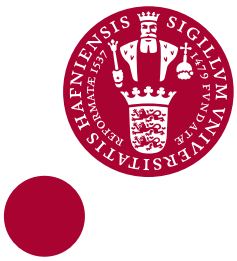

Drosophila Stardust is a partner of Crumbs in the control of epithelial cell polarity

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Published in:
Nature

DOI:
10.1038/414638a

Publication date:
2001

Document version
Publisher's PDF, also known as Version of record

the epithelial but not neuroblast polarity, the Baz–DmPar-6–aPKC complex affects both epithelial and neuroblast polarity. Proteins in both pathways are expressed in epithelial cells with identical apicolateral localization patterns[7,8,12,21] (data not shown) but only Baz–DmPar-6–aPKC are expressed in neuroblasts. In epithelia, these two pathways are probably partially redundant in controlling apicobasal polarity. Their role in regulating ZA formation seems to be the key to their epithelial and cuticle phenotypes. Sdt and Crb colocalize in epithelia and together serve as apical determinants, but only partially overlap and are likely to serve different functions in sensory organs. A mutation in a human Crb homologue has been implicated as the cause of one form of retinitis pigmentosa[27], suggesting a potential role for the Crb family of proteins in the localization of a sensory transduction complex[28]. The localization of Sdt to the dendritic tip and scolopale cells, the probable sites for sensory transduction, raises the possibility that Sdt may have a role in the development or function of a sensory transduction apparatus. □

Methods

Fly stocks and molecular biology

y w was used as the wild-type stock in all experiments. For mutant phenotype analysis, sdt^{XN05} and sdt^{XP96} were balanced over *FM7,P{ftz/lacZ}* and crb^{11A22} was balanced over *TM3, P{w*$^{+mC}$ *= 35UZ} Sb*^{1}. sdt^{XP96} germline clone embryos were produced using the FLP/DFS technique as described[16]. Overexpression of Crb-intra was achieved by crossing upstream activating sequence (UAS)-Crb-intra[23] with the maternal GAL4 driver V32A (provided by D. St Johnston) at 25 °C. Crb-intra fragments were amplified by PCR and inserted into pGEX-4T-1 vector between *Eco*RI and *Sal*I to make GST fusion constructs. The GST pull-down assay was carried out as described[22].

Allele sequencing

Each *sdt* allele was rebalanced with a green fluorescent protein (GFP) X-chromosome balancer[29]. About 40 GFP-deficient late-stage embryos were picked for DNA preparation. Genomic DNA fragments of *sdt* mutants were amplified with Roche Expand 20kb^{PLUS} PCR kit from two embryo equivalents of DNA (pre-denatured before PCR). PCR reactions were set up in duplicate or triplicate and each PCR fragment was independently cloned into pGEM-T Easy+ vector and both strands were fully sequenced.

Immunofluorescence staining of embryos

Rabbit anti-Sdt serum was affinity purified using Bio-Rad Affi-Gel bound with the same GST:Sdt C-terminal fusion protein used for immunization. Both crude and purified Sdt antiserum failed to detect specific signals in immunoblot experiments. Embryos were fixed with either 4% formaldehyde or 4% paraformaldehyde in 1× PBS buffer. Dilution factors for primary antibodies are given in Supplementary Information. Images were taken on a Bio-Rad MRC600 and a Bio-Rad 1024MP confocal microscope and processed with Adobe Photoshop software.

Received 5 June; accepted 24 September 2001.

1. Nüsslein-Volhard, C. & Wieschaus, E. Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287,** 795–801 (1980).
2. Jurgens, G., Wieschaus, E., Nüsslein-Volhart, C. & Kluding, H. Mutation affecting the pattern of the larval cuticle in *Drosophila melanogaster*: II. Zygotic loci on the third chromosome. *Roux Arch. Dev. Biol.* **193,** 283–295 (1984).
3. Nüsslein-Volhart, C., Wieschaus, E. & Kluding, H. Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*: I. Zygotic loci on the second chromosome. *Roux Arch. Dev. Biol.* **193,** 267–282 (1984).
4. Wieschaus, E., Nüsslein-Volhart, C. & Jurgens, G. Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*: III. Zygotic loci on the X chromosome and fourth chromosome. *Roux Arch. Dev. Biol.* **193,** 296–307 (1984).
5. Müller, H.-A. J. Genetic control of epithelial cell polarity: lessons from *Drosophila*. *Dev. Dyn.* **218,** 52–67 (2000).
6. Tepass, U., Theres, C. & Knust, E. *crumbs* encodes an EGF-like protein expressed on apical membranes of *Drosophila* epithelial cells and required for organization of epithelia. *Cell* **61,** 787–799 (1990).
7. Schober, M., Schaefer, M. & Knoblich, J. A. Bazooka recruits Inscuteable to orient asymmetric cell divisions in *Drosophila* neuroblasts. *Nature* **402,** 548–551 (1999).
8. Wodarz, A., Ramrath, A., Kuchinke, U. & Knust, E. Bazooka provides an apical cue for Inscuteable localization in *Drosophila* neuroblasts. *Nature* **402,** 544–547 (1999).
9. Tepass, U. & Knust, E. *crumbs* and *stardust* act in a genetic pathway that controls the organization of epithelia in *Drosophila melanogaster*. *Dev. Biol.* **159,** 311–326 (1993).
10. Dimitratos, S. D., Woods, D. F., Stathakis, D. G. & Bryant, P. J. Signalling pathways are focused at specialized regions of the plasma membrane by scaffolding proteins of the MAGUK family. *BioEssays* **21,** 912–921 (1999).
11. Gonzalez-Mariscal, L., Betanzos, A. & Avila-Flores, A. MAGUK proteins: structure and role in the tight junction. *Cell Dev. Biol.* **11,** 315–324 (2000).
12. Petronczki, M. & Knoblich, J. A. DmPAR-6 directs epithelial polarity and asymmetric cell division of neuroblasts in *Drosophila*. *Nature Cell Biol.* **3,** 43–49 (2001).
13. Bhat, M. *et al.* Discs Lost, a novel multi-PDZ domain protein establishes and maintains epithelial polarity. *Cell* **96,** 833–845 (1999).
14. Bilder, D. & Perrimon, N. Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. *Nature* **403,** 676–680 (2000).
15. Kamberov, E. *et al.* Molecular cloning and characterization of Pals, proteins associated with mLin-7. *J. Biol. Chem.* **275,** 11425–11431 (2000).
16. Müller, H.-A. J. & Wieschaus, E. *armadillo*, *bazooka*, and *stardust* are critical for early stages in formation of the zonula adherens and maintenance of the polarized blastoderm epithelium in *Drosophila*. *J. Cell Biol.* **134,** 149–163 (1996).
17. Tepass, U. Epithelial differentiation in *Drosophila*. *BioEssays* **19,** 673–682 (1997).
18. Tepass, U. & Hartenstein, V. The development of cellular junctions in the *Drosophila* embryo. *Dev. Biol.* **161,** 563–596 (1994).
19. Tepass, U. Crumbs, a component of the apical membrane, is required for zonula adherens formation in primary epithelia of *Drosophila*. *Dev. Biol.* **177,** 217–225 (1996).
20. Grawe, F., Wodarz, A., Lee, B., Knust, E. & Skaer, H. The *Drosophila* genes *crumbs* and *stardust* are involved in the biogenesis of adherens junctions. *Development* **122,** 951–959 (1996).
21. Wodarz, A., Ramrath, A., Grimm, A. & Knust, E. *Drosophila* atypical portein kinase C associates with Bazooka and controls polarity of epithelia and neuroblasts. *J. Cell Biol.* **150,** 1361–1374 (2000).
22. Shen, C.-P., Jan, L. Y. & Jan, Y. N. Miranda is required for the asymmetric localization of Prospero during mitosis in *Drosophila*. *Cell* **90,** 449–458 (1997).
23. Klebes, A. & Knust, E. A conserved motif in Crumbs is required for E-cadherin localisation and zonula adherens formation in *Drosophila*. *Curr. Biol.* **10,** 76–85 (2000).
24. Wodarz, A. Hinz, U., Engelbert, M. & Knust, E. Expression of Crumbs confers apical character on plasma membrane domains of ectodermal epithelia of *Drosophila*. *Cell* **82,** 67–76 (1995).
25. Brenman, J. E. *et al.* Localization of postsynaptic density-93 to dendritic microtubules and interaction with microtubule-associated protein 1A. *J. Neurosci.* **18,** 8805–8813 (1998).
26. Shin, H., Hsueh, Y.-P., Yang, F.-C., Kim, E. & Sheng, M. An intramolecular interaction between Src homology 3 domain and guanylate kinase-like domain required for channel clustering by Post-synaptic Density-95/SAP90. *J. Neurosci.* **20,** 3580–3587 (2000).
27. Den Hollander, A. I. *et al.* Mutations in a human homologue of *Drosophila crumbs* cause retinitis pigmentosa (RP12). *Nature Genet.* **23,** 217–221 (1999).
28. Rashbass, P. & Skaer, H. Cell polarity: nailing Crumbs to the scaffold. *Curr. Biol.* **10,** R234–R236 (2000).
29. Casso, D., Ramirez-Weber, F., & Kornberg, T. B. GFP-tagged balancer chromosomes for *Drosophila melanogaster*. *Mech. Dev.* **91,** 451–454 (2000).

Supplementary Information accompanies the paper on *Nature*'s website (http://www.nature.com).

Acknowledgements

We are grateful to D. Bilder, M. Bhat, T. Uemura, P. J. Bryant, U. Tepass, E. Knust, E. Wieschaus, Developmental Studies Hybridoma Bank and Bloomington Stock Center for providing fly stocks, antibodies and constructs. We thank S. Barbel, L. Ackerman and S. Younger-Shepherd for technical assistance and M. Rothenberg, D. Cox, S. Abdelilah and U. Tepass for critical comments on the manuscript. B.S. is supported by a grant from the National Institutes of Health. Y.H. is a Research Associate and N.P., L.Y.J. and Y.N.J are Investigators of the Howard Hughes Medical Institute.

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Drosophila Stardust is a partner of Crumbs in the control of epithelial cell polarity

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The polarized architecture of epithelial cells depends on the highly stereotypic distribution of cellular junctions and other membrane-associated protein complexes. In epithelial cells of the *Drosophila* embryo, three distinct domains subdivide the lateral plasma membrane. The most apical one comprises the subapical complex (SAC). It is followed by the zonula adherens (ZA) and, further basally, by the septate junction[1]. A core component of the SAC is the transmembrane protein Crumbs, the cytoplasmic domain of which recruits the PDZ-protein Discs Lost into the complex[2,3]. Cells lacking *crumbs* or the functionally related gene *stardust* fail to organize a continuous ZA and to maintain cell

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polarity[4–6]. Here we show that *stardust* provides an essential component of the SAC. Stardust proteins colocalize with Crumbs and bind to the carboxy-terminal amino acids of its cytoplasmic tail. We introduce two different Stardust proteins here: one MAGUK protein, characterized by a PDZ domain, an SH3 domain and a guanylate kinase domain; and a second isoform comprising only the guanylate kinase domain. The Stardust proteins represent versatile candidates as structural and possibly regulatory constituents of the SAC, a crucial element in the control of epithelial cell polarity.

The allele sdt^{P10} was induced by mobilization of the P-element of the viable and fertile insertion line $Is(1)P[ry^{+}]^{334}$. sdt^{P10} exhibits an intermediate *sdt* phenotype and fails to complement other *sdt* alleles (Fig. 1a–c). Molecular cloning of flanking DNA allowed to map the P-element of $Is(1)P[ry^{+}]^{334}$ about 20 kilobases (kb) distal to that of sdt^{P10} and very close to the enhancer-promoter (EP)-element of the homozygous viable EP-line *EP(X)1143* (Fig. 2a). Imprecise excision of the EP-element induced additional *sdt* alleles (data not shown).

We recovered more than ten related but incomplete complementary DNAs and used them to isolate additional cDNAs. The 5.7-kb

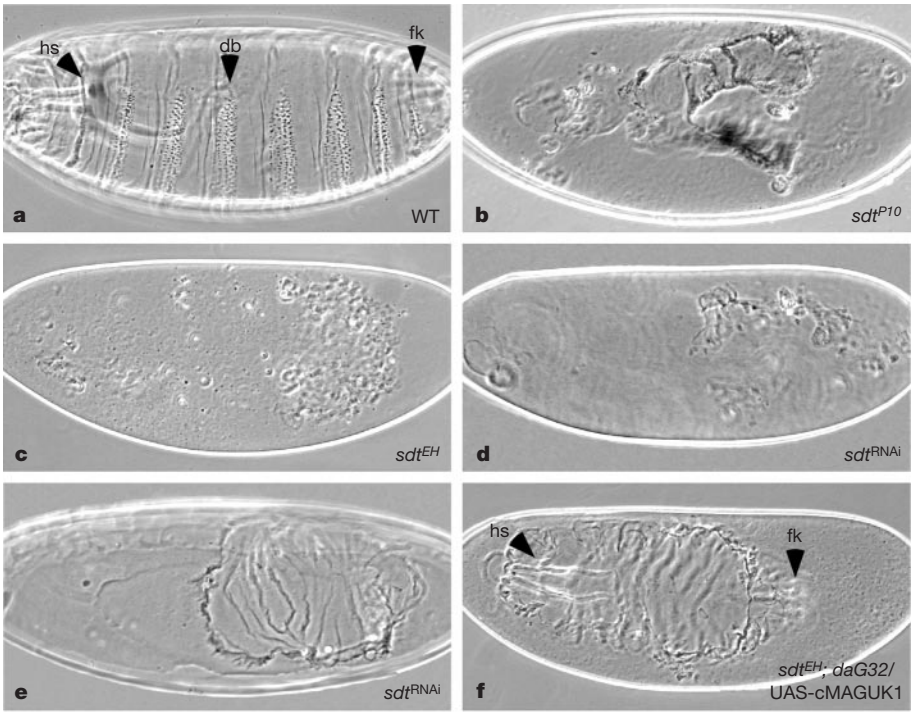
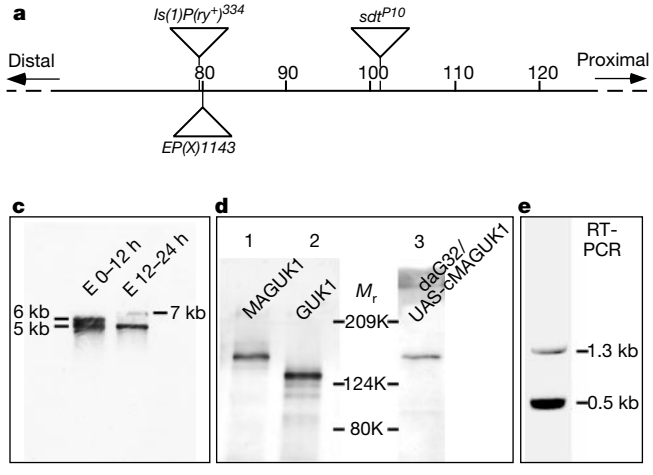


Figure 1 Cuticle preparations of embryos. **a**, Wild-type embryo. **b**, sdt^{P10}/Y embryo with an intermediate phenotype. **c**, sdt^{EH}/Y embryo with a strong phenotype. **d**, **e**, Wild-type embryos injected with MAGUK dsRNA (RNAi). Embryos show a variable expression level of the mutant phenotype (42% weak, 40% intermediate (**e**) and 18% strong (**d**) phenotypes). The same classes of mutant phenotypes were achieved on injection of either SH3 dsRNA or N-term dsRNA. **f**, sdt^{EH}/Y;*daG32*/UAS-cMAGUK1 embryo. Large parts of continuous cuticle and the head skeleton are restored. hs, head skeleton; db, denticle belts; fk, filzkörper. Anterior is to the left.



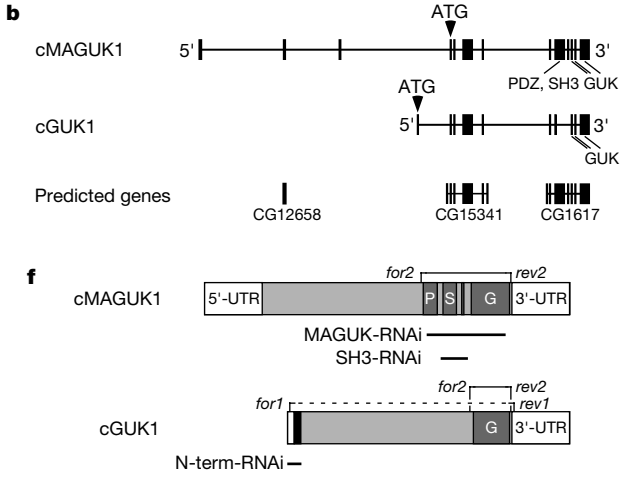


Figure 2 Molecular analysis of the *sdt* locus. **a**, Genomic region from the cytological interval 7D18. Triangles are P-element inserts. Numbering refers to genomic scaffold AE003443. **b**, cDNAs cMAGUK1 and cGUK1. Predicted genes refer to those assumed by the Berkeley Drosophila Genome Project. ATG, putative translation initiation codon. **c**, Northern blot of embryonic poly(A)$^{+}$ RNA, hybridized with a probe covering the MAGUK domain. **d**, *In vitro* translated Sdt-MAGUK1 (lane 1) and Sdt-GUK1 (lane 2) and extracts of embryos (*daG32*/UAS-cMAGUK1) (lane 3), detected with the anti-Sdt^{pep153} antiserum. **e**, Products of RT-PCR (see Methods). The amplified fragments correspond to those coding for the MAGUK (1.3 kb) and GUK (0.5 kb) domains, respectively. **f**, cMAGUK1 and cGUK1. Grey, ORF; black bar in cGUK1, cGUK1-specific N-terminal sequence (N-term-RNAi). P, PDZ domain; S, SH3 domain; G, GUK domain. Bold bars, regions used for RNAi; *for* and *rev*, primers used for RT-PCR.

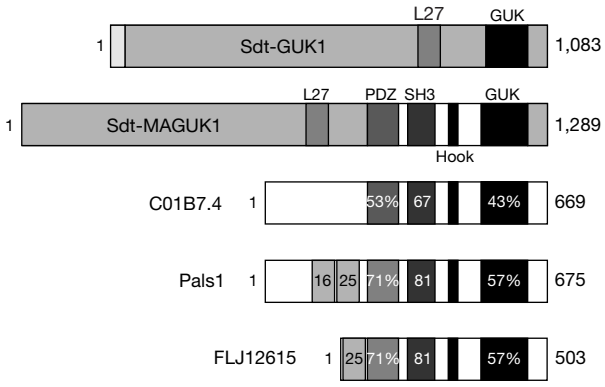


Figure 3 Proteins predicted from the *sdt* locus. Proteins predicted by cGUK1 (GenBank accession number AF414118) and cMAGUK1 (AF414117), *C. elegans* ORF CO1B7.4 (AAA96115), mouse Pals1 (AF199008)[8] and the human ORF FLJ12615 (BAB14172). Varying shades of grey indicate different protein domains. Amino-acid identities of 71, 81 and 57% in the PDZ, SH3 and GUK domains, respectively, suggest that Sdt MAGUK1 and the mammalian proteins are true orthologues.

cDNA cMAGUK1 (Fig. 2b) detects three developmentally regulated transcripts of about 5, 6 and 7 kb, respectively (Fig. 2c). Data from *in vitro* transcription/translation and overexpression of an upstream activating sequence (UAS)-cMAGUK1 transgene in wild-type embryos suggest that the first AUG codon, which complies to the Kozak consensus sequence, is used as the start site, resulting in a protein product (Sdt-MAGUK1) of relative molecular mass about 140,000 (M_r ~140K) (Fig. 2d, lanes 1 and 3). The protein contains a single PDZ (PSD-95, Discs Large, ZO-1) domain, an SH3 (Src homology region 3) domain and a GUK (guanylate kinase) domain (Fig. 3 and Supplementary Information), which, together with a Hook domain, classify Sdt-MAGUK1 as a member of the p55 subfamily of the MAGUK (membrane-associated guanylate kinase homologues) family of scaffolding proteins[7]. The MAGUK domain exhibits a high degree of similarity to that of a predicted open reading frame (ORF) from *Caenorhabditis elegans*, to mouse Pals1 (ref. 8) and to a human predicted ORF (Fig. 3). Sdt-MAGUK1 also shares the putative Lin-7-binding domain (L27) with the mammalian proteins.

Using cMAGUK1 as probe, the 4.5-kb cDNA cGUK1 was isolated, which starts about 25 kb downstream of the start site of cMAGUK1

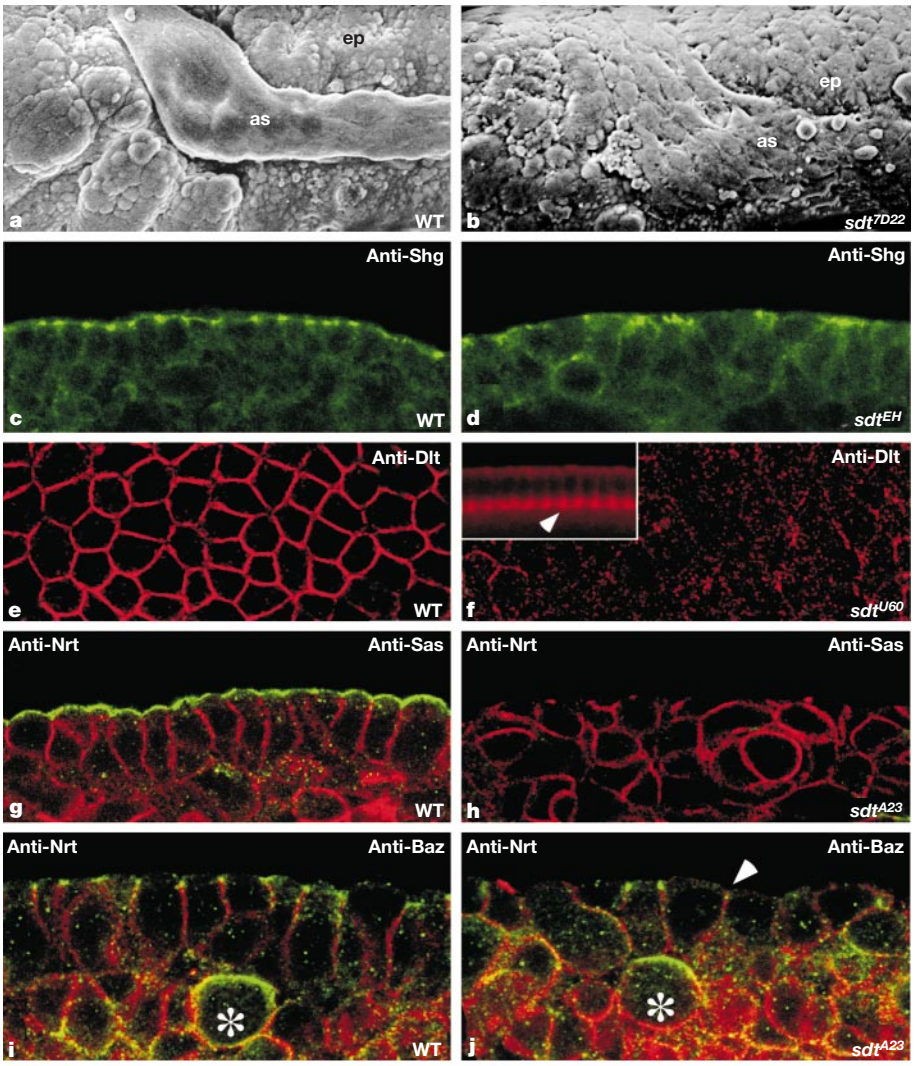


Figure 4 Phenotypic characteristics of *sdt* mutant embryos. **a**, **c**, **e**, **g**, **i**, Wild type (WT). **b**, **d**, **f**, **h**, **j**, *sdt* mutant embryos. **a**, **b**, Scanning electron micrographs of lateral views of stage 11 embryos. ep, epidermis; as, amnioserosa. **c**, **d**, Lateral optical sections of stage 11 embryos stained with anti-Shotgun/DE-cadherin, a constituent of the ZA. **e**, **f**, Surface views of stage 9 embryos stained with anti-Discs Lost, a component of the SAC. Inset (**f**), Dlt localization during cellularization. **g**, **h**, Lateral optical sections of stage 12 embryos stained with anti-Neurotactin (Nrt; red), and anti-Stranded at Second (Sas; green) basolateral and apical markers, respectively. **i**, **j**, Lateral optical section of stage 11 embryos, stained with anti-Neurotactin (Nrt; red) and anti-Bazooka (Baz; green). Asterisks indicate delaminated neuroblasts. In **a** and **b**, anterior is left, ventral down. In **c**, **d** and **g**–**j**, apical is up, basal down.

(Fig. 2a, b). A protein product (Sdt-GUK1) with M_r about 120K could be synthesized from cGUK1 *in vitro* (Fig. 2d, lane 2). It differs from Sdt-MAGUK1 by 78 additional amino-terminal amino acids and the lack of 284 internal amino acids, removing the PDZ, SH3 and Hook domains (Fig. 3 and Supplementary Information). Polymerase chain reaction with reverse transcription (RT-PCR) on embryonic poly(A)$^+$ RNA was performed to verify the transcript represented by cGUK1. RT-PCR with primers from the cGUK1-specific 5′ exon and the common 3′ untranslated region (UTR), followed by a nested PCR with a primer pair flanking the MAGUK/GUK domain (Fig. 2f), yielded, besides a minor band of 1.3 kb that corresponds to a MAGUK transcript, one major product with the expected size of 0.5 kb (Fig. 2e). Sequencing of this fragment revealed a slight difference compared with cGUK1 in the region between the Lin-7-binding domain and the GUK domain. From the sequence, a protein is predicted (named Sdt-GUK2) with a complete GUK domain and a deletion of 271 amino acids compared with Sdt-MAGUK1 (see Supplementary Information). These data suggest that *sdt* transcripts encode proteins with either a MAGUK or a GUK domain.

To link the transcripts characterized above to the *sdt* phenotype, injections of double-stranded RNA (dsRNA) into wild-type embryos (RNAi) were performed, using dsRNA from three different regions (Fig. 2f). In all three cases, weak to strong *sdt* phenotypes

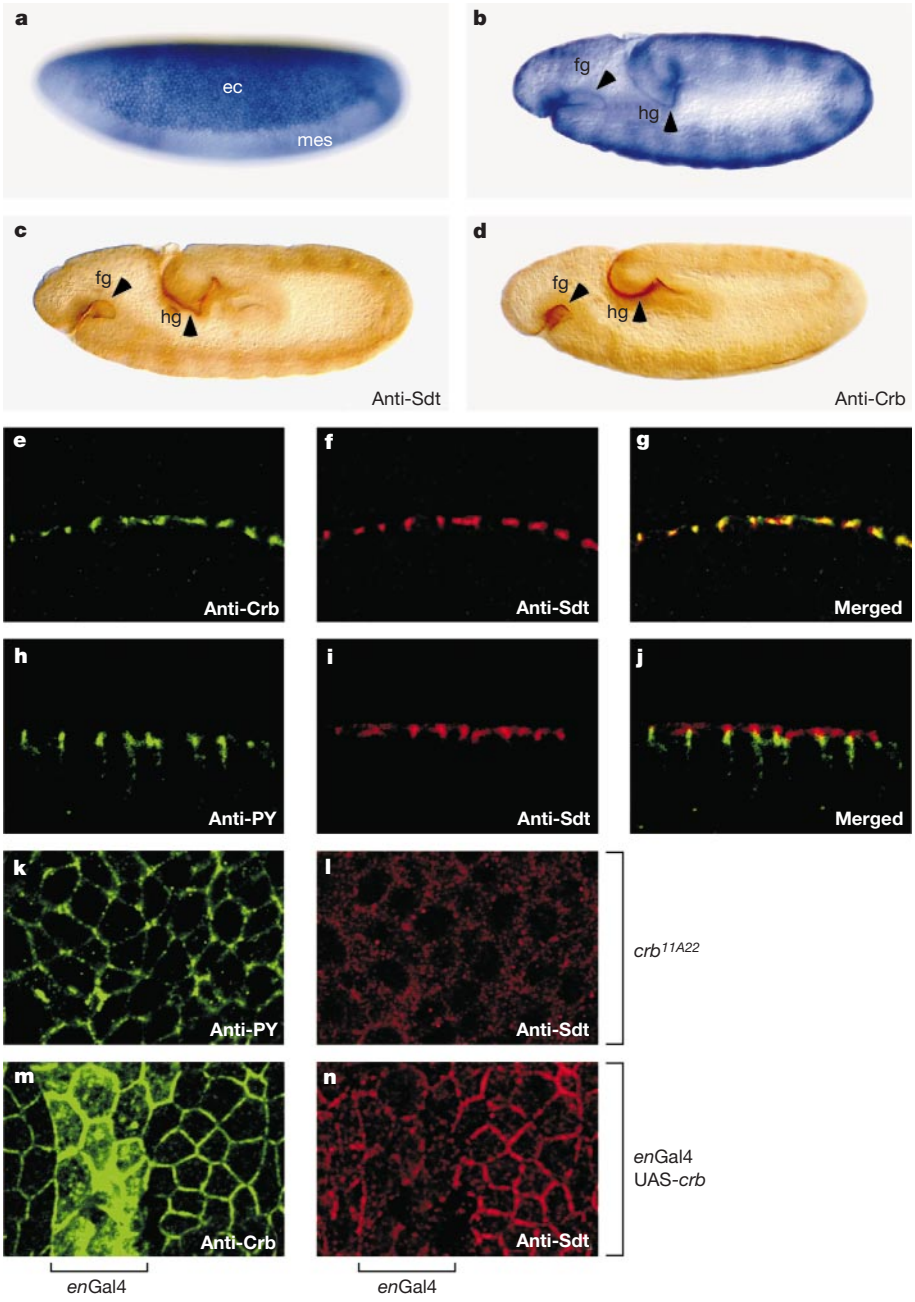


Figure 5 Relationship between *sdt* and *crb* expression. **a**–**d**, Expression of *sdt* RNA (**a**, **b**), Sdt protein (**c**) and Crb protein (**d**) in wild-type embryos of stages 6 (**a**) and 10 (**b**–**d**). ec, ectoderm; mes, mesoderm; fg, foregut; hg, hindgut. Arrowheads point to apical localization of RNA and proteins. **e**–**j**, Stage 13 (**e**–**g**) and stage 11 (**h**–**j**) wild-type embryos stained with anti-Crb (**e**; green), anti-Sdt (**f**, **i**; red) and anti-phosphotyrosine (**h**; green), a marker for the ZA. Sdt is colocalized with Crb (**g**) apical to the ZA (**j**). **k**–**n**, Stage 12 crb^{11A22} embryos (**k**, **l**) and wild-type embryos overexpressing Crb in the posterior compartment of each segment (bar; **m**, **n**), stained with anti-phosphotyrosine (**k**; green), anti-Crb (**m**; green) and anti-Sdt (**l**, **n**; red) antibodies. In **a**–**d**, anterior is left, ventral down. In **e**–**j**, apical is up, basal down.

were observed, which ranged from holes in the cuticle to only grains of cuticle (Fig. 1d, e). To further assay that the cloned gene corresponded to *sdt*, we carried out rescue experiments. GAL4-mediated overexpression of UAS-cGUK1 did not rescue the *sdt* loss-of-function phenotype, whereas expression of UAS-cMAGUK1 gave considerable rescue (Fig. 1f), which was not significantly improved on overexpression of both transgenes. In summary, the data show that MAGUK1 represents a *sdt* gene product, whereas the function of the GUK proteins remains elusive.

The *sdt* mutant phenotype is characterized by the loss of epithelial integrity and cell shape (Fig. 4a, b). *sdt* mutant embryos fail to concentrate the scattered spot adherens junctions into a continuous ZA[5,6,9] (Fig. 4c, d). As a consequence, cells lose contact and the epithelium becomes multilayered (Fig. 4a–d and g–j). As previously shown, the assembly of the ZA requires a functional SAC. Formation of the SAC depends on an intact cytoplasmic tail of Crumbs, which recruits the PDZ protein Discs Lost (Dlt) into the complex[3]. In *sdt* mutant embryos, the SAC components Crumbs (Crb)[4] and Dlt (Fig. 4e, f) fail to localize apically from stage 8 onwards. Because Dlt is correctly associated with the ingrowing plasma membrane during cellularization in *sdt* embryos (Fig. 4f, inset), we suggest that *sdt* is, similarly to *crb*, essential for maintenance, but not for initiation of Dlt localization. The transmembrane protein Stranded at Second (Sas) is lost from the apical pole, whereas the basolateral marker Neurotactin, initially retained at the lateral membranes, is delocalized in later stages (Fig. 4g, h). Lack of *sdt* also affects localization of the PDZ protein Bazooka (Baz) in epithelial cells (Fig. 4i, j). Baz forms, together with the PDZ protein DmPar-6 and an atypical protein kinase C (DaPKC), another apical protein complex. This complex is essential for the polarity of epithelial cells and neuroblasts, the progenitors of the central nervous system, where it controls spindle orientation and localization of determinants[10–13]. Lack of *sdt* function does not affect Baz localization in neuroblasts (Fig. 4j), showing that *sdt*, unlike *baz*[10,11], is not required for the apicobasal polarity of neuroblasts.

In the embryo, *sdt* transcripts can be detected from the cellular blastoderm onwards, where they are restricted to the anlage of the ectoderm (prospective ectoderm) and excluded from that of the mesoderm (Fig. 5a). Later on, *sdt* transcripts are present in epithelial derivatives of the ectoderm, that is, the epidermis, the foregut and hindgut, the tracheae, the salivary glands and the Malpighian tubules, where they are localized apically (Fig. 5b). At no time is *sdt* RNA detectable in neuroblasts or other cells of the central nervous system. Both the tissue distribution and the subcellular localization of *sdt* transcripts are similar to those of *crb* transcripts[14].

We raised an antiserum against two synthetic peptides of the GUK domain, which thus should recognize both Sdt-MAGUK1 and Sdt-GUK1/2. It is specific for Sdt, because no staining was observed in several *sdt* alleles (data not shown). Apical staining was detected from gastrulation (stage 7) onwards in all epithelia derived from the ectoderm. Hence, Sdt shows the same temporal and spatial expression pattern as Crb (Fig. 5c, d). Sdt is colocalized with Crb in the SAC, apical to the ZA (Fig. 5e–j). In *crb* mutants, Sdt protein is lost from the plasma membrane (Fig. 5l), whereas some residual ZA components show a scattered distribution on all membranes (Fig. 5k). Sdt in *crb* mutants thus behaves similarly to Crb protein in *sdt* mutants[4]. Overexpression of Crb results in the depletion of Sdt from the apical surface (Fig. 5m, n).

These results and the previously shown genetic interaction between *crb* and *sdt*[4] prompted us to look for a possible direct interaction between the two proteins using the yeast two-hybrid system. A fusion protein consisting of the MAGUK domain of Sdt and the transactivation domain of GAL4 was co-expressed in yeast cells with various forms of the Crb cytoplasmic domain, fused to the DNA-binding domain of GAL4. The full-length intracellular domain of Crb and those with mutations in a region close to the membrane (Crb-intra^{WT}, Crb-intra^{Y10A} and Crb-intra^{E16A}) showed strong interaction with the MAGUK domain of Sdt. In contrast, lack of the four C-terminal amino acids ERLI (Crb-intra$^{\Delta ERLI}$) completely abolished the interaction (see Supplementary Information). This suggests that binding of the MAGUK domain of Sdt depends on the four C-terminal amino acids of Crb.

The data presented here suggest that *sdt* encodes several isoforms, which are constituents of the SAC. The presence of Sdt proteins containing either a MAGUK or a GUK domain has, to our knowledge, no precedent among other MAGUK proteins. Recent results have shown that the GUK domain of the rat MAGUK proteins SAP97 and PSD-95/SAP-90 (refs 15–17) and of hCASK and hDlg[18] can form intra- as well as intermolecular interactions with the SH3 domain of the same or other MAGUK proteins, respectively. It has also been demonstrated that intramolecular interactions between the SH3 and the GUK domain of SAP97 modulate binding of its GUK domain to its partner GKAP.

Several issues are yet to be solved: how Crb, Dlt and Sdt participate in the organization of the SAC; in which temporal sequence they are recruited; and whether Dlt and Sdt compete for the Crb binding site. Sdt and Dlt behave differently on overexpression of Crb, in that Dlt is redistributed around the plasma membrane[3], whereas Sdt is depleted from the apical membrane. The different Sdt isoforms now make it possible to isolate additional components of the SAC. The mouse homologue Pals1 has been isolated by virtue of its interaction with mouse Lin-7 (ref. 8), another PDZ protein. Homologues of Lin-7 and its *C. elegans* binding partners Lin-2 and Lin-10 have been identified in *Drosophila*[19] (A.B. and E.K., unpublished data) and we are interested to see whether they form part of the SAC. The analysis of all the interactions necessary to establish a functional SAC, the control of its assembly and its role in the establishment of the ZA will allow further unravelling of the mechanisms involved in the control of apicobasal polarity of epithelial cells. □

Methods

Fly stocks

The following fly stocks were used: wild type (Oregon R), *crb*^{11A22}, *sdt*^{7D22}, *sdt*^{EH} (ref. 4), *sdt*^{U60}, *sdt*^{A23} (ref. 20), *sdt*^{P10} (this work), *EP(X)1143* (ref. 21), *Is(1)P(ry*$^{+}$*)*^{334} (ref. 22), Gal4^{daG32}, UAS-*crb*$^{30.12e}$ (ref. 23), *en*Gal4 (ref. 24), UAS-cMAGUK1 and UAS-cGUK1 (this work).

Molecular analysis of *stardust*

The homozygous viable P-element insertion line *Is(1)P(ry*$^{+}$*)*^{334} was used as a starter strain to initiate a local hop mutagenesis with Δ2–3 as the source of transposase[25]. A total of 7,250 single crosses were performed and 20 lethal P-element insertion lines could be isolated. By phenotypic and complementation analysis one P-element insertion turned out to be a new *sdt* allele, named *sdt*^{P10}. Mobilization of this P-element reverted the *sdt* mutant phenotype to wild type, demonstrating that the *sdt* mutation is caused by the P-element insertion.

Genomic DNA adjacent to the P-element insertion site in *sdt*^{P10} was isolated from a partial genomic phage library by screening with a P-element-specific probe. A chromosomal walk was initiated, isolating about 60 kb of flanking genomic DNA. Complementary DNAs from this region were recovered by screening the following cDNA libraries with various genomic fragments: NB 4–8-h embryonic library[26], Hovemann 0–16-h embryonic library[27], LD 0–22-h embryonic library (BDGP, provided by C. M. Schuster), and testis-specific library (provided by M. Schäfer). Two nearly full-length cDNAs were recovered: cMAGUK1 is 5,758 nucleotides long and has an ORF of 3,870 nucleotides; cGUK1 is 4,548 bp long and includes an ORF of 3,252 nucleotides.

RT-PCR, *in vitro* transcription/translation and RNAi

We performed RT-PCR using the Qiagen OneStep RT-PCR Kit with primers *for1* and *rev1*, followed by nested PCR with primers *for2* and *rev2* (Fig. 2f; for primer sequences see Supplementary Information). *In vitro* transcription/translation was carried out with the TNT Coupled Reticulocyte Lysate Systems (Promega). Double-stranded RNA for RNAi was produced according to the PCR template method and injected into the posteroventral region of preblastoderm embryos[28]. The following primers were used for generation of dsRNA (Fig. 2f; for primer sequences see Supplementary Information): MAGUK-RNAi-5 and MAGUK-RNAi-3 (MAGUK-RNAi dsRNA inactivates proteins containing either a MAGUK or a GUK domain); SH3-RNAi-5 and SH3-RNAi-3 (SH3-RNAi dsRNA inactivates proteins containing a MAGUK domain); N-term-RNAi-5 and N-term-RNAi-3 (N-term-RNAi dsRNA inactivates proteins containing the cGUK1-specific 5′ exon).

Germline transformation

Transgenic UAS-cMAGUK1 and UAS-cGUK1 flies were generated by introducing the corresponding cDNAs into w^- flies[29]. Several transgenic lines were established for each construct.

In situ hybridization

A digoxygenin-labelled RNA antisense probe that recognizes a 3′ part of cMAGUK1 encoding the PDZ, SH3 and GUK domains was generated using the DIG RNA Labeling Kit (Roche). *In situ* hybridization was performed following standard procedures.

Antibody production and immunohistochemistry

Two peptides from the C terminus of Sdt that are present in Sdt-MAGUK1 and Sdt-GUK1/2 (DKLRQKKLRNGEPFK and QWVPAQWVHNNRDES) were used to immunize rabbits (Eurogentec). For immunohistochemistry and western analysis anti-Sdt^{pep153} antibody was affinity purified and used at a dilution of 1:100 and 1:500, respectively. Enhancement of anti-Sdt^{pep153} antibody staining was accomplished with the Vectastain Elite ABC kit (Vector).

For immunohistochemistry, embryos were fixed by standard protocols[3]. The following antibodies were used for antibody staining: mouse anti-Crb Cq4 (ref. 4) (1:2), rabbit anti-Dlt[2] (1:1,000), mouse anti-phosphotyrosine PY20 (1:500, Transduction Laboratories), rabbit anti-Sas (1:500, E. Organ and D. Cavener, unpublished material), mouse anti-Nrt[9] (1:10; Developmental Studies Hybridoma Bank), rat anti-Baz N-term[11] (1:200), rat anti-Shg[30] (DCad2, 1:20) and rabbit anti-Sdt^{pep153} (this work). Cy2- and Cy3-conjugated secondary antibodies were from Jackson Immunoresearch. HRP-conjugated secondary antibodies were from Dianova. Embryos were analysed with a Leica TCS NT confocal microscope and images were processed and mounted using Photoshop 5.5 (Adobe) and Canvas 6.0 (Deneba).

Scanning electron microscopy

Embryos were initially fixed in 25% glutaraldehyde in 0.05 M phosphate buffer and heptane (1:1). After extensive washing with 0.05 M phosphate buffer, embryos were further fixed on ice for 30 min with 2% osmiumtetroxide in PBS, followed by several washes with PBS and dehydration with ethanol. Embryos were critical-point dried and covered with a gold-palladium layer 40–50 Å thick. Pictures were taken with a Hitachi S-520 scanning electron microscope.

Yeast two-hybrid interaction assays

A fragment from cMAGUK1 covering PDZ, SH3 and GUK domains was amplified by PCR using primers M5-Prot.5 and M5-Prot.3 (for primer sequences see Supplementary Information) and cloned in frame with the Gal4 transactivation domain into pACT2 (Clontech). The intracellular domain of Crb as well as mutant versions of it[3] were amplified by PCR using primers crb5-intra and crb3-intra (for primer sequences see Supplementary Information) and cloned in frame with the Gal4 DNA-binding domain into pGBT9 (Clontech). Two-hybrid interaction assays were performed according to the manufacturer's instructions.

Received 19 June; accepted 24 September 2001.

1. Müller, H. A. J. Genetic control of epithelial cell polarity: lessons from *Drosophila*. *Dev. Dyn.* **218,** 52–67 (2000).
2. Bhat, M. A. *et al.* Discs Lost, a novel multi-PDZ domain protein, establishes and maintains epithelial polarity. *Cell* **96,** 833–845 (1999).
3. Klebes, A. & Knust, E. A conserved motif in Crumbs is required for E-cadherin localisation and zonula adherens formation in *Drosophila*. *Curr. Biol.* **10,** 76–85 (2000).
4. Tepass, U. & Knust, E. Crumbs and stardust act in a genetic pathway that controls the organization of epithelia in *Drosophila melanogaster*. *Dev. Biol.* **159,** 311–326 (1993).
5. Grawe, F., Wodarz, A., Lee, B., Knust, E. & Skaer, H. The *Drosophila* genes *crumbs* and *stardust* are involved in the biogenesis of adherens junctions. *Development* **122,** 951–959 (1996).
6. Tepass, U. Crumbs, a component of the apical membrane, is required for zonula adherens formation in primary epithelia of *Drosophila*. *Dev. Biol.* **177,** 217–225 (1996).
7. Dimitratos, S. D., Woods, D. F., Stathakis, D. G. & Bryant, P. J. Signaling pathways are focused at specialized regions of the plasma membrane by scaffolding proteins of the MAGUK family. *Bioessays* **21,** 912–921 (1999).
8. Kamberov, E. *et al.* Molecular cloning and characterization of Pals, proteins associated with mLin-7. *J. Biol. Chem.* **275,** 11425–11431 (2000).
9. Müller, H. A. & Wieschaus, E. *armadillo*, *bazooka*, and *stardust* are critical for early stages in formation of the zonula adherens and maintenance of the polarized blastoderm epithelium in *Drosophila*. *J. Cell Biol.* **134,** 149–163 (1996).
10. Schober, M., Schaefer, M. & Knoblich, J. A. Bazooka recruits Inscuteable to orient asymmetric cell divisions in *Drosophila* neuroblasts. *Nature* **402,** 548–551 (1999).
11. Wodarz, A., Ramrath, A., Kuchinke, U. & Knust, E. Bazooka provides an apical cue for Inscuteable localization in *Drosophila* neuroblasts. *Nature* **402,** 544–547 (1999).
12. Wodarz, A., Ramrath, A., Grimm, A. & Knust, E. *Drosophila* atypical protein kinase C associates with Bazooka and controls polarity of epithelia and neuroblasts. *J. Cell Biol.* **150,** 1361–1374 (2000).
13. Petronczki, M. & Knoblich, J. A. DmPAR-6 directs epithelial polarity and asymmetric cell division of neuroblasts in *Drosophila*. *Nature Cell Biol.* **3,** 43–49 (2001).
14. Tepass, U., Theres, C. & Knust, E. *crumbs* encodes an EGF-like protein expressed on apical membranes of *Drosophila* epithelial cells and required for organization of epithelia. *Cell* **61,** 787–799 (1990).
15. McGee, A. W. & Bredt, D. S. Identification of an intramolecular interaction between the SH3 and guanylate kinase domains of PSD-95. *J. Biol. Chem.* **274,** 17431–17436 (1999).
16. Shin, H., Hsueh, Y.-I., Yang, F.-C., Kim, E. & Sheng, M. An intramolecular interaction between Src homology 3 domain and guanylate kinase-like domain required for channel clustering by post-synaptic density-95/SAP90. *J. Neurosci.* **20,** 3580–3587 (2000).
17. Wu, H. *et al.* Intramolecular interactions regulate SAP97 binding to GKAP. *EMBO J.* **19,** 5740–5751 (2000).
18. Nix, S. L., Chishti, A. H., Anderson, J. M. & Walther, Z. hCASK and hDlg associate in epithelia, and their Src homology 3 and guanylate kinase domains participate in both intramolecular and intermolecular interactions. *J. Biol. Chem.* **275,** 41192–41200 (2000).
19. Dimitratos, S. D., Woods, D. F. & Bryant, P. J. Camguk, Lin-2, and CASK: novel membrane-associated guanylate kinase homologs that also contain CaM kinase domains. *Mech. Dev.* **63,** 127–130 (1997).
20. Lammel, U. & Saumweber, H. X-linked loci of *Drosophila melanogaster* causing defects in the morphology of the embryonic salivary gland. *Dev. Genes Evol.* **10,** 525–535 (2000).
21. Rorth, P. A modular misexpression screen in *Drosophila* detecting tissue-specific phenotypes. *Proc. Natl Acad. Sci. USA* **93,** 12418–12422 (1996).
22. Rubin, G. M. & Spradling, A. C. Vectors for P-element-mediated gene transfer in *Drosophila*. *Nucleic Acids Res.* **11,** 6341–6351 (1983).
23. Wodarz, A., Hinz, U., Engelbert, M. & Knust, E. Expression of Crumbs confers apical character on plasma membrane domains of ectodermal epithelia of *Drosophila*. *Cell* **82,** 67–76 (1995).
24. Han, K. & Manley, J. L. Functional domains of the *Drosophila* Engrailed protein. *EMBO J.* **12,** 2723–2733 (1993).
25. Tower, J., Karpen, G. H., Craig, N. & Spradling, A. C. Preferential transposition of *Drosophila* P-elements to nearby chromosomal sites. *Genetics* **133,** 347–359 (1993).
26. Brown, N. H. & Kafatos, F. C. Functional cDNA libraries from *Drosophila* embryos. *J. Mol. Biol.* **203,** 425–437 (1988).
27. Hovemann, B. T., Dessen, E., Mechler, H. & Mack, E. *Drosophila* snRNP associated protein P11 which specifically binds to heat shock puff 93D reveals strong homology with hnRNP core protein A1. *Nucleic Acids Res.* **19,** 4909–4914 (1991).
28. Kennerdell, J. R. & Carthew, R. W. Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the Wingless pathway. *Cell* **95,** 1017–1026 (1998).
29. Brand, A. H. & Perrimon, N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118,** 401–415 (1993).
30. Oda, H., Uemura, T., Harada, Y., Iwai, Y. & Takeichi, M. A *Drosophila* homolog of cadherin associated with armadillo and essential for embryonic cell–cell adhesion. *Dev. Biol.* **165,** 716–726 (1994).

Supplementary Information accompanies the paper on *Nature*'s website (http://www.nature.com).

Acknowledgements

We thank N. Brown, B. Hovemann, M. Schäfer and C. M. Schuster for cDNA libraries, M. A. Bhat, D. Cavener and T. Uemura for antibodies, A. Ramrath for help with the yeast two-hybrid assay, and J. A. Campos-Ortega, K. Johnson, A. Müller and A. Wodarz for discussions and critical reading of the manuscript. The work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

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Kinesin-mediated axonal transport of a membrane compartment containing β-secretase and presenilin-1 requires APP

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Proteolytic processing of amyloid precursor protein (APP) generates amyloid-β peptide and has been implicated in the pathogenesis of Alzheimer's disease[1]. However, the normal function of APP, whether this function is related to the proteolytic processing of APP, and where this processing takes place in neurons *in vivo* remain unknown. We have previously shown that the axonal transport of APP in neurons is mediated by the direct binding

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