

Cooperative Regulation of Cell Polarity and Growth by *Drosophila* Tumor Suppressors

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Loss of cell polarity and tissue architecture are characteristics of malignant cancers derived from epithelial tissues. We provide evidence from *Drosophila* that a group of membrane-associated proteins act in concert to regulate both epithelial structure and cell proliferation. Scribble (Scrib) is a cell junction-localized protein required for polarization of embryonic and, as demonstrated here, imaginal disc and follicular epithelia. We show that the tumor suppressors *lethal giant larvae* (*lgl*) and *discs-large* (*dlg*) have identical effects on all three epithelia, and that *scrib* also acts as a tumor suppressor. Scrib and Dlg colocalize and overlap with Lgl in epithelia; activity of all three genes is required for cortical localization of Lgl and junctional localization of Scrib and Dlg. *scrib*, *dlg*, and *lgl* show strong genetic interactions. Our data indicate that the three tumor suppressors act together in a common pathway to regulate cell polarity and growth control.

Cells in epithelial sheets are characterized by columnar or cuboidal shape, strong cell-cell adhesion, and pronounced apicobasal polarity. However, tumors of epithelial origin lose these characteristics as they progress from benign growth to malignant carcinoma, and this loss is associated with poor clinical prognosis. We have used *Drosophila* genetics to study the morphogenesis of epithelia, in order to illuminate the relation between epithelial organization and oncogenesis.

The multi-PDZ (PSD-95, Dlg, ZO-1) domain protein Scrib is required for proper polarity and morphogenesis of embryonic epithelia (1). To test whether the requirement for Scrib is limited to the embryo, we examined the role of *scrib* in follicle cells, a monolayered epithelium of somatic cells that encases the germ line in the adult female ovary. Scrib was localized to lateral follicle cell membranes (Fig. 1A), and clones of cells (2) that lack *scrib* function became round and multilayered (Fig. 1C) with polarity defects, similar to the phenotype of embryonic epithelia lacking *scrib* function. The epithelial defects of *scrib* mutant follicle cells are cell autonomous. These data indicate that *scrib* is required within cells from multiple tissues for proper epithelial structure.

In a screen to identify additional mutations causing epithelial defects in follicle cell clones (FCCs), we isolated a mutation (*4w3*) that produces a phenotype similar to that of *scrib*. Mapping and complementation analysis demonstrated that *4w3* is a strong allele of *lgl*, which encodes a cytoplasmic, myosin-

associated protein with WD-40 repeats (3, 4). FCCs of the null allele *lgl^d* showed loss of cell shape and monolayer organization as seen in *lgl^{4w3}* FCCs (Fig. 2B). Furthermore, this phenotype resembles that seen in FCCs for strong alleles of *dlg* (Fig. 2C) (5), which encodes a multi-PDZ domain protein (6). The phenotypes of *scrib*, *lgl*, and *dlg* FCCs are indistinguishable.

Because follicle cell epithelia require *scrib*, *lgl*, and *dlg*, we examined the function of *lgl* and *dlg* in the embryonic epidermis, where *scrib* acts to restrict apical proteins and adherens junctions to their appropriate positions within the cell membrane (1). We stained embryos lacking both maternal and zygotic contributions of *lgl* and *dlg* [(7), hereafter referred to as *lgl* and *dlg* embryos] with antibodies to polarized proteins and cellular junction components (8). During mid-embryogenesis, *lgl* and *dlg* embryos showed defects in apicobasal polarity, revealed by aberrant distribution of the apical protein Crumbs (Crb) (9) (Fig. 2, H and I) and disruption of adherens junctions (Fig. 2, E and F). These defects are similar to those of *scrib* embryos; the terminal phenotypes of *scrib*,

lgl, and *dlg* embryos, as indicated by cuticle deposition, are also nearly identical (Fig. 2, K and L) (6, 10). Thus, *lgl* and *dlg*, like *scrib*, act to properly localize apical proteins and adherens junctions to organize epithelial architecture in embryos.

The similarity of mutant phenotypes in different epithelia suggests that the three proteins are components of the fundamental machinery that creates the distinctive architecture of epithelial cells and tissues. To test this assertion, we compared the *scrib* phenotype to that of *lgl* and *dlg* in a third major epithelium, the larval imaginal disc. Discs isolated from late third instar larvae zygotically mutant for *scrib* (11) were profoundly disorganized and also massively overgrown. *scrib* discs (Fig. 3, C and D) contained 4.7 times as many cells as wild-type (WT) discs (4.17×10^5 versus 8.82×10^4) (11) and consisted of spherical masses of tightly packed cells as opposed to the folded monolayer epithelium seen in WT larvae. The apical polarization of actin evident in WT discs (Fig. 3E) was absent in *scrib* discs (Fig. 3F). This loss of epithelial organization accompanied by overproliferation corresponds to the phenotype described for *lgl* and *dlg* zygotic mutant discs (12, 13). Additional features of *lgl* and *dlg* larval phenotypes, such as overgrowth of brain tissue, were also present in *scrib* larvae (Fig. 3H). Together, these data indicate that *scrib* and the two previously characterized *Drosophila* malignant neoplastic tumor suppressors, *lgl* and *dlg* (12), share a role in growth control as well as epithelial polarity.

The equivalent requirements for *scrib*, *lgl*, and *dlg* in epithelial development could result from independent activity of each gene in a separate pathway or from collaborative activity of the three genes in a single pathway. To address this issue, we tested for genetic interactions between the three mutations and found strong interactions of *dlg* and *lgl* with *scrib*. Most embryos zygotically mutant for *scrib* hatch and survive into late larval stages (see above). However, embryos homozygous for *scrib* and additionally heterozygous for *dlg* died before hatching, with evident defects

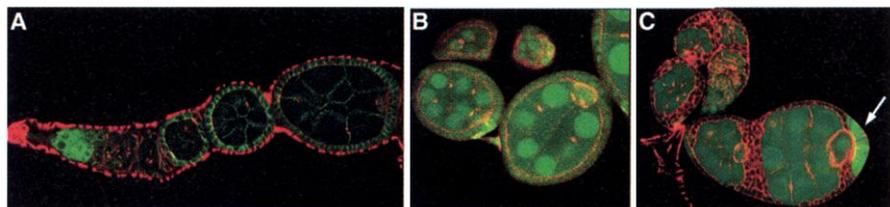


Fig. 1. Scrib is required for follicular epithelium morphogenesis. (A) Ovary costained for Scrib (green) and cortical actin (red) reveals expression in the lateral membranes of the follicle cell epithelium from stages 2b to 6. Scrib is also found in region 1 of the germarium and at low levels on germ cell membranes. (B) Wild-type ovary expressing green fluorescent protein (GFP) stained with rhodamine phalloidin displays the regular monolayered cuboidal epithelium. (C) Follicle cells homozygous for *scrib* (lacking GFP) (2) are rounded and often multilayered. Groups of cells carrying a WT *scrib* allele (arrow) maintain the WT epithelial monolayer.

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in dorsal closure (Fig. 4A). Dorsal closure phenotypes are characteristic of reduced activity of both *dlg* (10) and *lgl* (14). Additionally, embryos homozygous for both *lgl* and *scrib* displayed a cuticle phenotype nearly as severe as those of *lgl* or *scrib* null embryos (Fig. 4B). Heterozygosity for *lgl* also enhanced the imaginal disc phenotype of *scrib* hypomorphic larvae. These dose-sensitive interactions of *dlg* and *lgl* with *scrib* suggest that the three genes function in a common pathway.

We further explored the relation between *scrib*, *lgl*, and *dlg* by comparing the subcellular localization of the gene products (8). Scrib and Dlg colocalized throughout development, in particular at the apical margin of the lateral membrane (ALM) of the embryonic epidermal epithelium (Fig. 5A). Colocalization at the ALM occurred after gastrulation and persisted in mature epithelia, where the ALM is the site of the septate junction (15). Lgl protein was not exclusively associated with the plasma membrane and was not polarized along it (4); however, it overlapped substantially with Dlg and Scrib at the ALM (Fig. 5B).

We investigated epistatic relations between *scrib*, *lgl*, and *dlg* by determining the localization of each protein in embryos mutant for the other two genes. We first assayed the localization of Dlg and Scrib to the ALM. In all mutant blastoderms, Scrib and Dlg were associated with ingrowing cell membranes, as in WT. However, after gastrulation, when WT embryos display an enrichment of Scrib in the ALM (Fig. 5C), *lgl* embryos showed Scrib (Fig. 5D) and Dlg (Fig. 5H) localized throughout the basolateral cell membrane, a misdistribution that persists into late embryogenesis. Like *lgl* embryos, *scrib* embryos failed to polarize Dlg to the ALM (Fig. 5E), while *dlg* embryos not only failed to polarize Scrib to the ALM (Fig. 5F), but also displayed a progressive loss of membrane-associated Scrib (Fig. 5G).

We next examined the distribution of Lgl, which normally has both a membrane-bound and a cytosolic component (4); reduction of activity in an *lgl* temperature-sensitive mutant correlates with loss of the membrane-bound pool (14). In WT embryos, Lgl was in close apposition to cell membranes (Fig. 5, I and J). However, in *scrib* blastoderms and embryos, Lgl was distributed throughout the cytoplasm (Fig. 5, K and L). *dlg* blastoderms showed intermediate defects in Lgl distribution (Fig. 5M), but by mid-embryogenesis loss of membrane-localized Lgl was evident (Fig. 5N). The dissociation of Lgl from the membranes of *dlg* embryos parallels the loss of Scrib seen in these embryos. These data indicate that *dlg* is required for the stable

association of Scrib with the cell membrane, and that *scrib* is required for the cortical association of Lgl; all three genes act to localize Scrib and Dlg to the ALM.

Our results provide strong evidence that Scrib, Dlg, and Lgl act in a common pathway to regulate cell architecture and cell proliferation control. Of the ~50 *Drosophila* genes in which mutation gives rise to overproliferation (16), only *scrib* shares with *dlg* and *lgl* the concomitant loss of

tissue organization that groups the three together as malignant neoplastic tumor suppressors (12). Previous analyses have described a role for *dlg* and *lgl* in imaginal disc polarity (17, 18); the demonstration in this work of genetic interactions with *scrib* and codependence for protein localization indicates a functional link between the three tumor suppressors. Furthermore, involvement of the tumor suppressors in embryonic epithelial polarity provides a well-

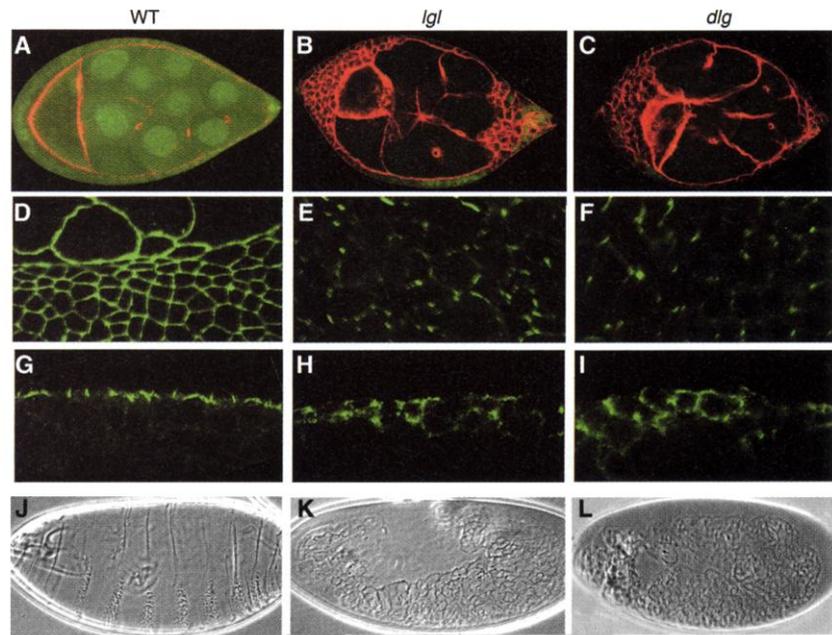


Fig. 2. *lgl* and *dlg* phenocopy *scrib* in follicular and embryonic epithelia. *lgl* and *dlg* mutations cause defects in follicle cell morphology (A to C), embryonic adherens junction formation at stage 10 (D to F), polarization of apical proteins at stage 11 (G to I), and cuticle deposition (J to L), phenotypes also exhibited by *scrib* mutations. Stains: (A to C) GFP, green; and phalloidin, red; (D to F) anti-Arm seen en face; (G to I) anti-Crb seen in cross section with apical surface oriented upward.

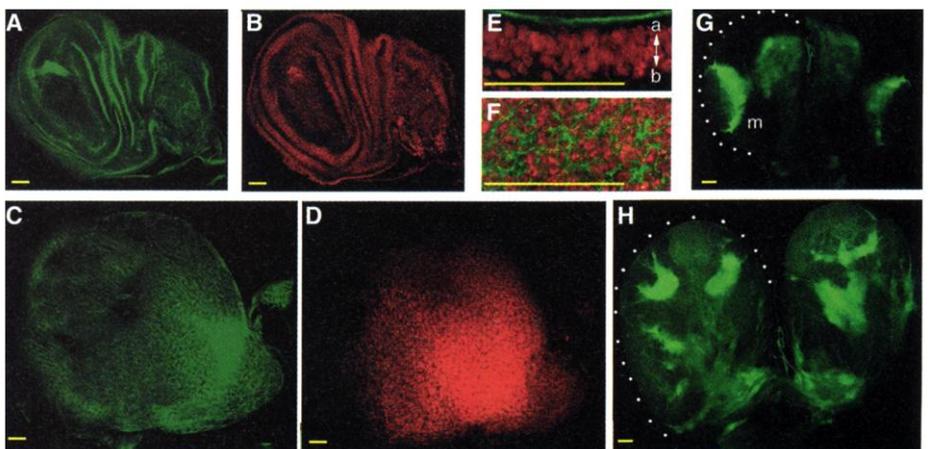


Fig. 3. *scrib* is an imaginal disc tumor suppressor. Confocal microscope sections of WT (A and B) and *scrib* (C and D) wing discs stained for phalloidin (green) and propidium iodide (red) to reveal cell shape and DNA, respectively. The well-organized, folded monolayer epithelium in WT is apparent; the much larger *scrib* disc shows no signs of organization. High-magnification images of WT (E) and *scrib* (F) discs stained as above demonstrate that the regular shape and apical polarization of actin seen in WT cells are totally absent in *scrib* (a↔b, apicobasal polarity). Wild-type (G) and *scrib* (H) larval brains stained with phalloidin. The *scrib* brain lobes (outlined in dots) are much larger than in WT, and the axon-rich medulla (m) is disorganized.

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studied context in which to understand their activities (19). Our findings suggest that, in the WT gastrula, intrinsic, perhaps adhesion-based cues localize Dlg at the ALM; Dlg stabilizes Scrib at this position, and finally Scrib acts on the cortical cytoskeleton to bring Lgl to the membrane. The three proteins may then collaborate to maintain the proper distribution of polarized factors, including themselves.

The correlation between loss of membrane-associated Lgl in *scrib* and *dlg* mutants and defective cell polarity suggests models of action for this group of proteins. Whereas the PDZ domains of Scrib and Dlg are likely to bind to transmembrane proteins that organize the epithelial cell surface, the role of Lgl in polarity determination may derive from its function in target-

ed secretion of membrane proteins. Lgl homologs from humans and yeast can bind to plasma membrane t-SNARE proteins and promote the fusion of cargo-carrying vesicles with target membranes (20, 21). In yeast undergoing polarized growth, the broadly distributed Lgl homologs function primarily at the bud tip, the site of the "exocyst" complex required for vesicle trafficking and addition (22). In vertebrate epithelia, exocyst components are found at the tight junction (23), a structure analogous to the septate junction where Dlg and Scrib localize (15). In *Drosophila* epithelia, recruitment of Lgl into the proximity of membrane t-SNAREs requires proper localization of Scrib and Dlg, thus potentially linking the transmembrane proteins that establish polarity to the protein-targeting system that preserves it.

In many epithelial-derived cancers, cytoarchitectural changes are hallmarks of oncogenic transformation. The disruption of epithelial architecture seen in *scrib*, *dlg*, and *lgl* animals could affect growth control by several mechanisms. Many growth factor receptors are polarized to a specific membrane domain, and mislocalization of such proteins may affect signaling pathways that maintain cells in a differentiated, nonproliferative state. Additionally, the aberrant cell-cell junctions formed in *scrib*, *dlg*, and *lgl* mutants could compromise contact inhibition. Finally, disruption of cell-cell contacts may release junction-localized signaling components, such as Arm or APC, that have been implicated in regulating cell proliferation (24); indeed, a human Dlg homolog has been shown to bind APC and associate with β -catenin, the human homolog of Arm (29). Because the modes of action of Scrib, Dlg, and Lgl are likely to be conserved between vertebrates and invertebrates, investigation into a tumorigenic role for the multiple human homologs of these genes (25–28) is warranted. Further analysis of the mechanisms by which Scrib, Dlg, and Lgl keep *Drosophila* cell growth in check will likely enhance our understanding of mammalian oncogenesis as well.

Fig. 4. *dlg* and *lgl* genetically interact with *scrib*. Zygotic *scrib/scrib* embryos hatch into larvae, but *dlg/+; scrib/scrib* embryos die with defects in dorsal closure (A, arrow). Zygotic *lgl/lgl; scrib/scrib* embryos (B) die exhibiting phenotypes similar to that of complete absence of maternal and zygotic *lgl* or *scrib* gene product.

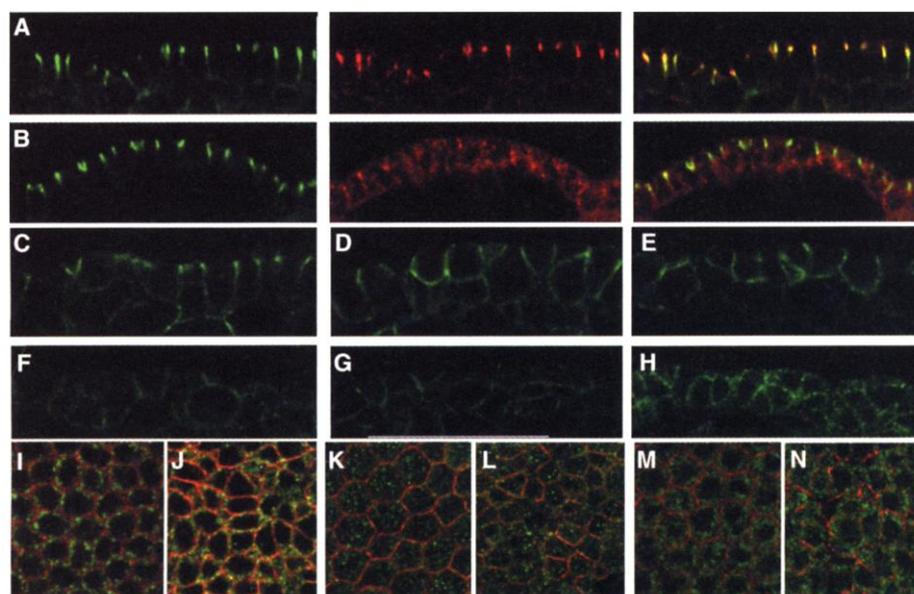
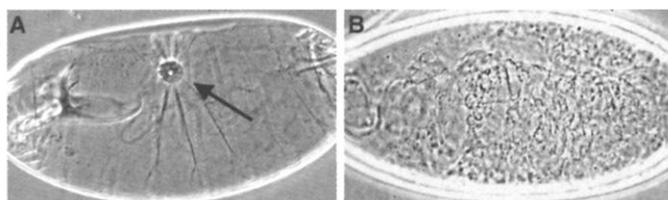


Fig. 5. Scrib, Dlg, and Lgl are mutually dependent for proper subcellular localization. (A) Stage 16 WT embryonic epidermis costained for Dlg (green, left) and Scrib (red, center) reveals that the two proteins colocalize at the ALM (yellow, right). (B) Stage 16 WT embryo costained for Dlg (green, left) and Lgl (red, center). Lgl is found predominantly at the plasma membrane, including the ALM (yellow, right). In WT stage 11 embryos (C), Scrib is enriched at the ALM. However, in *lgl* embryos (D), Scrib is mislocalized throughout the basolateral membrane. *scrib* embryos show a similar basolateral misdistribution of Dlg (E), whereas *dlg* embryos (F) both mislocalize Scrib and display reduced levels of Scrib at the membrane. By stage 16 little Scrib is visible in *dlg* embryos (G); in contrast, stage 16 *lgl* embryos (H) retain ~WT levels of misdistributed Dlg. En face sections of WT blastoderm (I) and stage 11 epidermis (J) demonstrate the localization of a significant proportion of Lgl (green) at cell membranes (marked with anti-Neurotactin in red). Similar sections of *scrib* embryos (K, blastoderm; L, stage 11) reveal that Lgl is almost exclusively cytoplasmic. In *dlg* embryos, Lgl is localized near to membranes at blastoderm (M) but becomes cytoplasmic by stage 11 (N).

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Molecular Identification of a Taste Receptor Gene for Trehalose in *Drosophila*

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The molecular nature of sweet taste receptors has not been fully explored. Employing a differential screening strategy, we identified a taste receptor gene, *Tre1*, that controls the taste sensitivity to trehalose in *Drosophila melanogaster*. The *Tre1* gene encodes a novel protein with similarity to G protein-coupled seven-transmembrane receptors. Disruption of the *Tre1* gene lowered the taste sensitivity to trehalose, whereas sensitivities to other sugars were unaltered. Overexpression of the *Tre1* gene restored the taste sensitivity to trehalose in the *Tre1* deletion mutant. The *Tre1* gene is expressed in taste sensory cells. These results provide direct evidence that *Tre1* encodes a putative taste receptor for trehalose in *Drosophila*.

Olfactory receptors are G protein-coupled seven-transmembrane proteins encoded by a divergent multigene family in vertebrates (1) and in *Drosophila* (2, 3). Taste receptors are also thought to belong to the superfamily of G protein-coupled receptors (GPCRs) (4), and several candidate genes have been reported (5–8), but their function as a taste receptor has not been proved. In *Drosophila*, taste sensilla are present on the labellum, tarsi, and wing margins (9, 10). In a typical chemosensillum on the labellum, there are four taste sensory cells each of which responds to either water, salt, or sugar. Previously, we showed that there are at least three separate receptor sites for sugars in the sugar receptor cell of *Drosophila* (11, 12). The *Tre* gene was identified through studies on natural variants (12). Because the *Tre* gene controls taste sensitivity to trehalose without affecting the responses to other sugars, the gene product of *Tre* should function in sugar receptor cells. The *Tre* gene is cytologically mapped to the region between 5A10 and 5B1-3 on the X chromosome (13). A P1 clone containing a 90-kb genomic region from 5A9 to 5B1 was used as a starting material to clone the taste receptor gene (Fig. 1A).

To identify the putative *Tre* gene, we per-

formed a differential screening for genes encoded in the P1 clone that might be specifically expressed in chemosensory cells. We took advantage of the *pox-neuro* (*poxn*) gene, which is involved in the developmental decision pathway between mechanosensory and chemosensory cell fates (14). In an adult-viable allele of the *poxn* mutant, all external chemosensilla are either transformed into mechanosensilla or are deleted (15). In the legs of the wild-type fly, chemosensilla exist on the tarsus, but there are no chemosensilla on the femur. We performed a differential screening with cDNA probes derived from labella, tarsi, and femurs of wild-type and *poxn* mutant flies (16).

Southern blot analysis of the subcloned P1 DNA fragments identified one clone that hybridized to the wild-type labella and tarsi probes, but not to the other probes (17). A portion of the 8.2-kb clone displayed conserved features of the superfamily of seven-transmembrane domain receptor proteins. The full-length putative *Tre1* cDNA was obtained by reverse transcriptase-dependent polymerase chain reaction (RT-PCR) and 5' and 3' rapid amplification of cDNA ends (RACE) (18). Sequence analysis revealed that the putative *Tre1* gene contained a 1179-base pair (bp) open reading frame that encodes 392 amino acid residues preceded by an in-frame termination codon. Hydrophathy analysis suggests that the *Tre1* cDNA sequence contains seven hydrophobic stretches that represent potential transmembrane do-

main (Fig. 1C). These domains constitute the regions of maximal sequence similarity to other seven-transmembrane receptors. Although several conserved regions are found between *Tre1* and other GPCRs, the structures of the third and fourth cytoplasmic domains may be unique, because they are longer than the corresponding domains of typical GPCRs. The *Tre1* gene has no similarity to other GPCRs recently identified by database search (8). We suggest that the *Tre1* gene may represent a new subclass of GPCRs.

By searching the *Drosophila* DNA database with the 5'-flanking genomic sequences of the putative *Tre1* gene, we found that flanking genomic sequences of the P-element in strains in one of the transposon-inserted strains [EP(X) strains (19)] completely matched our genomic sequence (Fig. 1B). The EP element is inserted 113 bp upstream to the transcription initiation site in the EP(X)0496 strain. The taste sensitivity of this strain to trehalose was tested with the two-choice preference test (12) and was found to be highly sensitive (high-sensitive). We expected that imprecise excision of the P-element should disrupt the promoter region of the *Tre1* gene, and this event might change the trehalose sensitivity. The EP element carries *w*⁺ as a genetic marker, and the element was jumped out by genetically supplying a transposase source (20). We tested *w* male flies by two-choice preference tests, using 30 mM trehalose and 2 mM sucrose as the choices. At this concentration of trehalose, nearly 98% of the parental EP(X)0496 flies preferred trehalose (Fig. 2B). Most of the *w* flies preferred trehalose, indicating that the precise excision of the P-element did not impair trehalose sensitivity. Flies that consumed the sucrose side were selected and individually crossed to *C(1)DX* attached-X females. Among about 3000 *w* flies, we isolated 90 lines that were confirmed as showing low sensitivity (low-sensitive) to trehalose. We determined the extent of deletion in all the 90 lines by PCR, using primers flanking the P-element insertion site. There were no amplification products in most of these lines, indicating that a deletion eliminated the primer site(s). Next, several lines were selected, and the extent of deletion was determined by Southern blotting. The results (Fig. 1B) indicated that the deletions removed the putative promoter region and the first exon. In fact,

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