correct eye development (Fischer-Vize *et al.* 1992; Huang *et al.* 1995); *apontic* [*EP(2)2373*], a gene involved in multiple processes, including head patterning (Gellon *et al.* 1997) and heart morphogenesis (Su *et al.* 1999); Drosophila *lim-domains only* [*EP(X)1306*, *EP(X)1383*, and *EP(X)1394*], a gene with a role in wing patterning (Milan *et al.* 1998; Shoresh *et al.* 1998; Zeng *et al.* 1998b), *longitudinals lacking* (*lola*) [*EP(2)0343*], which is required for correct axonal projection (Giniger *et al.* 1994); and hnRNP 27C [*EP(2)0748*], which encodes a heterogeneous nuclear RNA-associated protein. Previous studies suggest that different heterogeneous nuclear RNA-associated proteins may play a role in the development of the es organ (Hammond *et al.* 1997; zur Lage *et al.* 1997).

This class includes insertions at 15 previously uncharacterized genes. Four of these insertions showed genetic interactions with *N* or *H* (see Table 2), indicating that they affect genes that are potentially in the N signaling pathway. These genes are therefore good candidates for future analyses.

Supernumerary es organs or support cells: Thirty-eight lines, carrying insertions at 28 loci, caused misexpression phenotypes with increased numbers of internal and external cell types. We further subdivided these lines into two subclasses. One subclass of lines produced ectopic (*i.e.*, spatially separate) es organs; these might arise from defective lateral inhibition or ectopic proneural activity. The other subclass of lines exhibited supernumerary support cells that were clustered together. This phenotype could be due to either increased cell numbers within an es organ or formation of several tightly associated es organs. Such phenotypes could result from defects in lateral inhibition or cell cycle regulation.

In this class, there are 16 previously uncharacterized genes (Table 1). To distinguish lines that affect lateral inhibition from those that affect other functions, we tested a subset of these lines for genetic interactions with *N* and *H*. Eight lines representing eight independent loci displayed significant genetic interactions (see Table 2).

Ectopic supernumerary es organs: This subclass includes *big brain* [*EP(2)2278*], a gene involved in lateral inhibition that encodes a channel-like transmembrane protein (Rao *et al.* 1990). Also in this subclass are two genes with a known function in eye development: *yan* [*EP(2)0598* and *EP(2)2500*], which encodes an ETS domain nuclear protein that has an essential function in photoreceptor cell development (Lai and Rubin 1992; O'Neill *et al.* 1994); and *hedgehog* [*EP(3)3521*], which is involved in multiple developmental processes including eye furrow progression (Heberlein *et al.* 1993; Ma *et al.* 1993). *hedgehog* has also been implicated in the correct patterning of es organs on the adult notum (Gomez-Skarmeta and Modolell 1996; Mullor *et al.* 1997). Another gene, *split ends* (*spen*) [*EP(2)2583*], resulted in a misexpression phenotype with increased numbers of scutellar and dorsocentral macrochaetae (Figure 4A).

TABLE 2

Genetic interactions with *N* and *H*

	Genetic interactions with			
	Notch		*Hairless*	
EP no.	*Notch* phenotype	Misexpression phenotype	*Hairless* phenotype	Misexpression phenotype
Class I				
EP(2)0383	Enhanced	Not affected	Suppressed	Suppresses misexpression phenotype (on wing margin)
EP(2)0595	Enhanced	Enhances balding on notum; suppresses loss of abdominal microchaetes	Not determined	Not determined
EP(2)2306	Not informative	Enhanced	Not informative	Enhanced
EP(2)2010	Enhanced	Not affected	Enhanced	Suppressed
EP(3)0415	Not determined	Not determined	Not affected	Enhanced
EP(3)3673	Enhanced	Not affected	Enhanced	Enhanced
EP(3)3519	Enhanced	Enhanced	Suppresses shaft-to-socket transformation; enhances loss of es organs	Enhanced
Class II				
EP(X)1408	Enhanced	Not affected	Suppressed	Not affected
EP(2)2583	Enhanced	Enhanced	Enhanced	Suppressed
EP(2)1229	Enhanced	Not affected	Not determined	Not determined
EP(2)0639	Not determined	Not determined	Enhanced	Suppressed
EP(2)0647	Enhanced	Alters misexpression phenotype	Not informative	Suppressed
EP(2)0954	Enhanced	Enhanced	Not informative	Not informative
EP(3)3622	Enhanced	Enhanced	Not affected	Suppressed
EP(3)2409	Enhanced	Enhanced	Not informative	Not informative
Class III				
EP(X)1503	Enhanced	Suppresses two socket/no shaft phenotype	Enhanced	Suppresses additional scutellar macrochaetae
EP(2)2478	Not determined	Not determined	Suppresses shaft-to-socket transformation; enhances loss of es organs	Enhances loss of es organs
EP(2)0386	Not affected	Suppressed	Enhanced	Suppresses shaft/no socket phenotype
EP(3)0596	Not informative	Not informative	Not informative	Enhances no shaft/one socket phenotype

A total of 76 EP lines (64 loci) were tested for genetic interactions with *N* and *H*. Misexpression of the genes targeted by 19 independent lines showed interpretable genetic interactions in heterozygous backgrounds of either *N* or *H*. Genetic interactions were scored as the effect of the EP misexpression on the haploinsufficient *N* or *H* phenotype and as the effect of these mutations on the EP misexpression phenotype. For *N*, EP misexpression enhanced the *N* phenotype when socket-to-shaft transformations occurred. Conversely, for *Hairless*, EP misexpression enhanced the *H* phenotype when the number of es organs with shaft-to-socket transformations was increased (relative to the dominant *Hairless* phenotype), while EP misexpression suppressed the *H* phenotype when reduced numbers of es organs with such transformations were found. An enhancement of *Hairless* is also associated with the loss of es organs. None, no genetic interactions observed.

spen has multiple developmental functions including correct axon formation (Kolodziej *et al.* 1995) and control of correct segment identity (Wiellette *et al.* 1999). Two insertions near *nuclear fallout* [*EP(3)3324* and *EP(3)3339*] resulted in additional scutellar macrochaetae and in one-socket/two-shaft phenotypes. This gene encodes a coiled-coil protein with a function in cortical actin organization and cytokinesis (Rothwell *et al.* 1998).

Several previously uncharacterized genes targeted by the EP element displayed genetic interactions with *N* and *H*. For example, *EP(3)3622* produced a misexpression phenotype with additional es organs and tufts (*i.e.*, a large number of clustered shafts; Figure 4B). The misexpression phenotype produced by *EP(3)3622* is enhanced by removing one copy of *N* and suppressed by removing one copy of *H* (Table 2).

Increased numbers of internal and external support cells:

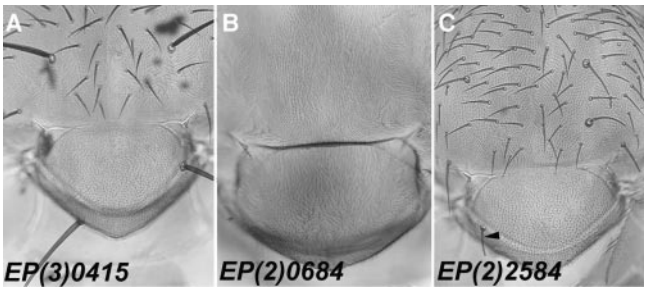

Figure 3.—Examples of class I misexpression phenotypes. (A) Misexpression of *EP(3)0415* at the *extra macrochaetae* locus resulted in the loss of scutellar and dorsocentral macro- and microchaetae. (B) Several insertions targeting *escargot*, including *EP(2)0684*, resulted in the loss of almost the entire population of macro- and microchaetae. (C) Misexpression of *EP(2)2584* at the *dacapo* locus resulted in the loss of external cells of scutellar and dorsocentral macrochaetae. The shaft cell morphology of many macrochaetae was abnormal. The arrowhead indicates an abnormal shaft cell morphology.

Supernumerary internal and external support cells could arise from ectopic cell divisions caused by altered cell cycle regulation. A previously uncharacterized gene targeted by *EP(3)3559* has sequence similarities with human regulatory subunits of protein phosphatase 2A (PP2A). Genes coding for the regulatory subunit B of PP2A (*abnormal anaphase*, *twins*) are involved in both cell cycle progression and cell fate determination (Gomes *et al.* 1993; Shiomi *et al.* 1994). *EP(3)3559* shows increased numbers of support cells in each es organ (Figure 4C). This misexpression phenotype mimics the phenotype observed in *twins*, a mutation in the regulatory B subunit of PP2A (Uemura *et al.* 1993). Regulatory subunits that are under temporal or tissue-specific control in turn regulate the activity of PP2A. It will be of interest to test how the newly identified regulatory subunit regulates the function of PP2A.

Three insertions at a novel locus, *EP(2)0639*, *EP(2)2148*, and *EP(2)2437*, produce supernumerary support cells in the es organ (Figure 4D). The orientation of the EP elements at this locus is such that they presumably generate a partial antisense transcript. Therefore, the phenotypes could be caused by lof or neomorphic effects.

Genetic interactions with *N* and *H* were found with *EP(2)0647*, an insertion at a gene that has sequence similarities with BTB-domain-containing proteins such as Pipsqueak. Misexpression of this gene resulted in, among other phenotypes, increased numbers of support cells associated with es organs.

Potential cell fate transformations: We expected to identify *P*-element insertions that target genes that function in the asymmetric divisions of the stereotyped es organ lineage. In total, 15 lines representing 10 loci resulted in apparent cell fate transformations. These lines fall into three subclasses. The first two subclasses are transformations within the IIA cell sublineage: (a) a socket-to-shaft cell transformation, which would result in a two-shaft/no-socket phenotype (twinned phenotype); and (b) a shaft-to-socket cell transformation, which would result in a no-shaft/two-socket phenotype. The third subclass is transformations from IIA to IIB, which would result in loss of external support cells (balding). However, mechanisms other than transformations may cause these phenotypes as well (*e.g.*, ectopic cell division of one type of support cell combined with the elimination of another type of support cell).

Potential transformations of socket cell to shaft cell: The misexpression of *numb* by *EP(2)2542* resulted in socket-to-shaft transformations similar to the *numb* overexpression phenotype (Figure 5A; Rhyu *et al.* 1994). The misexpression phenotype of *EP(2)2542* also included the loss of external structures of macrochaetae. This phenotype might be the result of IIA-to-IIB transformations.

Each of the two insertions [*EP(X)1149* and *EP(X)1179*] that target the same unknown gene produced both socket-to-shaft and reciprocal shaft-to-socket transformations (Figure 6C). Both lines also caused a loss of external support cells on the notum.

Potential transformations of shaft cell to socket cell: This subclass includes *string*, *twine*, and *grapes*, three genes with a function in mitotic or meiotic cell cycle regulation (Edgar and O'Farrell 1989; Alphey *et al.* 1992; Courtot *et al.* 1992; Fogarty *et al.* 1994, 1997). We identified four independent insertions at or near the *string* locus [*EP(3)1213*, *EP(3)3261*, *EP(3)3426*, and *EP(3)3432*].

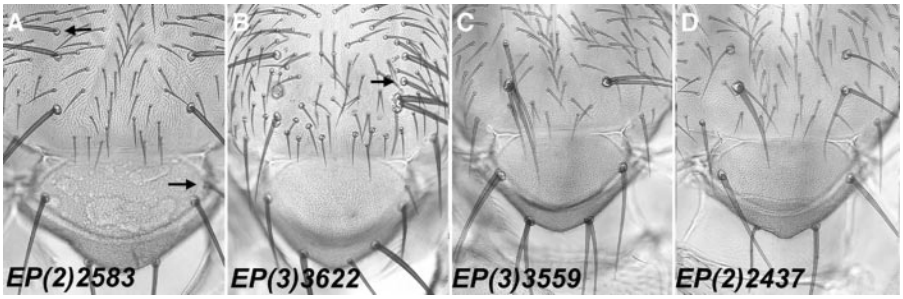

Figure 4.—Examples of class II misexpression phenotypes. (A) Misexpression of *EP(2)2583* at the *split ends* locus resulted in ectopic additional scutellar and dorsocentral macrochaetae (arrowheads). (B) Insertion *EP(3)3622* resulted in tufting, a phenotype with clustered shafts, and ectopic scutellar and dorsocentral macrochaetae. (C) Misexpression of *EP(3)3559*, which targets a new regulatory subunit of protein phosphatase2A, resulted in increased numbers of support cells. (D) Similarly, misexpression of *EP(2)2437* resulted in increased numbers of internal and external cell types. *EP(2)2437* is an insertion in antisense orientation within EST SD02913 and may cause lof effects. Arrows indicate ectopic macrochaetae.

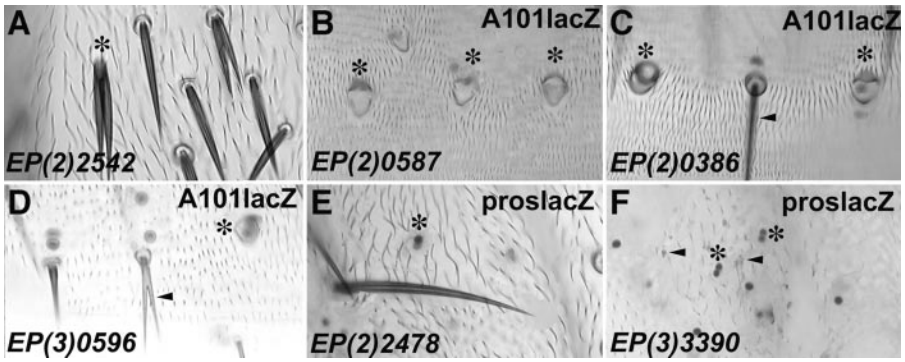

Figure 5.—Examples of class III misexpression phenotypes. (A) Misexpression of *EP(2)2542* at the *numb* locus resulted in apparent socket-to-shaft transformations. (B) *EP(2)0587* at the *grapes* locus caused apparent shaft-to-socket transformations on the abdomen. Double sockets are indicated by the presence of two large *A101 lacZ*-positive nuclear stains. (C) Misexpression of *EP(2)0386* produced apparent shaft-to-socket transformations on the abdomen, as indicated by the presence of two large *A101 lacZ*-positive nuclear stains. (D) The abdominal misexpression phenotypes of *EP(3)0596* were apparent shaft-to-socket transformations (asterisk) and branching of shaft cells (arrowhead). (E) Misexpression of *EP(2)2478* resulted in apparent IIA-to-IIB or neuron-to-sheath transformations. In the absence of external support cells, two *proslacZ*- positive sheath cells were tightly associated (asterisk). (F) Similarly, misexpression of *EP(3)3390* resulted in apparent IIA-to-IIB or neuron-to-sheath transformations. Two associated *proslacZ*-positive sheath cells were commonly scored in the absence of differentiated external structures (asterisk). However, abnormal cuticular structures were visible (arrowheads). Potential transformation phenotypes are indicated with an asterisk.

With the exception of *EP(3)1213*, which carries an insertion ~1.5 kb upstream of the normal transcript, the other three insertions lie close to the transcription initiation site (see Table 1). However, only *EP(3)1213* resulted in possible shaft-to-socket transformations, raising the question whether a gene other than *string* is affected in this line. The misexpression by *EP(3)3261* produced increased numbers of internal and external support cells. X-gal staining with enhancer trap lines *A101 lacZ* and *prospero lacZ*, which mark the external and the sheath cells, respectively, showed an approximate doubling of the cell number in many es organs (not shown).

Insertions near *grapes* [*EP(2)0587*] and *twine* [*EP(2)0613*] resulted in potential shaft-to-socket transformations on the abdomen and notum, respectively (Figure 5B). Mutations in *grapes*, a protein kinase with homologies to *Saccharomyces cerevisiae* CHK1, have been shown to interfere with the DNA replication checkpoint control of the cell cycle (Fogarty *et al.* 1997). In addition, embryos mutant in *grapes* exhibit cortical cytoskeletal defects during syncytial divisions (Sullivan *et al.* 1993). Misexpression of *twine* caused, in addition to possible shaft-to-socket transformations, a four-socket phenotype. *twine*, a *cdc25* homolog, has a function during male and female meiotic divisions and participates in some aspects of mitotic control at the syncytial embryo stage (Alphey *et al.* 1992; Courtot *et al.* 1992; Edgar and Datar 1996).

The most prominent phenotype found with two other lines, [*EP(2)0386* and *EP(2)0988*], was apparent shaft-to-socket cell transformations on the abdomen. X-gal staining with enhancer trap line *A101 lacZ*, which predominantly marks two large nuclei of the two external cells of the es organ, confirmed the presence of two socket cells (Figure 5C). A third line, *EP(3)0596*, produced a similar misexpression phenotype (Figure 5D).

Potential transformations of IIA to IIB: Two insertions at two independent loci each produced potential IIA-to-IIB cell fate transformations, with two or more prospero-positive cells in the absence of external support cells. With *EP(2)2478*, both macro- and microchaetae exhibited a loss of external support cells as well as a duplication of presumptive sheath cells (Figure 5E). Similarly, the misexpression caused by *EP(3)3390* resulted in a loss of external support cells of macro- and microchaetae as well as duplication of prospero-positive sheath cells (Figure 5F). In rare cases, up to four sheath cells were present.

Defective morphology of the es organ: At least 41 lines, representing 38 loci, identified in this screen produced aberrant morphology of either the socket or the shaft cell. The following are examples of different morphology phenotypes observed.

Misexpression driven by *EP(2)2356* produced an abnormal shaft cell morphology. Most prominently, the shaft cell was short and branched into many distal tips (Figure 6A). Branching of the shaft cell into two distal tips was observed in several lines [*i.e.*, in *EP(3)0596*, Figure 5D].

Morphologically abnormal socket cells were produced by *EP(3)3463*. Among other phenotypes, the socket cells frequently were large and flattened (Figure 6B). *EP(X)1149* (see also phenotype in class III) produced an abnormal socket cell morphology with a protruding tip similar to a short shaft (Figure 6C).

We observed a massive reduction in the size of shaft cells and morphologically abnormal socket cells with *EP(2)2317*, an insertion at *eIF-4A* (Figure 6D). Similar phenotypes were seen with several other lines.

The sensitivity of cell morphology to the misexpression of candidate genes might yield an entry point to identify genetic components involved in differentiation and morphogenesis. Several of the phenotypes described here resemble phenotypes caused by mutations of genes that function in cytoskeletal assembly (Cant *et al.* 1994; Tilney *et al.* 1995, 1996).

DISCUSSION

Analyzing development of the es organ using a gain-of-function approach: Traditionally, genetic screens have been based on the isolation of lof mutations. This approach has been invaluable in unraveling the mechanisms underlying many biological processes, including the formation of the peripheral nervous system (Salzberg *et al.* 1994; Kania *et al.* 1995; Go *et al.* 1998). However, lof screens have several limitations. Redundancy between genes that have overlapping functions might partially or completely mask gene function. In such cases, it is necessary to make double or multiple mutant combinations to produce a phenotype, an approach that is not generally applicable during lof screens. Moreover, early phenotypes caused by a mutation might prevent the detection of later phenotypes (Miklos and Rubin 1996). Such limitations can be partially circumvented by screens that are based on analyzing the phenotypes of clones of mutant tissue generated by somatic recombination (Xu and Rubin 1993) or by screens for enhancers or suppressors of a particular mutant phenotype (Simon *et al.* 1991). Nevertheless, many genes might have escaped detection by lof approaches.

The gof screening system devised by P. Rørth complements lof approaches. This system is based on the analysis of phenotypes generated by tissue-specific misexpression of genes using the UAS-Gal4 system. Any gene that produces a misexpression phenotype is detectable by the system in spite of possible functional redundancy and pleiotropy of gene function (Rørth 1996; Rørth *et al.* 1998). In addition, the tissue specificity of the UAS-Gal4 system allows the examination of misexpression phenotypes in the biological context of choice. In various screens, phenotypes that affected eye development, wing development, and follicle cell migration were analyzed (Rørth *et al.* 1998).

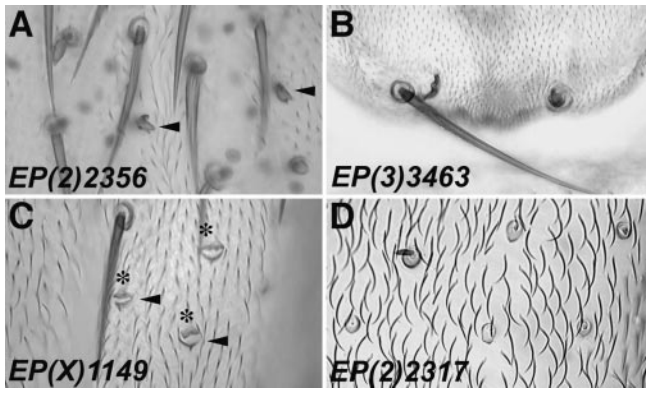

Figure 6.—A group of 41 EP lines carry insertions near genes that when misexpressed, produced an abnormal es organ morphology. Examples are as follows: (A) *EP(2)2356* caused branching of shafts into multiple tips (arrowheads). (B) Flattened and enlarged socket cells were commonly scored with *EP(3)3463*. (C) *EP(X)1149* resulted in potential shaft-to-socket transformations. Socket cells frequently displayed protruding shaft-like tips. (D) *EP(2)2317* resulted in the severe reduction of shaft cells into shortened or dot-like structures. Arrowheads indicate abnormal cell morphology. Potential transformation phenotypes are indicated with an asterisk.

In this study, these 2293 randomly inserted *P* elements were each driven by a sensory-organ-specific Gal4 driver and any resulting misexpression phenotypes in the es organ were analyzed. Of these lines, 105 produced es organ phenotypes. Our preliminary phenotypic and molecular analyses suggest that we have identified genes that are involved in lateral inhibition, cell cycle control, cell fate specification, and cell differentiation. A subset of these genes is likely to play a role in es organ formation.

One potential drawback of gof screens is that misexpression of a gene may affect the development of tissues in which that gene is not normally expressed. In some cases, misexpression of a gene may ectopically effect a signaling pathway that functions in multiple developmental processes. Another concern is that phenotypes may be artificial. For example, the phenotype caused by misexpression of a gene at levels much higher than normal may interfere with development, even if that gene does not have a function in development.

To identify those genes that normally function in es organ development, it will be important to examine the lof phenotype, the expression pattern, and genetic interactions with genes known to be involved in es organ development.

The systematic misexpression screen identifies candidate genes that interfere with distinct developmental aspects of es organ formation: Among the 105 lines (78 loci) identified in the screen, 49 lines (37 loci) correspond to previously characterized genes. A subset of these genes has been shown to have roles during es organ development. Some, such as *emc* and *big brain*, have a function in lateral inhibition (Skeath and Carroll 1991; Rao *et al.* 1992). Several are genes with a function in cell cycle regulation, including *dacapo* and *string*, and thus might be required during es organ cell division. Others, such as *numb*, are known to be involved in asymmetric cell division (Rhyu *et al.* 1994). Moreover, a large group of genes with essential roles in other developmental processes were identified. Some of these genes, such as *hedgehog* and *yan*, have not been tested for their role in es organ development, but it is possible that they are involved in this developmental process as well. Since many of the known genes identified in this screen are likely to have normal functions in es organ development, the concern of the potentially artificial nature of the gof screen may be alleviated. It thus seems likely that at least a substantial subset of the new genes identified in our screen will turn out to be important for the formation of es organs, perhaps in some of the less understood aspects of es organ development, including the following:

Context-specific components of the N-signaling pathway:

The transducers of *N* signaling in IIB and her daughters are currently not known (Wang *et al.* 1997). *EP(2)2478* and *EP(3)3390* target genes with possible functions in IIB and her daughters. Misexpression of those genes was sufficient to generate potential IIA-to-IIB or neuron-to-sheath transformations. One possible explanation for this phenotype is ectopic activation of IIB-specific target genes (*e.g.*, by IIB or sheath-cell-specific N-signaling components).

Cell cycle regulation of stereotyped lineage events: One likely link between cell cycle regulation and asymmetric cell division is the cell-cycle-dependent asymmetric localization of cell fate determinants and adaptor proteins (Hirata *et al.* 1995; Knoblich *et al.* 1995; Spana and Doe 1995; Kraut *et al.* 1996; Ikeshima-Kataoka *et al.* 1997; Shen *et al.* 1997; Lu *et al.* 1998, 1999; Schuldt *et al.* 1998). Untimely cell cycle progression or defective integration of cell cycle with the localization of Numb protein may create a phenotype reminiscent of *numb* lof, a phenotype that was observed with misexpression of the cell cycle regulatory genes *grapes* and *twine*.

In addition, cell cycle regulatory genes may serve additional functions that affect cell fate specification. *grapes*, for example, is essential for the normal formation of the cortical cytoskeleton during syncytial divisions (Sullivan *et al.* 1993). Given the importance of the cortical cytoskeleton during asymmetric division (Broadus and Doe 1997; Knoblich *et al.* 1997), genes that regulate the dynamics of this structure may also turn out to be essential during cell fate decisions.

Highly stereotyped division patterns occur throughout Drosophila development (Foe 1989; Gho *et al.* 1999). Several cell cycle regulators, including *dacapo*, are required to control the cell division patterns in the neural lineages of the embryonic nervous system (Cui and Doe 1995; Weigmann and Lehner 1995; de Nooij *et al.* 1996; Lane *et al.* 1996; Hassan and Vaessin 1997). It is not known at this time whether *dacapo* normally functions during the development of the es organ to control precise cell division patterns.

Execution of morphogenesis: There are different types of genes that when misexpressed could give rise to morphology defects. These include genes that affect differentiation of a single cell type (*e.g.*, shaft cell differentiation controlled by *pax2*; Kavaler *et al.* 1999) or that affect proper regulation of cytoskeletal dynamics. We found a large number of lines that, when misexpressed, resulted in aberrant morphogenesis of the socket or shaft cell. One phenotype observed was the branching of shafts. It has been suggested that mutations causing branched hairs are in genes that regulate the actin cytoskeleton (Turner and Adler 1998). Consistent with this prediction, mutations of genes with a function in actin bundle formation display similar branching phenotypes (Cant *et al.* 1994; Tilney *et al.* 1995, 1996). Several of the lines identified in this screen might provide additional components involved in executing shaft cell morphology or in regulating the actin cytoskeleton in other tissues. Less is known about the morphogenesis of socket cells. EP lines that affected predominantly socket cell morphology might provide clues to this process.

Genomic considerations and perspectives: Genome sequencing by the European and Berkeley *Drosophila* Genome Projects (EDGP and BDGP) and the ease with which genomic sequences flanking the EP element can be cloned have greatly facilitated the identification of targeted genes. Of the insertion sites we sequenced, 49 (37 loci; 46.7% of all lines) matched known genes, 34 (28 loci; 32.4% of all lines) matched EST, and 22 (13 loci; 20.9% of all lines) matched sequenced genomic regions but still have no candidate transcripts.

Altogether, 105 lines or 4.5% of the lines tested gave rise to misexpression phenotypes. Rørth *et al.* (1998) reported comparable frequencies of misexpression phenotypes: 7% with *ombGal4*, 4% with *dppGal4*, 3% with *slboGal4*, and 2% with *sevGal4*. Among the few genes that were reported from those screens, we have isolated *escargot*, *hedgehog*, *yan*, *scalloped*, and *big brain*. It will be interesting to compare those screens to obtain an estimate of the overlap of the genes used in those different developmental processes.

In a separate database analysis, we searched for EP element insertions that target genes with a known func-

TABLE 3

Summary of EP element insertions

Locus	Map position	EP no.	Insertion site
kuzbanian (*kuz*)	34D4	*EP(2)2503*	−916 bp of transcript
neuralized (*neur*)	85D	*EP(3)3026*	+466 bp of transcript (CDS at +278 bp)
Enhancer of split transcript m2 (*E(spl)m2*)	96F9	*EP(3)3635*	−2702 bp of transcript
Enhancer of split transcript m7 (*E(spl)m7*)	96F9	*EP(3)3272* *EP(3)3587*	−11 bp of transcript −646 bp of transcript

Summary of those EP element insertions near genes with a function in neurogenesis or es organ development that did not result in misexpression phenotypes. The EP element insertions were identified by database analysis.

tion in neurogenesis and sensory organ development. Among seven EP element insertions that target six genes (*extra macrochaetae*, *big brain*, *kuzbanian*, *neuralized*, and *Enhancer of split* transcripts *m2* and *m7*), only two insertions near two loci yielded misexpression phenotypes in our assay (*extra macrochaetae*, *big brain*). Five insertions near four loci did not cause obvious misexpression phenotypes (Table 3). Therefore, the misexpression screen was not fully efficient. Similarily, there may be other unknown genes with a function in es organ development that escaped detection even with an EP element inserted nearby.

Determining the exact insertion site and orientation of the EP element is essential to the interpretation of misexpression phenotypes. In the lines for which we identified a transcript, most of the EP transposons were inserted between −850 bp upstream and +800 bp downstream of the transcription start site (61/83 = 73.5%). Seven lines (8.4%) were identified with insertions at greater distances from the transcription start site of putative target genes. In these cases it is possible that additional transcripts that have not been identified might be located closer to the EP element. One example is *EP(3)1213*, which carries an insertion ~1.5 kb 5′ of the transcriptional start site of *string*. The misexpression phenotype produced by this line was qualitatively different from other EP insertions closer to the *string* transcriptional start site. Whether these differences are attributable to different levels of expression or are caused by an unidentified transcript needs to be determined. Another 9 lines (10.8%) carried EP elements with an apparent antisense orientation and might generate partial antisense transcripts. How these antisense messages might cause phenotypes is not clear. In addition, there are several lines (6/83 = 7.2%) that carried insertions 3′ of the CDS, or insertions within new transcripts for which the CDS is not known. In these cases, the phenotypes might be caused by truncated transcripts.

The EP transposon allows only the unidirectional transcription of potential target genes. Therefore, ~50% of the EP lines are expected to be in the correct orientation to drive misexpression of a sense transcript [only nine of the lines that gave rise to phenotypes with *sca-Gal4* (8.6%) had an inverted or antisense orientation]. Thus, the total number of genes targeted for overexpression in the screen might be no more than 1150. The number of targeted genes is further reduced by multiple lines targeting the same gene (1.33 insertions/locus) and by insertions that lie too distantly to drive sufficient transcriptional activation.

The current estimate for the number of genes in the Drosophila genome by the BDGP is around 14,000 (based on Miklos and Rubin 1996). Therefore, the EP collection targets ~10% of the genome. In an extrapolation, for a genome-wide saturation screen we would expect ≥800 different loci or ~5–6% of all genes to give rise to misexpression phenotypes. The future challenge will be to determine the biological significance of the genes identified during this screen.

We are most grateful to P. Rørth for the generous gift of EP lines. We thank Todd Laverty and G. Rubin for kindly providing us with the EP lines, and the lab of J. Campos-Ortega and the Bloomington *Drosophila* Stock Center for fly strains. Our sequence analysis was helped by the sequencing efforts of BDGP and EDGP. Thanks to B. Lu and S. Zhou for critical reading of the manuscript; to D. Doherty for providing us with Figure 2; and to other current and former members of the Jan lab for discussion, suggestions, and help. S.A.-S. was supported by a fellowship from the Deutsche Forschungsgemeinschaft. Y.-M. C. currently is supported by the Program in Biological Sciences Markey Grant and the Herb Boyer Fund. C.Z. is a postdoctoral associate, N.J.J. is a predoctoral associate, and L.Y.J. and Y.N.J. are investigators of the Howard Hughes Medical Institute.

LITERATURE CITED

Alphey, L., J. Jimenez, H. White-Cooper, I. Dawson, P. Nurse *et al.*, 1992 *twine*, a *cdc25* homolog that functions in the male and female germline of *Drosophila*. Cell **69:** 977–988.

Artavanis-Tsakonas, S., M. D. Rand and R. J. Lake, 1999 *Notch* signaling: cell fate control and signal integration in development. Science **284:** 770–776.

Auld, V. J., R. D. Fetter, K. Broadie and C. S. Goodman, 1995 Gliotactin, a novel transmembrane protein on peripheral glia, is required to form the blood-nerve barrier in *Drosophila*. Cell **81:** 757–767.

Bailey, A. M., and J. W. Posakony, 1995 Suppressor of hairless directly activates transcription of *enhancer of split* complex genes in response to Notch receptor activity. Genes Dev. **9:** 2609–2622.

Bang, A. G., and J. W. Posakony, 1992 The *Drosophila* gene *Hairless* encodes a novel basic protein that controls alternative cell fates in adult sensory organ development. Genes Dev. **6:** 1752–1769.

Bang, A. G., A. M. Bailey and J. W. Posakony, 1995 *Hairless* promotes stable commitment to the sensory organ precursor cell fate by negatively regulating the activity of the *Notch* signaling pathway. Dev. Biol. **172:** 479–494.

Blochlinger, K., L. Y. Jan and Y. N. Jan, 1993 Postembryonic patterns of expression of *cut*, a locus regulating sensory organ identity in *Drosophila*. Development **117:** 441–450.

Brand, A. H., and N. Perrimon, 1993 Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development **118:** 401–415.

Broadus, J., and C. Q. Doe, 1997 Extrinsic cues, intrinsic cues and microfilaments regulate asymmetric protein localization in *Drosophila* neuroblasts. Curr. Biol. **7:** 827–835.

Brou, C., F. Logeat, M. Lecourtois, J. Vandekerckhove, P. Kourilsky *et al.*, 1994 Inhibition of the DNA-binding activity of *Drosophila* suppressor of hairless and of its human homolog, KBF2/RBP-J kappa, by direct protein-protein interaction with *Drosophila* hairless. Genes Dev. **8:** 2491–2503.

Campos-Ortega, J., 1996 Numb diverts notch pathway off the tramtrack. Neuron **17:** 1–4.

Cant, K., B. A. Knowles, M. S. Mooseker and L. Cooley, 1994 *Drosophila singed*, a *fascin* homolog, is required for actin bundle formation during oogenesis and bristle extension. J. Cell Biol. **125:** 369–380.

Carmena, A., B. Murugasu-Oei, D. Menon, F. Jimenez and W. Chia, 1998 *Inscuteable* and *numb* mediate asymmetric muscle progenitor cell divisions during *Drosophila* myogenesis. Genes Dev. **12:** 304–315.

Courtot, C., C. Fankhauser, V. Simanis and C. F. Lehner, 1992 The *Drosophila cdc25* homolog *twine* is required for meiosis. Development **116:** 405–416.

Craymer, L., 1980 [New mutants report.] Dros. Inf. Serv. **55:** 197–200.

Cui, X., and C. Q. Doe, 1995 The role of the cell cycle and cytokinesis in regulating neuroblast sublineage gene expression in the *Drosophila* CNS. Development **121:** 3233–3243.

De Nooij, J. C., M. A. Letendre and I. K. Hariharan, 1996 A

cyclin-dependent kinase inhibitor, Dacapo, is necessary for timely exit from the cell cycle during *Drosophila* embryogenesis. Cell **87:** 1237–1247.

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.

Doherty, D., L. Y. Jan and Y. N. Jan, 1997 The *Drosophila* neurogenic gene *big brain*, which encodes a membrane-associated protein, acts cell autonomously and can act synergistically with *Notch* and *Delta*. Development **124:** 3881–3893.

Dye, C. A., J. Lee, R. C. Atkinson, R. Brewster, P. Han *et al.*, 1998 The *Drosophila sanpodo* gene controls sibling cell fate and encodes a Tropomodulin homolog, an actin/tropomyosin associated protein. Development **125:** 1845–1856.

Edgar, B. A., and P. H. O'Farrell, 1989 Genetic control of cell division patterns in the *Drosophila* embryo. Cell **57:** 177–187.

Edgar, B. A., and S. A. Datar, 1996 Zygotic degradation of two maternal *Cdc25* mRNAs terminates *Drosophila's* early cell cycle program. Genes Dev. **10:** 1966–1977.

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.

Fischer-Vize, J. A., G. M. Rubin and R. Lehmann, 1992 The *fat facets* gene is required for *Drosophila* eye and embryo development. Development **116:** 985–1000.

Foe, V. E., 1989 Mitotic domains reveal early commitment of cells in *Drosophila* embryos. Development **107:** 1–22.

Fogarty, P., R. F. Kalpin and W. Sullivan, 1994 The *Drosophila* maternal-effect mutation *grapes* causes a metaphase arrest at nuclear cycle 13. Development **120:** 2131–2142.

Fogarty, P., S. D. Campbell, R. Abu-Shumays, B. De Saint Phalle, K. R. Yu *et al.*, 1997 The *Drosophila grapes* gene is related to checkpoint gene *chk-1 rad27* and is required for late syncytial division fidelity. Curr. Biol. **7:** 418–426.

Fortini, M. E., and S. Artavanis-Tsakonas, 1994 The suppressor of Hairless protein participates in Notch receptor signaling. Cell **79:** 273–282.

Frise, E., J. A. Knoblich, S. Younger-Shepherd, L. Y. Jan and Y. N. Jan, 1996 The *Drosophila* Numb protein inhibits signaling of the Notch receptor during cell-cell interaction in sensory organ lineage. Proc. Natl. Acad. Sci. USA **93:** 11925–11932.

Fuse, N., S. Hirose and S. Hayashi, 1994 Diploidy of *Drosophila* imaginal cells is maintained by a transcriptional repressor encoded by *escargot*. Genes Dev. **8:** 2270–2281.

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.

Gellon, G., K. W. Harding, N. McGinnis, M. M. Martin and W. McGinnis, 1997 A genetic screen for modifiers of *Deformed* homeotic function identifies novel genes required for head development. Development **124:** 3321–3331.

Gho, M., Y. Bellaiche and F. Schweisguth, 1999 Revisiting the *Drosophila* microchaete lineage: a novel intrinsically asymmetric cell division generates a glia cell. Development **126:** 3573–3584.

Ghysen, A., and C. Dambly-Chaudiere, 1989 Genesis of the *Drosophila* peripheral nervous system. Trends Genet. **5:** 251–255.

Giniger, E., K. Tietje, L. Y. Jan and Y. N. Jan, 1994 *lola* encodes a putative transcription factor required for axon growth and guidance in *Drosophila*. Development **120:** 1385–1398.

Go, M. J., and S. Artavanis-Tsakonas, 1998 A genetic screen for novel components of the *notch* signaling pathway during *Drosophila* bristle development. Genetics **150:** 211–220.

Gomes, R., R. E. Karess, H. Ohkura, D. M. Glover and C. E. Sunkel, 1993 *abnormal anaphase resolution* (*aar*): a locus required for progression through mitosis in *Drosophila*. J. Cell Sci. **104:** 583–593.

Gomez-Skarmeta, J. L., and J. Modolell, 1996 *araucan* and *caupolican* provide a link between compartment subdivisions and patterning of sensory organs and veins in the *Drosophila* wing. Genes Dev. **10:** 2935–2945.

Guo, M., E. Bier, L. Y. Jan and Y. N. Jan, 1995 *tramtrack* acts downstream of *numb* to specify distinct daughter cell fates during asymmetric cell divisions in the *Drosophila* PNS. Neuron **14:** 913–925.

Guo, M., L. Y. Jan and Y. N. Jan, 1996 Control of daughter cell fates during asymmetric division: interaction of Numb and Notch. Neuron **17:** 27–41.

Hammond, L. E., D. Z. Rudner, R. Kanaar and D. C. Rio, 1997 Mutations in the hrp48 gene, which encodes a *Drosophila* heterogeneous nuclear ribonucleoprotein particle protein, cause lethality and developmental defects and affect P-element third-intron splicing in vivo. Mol. Cell. Biol. **17:** 7260–7267.

Hartenstein, V., and J. W. Posakony, 1989 Development of adult sensilla on the wing and notum of *Drosophila melanogaster*. Development **107:** 389–405.

Hassan, B., and H. Vaessin, 1997 *Daughterless* is required for the expression of cell cycle genes in peripheral nervous system precursors of *Drosophila* embryos. Dev. Genet. **21:** 117–122.

Hassan, B. A., S. N. Prokopenko, S. Breuer, B. Zhang, A. Paululat *et al.*, 1998 *skittles*, a *Drosophila* phosphatidylinositol 4-phosphate 5-kinase, is required for cell viability, germline development and bristle morphology, but not for neurotransmitter release. Genetics **150:** 1527–1537.

Hayashi, S., 1996 A *Cdc2* dependent checkpoint maintains diploidy in *Drosophila*. Development **122:** 1051–1058.

Hayashi, S., S. Hirose, T. Metcalfe and A. D. Shirras, 1993 Control of imaginal cell development by the *escargot* gene of *Drosophila*. Development **118:** 105–115.

Heberlein, U., T. Wolff and G. M. Rubin, 1993 The TGF beta homolog *dpp* and the segment polarity gene *hedgehog* are required for propagation of a morphogenetic wave in the *Drosophila* retina. Cell **75:** 913–926.

Hirata, J., H. Nakagoshi, Y. Nabeshima and F. Matsuzaki, 1995 Asymmetric segregation of a homeoprotein, *prospero*, during cell divisions in neural and endodermal development. Nature **377:** 627–630.

Huang, Y., R. T. Baker and J. A. Fischer-Vize, 1995 Control of cell fate by a deubiquitinating enzyme encoded by the *fat facets* gene. Science **270:** 1828–1831.

Ikeshima-Kataoka, H., J. B. Skeath, Y. Nabeshima, C. Q. Doe and F. Matsuzaki, 1997 Miranda directs Prospero to a daughter cell during *Drosophila* asymmetric divisions. Nature **390:** 625–629.

Jan, L. Y., and Y. N. Jan, 1995 Maggot's hair and bug's eye: role of cell interactions and intrinsic factors in cell fate specification. Neuron **14:** 1–5.

Jarriault, S., C. Brou, F. Logeat, E. H. Schroeter, R. Kopan *et al.*, 1995 Signalling downstream of activated mammalian Notch. Nature **377:** 355–358.

Kania, A., A. Salzberg, M. Bhat, D. D'Evelyn, Y. He *et al.*, 1995 P-element mutations affecting embryonic peripheral nervous system development in *Drosophila melanogaster*. Genetics **139:** 1663–1678.

Kavaler, J., W. Fu, H. Duan, M. Noll and J. W. Posakony, 1999 An essential role for the *Drosophila Pax2* homolog in the differentiation of adult sensory organs. Development **126:** 2261–2272.

Knoblich, J. A., L. Y. Jan and Y. N. Jan, 1995 Asymmetric segregation of Numb and Prospero during cell division. Nature **377:** 624–627.

Knoblich, J. A., L. Y. Jan and Y. N. Jan, 1997 The N terminus of the *Drosophila* Numb protein directs membrane association and actin-dependent asymmetric localization. Proc. Natl. Acad. Sci. USA **94:** 13005–13010.

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.

Kraut, R., W. Chia, L. Y. Jan, Y. N. Jan and J. A. Knoblich, 1996 Role of *inscuteable* in orienting the asymmetric cell divisions in *Drosophila*. Nature **383:** 50–55.

Lai, Z. C., and G. M. Rubin, 1992 Negative control of photoreceptor development in *Drosophila* by the product of the *yan* gene, an ETS domain protein. Cell **70:** 609–620.

Lane, M. E., K. Sauer, K. Wallace, Y. N. Jan, C. F. Lehner *et al.*, 1996 Dacapo, a cyclin-dependent kinase inhibitor, stops cell proliferation during *Drosophila* development. Cell **87:** 1225–1235.

Lecourtois, M., and F. Schweisguth, 1995 The neurogenic suppressor of hairless DNA-binding protein mediates the transcriptional activation of the *enhancer of split* complex genes triggered by *Notch* signaling. Genes Dev. **9:** 2598–2608.

Lehmann, R., U. Dietrich, F. Jimenez and J. A. Campos-Ortega, 1981 Mutations of early neurogenesis in *Drosophila*. Roux's Arch. Dev. Biol. **190:** 226–229.

Lehmann, R., F. Jimenez, U. Dietrich and J. A. Campos-Ortega, 1983 On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster*. Roux's Arch. Dev. Biol. **192:** 62–74.

Lieber, T., S. Kidd, E. Alcamo, V. Corbin and M. W. Young, 1993 Antineurogenic phenotypes induced by truncated Notch proteins indicate a role in signal transduction and may point to a novel function for Notch in nuclei. Genes Dev. **7:** 1949–1965.

Lin, X., and N. Perrimon, 1999 *Dally* cooperates with *Drosophila Frizzled 2* to transduce *Wingless* signalling. Nature **400:** 281–284.

Lu, B., M. Rothenberg, L. Y. Jan and Y. N. Jan, 1998 Partner of Numb colocalizes with Numb during mitosis and directs Numb asymmetric localization in *Drosophila* neural and muscle progenitors. Cell **95:** 225–235.

Lu, B., L. Ackerman, L. Y. Jan and Y. N. Jan, 1999 Modes of protein movement that lead to the asymmetric localization of Partner of Numb during neuroblast division in *Drosophila*. Mol. Cell **4:** 883–891.

Ma, C., Y. Zhou, P. A. Beachy and K. Moses, 1993 The segment polarity gene *hedgehog* is required for progression of the morphogenetic furrow in the developing *Drosophila* eye. Cell **75:** 927–938.

Miklos, G. L., and G. M. Rubin, 1996 The role of the genome project in determining gene function: insights from model organisms. Cell **86:** 521–529.

Milan, M., F. J. Diaz-Benjumea and S. M. Cohen, 1998 *Beadex* encodes an LMO protein that regulates Apterous LIM-homeodomain activity in *Drosophila* wing development: a model for LMO oncogene function. Genes Dev. **12:** 2912–2920.

Mullor, J. L., M. Calleja, J. Capdevila and I. Guerrero, 1997 *Hedgehog* activity, independent of *decapentaplegic*, participates in wing disc patterning. Development **124:** 1227–1237.

Nakao, K., and J. A. Campos-Ortega, 1996 Persistent expression of genes of the *enhancer of split* complex suppresses neural development in *Drosophila*. Neuron **16:** 275–286.

Nakato, H., T. A. Futch and S. B. Selleck, 1995 The *division abnormally delayed* (*dally*) gene: a putative integral membrane proteoglycan required for cell division patterning during postembryonic development of the nervous system in *Drosophila*. Development **121:** 3687–3702.

O'Neill, E. M., I. Rebay, R. Tjian and G. M. Rubin, 1994 The activities of two Ets-related transcription factors required for *Drosophila* eye development are modulated by the Ras/MAPK pathway. Cell **78:** 137–147.

Phillips, R. G., and J. R. Whittle, 1993 wingless expression mediates determination of peripheral nervous system elements in late stages of *Drosophila* wing disc development. Development **118:** 427–438.

Pirotta, V., 1986 Cloning *Drosophila* genes, pp. 83–110 in *Drosophila: A Practical Approach*, edited by D. B. Roberts. IRL Press, Oxford.

Posakony, J. W., 1994 Nature versus nurture: asymmetric cell divisions in *Drosophila* bristle development. Cell **76:** 415–418.

Rao, Y., L. Y. Jan and Y. N. Jan, 1990 Similarity of the product of the *Drosophila* neurogenic gene *big brain* to transmembrane channel proteins. Nature **345:** 163–167.

Rao, Y., R. Bodmer, L. Y. Jan and Y. N. Jan, 1992 The *big brain* gene of *Drosophila* functions to control the number of neuronal precursors in the peripheral nervous system. Development **116:** 31–40.

Rhyu, M. S., L. Y. Jan and Y. N. Jan, 1994 Asymmetric distribution of numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. Cell **76:** 477–491.

Rørth, P., 1996 A modular misexpression screen in *Drosophila* detecting tissue specific phenotypes. Proc. Natl. Acad. Sci. USA **93:** 12418–12422.

Rørth, P., K. Szabo, A. Bailey, T. Laverty, J. Rehm *et al.*, 1998 Systematic gain-of-function genetics in *Drosophila*. Development **125:** 1049–1057.

Rothwell, W. F., P. Fogarty, C. M. Field and W. Sullivan, 1998 Nuclear-fallout, a *Drosophila* protein that cycles from the cytoplasm to the centrosomes, regulates cortical microfilament organization. Development **125:** 1295–1303.

Salzberg, A., D. D'Evelyn, K. L. Schulze, J. 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.

Schuldt, A. J., J. H. Adams, C. M. Davidson, D. R. Micklem, J. Haseloff *et al.*, 1998 Miranda mediates asymmetric protein and RNA localization in the developing nervous system. Genes Dev. **12:** 1847–1857.

Schweisguth, F., and J. W. Posakony, 1992 *Suppressor of Hairless*, the *Drosophila* homolog of the mouse recombination signal-binding protein gene, controls sensory organ cell fates. Cell **69:** 1199–1212.

Schweisguth, F., and J. W. Posakony, 1994 Antagonistic activities of *Suppressor of Hairless* and *Hairless* control alternative cell fates in the *Drosophila* adult epidermis. Development **120:** 1433–1441.

Shen, C. P., L. Y. Jan and Y. N. Jan, 1997 Miranda is required for the asymmetric localization of Prospero during mitosis in *Drosophila*. Cell **90:** 449–458.

Shiomi, K., M. Takeichi, Y. Nishida, Y. Nishi and T. Uemura, 1994 Alternative cell fate choice induced by low-level expression of a regulator of protein phosphatase 2A in the *Drosophila* peripheral nervous system. Development **120:** 1591–1599.

Shoresh, M., S. Orgad, O. Shmueli, R. Werczberger, D. Gelbaum *et al.*, 1998 Overexpression *Beadex* mutations and loss-of-function *heldup-a* mutations in *Drosophila* affect the 3′ regulatory and coding components, respectively, of the *Dlmo* gene. Genetics **150:** 283–299.

Simon, M. A., D. D. Bowtell, G. S. Dodson, T. R. Laverty and G. M. Rubin, 1991 Ras1 and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the sevenless protein tyrosine kinase. Cell **67:** 701–716.

Simpson, P., R. Woehl and K. Usui, 1999 The development and evolution of bristle patterns in Diptera. Development **126:** 1349–1364.

Skeath, J. B., and S. B. Carroll, 1991 Regulation of *achaete-scute* gene expression and sensory organ pattern formation in the *Drosophila* wing. Genes Dev. **5:** 984–995.

Skeath, J. B., and C. Q. Doe, 1998 *sanpodo* and *Notch* act in opposition to *numb* to distinguish sibling neuron fates in the *Drosophila* central nervous system. Development **125:** 1857–1865.

Spana, E. P., and C. Q. Doe, 1995 The prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in *Drosophila*. Development **121:** 3187–3195.

Su, M. T., T. V. Venkatesh, X. Wu, K. Golden and R. Bodmer, 1999 The pioneer gene, *apontic*, is required for morphogenesis and function of the *Drosophila* heart. Mech. Dev. **80:** 125–132.

Sullivan, W., P. Fogarty and W. Theurkauf, 1993 Mutations affecting the cytoskeletal organization of syncytial *Drosophila* embryos. Development **118:** 1245–1254.

Tilney, L. G., M. S. Tilney and G. M. Guild, 1995 F actin bundles in *Drosophila* bristles. I. Two filament cross-links are involved in bundling. J. Cell Biol. **130:** 629–638.

Tilney, L. G., P. Connelly, S. Smith and G. M. Guild, 1996 F-actin bundles in *Drosophila* bristles are assembled from modules composed of short filaments. J. Cell Biol. **135:** 1291–1308.

Tsuda, M., K. Kamimura, H. Nakato, M. Archer, W. Staatz *et al.*, 1999 The cell-surface proteoglycan Dally regulates Wingless signalling in *Drosophila*. Nature **400:** 276–280.

Turner, C. M., and P. N. Adler, 1998 Distinct roles for the actin and microtubule cytoskeletons in the morphogenesis of epidermal hairs during wing development in *Drosophila*. Mech. Dev. **70:** 181–192.

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 **58:** 349–360.

Uemura, T., K. Shiomi, S. Togashi and M. Takeishi, 1993 Mutation of *twins* encoding a regulator of protein phosphatase 2A leads to pattern duplication in *Drosophila* imaginal discs. Genes Dev. **7:** 429–440.

Usui, K., and K.-I. Kimura, 1993 Sequential emergence of the evenly spaced microchaetae on the notum of *Drosophila*. Roux's Arch. Dev. Biol. **203:** 151–158.

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 Doren, M., H. M. Ellis and J. W. Posakony, 1991 The *Drosophila* extramacrochaetae protein antagonizes sequence-specific DNA binding by daughterless/achaete-scute protein complexes. Development **113:** 245–255.

Wang, S., S. Younger-Shepherd, L. Y. Jan and Y. N. Jan, 1997 Only

a subset of the binary cell fate decisions mediated by *Numb*/*Notch* signaling in *Drosophila* sensory organ lineage requires *Suppressor of Hairless*. Development **124:** 4435–4446.

Weigmann, K., and C. F. Lehner, 1995 Cell fate specification by *even-skipped* expression in the *Drosophila* nervous system is coupled to cell cycle progression. Development **121:** 3713–3721.

Whiteley, M., P. D. Noguchi, S. M. Sensabaugh, W. F. Odenwald and J. A. Kassis, 1992 The *Drosophila* gene *escargot* encodes a zinc finger motif found in *snail*-related genes. Mech. Dev. **36:** 117–127.

Wiellette, E. L., K. W. Harding, K. A. Mace, M. R. Ronshaugen, F. Y. Wang *et al.*, 1999 *spen* encodes an RNP motif protein that interacts with Hox pathways to repress the development of head-like sclerites in the *Drosophila* trunk. Development **126:** 5373–5385.

Xiong, W. C., and C. Montell, 1991 *tramtrack* is a transcriptional repressor required for cell fate determination in the *Drosophila* eye. Genes Dev. **7:** 1085–1096.

Xu, T., and G. M. Rubin, 1993 Analysis of genetic mosaics in developing and adult *Drosophila* tissues. Development **117:** 1223–1237.

Zeng, C., S. Younger-Shepherd, L. Y. Jan and Y. N. Jan, 1998a *Delta* and *Serrate* are redundant *Notch* ligands required for asymmetric cell divisions within the *Drosophila* sensory organ lineage. Genes Dev. **12:** 1086–1091.

Zeng, C., N. J. Justice, S. Abdelilah, Y. M. Chan, L. Y. Jan *et al.*, 1998b The *Drosophila* LIM-only gene, *dLMO*, is mutated in *Beadex* alleles and might represent an evolutionarily conserved function in appendage development. Proc. Natl. Acad. Sci. USA **95:** 10637–10642.

Zur Lage, P., A. D. Shrimpton, A. J. Flavell, T. F. MacKay and A. J. Brown, 1997 Genetic and molecular analysis of *smooth*, a quantitative trait locus affecting bristle number in *Drosophila melanogaster*. Genetics **146:** 607–618.

Communicating editor: T. Schüpbach