Drosophila melanogaster chemosensory and muscle development: identification and properties of a novel allele of *scalloped* and of a new locus, SG18.1, in a Gal4 enhancer trap screen

B. V. SHYAMALA[1,2,*] and A. CHOPRA[3]

[1]*Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore 570 006, India*
[2]*National Centre for Biological Sciences, Tata Institute of Fundamental Research, GKVK campus, Bangalore 560 065, India*
[3]*Molecular Biology Unit, Tata Institute of Fundamental Research, Homi Bhabha Road, Mumbai 400 005, India*

Abstract

Our primary interest is to probe into the genetic and molecular mechanisms underlying the development of the chemosensory and neuromuscular systems in *Drosophila melanogaster*. We have generated and characterized 40 Gal4 enhancer trap lines with P-*Gal4* insertion as an attempt to identify genes with a likely role in the development and differentiation of chemosensory and neuromuscular tissues, and at the same time to obtain Gal4 drivers that would facilitate targeted ectopic expression of genes in these tissues. Insertion strain SG18.1 has reporter gene activity in major olfactory components of the adult fly and in their presumptive areas in the imaginal discs. SG29.1 has an insertion in the *scalloped* gene and has been useful in understanding genetic interactions that pattern the wing and in defining the role of *scalloped* in muscle development in flies.

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Introduction

Development of adult tissues in *Drosophila* involves progressive decisions beginning during embryogenesis. These involve cell-fate choice, mediated by intrinsic factors and cell–cell communication, followed by differentiation events which also require recognition of and response to the cellular environment. Chemosensory structures and adult thoracic muscle in *Drosophila melanogaster* provide two distinct and sensitive model systems, wherein a combination of structural, genetic, molecular and behavioural analysis can be used to address basic questions of development and differentiation (VijayRaghavan *et al.* 1991). Unlike the well-studied eye, where neuronal structures are arranged in a highly stereotyped fashion, the olfactory sense organ on the third segment of the antenna bears three different types of sensory bristles—the trichoid, the coeloconic and the basiconic sensilla—distributed as broad domains (Venkatesh and Singh 1984). Each of these sensory bristles is poly-innervated and consists of other support cells, trichogen forming the shaft, tormogen forming the socket, and thecogen forming the sheath cell (Falk *et al*. 1976; Nayak and Singh 1983; Venkatesh and Singh 1984). Studies on the cellular events occurring during olfactory sense organ development (Ray and Rodrigues 1995; Reddy *et al*. 1997) have shown that it is a composite process exhibiting features of development of the eye as well as of the mechanosensory bristles (Dickson and Hafen 1993; Hartenstein and Posakony 1989). There is a primary selection of a founder cell, as seen in bristle development, and then recruitment of neighbouring cells to form sensory neurons and the supporting structures of a chemosensory bristle.

Development of adult thoracic muscles in flies involves specification of cell fate in the adepithelial cells of wing

* For correspondence. E-mail: shyamala@cellbio.emory.edu.

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imaginal discs, their release from the everting discs, their migration to the appropriate position in thorax, and subsequent fusion to form the muscle fibres. Some of these muscles are formed on remnants of larval muscles which serve as templates, whereas others form *de novo* (Fernandes *et al*. 1991; Bate 1993). In other words, thoracic muscle development involves a sequence of precisely programmed events involving various cues and cell–cell interactions.

We are interested in understanding the intricate steps and the mechanisms involved in development and cell-fate specification of the chemosensory pathway as well as the neuromuscular tissue in *Drosophila melanogaster.* The molecules used in these pathways are, most likely, also used in earlier developmental events. Thus, loss-of-function mutations that affect these processes will more often lead to lethality at an earlier stage and their role in development of adult structures might go unidentified. The ability to identify and isolate genes based on their expression pattern has been made possible by the 'enhancer trap technique' developed by O'Kane and Gehring (1987). Many such enhancer trap screens have been carried out since then which have served as a means to isolate new genes and understand their role in development and differentiation in *Drosophila melanogaster* (Bellen *et al*. 1989; Bier *et al.* 1989; Grossniklaus *et al*. 1989; Wilson *et al*. 1989; Anand *et al*. 1990). This enhancer trap technique has been modified by Brand and Perrimon (1993), with the basic intention of achieving targeted expression of a gene of interest in a tissue-specific and cell-type-specific manner. This is a two-part system with the activator, the yeast transcription factor Gal4, in one transgenic strain and the target gene, cloned downstream of the upstream activator sequence (UAS) specific for GAl4, in another strain. Gal4 does not appear to have any endogenous target sites in *Drosophila*, and hence can activate only the gene cloned downstream of the UAS promoter (Fischer *et al*. 1988). Thus the Gal4 method not only allows the identification of genes based on their expression pattern, but also facilitates ectopic expression of genes in defined pattern. Assays can be made readily for dominant, gain-of-function phenotypes that would otherwise be lethal. In this paper we report the generation of 40 *Gal4* insertion strains, analysis of their expression pattern, and chromosomal localization of a subset of them. These strains will be useful to *Drosophila* workers for ectopic expression studies.

We also discuss two insertion strains in greater detail. Their expression pattern and developmental profile make these strains important in the context of our interest in chemosensory pathway and neuromuscular development. SG18.1 is a strain with antennal specific reporter gene expression. The reporter gene in SG18.1 is expressed in the key components of reception, conduction and processing of the olfactory input in adult flies and has a developmental profile of expression. SG29.1 is a *Gal4* insertion in the *scalloped (sd)* gene (Gruneberg 1929). *sd* codes for a TEA family transcription factor, highly conserved from yeast to humans (Campbell *et al*. 1992). The phenotype of this insertion is different from that of all hitherto examined hypomorphic *sd* mutants. This is brought out best in studies on the interaction of the $sd^{29.1}$ mutant with the *Serrate (Ser)* and *Notch (N)* mutants. SG29.1 shows a partial complementation of the wing phenotype and lethality of other *sd* alleles. The human homologue of the product of *sd,* TEF-1, has been shown to interact with the enhancer sequences of HPV16 and SV40 viruses in culture cells (Xiao *et al.* 1991; Ishiji *et al.* 1992). The $sd^{29.1}$ allele will be useful in identifying the other genes that interact with *sd* in *Drosophila* and can give us clues about the probable factors involved in TEF-1 function.

Materials and methods

Enhancer trap screening: The P-*Gal4* insertion strain that was used to mobilize and generate new P-insertion strains was kindly sent by Andrea Brand. The description of the *Gal4* vector and the details of the generation of this transgenic strain is given in Brand and Perrimon (1993). This strain carries a single X-linked *Gal4* insertion, is hemizygous lethal, and is maintained over the FM7 balancer: *w*, P(*Gal4*-lethal; w^+)/FM7. This P-*Gal4* insertion was mobilized to new random sites by crossing to a 'Jumpstarter' strain (Cooley *et al.* 1988): w/w; $+/+$; P(ry^+; Δ2–3) *Sb*/TM2, P(ry^+; Δ2–3), *Ubx*. New P-*Gal4* mobilizations were detected by the presence of w^+ marker. The insertions segregating on X, 2nd and 3rd chromosomes were mapped by classical genetic methods using (i) FM7; $+/+$; $+/+$, (ii) *w*; *Cyo*/*Tft*; $+/+$, and (iii) *w*; $+/+$; TM3, *Sb*, *e*/TM6, *Tb*, *Ubx*, *e* balancer stocks respectively. Descriptions of the markers can be found in Lindsley and Zimm (1992).

Histochemical detection of activator gene expression: Each of the *Gal4* insertion strains was crossed to a transgenic strain with UAS *lacZ* (Brand and Perrimon 1993) on the 2nd chromosome. The F_1 adults were stained for β-galactosidase activity as described in VijayRaghavan *et al.* (1986). Flies were taken on a slide, snap frozen in liquid nitrogen, and cut into longitudinal halves with a razor blade. The thoracic and the head segments were incubated at 37°C in Ficoll containing X-gal staining solution (0.060 ml 5% X-gal, 0.020 ml 100 mM potassium ferrocyanide, 0.020 ml 100 mM potassium ferricyanide, 0.050 ml 1 M sodium phosphate pH 8.0, 0.850 ml 35% Ficoll-100) for 30 min to several hours. The tissue after staining was dehydrated in an ethanol series and mounted in Canada Balsam. Embryos and discs were stained according to the standard protocol of Wilson *et al.* (1989). Dechorionated embroys were fixed with a 1 : 1 mixture of 4% paraformaldehyde and heptane. Following washes with PBS, the embryos were incubated in X-gal staining solution without Ficoll. Larval imaginal discs were fixed in 4% paraformaldehyde prior to staining. Stained embryos and discs were washed in PBS and

mounted in 50% glycerol. Antibody staining on cryosections was done as described in Ashburner (1989). Monoclonal antibodies to *E. coli* β-galactosidase (Promega) were used to detect the enzyme. Antigen–antibody reaction was revealed using a biotin–avidin-coupled horseradish peroxidase kit (Vector Laboratories).

Cytological mapping: Chromosomal localization of the *Gal4* insertion was done by *in situ* hybridization on larval polytene chromosomes as described in Ashburner (1989). Briefly, single-stranded w^+ mini gene fragments were labelled with Bio-16-dUTP (Boehringer Mannheim random primed DNA labelling kit). The labelled fragments were used as probe in hybridization to salivary gland polytene chromosomes, following which the biotinylated DNA was labelled by streptavidin conjugated with horseradish peroxidase (detek-hrp kit, ENZO Bio Labs). The enzyme was detected by a colour reaction using diaminobenzidine in presence of hydrogen peroxide.

Fly stocks for analysing sd Gal4 allele: For complementation test, two P-*lacZ* insertion alleles of *sd*, sd^{ETX4} and sd^{ETX81} (Anand *et al.* 1990), were used. A dominant homozygous viable mutation Ser^D at the Beaded (Serrate) locus (Lindsley and Zimm 1992) was used for analysing the interaction with *Ser*. To study the interaction with *N*, a null allele, N^{XK11} (y, N^{XK11} FRT101/FM7c, *ftz-lacZ*), was used (Lindsley and Zimm 1992).

Wings for analysis: Wings from etherized flies were excised and mounted in Canada Balsam. They were flattened using paper clips while drying.

Molecular mapping of scalloped Gal4 insertion: Polymerase chain reactions using different *sd*-specific and P-element-end-specific primers, with SG29.1 ($sd^{29.1}$) genomic DNA as template, were used to map the *Gal4* insertion in the *sd* genomic region.

Results and discussion

Generation and characterization of Gal4 insertion strains

For the generation of new *Gal4* insertion strains, single-pair crosses were set up with 400 jumpstarter males. Forty new *Gal4* insertions were obtained out of which three were homozygous lethal and two male sterile. The distribution of these new *Gal4* insertions among the three major chromosomes is given in table 1. A general summary of the reporter gene expression pattern in the new *Gal4* insertion strains is shown in table 2. Out of the 40 strains 23 showed expression of the activator gene in muscles and 21 showed expression in the central nervous system, viz. cephalic and the ventral thoracic ganglion. Some of the strains showed specific staining in antenna, proboscis and maxillary palp. All the strains invariably stained in the salivary glands. A similar situation was seen in other *Gal4* strains (Brand and Perrimon 1993), and is thought to be because of a position-dependent salivary gland enhancer that might have been generated during construction of the *Gal4* vector. The expression pattern of the reporter gene in a few representative strains is showed in figure 1 (A–I).

Table 2. Summary of reporter gene activity pattern in the *Gal4* insertion strains.

Tissue with reporter gene expression	No. of enhancer trap strains
Muscles	23
Brain	20
Ventral ganglion	21
Antenna	4
Proboscis	5
Maxillary palp	3
No expression in adults	3

For some of the strains with an 'interesting' expression pattern/phenotype, *in situ* hybridization on polytene chromosomes was done with P-element-specific probes to find the chromosomal position of the *Gal4* insertion. The positions of *Gal4* insertion in these lines are given in table 3. The position of the insertion in SG29.1 and its interaction with *Ser* mutation (see below) suggested that it could be an insertion in the *sd* gene.

SG18.1, a useful strain for studying the development and differentiation of the chemosensory pathway in Drosophila

The genetic factors functioning in specification of cell fate at different steps of development of chemosensory bristles, sensillary type specification, and pattern formation in antenna are a subject of interest. A recent study (Reddy *et al.* 1997) has shown that during the development of an olfactory bristle there is a primary selection of a single founder cell mediated by a *Notch*-gene-dependent lateral inhibition process. The next step is non-lineage-dependent recruitment of cells, which requires cell–cell interactions and receptor-mediated endocytosis, as evidenced by the effect of the *shibire* (*shi*) mutation on the formation of primary sensillar clusters. Following this is at least one round of cell division, differentiation and programmed cell death. *Notch* and *scabrous* (*sca*) mutants have been shown

Table 1. Distribution of *Gal4* insertions in the three major chromosomes.

	Chromosome X	Chromosome 2	Chromosome 3	Homozygous lethals	Sterile
No. of strains	14	17	7	3	2

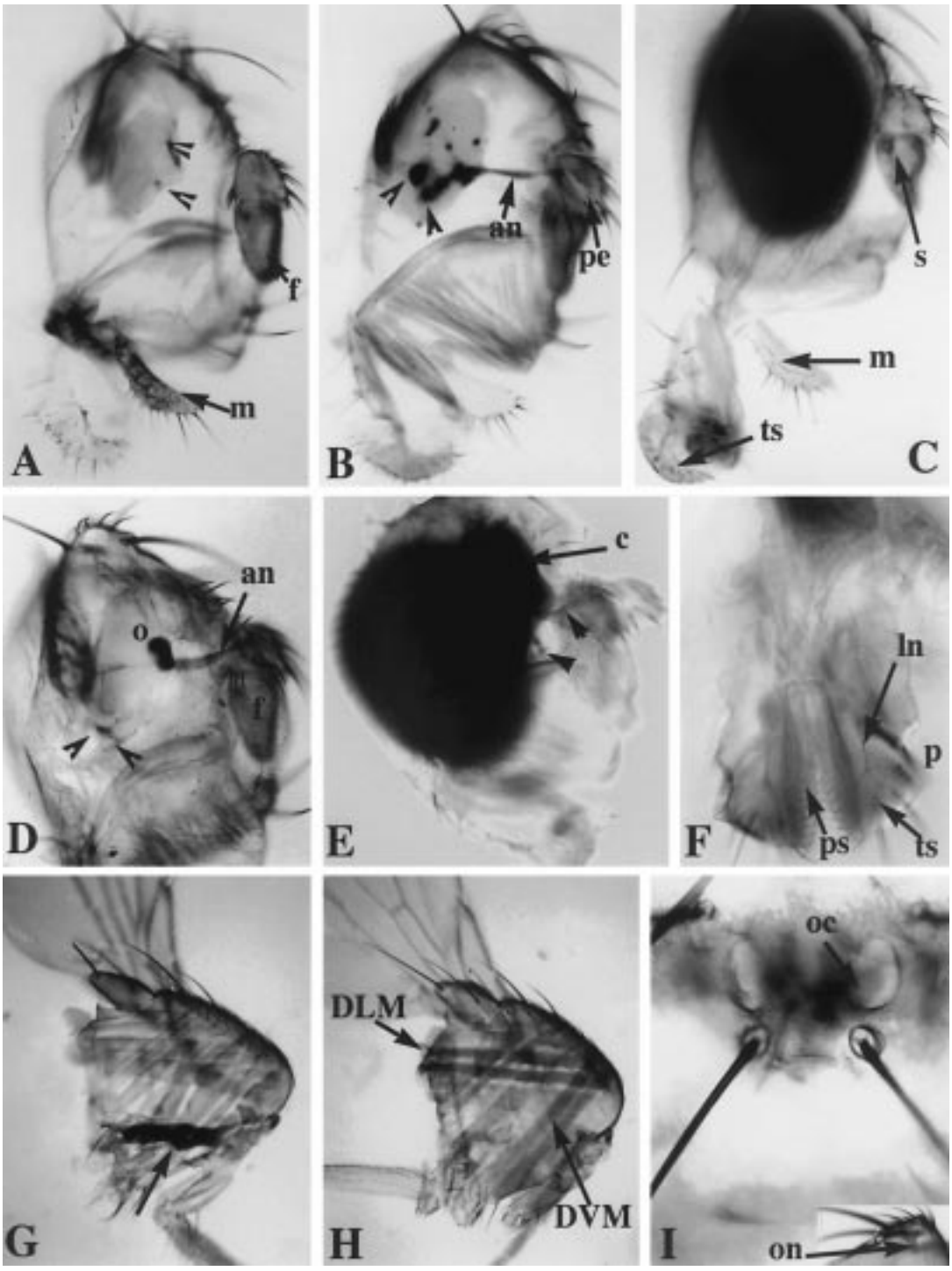


Figure 1. Gal4 expression pattern in some representative Gal4 enhancer trap strains. Transgenic strains with P-*Gal4* insertion were crossed to UAS-*lacZ* strain and the F_1 adults were assayed for β-galactosidase enzyme activity. **A**, SG17.1: Longitudinal section through the cephalic region showing expression in funiculus (f), maxillary nerve (m) and clusters of cells in the brain (arrowheads). **B**, SG26.1: Longitudinal section through the cephalic region; expression is seen in the pedicel of the antenna (pe), antennal nerve (an), and the mechanosensory and motor centres in the brain (arrowheads). **C**, SG14.1: Longitudinal section through the cephalic region; β-galactosidase is expressed in the labellar taste sensilla (ts), maxillary nerve (m), and the sacculus (s). **D**, SG18.1: Longitudinal section through the cephalic region; enzyme activity is seen in the funiculus (f), antennal nerve (an), olfactory lobe (o) and calyx of mushroom body (arrowheads). **E**, SG28.1: Longitudinal section through the cephalic region; the cephalic ganglion (c), and the projections from peripheral afferents and efferents to the cephalic ganglion (arrowheads) show enzyme activity. **F**, SG34.1: The distal proboscis region (p) showing expression in the pseudotracheal ridges (ps), the labellar taste sensilla (ts) and their projections into the labial nerve (ln). **G**, SG28.1: Longitudinal section through the thoracic region; the ventral neural ganglion (arrow) shows strong enzyme activity. **H**, SG5.1: Longitudinal section through the thoracic region of a female fly showing β-galactosidase expression in second set of dorsal longitudinal muscles (DLM) and first set of dorsoventral muscles (DVM); in males both the second and the third sets of DLMs show reporter activity (not shown in the figure). **I**, SG19.1: Frontal region of the head showing expression in the three ocelli (oc); inset shows enzyme activity in the ocellar nerve (on). **F** and **I** are frontal views; in all the other figures, the anterior is to the right.

Table 3. Chromosomal position of *Gal4* insertions in some of the interesting strains.

Strain	Chromosomal position	Features
SG18.1	57A	Antennal specific
SG29.1	13F	Genetic interaction with *Serrate* and *Notch* (insertion in *sd*)
SG5.1	8C	Specific sets of indirect flight muscles
SG17.1	20A	Maxillary palp specific
SG26.1	62B1-2	Mechanosensory centres specific

to affect the differentiation step. Selection of the founder cell is from an equivalent group of cells that delaminate from the epidermal layer. Genes of the *achaete-scute* (*AS-C*) complex function as proneural genes in external sensory bristle development, and *atonal* (*ato*) in photoreceptor and chordotonal organ development (Rodriguez *et al.* 1990; Campuzano and Modolell 1992; Jarman *et al.* 1994). Reddy *et al.* (1997) have shown that *ato* is the proneural gene for the coeloconic sensilla of the olfactory bristles. The proneural gene(s) required for specification of trichoid and basiconic sensilla remain to be discovered. Different alleles of *lozenge* (*lz*) differentially affect the sensilla basiconica and trichoidea, the effect being dependent on the strength of the allele (B. P. Gupta, G. Shirey, U. Banerjee and V. Rodrigues 1997 Role of early patterning gene *lozenge* in the specification of antennal sense organs. Abstract, 38th Annual Drosophila Research Conference, Chicago, USA). Identification of the genetic factors involved in type specification of the olfactory bristles awaits availability of markers specific for different cell types (Reddy *et al.* 1997). This situation requires identification of more genes with probable role in development of chemosensory structures in flies. The SG18.1 strain isolated in the present screen is important in this regard. In this strain, we see reporter gene activity in the olfactory receptors and their processing centres in the adult central nervous system as well as in their probable precursor cells in the imaginal discs during development. In the adult, reporter gene activity is seen in the funiculus, sensory neurons in the maxillary palp, afferents from these sensilla projecting into the antennal lobe which is the primary olfactory association centre in the brain, and their interneurons. Further the calyx of the mushroom body and the relay interneurons extending from the antennal glomeruli to it also show strong reporter gene activity (figure 2, A–C).

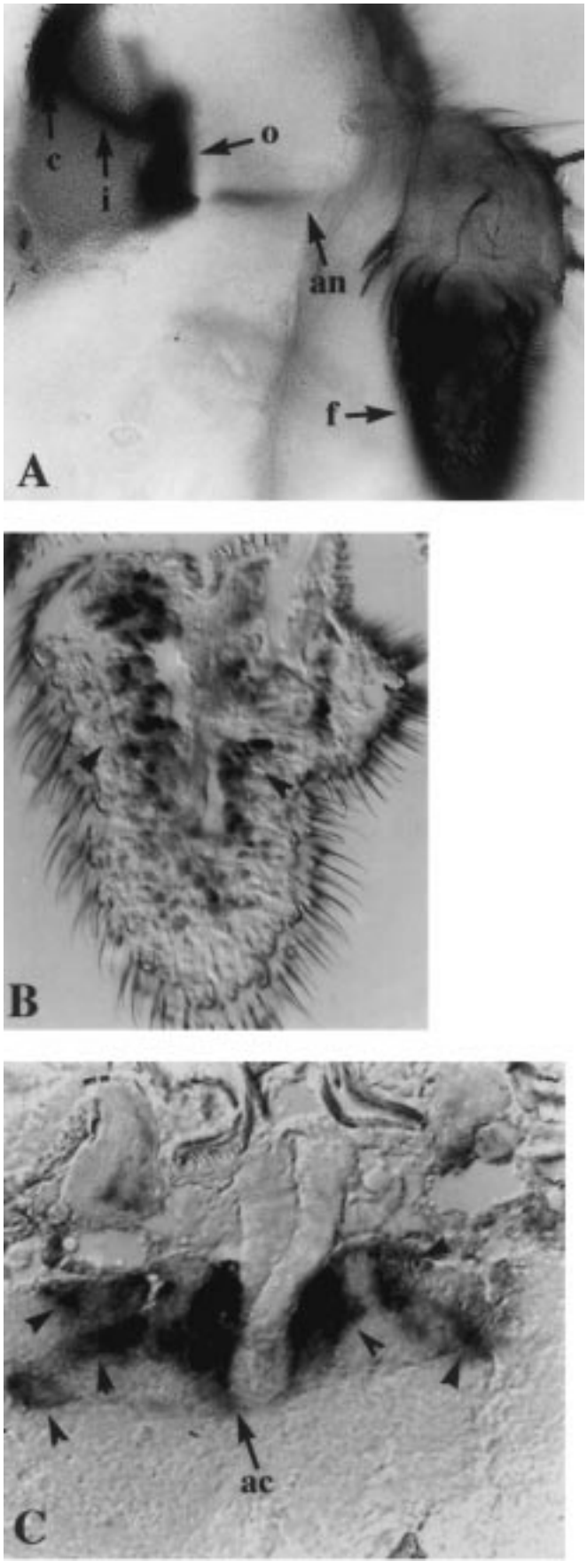
Following the developmental profile of reporter gene activity, we see expression very early, starting from the embryo where it is expressed in the sensory neurons of the peripheral nervous system (figure 3, A & B). The reporter gene is expressed in a ring pattern in the leg disc, and in groups of cells in the dorsal and ventral surfaces of the haltere disc (figure 3, C & D). In the wing imaginal disc, we see staining in the dorsal and the ventral wing blade region (figure 3E). In the eye antennal disc of third instar larva, clusters of cells in the presumptive funiculus and the maxillary palp region show expression (figure 3F). The sensory neurons and antennal nerve show expression of the reporter gene at 54 hours after puparium formation (APF) (figure 3G). The *Gal4* insertion in this line maps to region 57A on the second chromosome. Owing to the fact that the reporter gene in this line is expressed in the neuronal precursors of the discs early during development, we suspect that the native gene might have a role in development of the chemosensory neurons and other key structures in the pathway. Molecular cloning of the native gene and generation of excision mutants for this line are in progress. Partial sequence data of cDNA clones for the native gene have confirmed that it is a new gene (B.V. Shyamala, unpublished data).

Besides carrying an insertion in a novel gene with a putative role in development of the chemosensory pathway, SG18.1 has potential use in ectopic expression of any gene in the chemosensory organs and their precursors. Expression pattern of the reporter gene in heterozygous combination (SG18.1, +/+, UAS-*lacZ*) differs from the expression pattern seen in homozygous combination (SG18.1, UAS-*lacZ*/SG18.1, UAS-*lacZ*) of the insertion. Expression in the embryo and in the antennal disc are seen in the homozygous state but are not detectable in heterozygotes. Expression domains in the leg and wing imaginal discs remain the same but are more restricted in heterozygotes, with reporter gene activity in small discrete pockets of cells in the respective domains. This allows us to obtain different degrees of ectopic expression of a gene, using the same *Gal4* insertion in homozygous or heterozygous combination. Furthermore, we can mutagenize these flies and screen for specific mutations that affect structures of the chemosensory pathway.

sd$^{29.1}$, *a new Gal4 insertion allele of sd*

The *sd* gene encodes a DNA-binding protein that contains a TEA domain, which is a highly conserved sequence and found in transcription factors like AbaA protein from *Aspergillus nidulans*, TEC1 protein from *Saccharomyces cervisiae* and the human TEF-1 (Campbell *et al.* 1992). Hypomorphic mutants of *sd* affect the morphology of the adult wing. They show scalloping of the wing margin caused by increased cell death in the wing disc and/or loss of sensory structures along the wing margin (James and Bryant 1981; Simpson *et al.* 1981). In the present enhancer screen we have isolated a new allele of *sd*, *sd*$^{29.1}$, an allele with P-*Gal4* insertion.

Insertion line SG29.1, with a weak wing scalloping phenotype (figure 4B), created interest because of the strong wing phenotype it gave with an allele of *Ser*, *Ser*^{D}, in transheterozygous condition. *In situ* hybridization with P-element-specific probes revealed that it maps to region 13F



on the X chromosome, which is the same as the cytological region of the *sd* gene. Complementation analysis was carried out with other *sd* alleles, sd^{ETX4} and sd^{ETX81} (Anand *et al.* 1990). sd^{ETX4} is a recessive, hypomorphic allele with extensive nicking of the wing blade (figure 4C). sd^{ETX81} is a pupal lethal allele. Both are P-*lacZ* insertion alleles. In trans-heterozygous condition with sd^{ETX4}, SG29.1 shows only partial complementation of the wing phenotype. The heterozygotes show slight nicking of the wing comparable to the phenotype of SG29.1 homozygotes (figure 4B). In sd^{ETX81}/SG29.1 heterozygotes, the lethality is rescued but the wing phenotype is not complemented (figure 4D). Thus complementation tests suggest that the *Gal4* insertion gene in SG29.1 is an allele of *sd*. Molecular mapping of the *Gal4* insertion within *sd* was done by PCR. A *sd* genomic-sequence-specific primer with P-specific primers gives an amplified fragment of 0.5 kb (figure 5). The *Gal4* insertion thus maps in the first large intron after the translational start site of *sd* cDNA, close to the 5′ splice site. In most of the viable insertion alleles of *sd*, the P insertions have been localized within 2 kb around the 5′ end and are thought to affect the regulatory region of the gene (Anand *et al.* 1990; Campbell *et al.* 1991, 1992), whereas in $sd^{29.1}$ the *Gal4* insertion maps downstream of the start codon in the ORF (figure 5).

$sd^{29.1}$ is expressed in adult muscle progenitors

While the expression pattern of the reporter gene in $sd^{29.1}$ insertion line is by and large comparable to that seen in the other *sd* P-*lacZ* alleles (Campbell *et al.* 1992), there are significant differences (figure 6, A–D). Unlike in *ETX4*, where subsets of neurons in the adult brain show expression (Campbell *et al.* 1992), in the adult flies here we see a strong expression of the reporter gene in the cephalic ganglion, afferents from the antenna to it, sensory neurons in the maxillary palp, antennal neurons, and the ventral thoracic ganglion. A special feature of $sd^{29.1}$ is its strong expression in the larval myoblasts and developing and adult muscles. Strong expression is seen in the muscles of the proboscis and the dilator muscles in the cibarial pump region of the head (figure 6A). In the thorax it strongly

Figure 2. Gal4 expression pattern in adult flies of the antennal specific strain SG18.1. *Gal4* insertion strain was crossed to UAS-*lacZ* strain and the F_1 adults were assayed for β-galactosidase enzyme activity as described in Materials and methods. **A**, Longitudinal section through the head (anterior to the right); enzyme activity is seen in funiculus (f), antennal nerve (an), olfactory lobe (o), interneurons between the olfactory lobe and calyx of mushroom body (i), and the calyx of mushroom body itself (c). **B**, Longitudinal section through the funiculus of antenna, showing strong enzyme activity in the trichoid sensory neurons (arrowheads). **C**, Horizontal section through the head at the level of the olfactory lobe; the β-galactosidase in this case was detected by monoclonal antibody staining (Materials and methods); activity is seen in specific olfactory glomeruli (arrowheads), and the antennal commissure (ac) connecting the contralateral olfactory glomeruli.

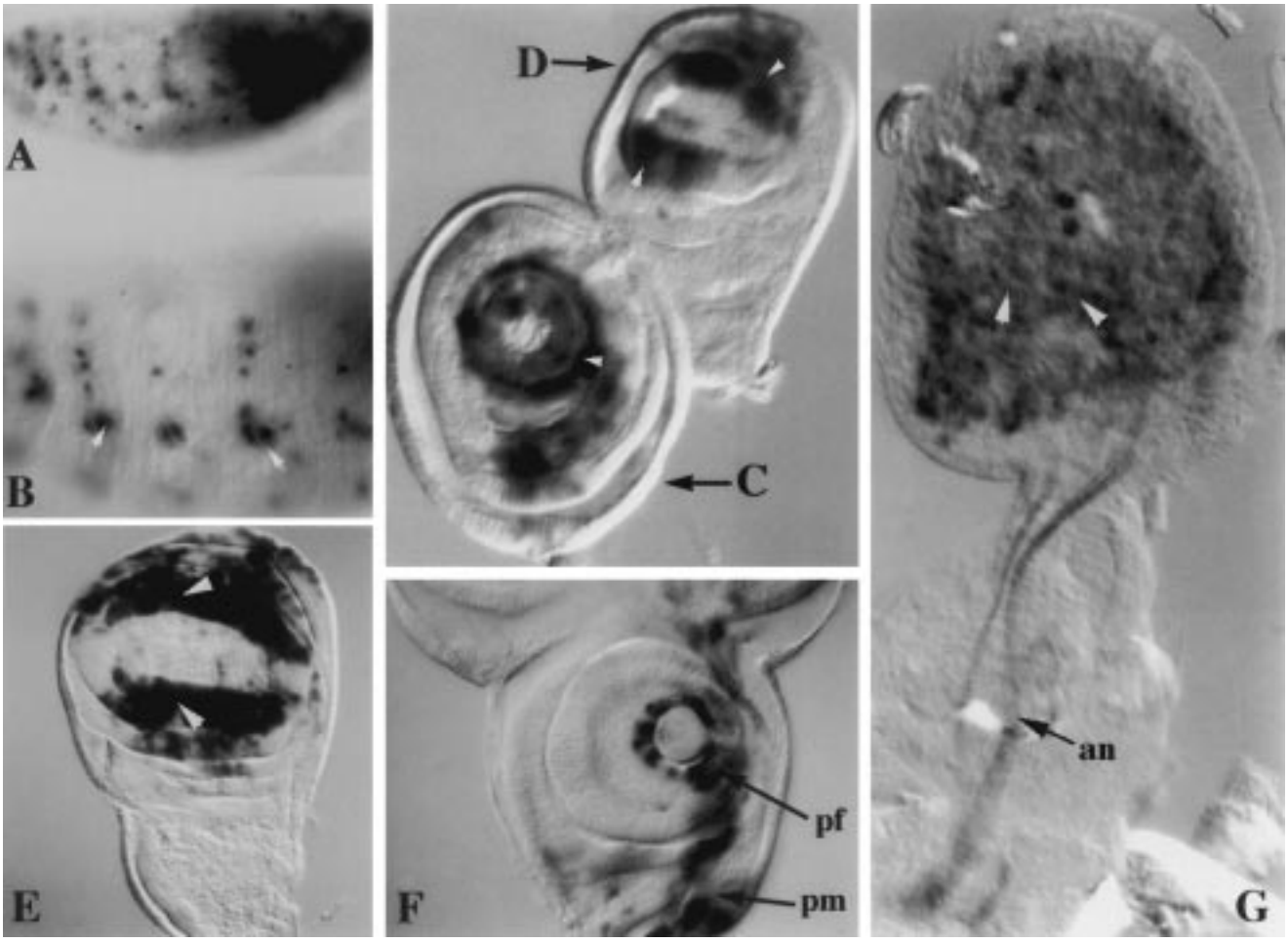


Figure 3. Gal4 expression pattern in SG18.1 during development. The *Gal4* insertion strain was crossed to UAS-*lacZ* strain and the progeny were assayed for β-galactosidase activity during development. **A**, Lateral view of a stage 13 embryo with anterior end to the left and dorsal surface up, and **B**, an enlarged portion of the same showing enzyme activity in peripheral nervous system (arrowheads). **C**, One of the leg discs from third instar larva showing expression in clusters of cells forming a ring pattern (arrowhead). **D**, Haltere disc from third instar larva with expression in the presumptive dorsal and the ventral surfaces (arrowheads). **E**, Wing imaginal disc from third instar larva; enzyme activity is seen in the presumptive dorsal and the ventral wing blade region. **F**, Antennal disc from third instar larva showing expression in clusters of cells in the presumptive funiculus (pf) and the maxillary palp (pm) region. **G**, Longitudinal section through the developing antenna at 54 h APF; strong β-galactosidase activity is seen in the sensory neurons (arrowheads) and the antennal nerve (an). **A–F** are from individuals in homozygous combination, **G** is from a heterozygous individual (see text for details).

stains the indirect flight muscles (figure 6B). The wing disc in the third instar larva shows expression in the presumptive wing blade and the scutellar region. More interestingly, we see strong expression in myoblasts in the presumptive notum of the wing disc, where the progenitors of the adult flight muscles are located (figure 6C). During the pupal stage, strong expression is seen in myoblasts of the developing indirect flight muscles (figure 6D).

sd had earlier been shown to be involved in peripheral nervous system and wing development. The muscle-specific expression of $sd^{29.1}$ during development and in adult suggests its putative role in muscle development. This strain gives us an access to understanding the role of *sd* in muscle development and differentiation by way of misexpression and genetic interaction studies (Roy *et al.* 1997). Three different transcripts have been identified so far for *sd* and it is predicted that there might be many more splice variants for the gene (Campbell *et al.* 1991, 1992). Further we also see allele-specific mutant phenotypes such as the wing scalloping, appearance of ectopic bristles (Campbell *et al.* 1992), gustatory phenotype (Anand *et al.* 1990; Inamdar *et al.* 1993), and interaction with other genes (M. Inamdar, unpublished; and present study). Scalloped as a transcription factor appears to have multiple roles in different cell types and different stages of development. Allele-specific expression of different enhancer trap strains also indicates that there might be many regulatory elements scattered along the length of the gene that drive the expression of different transcripts in a tissue-specific manner.

sd-Gal4 allele shows strong genetic interactions with Ser and N

Yet another feature of $sd^{29.1}$ is the interaction phenotype it shows with *Ser* and *N* mutants. $sd^{29.1}/+$ individuals

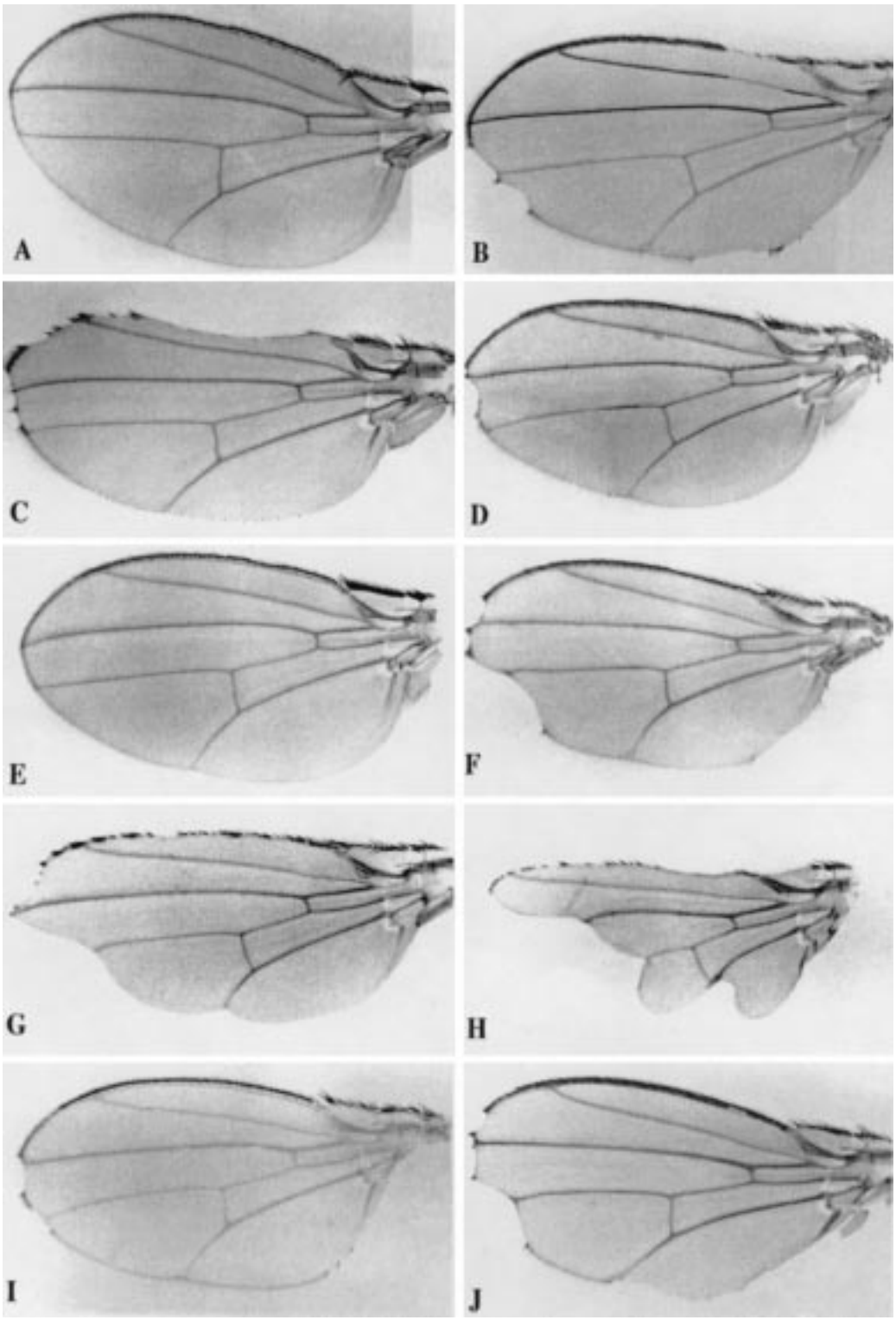


Figure 4. Different wing phenotypes demonstrating partial complementation and the strong interaction effects of $sd^{29.1}$ allele with other mutants. **A**, Canton S, wild type. **B**, $sd^{29.1}/sd^{29.1}$ wing; note the slight nicking at the tip of the wing. **C**, sd^{ETX4}/sd^{ETX4} wing, showing typical wing scalloping at the anterior margin and the tip. **D**, $sd^{29.1}/sd^{ETX4}$ wing, showing partial complementation; the phenotype is reduced to slight nicking at the tip. **E**, $sd^{29.1}/+$: in heterozygous condition $sd^{29.1}$ wing shows a wild-type phenotype. **F**, $Ser^{D}/+$: a dominant gain-of-function mutant of *Ser* in heterozygous condition shows slight scalloping of the wing blade along the tip and towards the posterior margin. **G**, $sd^{29.1}/+; ser^{D}/+$ wing showing the interaction effect; there is an enhancement of scalloping with total loss of sensory bristles along the posterior margin and some bristles along the anterior margin also missing. **H**, Hemizygous $sd^{29.1}$ with $Ser^{D}/+$ has much stronger interaction phenotype with the posterior margin deeply scalloped; sensory hairs are completely missing from the posterior margin and anterior margin has very few of them. **I**, Wing phenotype of *N* null heterozygote y, N^{XK11}, FRT101/FM7c, *ftz-lacZ*; note small notches at the tip of the wing. **J**, $sd^{29.1}/y, N^{XK11}$, FRT101 transheterozygote showing the interaction phenotype; there is a striking enhancement of the nicking along the tip and the posterior margin of the wing and the veins show delta-like thickening at the end.

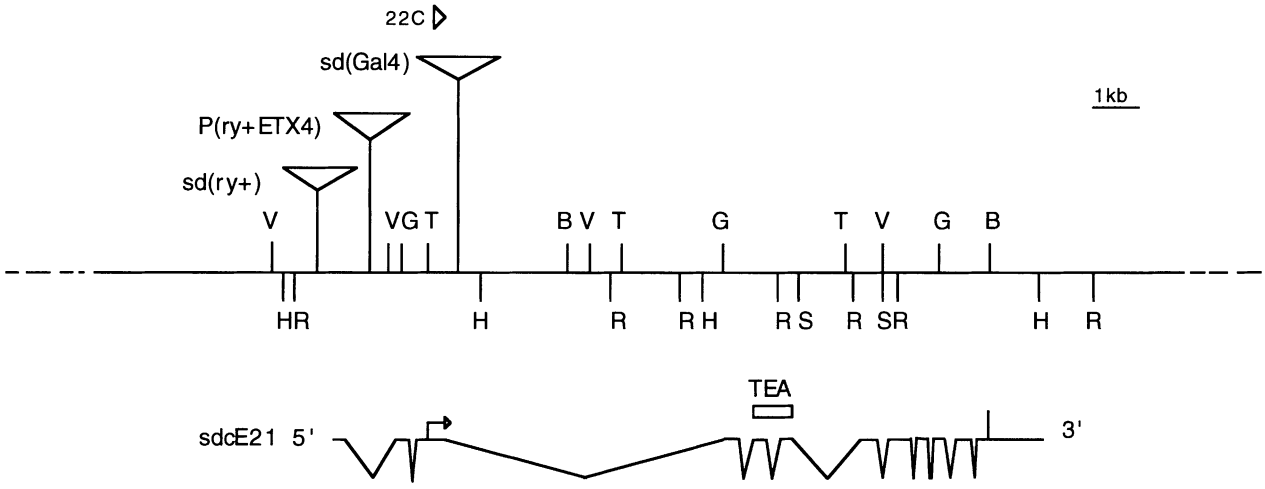


Figure 5. Molecular mapping of the *Gal4* insertion in the *sd* genomic region. Figure shows map of intron/exon structure of *sd* cDNA clone sdcE21, with relative positions of exons (horizontal portions) aligned with the *sd* genomic clone *cos*20. Selected restriction sites are indicated on the genomic clone. Abreviations used are B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; G, *Bgl*I; S, *Sal*I; T, *Pst*I; V, *Pvu*II. The position of the forward primer 22C specific for *sd* genomic region is aligned with the genomic clone. (This figure is drawn according to the details given in Inamdar 1994.) The start and the stop codons of the long open reading frame (ORF) for the cDNA clone sequenced by Campbell (1990) are shown and 5′–3′ orientation is indicated. The positions of insertion of two P elements, sd^{ETX4} (Anand *et al*. 1990) and $sd^{(ry+)}$ (Daniels *et al.* 1985) are indicated as shown in Campbell *et al.* (1992). Mapping of the *Gal4* insertion in the *sd* genomic region was done by carrying out PCRs with *sd* genomic-sequence-specific primers and P-element-specific primer, using genomic DNA from SG29.1 strain as template. The forward primer 22C with P-specific primer gives an amplified fragment of 0.5 kb. Thus the position of the insertion maps at the beginning of the first intron after the translational start site.

have wild-type wing phenotype (figure 4E). Flies that are transheterozygotes for Ser^D and $sd^{29.1}$ (figure 4G) show a strong enhancement of the wing phenotype which now looks similar to that of Ser^D/Ser^D. There is a deep scalloping of the wing blade along the posterior margin. All the hairs on the posterior margin, and some of the bristles on the anterior margin, are missing. In $sd^{29.1}$ in hemizygous state and Ser^D heterozygotes, the phenotype is much more pronounced. Scalloping is deeper so as to give a somewhat lobed appearance to the posterior margin. There are no hairs along the posterior margin and very few bristles along the anterior margin (figure 4D). To study the interaction of $sd^{29.1}$ with *N*, a $sd^{29.1}$ male was crossed to *N* null female balanced over FM7 (*N*/FM7). This allele of *N* in heterozygous condition shows slight nicking of the wing margin (figure 4I). The transheterozygotes (+, $sd^{29.1}/N$, +) on the contrary showed more enhanced nicking of the wing margin along with a delta-like phenotype of the wing veins (figure 4J).

The interaction phenotypes shown by $sd^{29.1}$ are much more dramatic and obvious than what is seen with the other hypomorphic *sd* alleles so far described (M. Inamdar, unpublished). $sd^{29.1}$ allele probably provides a more sensitized background to study such interactions. *sd* has

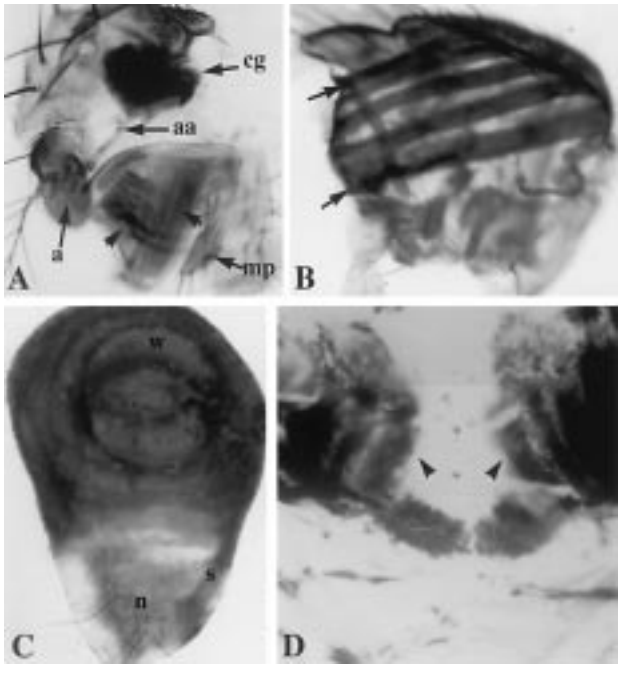


Figure 6. *Gal4* expression pattern in the *sd-Gal4* insertion strain SG29.1. The SG29.1 strain was crossed to UAS-*lacZ* strain and the F_1 were assayed for β-galactosidase enzyme activity during development and in adult. **A**, Longitudinal section through the cephalic region, with anterior end to the left; strong expression of the reporter gene is seen in the cephalic ganglion (cg), afferents from antenna to it (aa), neurons in the maxillary palp (mp) and the antennal neurons (a); muscles in the proboscis (arrowheads) show strong enzyme activity. **B**, Longitudinal section through the thoracic region with strong expression in the indirect flight muscles (arrows). **C**, Wing imaginal disc of third instar larva; expression is seen in the presumptive wing blade (w), and the scutellar (s) and notum (n) regions. **D**, 12 h APF pupa dissected out, showing strong enzyme activity in the myoblasts that are going to form the adult indirect flight muscles (arrowheads).

been shown to be involved in various *N*-mediated cell signalling pathways in cell fate specification in wing blade development in flies (Cohen 1993). $sd^{29.1}$ becomes important in this context to understand the exact nature and different components of interactions and to place the *sd* gene in its position in these pathways. Vertebrate homologues of several genes of the *Notch* pathways have been identified and studies indicate that the molecular mechanisms in flies and mammals are not too different from one another (Holland *et al.* 1992; Scott 1994). Functional homology of human TEF-1 to *Drosophila* Scalloped has been recently shown by Deshapande *et al.* (1997). Understanding the precise role of *sd* in development and the interaction mechanisms in flies might throw light on the nature of *in vivo* functions and interactions of TEF-1. Further, a large number of 'local hops' of this strain have also been generated (T.K. Rajendra and B.V. Shyamala, unpublished). These lines provide us with *sd-Gal4* alleles of various degrees of severity in phenotype, and also allow us to express other genes under different *sd* regulatory elements, specially the *sd* homologues from other species, and to study their functional homology.

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