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Unraveling the Genetic Complexity of Drosophila *stardust* During Photoreceptor Morphogenesis and Prevention of Light-Induced Degeneration

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ABSTRACT

Drosophila Stardust, a membrane-associated guanylate kinase (MAGUK), recruits the transmembrane protein Crumbs and the cytoplasmic proteins *D*PATJ and *D*Lin-7 into an apically localized protein scaffold. This evolutionarily conserved complex is required for epithelial cell polarity in Drosophila embryos and mammalian cells in culture. In addition, mutations in Drosophila *crumbs* and *DPATJ* impair morphogenesis of photoreceptor cells (PRCs) and result in light-dependent retinal degeneration. Here we show that *stardust* is a genetically complex locus. While all alleles tested perturb epithelial cell polarity in the embryo, only a subset of them affects morphogenesis of PRCs or induces light-dependent retinal degeneration. Alleles retaining particular postembryonic functions still express some Stardust protein in pupal and/or adult eyes. The phenotypic complexity is reflected by the expression of distinct splice variants at different developmental stages. All proteins expressed in the retina contain the PSD95, Discs Large, ZO-1 (PDZ), Src homology 3 (SH3), and guanylate kinase (GUK) domain, but lack a large region in the N terminus encoded by one exon. These results suggest that Stardust-based protein scaffolds are dynamic, which is not only mediated by multiple interaction partners, but in addition by various forms of the Stardust protein itself.

THE apico-basal polarity of epithelial cells is manifested by a patterned distribution of membrane-associated multiprotein complexes at distinct sites within the cell. Common to all epithelia is the *zonula adherens* (ZA), a belt-like structure encircling the apex of the cell, which harbors the homophilic adhesion protein E-cadherin. Its proper organization and function is essential for cell polarity, cell adhesion, and cell shape and hence crucial for many aspects of epithelial development and morphogenesis. In the Drosophila embryonic epithelium, three multiprotein complexes jointly act to establish and maintain the ZA during embryogenesis: members of the Bazooka (Baz)/*D*Par-6/atypical protein kinase C (*D*aPKC) complex play an important role in initiating polarity (Harris and Peifer 2004, 2005), while the Crumbs (Crb)/Stardust (Sdt)/PALS-1-associated TJ protein (*D*PATJ) complex maintains the ZA and epithelial tissue structure by counteracting the activity of the laterally localized Discs-large (Dlg)/Lethal giant larvae (Lgl)/Scribble (Scrib) complex (Bilder *et al.* 2003; Tanentzapf and Tepass 2003). In the Drosophila embryo, lack of individual components of these complexes results in the loss of polarity and integrity of most epithelial tissues and eventually leads to widespread apoptosis.

Recent studies have shown that some of the proteins required for epithelial polarization and integrity in the Drosophila embryo also play a role in the morphogenesis and polarity of photoreceptor cells (PRCs). PRCs develop from epithelial cells of the eye imaginal disc by undergoing a complex morphogenetic process during pupal development. During this process, the apical surface adopts a lateral position and becomes subdivided into the stalk, a supporting membrane just apical to the ZA, and the rhabdomere, a highly pleated array of microvilli, which harbors the light-sensitive photopigment rhodopsin. The formation of the rhabdomere is associated with a conspicuous elongation of the cell (Longley and Ready 1995). PRCs mutant for *crb*, *DPATJ*, or the *sdt* allele sdt^{XP96} show defects in morphogenesis during pupal development. This is manifested by a defective shape of the PRCs, a failure to properly extend the rhabdomere and position the ZA along the proximo-distal axis during pupal development, and a reduction of the length of the stalk membrane. Neither *crb* nor *sdt* or *DPATJ* are required for the maintenance of apico-basal polarity of PRCs. This function is mediated

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by *baz* (Izaddoost *et al.* 2002; Johnson *et al.* 2002; Pellikka *et al.* 2002; Hong *et al.* 2003; Nam and Choi 2003, 2006; Richard *et al.* 2006a). In addition, loss of *crb* and *D*PATJ in the Drosophila eye leads to progressive light-induced PRC degeneration (Johnson *et al.* 2002; Richard *et al.* 2006a).

In mammals, a similar protein complex, composed of Crb3, Pals1/MPP5, and Patj or Mupp1, localizes to the tight junctions of epithelial cells (Lemmers *et al.* 2002; Roh *et al.* 2002; Hurd *et al.* 2003), where it is required for their stabilization and the maintenance of cell adhesion and polarity (Straight *et al.* 2004; Michel *et al.* 2005; Shin *et al.* 2005; Wang *et al.* 2006). In addition, two orthologs can be found in the genome, Crb1, and Crb2. Crb2, Crb3, Patj, Pals1, and Mupp1 are expressed in both PRCs and adjacent Müller glia cells, where they form a complex at the outer limiting membrane, a region immediately apical to the ZA (van de Pavert *et al.* 2004; Stohr *et al.* 2005; van Rossum *et al.* 2006). In contrast, Crb1 expression is restricted to Müller glia cells (van Rossum *et al.* 2006). Mutations in human *CRB1* are associated with retinitis pigmentosa 12 (RP12) and Leber congenital amaurosis (LCA), severe forms of retinal dystrophy (den Hollander *et al.* 1999, 2001; Lotery *et al.* 2001a,b; recently reviewed in Richard *et al.* 2006b). In the zebrafish *Danio rerio*, mutations in the *crb* ortholog *oko meduzy* result in defects in apico-basal polarity of the neuroepithelium, and inactivation of its paralogs *crb2b* or *crb3a* affects the differentiation of apical characteristics of PRCs and renal cells (Malicki and Driever 1999; Omori and Malicki 2006). The zebrafish *sdt* ortholog *nagie oko* is essential for the cellular patterning of the retina (Wei and Malicki 2002; Wei *et al.* 2006). These results suggest that this protein complex serves conserved functions during cell polarization in different species.

Results based on cDNA analysis showed that Drosophila *sdt* encodes at least three protein isoforms in the fly embryo, Sdt-A, Sdt-GUK1, and Sdt-B1 (Figure 1A) (Bachmann *et al.* 2001; Hong *et al.* 2001; Wang *et al.* 2004). Two of them, Sdt-A (named Sdt-MAGUK1 in Bachmann *et al.* 2001) and Sdt-B1, are scaffolding proteins of the MAGUK family characterized by the presence of two L27 (Lin-2/Lin7) domains, a *P*SD95, *D*iscs Large, *Z*O-1 (PDZ), Src homology 3 (SH3), Hook and guanylate kinase (GUK) domain. Sdt-A and Sdt-B differ with respect to the presence or absence of a large exon, which encodes a region of 433 amino acids in the N terminus without any obvious domain structure. In contrast, the third known isoform, Sdt-GUK1, lacks most of the PDZ domain, the SH3 and Hook domain, and part of the GUK domain (Bachmann *et al.* 2001). *In vitro* binding assays and yeast two-hybrid analyses have shown that the PDZ domain of Sdt binds to the four C-terminal amino acids of the transmembrane protein Crumbs, while the two L27 domains interact with the L27 domains of *D*PATJ and *D*Lin-7, respectively, and the N-terminal ECR1 and ECR2 domains can bind to *D*Par-6 (Bachmann *et al.* 2001, 2004; Hong *et al.* 2001; Wang *et al.* 2004; Kempkens *et al.* 2006). Three embryonic lethal alleles, sdt^{EH681}, sdt^{N5}, and sdt^{XP96}, have been characterized molecularly and shown to carry single point mutations at different positions, which all result in premature termination codons (Hong *et al.* 2001) (Figure 1A). Here we show for the first time that mutations in *sdt* can result in light-dependent retinal degeneration, similar to those in *crb* or *DPATJ*. We provide further evidence that Drosophila *sdt* is a complex locus, mutations in which differentially affect epithelial polarity in the embryo, photoreceptor morphogenesis during pupal development, and light-induced retinal degeneration.

MATERIALS AND METHODS

Fly strains and clonal analysis: Flies were kept at 25°. The *sdt* alleles used are listed in Table 1. Small *sdt* clones in the eye were generated crossing *w* sdt^{x} *FRT19A/FM7* females to w^{+} *P{ry[+t7.2]=neoFRT}19A; P{ry[+t7.2]=eyFLP.N}5* males. Large *sdt* clones were generated by crossing w^{+} *GMR::hid cl FRT19A*/FM7; UAS::FLP ey::Gal4 females (Newsome *et al.* 2000) to *w* sdt^{x} FRT19A/Y; *Tp(1;2)* sn^{+} *72d/CyO* males (where *x* is any *sdt* allele). *m*osaic *a*nalysis with a *r*epressible *c*ell *m*arker (MARCM) clones, in which mutant cells are marked with GFP (Lee and Luo 2001), were induced by a 2-hr heat shock (37°) at 48–72 hr and 72–96 hr of development in the offspring of sdt^{x} *FRT19A/FM7* females crossed to *hsFLP, tubG80 FRT19A/Y; Act-Gal4 UAS-CD8::GFP/CyO* males. Light-induced retinal degeneration and vitamin A depletion experiments were performed as previously described (Johnson *et al.* 2002).

Generation of anti-Sdt antibodies and Western analysis: The PDZ domain-encoding region corresponding to amino acids 834–913 of the Sdt-A isoform was cloned into the expression vector pGEX-4T-2 (details can be obtained upon request). Antisera against GST fusion proteins were obtained by repeated immunization of rabbits with affinity-purified proteins (Eurogentech, Seraing, Belgium).

For Western analysis, embryos (overnight egg collection), heads, and adult or pupal retinas of the respective genotype were homogenized in lysis buffer containing 50 mm Tris-HCl pH 8, 150 mm NaCl, 0.5% Triton, 1 mm $MgCl_2$, 1 μm Pefabloc, 5 μm leupeptin, 1 μm pepstatin, and 0.3 μm aprotinin. Protein concentrations were determined with BioPhotometer (Eppendorf) and Ponceau staining of the membranes. Aliquots (heads, ~0.3 μg, corresponding to four heads; embryos, ~4 μg) of protein lysates were separated by SDS–PAGE and blotted onto nitrocellulose membranes. Membranes were blocked in TBST/5% dry milk and incubated overnight with rabbit anti-SdtPDZ antibody at 1:10,000. Peroxidase-conjugated secondary antibodies (1:1000) in combination with the ECL system (Amersham Pharmacia Biotech) were employed to detect immunoreactive bands.

Northern analysis and RT–PCR: Poly(A)$^{+}$ RNA was isolated from heads of adult *white* flies by using the MACS Kit (Miltenyi Biotec). Northern blotting was performed following standard procedures. Digoxygenin-labeled RNA antisense probes were generated using the DIG RNA labeling kit (Roche, Indianapolis). RT–PCR was performed using the One-Step RT–PCR Kit (QIAGEN, Valencia, CA) with different *Sdt*-specific primer pairs. The primers used for amplification of the region encoding the SH3 domain were 5′Sdt-PDZSH3 (GAAGGTGAT GAGATACTGGAGG) and R3 (GCGTGATGAAGTGGTAG TCC). The region encoding the N terminus, PDZ, SH3, and

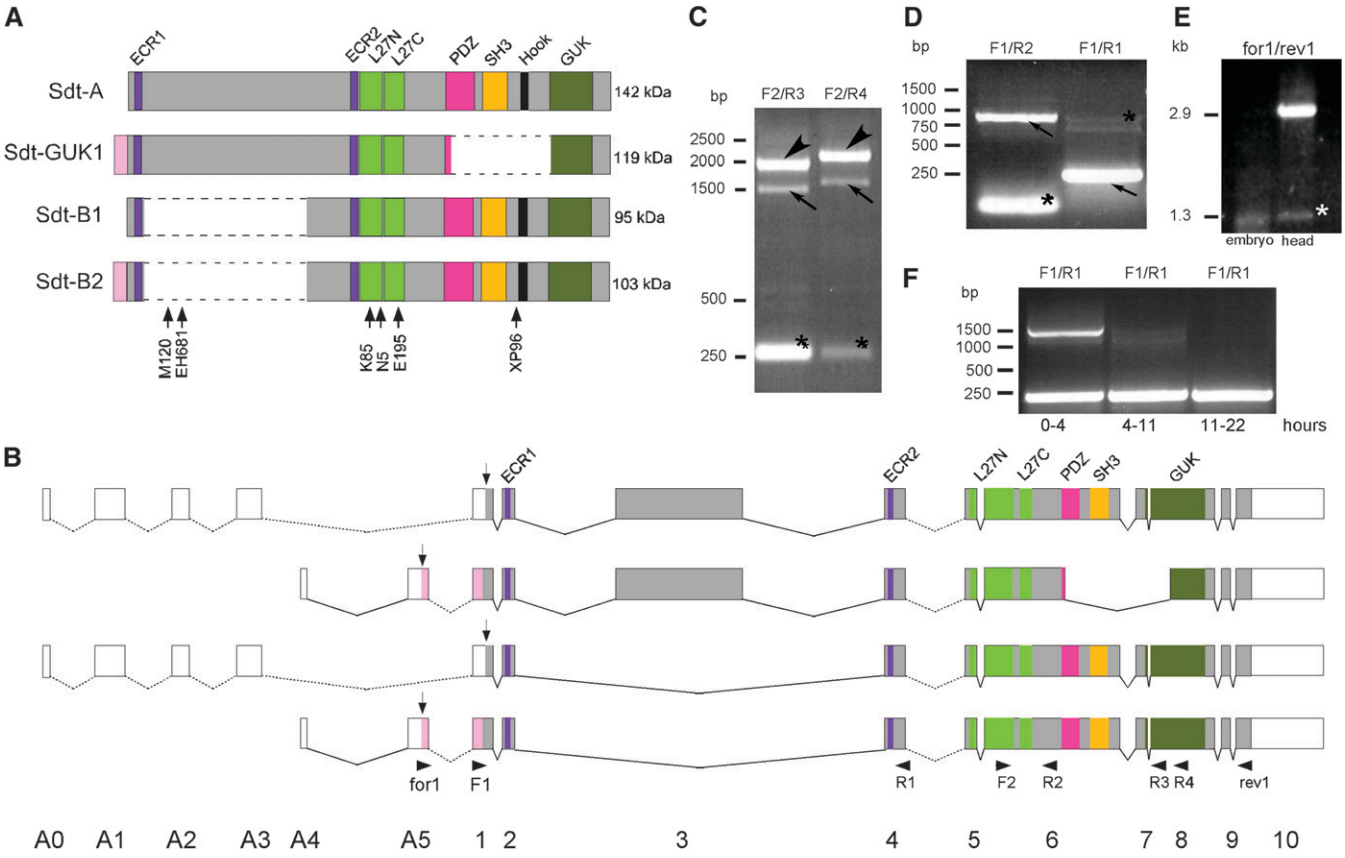

FIGURE 1.—Structure of Stardust isoforms and their expression. (A) Four different Sdt isoforms have been identified in embryos: SdtA (also called Sdt-MAGUK1), Sdt-GUK1, and SdtB1 (BACHMANN *et al.* 2001; HONG *et al.* 2001; WANG *et al.* 2004). SdtB2 is an isoform expressed in the adult head. Point mutations have been mapped in sdt^{M120} at position 32639, AAG**C**AACAT → AAG**T**AACAT (Q → stop); in sdt^{K85} at position 44683, CTG**C**AAA → CTG**T**AAA (Q → stop); and in sdt^{E195} at position 44881, ACG**C**AATC → ACG**T**AATC (Q → stop) (numbering according to FlyBase). Molecular defects in all alleles sequenced so far lead to the introduction of premature stop codons. (B) Schematic representation of the *sdt* splice variants discussed here and locations of primers used for RT–PCR (arrowheads). The colors correspond to those in A. Exons with numbers are according to HONG *et al.* (2001), novel exons are marked in uppercase letters, and untranslated 5′ and 3′ exons are in white. Sequences of primers are depicted in MATERIALS AND METHODS. (C–F) Results from RT–PCR experiments using poly(A)$^+$-RNA from adult heads (C–E, lane 2) or embryos (E, lane 1, and F). Primers used for amplification are indicated on top of each lane (for genomic positions see B). In F, developmental stages of embryos are indicated at the bottom of each lane (in hours of development at 25°). (C) Only variants encoding the complete MAGUK domain are expressed in the head. Amplification with the two primer pairs used leads in both cases to a fragment of ~1.500 bp (arrows), indicating that no RNA variant encoding the GUK isoform is present in the head (which would result in a fragment of ~300 bp). The larger bands (arrowheads) still contain the intron. (D) All RNA variants expressed in the adult head (arrows) lack exon 3, as revealed by amplification with two different primer pairs. Fragments containing exon 3 would be larger (exon 3 is 1.309 bp in length). (E) RNA encoding the SdtB variant SdtB2 can be detected only in RNA of adult heads, but not in embryos. (F) In the embryo, RNAs containing exon 3 are detectable only between 0 and 4 hr of development (larger band), but not in later stages. RNAs lacking exon 3 are present throughout embryonic development (smaller band). The sequence of all PCR products was determined. Bands marked by asterisks are nonspecific products.

GUK domains was obtained with the previously published primers (for1, GTCAGACCTCGAGAGGGAAAGACG; rev1, GTTGATGGATTCTAGAGAAGATGG) (BACHMANN *et al.* 2001). For the analysis of splice variants by RT–PCR, various combinations of the following primers were used: F1, GCAACAC TATCGCACGCACTC; F2, GCGAACTGATAGCGGCCCTTA CCC; R1, CGACCATCCTTCTCCACCC; R2, CAATGTCCTT CTGCAATCCGAG; R3, GCGTGATGAAGTGGTAGTCC; and R4, CCACCACCGTGCGTATGGCCTC (see Figure 1B). For amplification of the region deleted in the Sdt-GUK1 isoform (see Figure 1A), the primer pairs F2/R3 and F2/R4 were used. For amplification of exon 3 the primer pairs F1/R1 and F1/R2 were used. Positions of primers are shown in Figure 1B (arrowheads).

Transmission electron microscopy, immunohistochemistry, and confocal microscopy: Preparation of sections for transmission electron microscopy and measurements of stalk membrane length (R1–R6) was done as described previously (RICHARD *et al.* 2006a). For immunofluorescence analyses, the following antisera were used with Cy2- and Cy3-conjugated secondary antibodies (1:200) (Jackson ImmunoResearch Laboratories, West Grove, PA): rabbit anti-Sdt-PDZ (1:500), rat anti-Crb (1:100), rabbit anti-*D*PATJ (1:1000) (RICHARD *et al.* 2006a), rat anti-*D*E-Cad (1:50) (Developmental Studies Hybridoma Bank), rabbit anti-GFP (1:500) (Invitrogen, Carlsbad, CA), mouse anti-GFP (1:50) (Invitrogen), and guinea pig DPar-6 (1:1000; kindly provided by A. Wodarz). Rhabdomeres were visualized by labeling F-actin with Alexa-660-phalloidin

TABLE 1

Classification and characteristics of *sdt* alleles analyzed

Class	Allele	Origin[a]	Morphogenetic phenotype[c]	Light-induced degeneration	Molecular defect
I	sdt^{EH681}	EH	No	No	Stop codon in exon 3[g]
	sdt^{XH21}	HK	No	No	No mutation found
	sdt^{M120}	HK	No	No[f]	Stop codon in exon 3
	sdt^{7D22}	W	Yes	No[f]	ND
II	sdt^{XP96}	W	Yes[d]	Weak	Stop codon at the end of exon 6[g]
III	sdt^{N5}	W[b]	No[e]	Yes	Stop codon in L27N[g]
IV	sdt^{K70}	HK	Yes	Yes	No mutation found
	sdt^{K85}	HK	Yes	Yes	Stop codon in L27N
	sdt^{E195}	HK	Yes	Yes	Stop codon in L27C

[a] EH, Eberl and Hilliker (1988); W, Wieschaus *et al.* (1984); HK, T. Hummel and C. Klämbt (unpublished data).

[b] This allele is also named sdt^{NO5} (Eberl and Hilliker 1988) or sdt^{XNO5} (Hong *et al.* 2001). The identity of these alleles was verified by sequence analysis.

[c] Revealed in the light microscope as defects in the shape of the rhabdomeres.

[d] Stalk membranes of mutant PRCs are reduced by 40% (Hong *et al.* 2003).

[e] Stalk membranes of mutant PRCs are reduced by 50% (see Figure 2).

[f] Occasionally, one PRC per ommatidium was absent.

[g] Hong *et al.* (2003).

(1:40) (Molecular Probes, Eugene, OR). Immunolabeling of adult eyes was done on whole mounts (Johnson *et al.* 2002) or on cryosections (Richard *et al.* 2006a). Specimens were examined with a Leica TCS NT confocal microscope and images were processed and mounted using Photoshop 7.0 (Adobe) and Canvas 9.0 (Deneba).

RESULTS

Effects of *sdt* mutations on photoreceptor morphogenesis and survival in constant light: In the Drosophila embryo, *stardust* (*sdt*) is essential for the maintenance of cell polarity and integrity of many epithelia. This function is mediated by the direct interaction of its PDZ domain with the cytoplasmic tail of Crumbs (Crb), thereby stabilizing the ZA (Tepass and Knust 1993; Grawe *et al.* 1996; Tepass 1996; Klebes and Knust 2000; Bachmann *et al.* 2001; Hong *et al.* 2001). Besides epithelial development in the embryo, the transmembrane protein Crb controls morphogenesis of PRCs and prevents light-induced retinal degeneration. To understand better the role of the Crb/Sdt protein complex in these three distinct processes, we set out to analyze the postembryonic function of *sdt* in more detail. We included a total of nine *sdt* alleles in our analysis. All were isolated as embryonic lethals and are characterized by a strong embryonic phenotype (Table 1; data not shown). None of them complements the embryonic lethality of the amorphic allele sdt^{7D22} and all are rescued to viability by a *sdt* duplication (*Tp(1;2)sn*$^+$ *72d*). Anti-Sdt antibodies fail to detect any Sdt protein in mutant embryos (data not shown), suggesting that all alleles are nulls or strong hypomorphs in the embryo.

For the analysis of *sdt* function in PRCs, mosaic mutant eyes were generated with each allele using the FLP/FRT technique (Xu and Rubin 1993; Newsome *et al.* 2000). Flies with mosaic eyes were kept in the dark or exposed to constant light to test for effects on PRC morphogenesis and survival, respectively. The morphogenetic phenotype of PRCs is manifested by thicker and irregularly shaped rhabdomeres, which frequently contact adjacent rhabdomeres and often do not reach the base of the retina. In addition, the stalk, the part of the apical membrane between the ZA and the rhabdomere, is reduced in length. The results obtained allowed us to group the nine *sdt* alleles into four classes (Table 1, Figure 2, data not shown). Class I comprises four alleles, sdt^{EH681}, sdt^{XH21}, sdt^{M120}, and sdt^{7D22}, none of which shows any obvious effects on PRC morphogenesis. Shape and length of rhabdomeres equals that of wild-type eyes. When exposed to light, they do not exhibit any sign of degeneration. The class II allele sdt^{XP96} reveals a morphogenetic phenotype, which is manifested by bulky and irregularly shaped rhabdomeres (Figure 2B). This phenotype, associated with a reduction in the length of the stalk, has already been documented for sdt^{XP96} mutant PRCs (Hong *et al.* 2003; Nam and Choi 2003). When exposed to constant light for 5 days, nearly all PRCs mutant for each of these alleles survive (Figure 2B′). In contrast, PRCs mutant for the single class III allele sdt^{N5} exhibit normally shaped rhabdomeres. Quantification of EM data revealed that the stalk membrane is reduced in length by ~50% (Figure 2, C, E–G). When exposed to constant light, sdt^{N5} mutant PRCs undergo retinal degeneration (Figure 2C′). The three class IV alleles sdt^{K70}, sdt^{K85}, and sdt^{E195} provoke both phenotypes: PRCs exhibit severe morphogenetic defects, including a shortened stalk membrane, and degenerate when exposed to constant light (Figure 2, D, D′, and G, and data

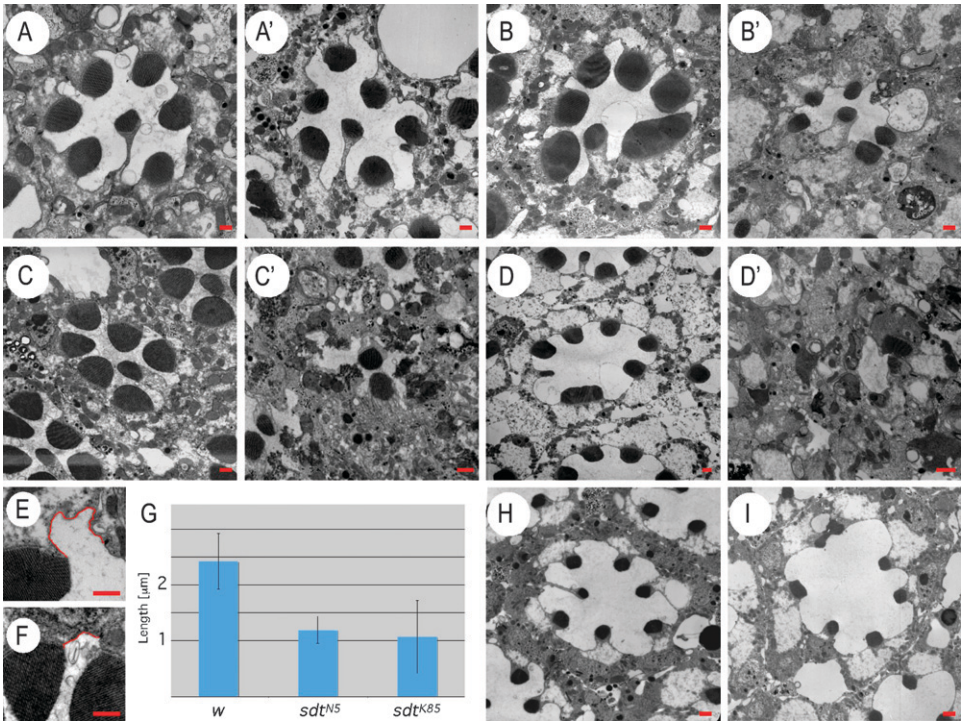

FIGURE 2.—Phenotypic defects in eye clones of different *sdt* alleles. (A–D′) Cross sections of ommatidia of *white* flies (A and A′) and flies carrying clones of sdt^{XP96} (B and B′), sdt^{N5} (C and C′), and sdt^{K85} (D and D′), kept for 7–14 days in the dark (A–D) or for 7 days in constant light (A′–D′). (E and F) Stalk membranes of *w* (E) and sdt^{N5} (F) photoreceptors, labeled in red. Those in sdt^{N5} are reduced in length. (G) The length of the stalk membranes in sdt^{K85} ($n = 140$) and sdt^{N5} ($n = 224$) is reduced by ~25% compared to that of *w* flies ($n = 210$). (H and I) Cross sections of sdt^{N5} (H) and sdt^{K70} (I) eyes from flies raised on a medium depleted of vitamin A and kept for 7 days in constant light. The rhabdomeres are smaller due to reduced amounts of rhodopsin, but the mutant PRCs do not degenerate. Bar, 1 μm.

not shown). As in the case of *crb* mutant PRCs, degeneration of *sdt* PRCs is a gradual process, characterized by the devolution of the rhabdomeres and followed by initial signs of apoptosis. Degeneration requires constant exposure to light for 5–7 days and is not observed when the flies are kept on a normal dark/light cycle.

In wild-type eyes, the light-induced signal transduction cascade is turned off by the formation of a complex between metarhodopsin, the activated form of rhodopsin, and arrestin2. The metarhodopsin-arrestin2 complex in turn dissociates upon further modification of its components. In a subset of retinal degeneration mutants, such as *arr2*, *norpA*, *rdgB*, or *rdgC*, the metarhodopsin-arrestin2 complex is abnormally stable, and its endocytosis induces apoptosis of the cells by a still unknown mechanism (ALLOWAY *et al.* 2000; KISELEV *et al.* 2000). Light-induced retinal degeneration in these mutants can be rescued by feeding larvae with a vitamin A-depleted medium (ALLOWAY *et al.* 2000; KISELEV *et al.* 2000), which reduces rhodopsin levels by ≥95% (NICHOLS and PAK 1985). Similarly, light-dependent degeneration of PRCs mutant for the class III allele sdt^{N5} and the class IV alleles sdt^{K70}, sdt^{K85}, and sdt^{E195} was strongly reduced when animals were raised and maintained on a vitamin A-depleted medium (Figure 2, H and I, and data not shown). The same degree of rescue was obtained in *crb* mutant PRCs (JOHNSON *et al.* 2002), suggesting that the light-dependent retinal degeneration seen in both mutants has a common cell biological origin.

Sdt protein expression and localization during photoreceptor development: To explore the molecular basis of the complex phenotypic behavior induced by the different *sdt* alleles, individual members of each phenotypic class were analyzed for the expression of Sdt protein in pupal and adult eyes. Photoreceptors develop from epithelial cells of the eye imaginal discs. In wild-type photoreceptors, Sdt is localized, together with Crumbs, *D*PATJ, and F-actin, at the apical membrane during the first half of pupal development (PD) (HONG *et al.* 2003; NAM and CHOI 2003; RICHARD *et al.* 2006a). From 55% of pupal development onward, a subdivision of the apical membrane into the stalk and the rhabdomere is obvious, with members of the Crumbs complex being restricted to the developing stalk, while F-actin accumulates in the forming rhabdomere, the most central region of the apical membrane (Figure 3A). In pupae of the class I alleles sdt^{EH681} and sdt^{M120}, Sdt protein is present in wild-type amounts and properly localized apically (not shown). In the class II allele sdt^{XP96}, Sdt protein is apically localized in pupae at 37% PD, although in reduced amounts (not shown). However, at 45% PD no localized protein can be detected (Figure 3A). In pupae of the class III allele sdt^{N5}, Sdt protein is still present and properly localized apically throughout pupal development (Figure 3, B–B″, and data not shown), but the amount of Sdt protein is reduced. In contrast, no Sdt protein is detectable in class IV alleles from early pupal stages onward (Figure 3, C–C″, and data not shown).

In PRCs of adult eyes, the amount and localization of Sdt protein is normal in the class I alleles sdt^{EH681} and sdt^{M120} (Table 1, data not shown). This observation is striking, given the fact that embryos mutant for either allele show a strong epithelial phenotype and that no

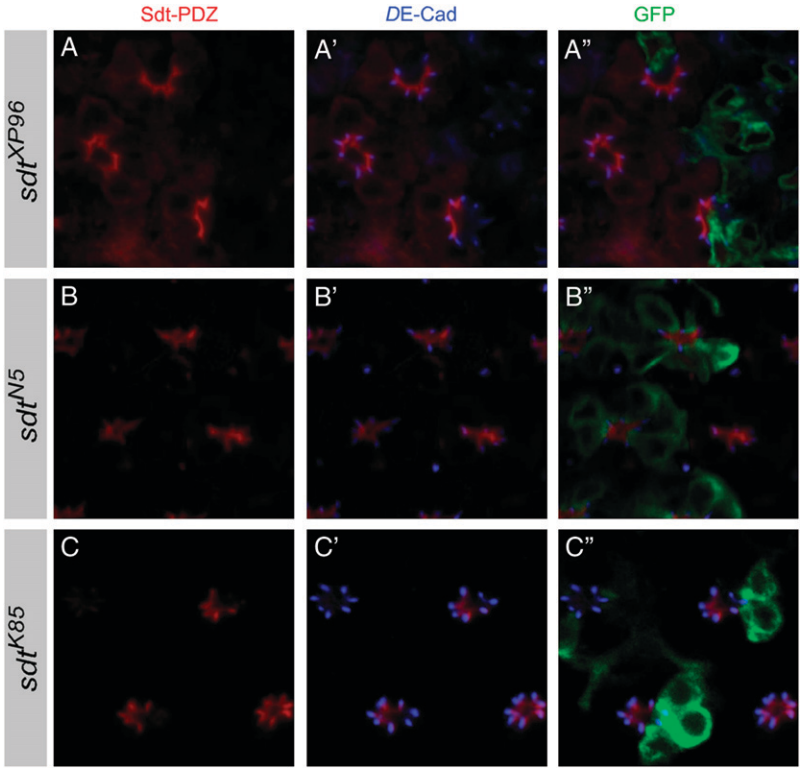

FIGURE 3.—Expression of Sdt protein in pupal eyes of different *sdt* alleles: optical cross sections of pupal eyes at 40–45% PD, before stalk membrane formation. MARCM clones of different *sdt* alleles were stained with anti-Sdt-PDZ (red) and anti-DE-cadherin to stain the *zonula adherens* (blue). Mutant cells are marked by GFP (green). Depending on the level of the optical section, the green staining is sometimes weaker. Cells not expressing GFP are wild type and serve as a control to demonstrate that Sdt localizes apically in PRCs at this stage. In sdt^{XP96} mutant PRCs (A–A″), Sdt protein is no longer detectable apically, although it is still localized earlier (not shown). sdt^{N5} mutant PRCs (B–B″) express reduced amounts of Sdt protein, which is, however, still localized apically. No Sdt protein can be detected in sdt^{K85} mutant PRCs (C and C′).

Sdt protein can be detected in these embryos. This, together with the wild-type phenotype of the PRCs indicates that sdt^{EH681} and sdt^{M120} are not null alleles in the eye. In sdt^{XP96} (class II) adult PRCs, Sdt antibodies fail to recognize a localized protein (Figure 4, B–B″). In sdt^{N5}, the only member of class III, Sdt protein can be detected at the stalk membrane, though in strongly reduced amounts (Figure 4, C–C″). Photoreceptor cells mutant for the class IV alleles sdt^{K70} and sdt^{K85} show no immunoreactivity with Sdt antibodies (Figure 4, D–D″, and data not shown). In adult PRCs mutant for the various *sdt* alleles, Crb and *D*PATJ proteins behave similarly to Sdt: they are normally localized in class I mutants, delocalized in class II mutants, localized in reduced amounts at the stalk in class III mutants, and absent in class IV mutants (not shown). Taken together, these data reveal that different *sdt* mutations have different consequences in embryos and PRCs. Particularly striking are the alleles of class I, which behave as null alleles in the embryo but are perfectly wild type in the adult eye.

One way to explain these findings is to predict that *sdt* encodes distinct isoforms that are expressed in a stage- and/or a tissue-specific manner and that the mutations examined here differentially affect various isoforms. To address this question, expression of Sdt proteins in different tissues and at different stages of development was analyzed by Western blots, using an antibody directed against the PDZ domain. In extracts from total heads of wild-type (*white*) flies, a doublet band of ~120 kDa can be distinguished (Figure 5A). In the pupal retina, the same doublet at 120 kDa and two additional bands between 130 and 140 kDa can be detected, all four of them in about the same ratio. All these forms are distinct from the major isoform expressed in the embryo (~100 kDa) (Figure 5A).

The size of the proteins detected in the eye did not allow us to determine unambiguously which of the Sdt isoforms shown in Figure 1 corresponds to the one detected in Western blots. To find out which of the isoforms is expressed in the eye, Northern analysis and RT–PCR experiments were performed. In heads of adult flies, three transcripts can be detected with *sdt*-specific probes (Figure 5B). If one of these encodes the Sdt-GUK1 isoform, this band should disappear when hybridizing the Northern blot with a probe encoding the SH3 domain, since this region is absent in the RNA encoding the Sdt-GUK1 isoform (see Figure 1A). However, this probe reveals the same three RNA bands (Figure 5B), suggesting that all transcripts include the SH3 domain and hence that Sdt-GUK1 is either not expressed or expressed only in very low amounts in the adult head. RT–PCR analysis performed on poly(A^+) RNA isolated from adult heads confirmed the absence of the GUK isoform in the head: all products amplified using various primer pairs contained the region coding for the L27, PDZ, SH3, and GUK domains (Figure 1C, arrows; for primers used see Figure 1B). In contrast, transcripts encoding Sdt-GUK1 isoforms are expressed in the embryo (BACHMANN *et al.* 2001). Extended RT–PCR studies indicate further that all transcripts expressed in the adult head lack exon 3 (Figure 1D),

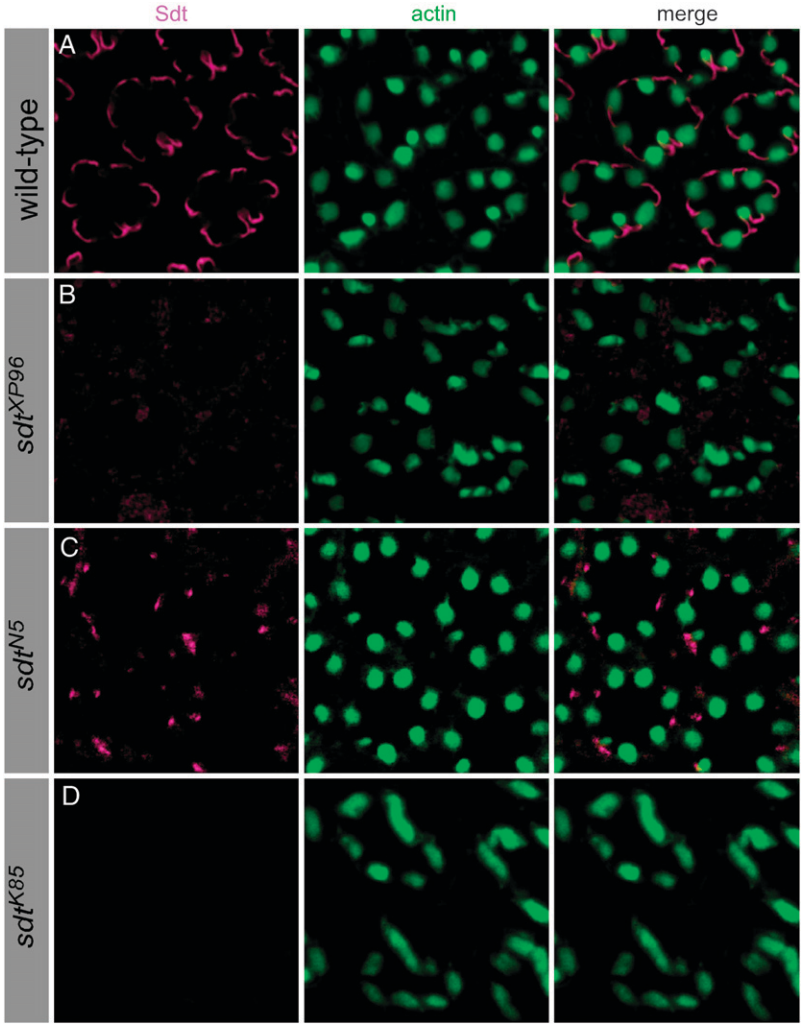

FIGURE 4.—Expression of Sdt protein in the adult eye of different *sdt* alleles. Optical cross sections of adult Drosophila eyes were stained with phalloidin to highlight the F-actin-rich rhabdomeres (green) and with anti-Sdt-PDZ (purple). (A) In wild-type (*w*) eyes, Sdt is restricted to the stalk membrane, adjacent to the rhabdomere. (B) In sdt^{XP96} eyes, Sdt protein is lost from the stalk membrane. Note the morphological defects in the rhabdomeres (compare the F-actin staining in A′ and B′). (C) In sdt^{N5} eyes, the shape of the rhabdomeres is normal and Sdt protein can be detected at the stalk membrane, albeit in reduced amounts. (D) sdt^{K85} eyes show morphological defects in the rhabdomeres and no Sdt protein can be detected.

which encodes for an extended N-terminal region, which is present in Sdt-A and Sdt-GUK1 (Figure 1A). Hence, all proteins expressed in the head resemble Sdt-B1 in that they are devoid of the extended N-terminal region. Using primers specific for exon A5 (for1), we detected the RNA for a novel variant expressed in the head but not in the embryo, named Sdt-B2, which carries the same N terminus as Sdt-GUK1, but lacks the region encoded by exon 3 (Figure 1, A and E). In the embryo, transcripts containing exon 3 are expressed, but only at early stages of development (Figure 1F). However, since the major protein isoform detected in extracts from embryos has a size of ~100 kDa (Figure 5A) and a Sdt-A variant would have a size of ~140 kDa (Figure 1A), we suggest that there are only minor amounts of Sdt-A variants present in embryos. To summarize, these data show that all the major Sdt isoforms expressed in the adult head lack the N-terminal portion of 433 amino acids encoded by exon 3 and that all contain a complete MAGUK region, composed of the PDZ, SH3, and GUK domains. Thus, the majority of the Sdt proteins in the adult head correspond to Sdt-B variants.

To determine the expression of Sdt protein in eyes of the different *sdt* alleles, Western blots with retina extracts prepared from adult mosaic flies were performed (Figure 5C). These flies carried *sdt* mutant clones that covered nearly the whole eye, with ~1% wild-type ommatidia (see MATERIALS AND METHODS). No Sdt protein can be recovered from sdt^{K85} mutant eyes. Eyes mutant for sdt^{N5} express proteins of the same size as those detected in the wild-type retina, though in reduced amounts, which is in agreement with the immunohistochemical data (compare with Figure 4, C–C″). Eyes mutant for sdt^{XP96} express very small amounts of proteins of about wild-type size (Figure 5C), although no localized protein could be detected in the mutant PRCs (compare with Figure 4, B–B″). This suggests that the mutant protein cannot be retained at the stalk membrane in adult flies and/or is unstable. To rule out the possibility that the Sdt protein detected in mosaic sdt^{XP96} and sdt^{N5} eyes is derived from the few wild-type ommatidia present in the mosaic eyes, we performed Western blots under the same conditions as in Figure 5C, using increasing amounts of protein extracts from wild-type eyes, which was prepared from 0.25, 0.5, 1, or 2 retinas,

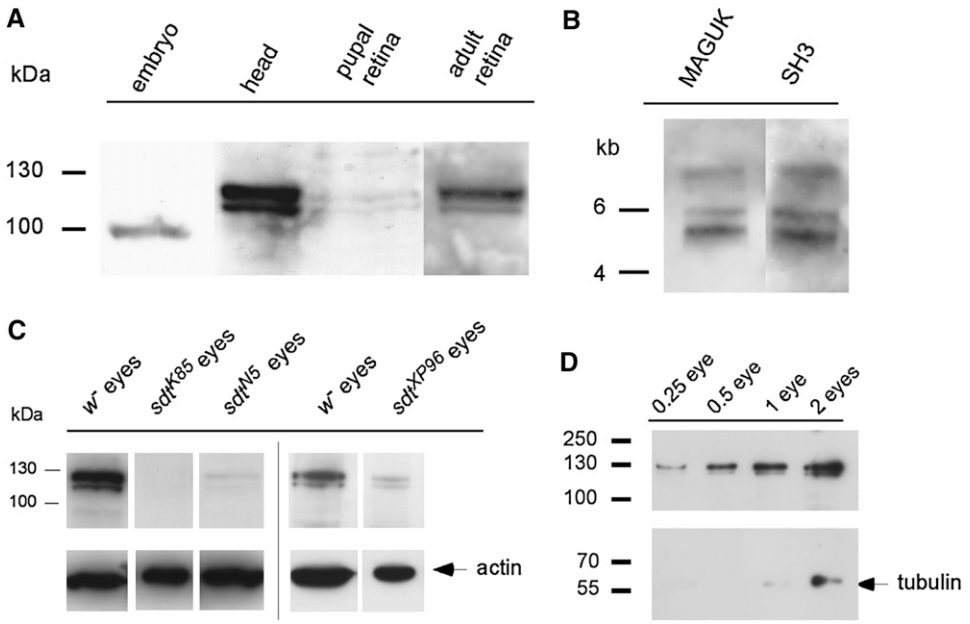

FIGURE 5.—Expression of Sdt protein and RNA. (A) Western blots of wild-type (*w*) protein extracts from different developmental stages and organs, probed with an antibody directed against the PDZ domain. (B) Northern blots of poly(A)$^+$-RNA from wild-type (*w*) heads hybridized with a probe encoding the full-length MAGUK domain (including PDZ, SH3, and GUK domain) or the SH3 domain only. (C) Western blots of extracts from wild-type (*w*) and mutant eyes (four eyes/lane), probed with anti-Sdt-PDZ antibody. Eyes used for this experiment contained large mutant clones with hardly any wild-type ommatidia (~1%), corresponding to ~32 ommatia. (D) Western blot of different amounts of wild-type eyes, probed with anti-Sdt-PDZ antibody. A faint band of Sdt protein can be detected in one-quarter of an eye, which corresponds to ~200 ommatidia.

corresponding to ~200, 400, 800, and 1600 ommatidia, respectively (assuming ~800 ommatidia/eye). The intensity of the band in extracts from 0.25 retinas was similar to that of extracts from four mosaic eyes (*i.e.*, sdt^{XP96} and sdt^{XP96}), which contained ~1% wild-type ommatidia, corresponding to ~32 ommatidia. The Sdt protein from these wild-type ommatidia would be below detection level. Therefore we conclude that the Sdt protein detected in the extracts of sdt^{XP96} and sdt^{N5} retinas is derived from the mutant cells, which is consistent with the data obtained by immunohistochemistry.

Molecular characterization of *sdt* alleles: sdt^{EH681}, sdt^{N5}, and sdt^{XP96} carry single nonsense mutations (HONG *et al.* 2001 and our results), which should cause premature translational termination (Figure 1A). To further characterize the molecular defects in the different *sdt* alleles analyzed here, we sequenced all exons and exon/intron boundaries (present in the Sdt-A and Sdt-B isoforms) in selected alleles from each group. No mutation could be detected in exons 1–6 in the class I allele sdt^{XH21}. The other allele of this class, sdt^{M120}, carries a single point mutation in exon 3, which results in a premature stop codon. Since all Sdt variants expressed in the eye lack exon 3, this mutation, similar to the one in sdt^{EH681}, does not affect any Sdt protein in the eye, which is consistent with the wild-type behavior of these alleles. From the three class IV alleles analyzed, no mutation was found in the sequenced region of sdt^{K70}. However, single point mutations were detected in sdt^{K85} and sdt^{E195} in regions coding for the N-terminal and C-terminal L27 domains, respectively. Both mutations introduce a premature stop codon and are expected to truncate all known Sdt isoforms (Figure 1). This is consistent with their complete loss-of-function phenotype.

Strikingly, sdt^{N5} also encodes a protein with a premature stop codon in the N-terminal L27 domain, yet we still detect Sdt protein with an antibody directed against the PDZ domain. To understand this difference in behavior, we analyzed the localization of *D*PATJ (which binds to the N-terminal L27 domain) and *D*Par-6 (which can interact with the ECR domains) in pupal eyes of sdt^{N5} and compared it to that in sdt^{K85}. *D*PATJ showed a wild-type localization in pupal ommatidia of both genotypes; *i.e.*, it was restricted apically (Figure 6A and data not shown). *D*Par-6, which localizes apically in wild-type ommatidia at 45% PD, was unchanged in its distribution in sdt^{N5} pupal ommatidia (Figure 6). In contrast, *D*Par-6 protein expanded to more basal positions, basal to the ZA in several PRCs of sdt^{K85} pupal ommatidia. A similar behavior has been described for *D*Par-6 and Bazooka in sdt^{XP96} mutant pupal photoreceptor cells (HONG *et al.* 2003; NAM and CHOI 2003), which also show a morphogenetic phenotype. Therefore the morphogenetic phenotype in sdt^{K85} and sdt^{XP96} can be explained by a mislocalization of Bazooka and *D*Par-6 and is in agreement with the observation that both proteins are required for proper development of PRCs (HONG *et al.* 2003; NAM and CHOI 2003). Morphogenesis of PRCs seems not to be affected if some Sdt protein is localized, as is the case in sdt^{N5}.

DISCUSSION

The data presented here provide evidence that the Drosophila *sdt* locus is a complex locus that encodes

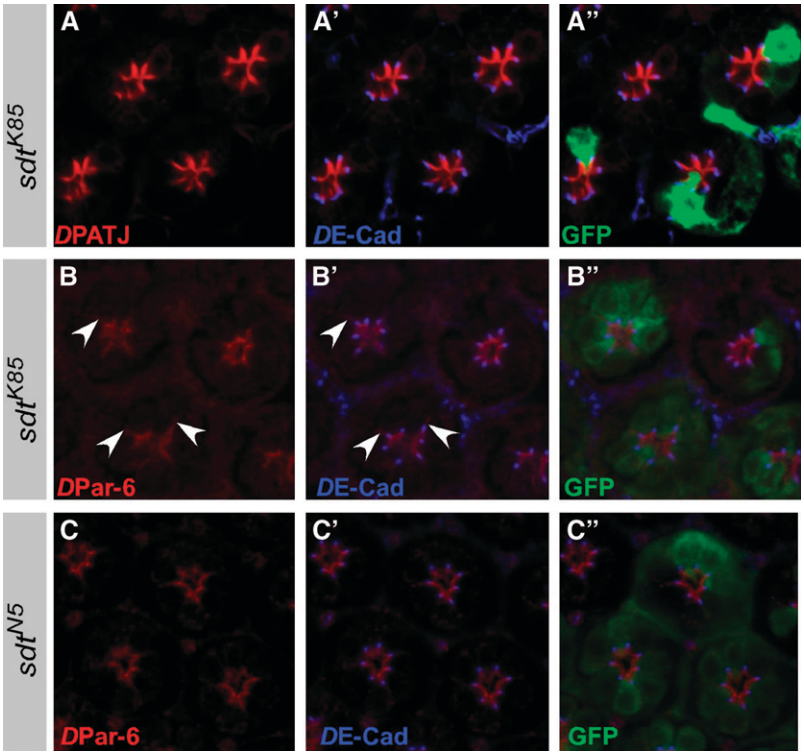

FIGURE 6.—Localization of *D*PATJ and *D*Par-6 in *sdt* mutant pupal photoreceptor cells. Optical cross sections of pupal eyes at 40–45% PD., before stalk membrane formation, are shown. MARCM clones of different *sdt* alleles were stained with anti-*D*PATJ (A) or anti *D*Par-6 (B) (red) and anti-*D*E-cadherin (C) to stain the *zonula adherens* (blue). Mutant cells are marked by GFP (green). Cells not expressing GFP are wild type and serve as a control.

several developmentally regulated protein isoforms, which control the integrity of embryonic epithelia and morphogenesis of pupal PRCs and prevent light-induced retinal degeneration. This finding suggests that the Crb/Sdt/*D*PATJ protein complex differs in its composition at different stages of development, at least with respect to the Sdt isoform included.

We have shown that of the previously published Sdt isoforms, neither Sdt-A nor Sdt-GUK1 is expressed in the eye. These two isoforms differ from Sdt-B variants by the presence of an extended N terminus, thus separating the two ECR domains more widely (see Figure 1). This region, encoded by exon 3, contains a stretch of 433 amino acids and does not exhibit any obvious domain structure. The molecular lesion in sdt^{M120} and sdt^{EH681} results in the replacement of a cytosine by a thymine, which changes the triplet encoding glutamine at positions 142 and 156 (HONG *et al.* 2001) of SdtA, respectively, into a stop codon. As a result, all Sdt-A isoforms containing this exon should be truncated, while Sdt-B variants should remain unaffected. This explains why sdt^{M120} and sdt^{EH681} PRCs behave perfectly wild type in the eye and supports our finding derived from RT–PCR analysis that all isoforms expressed in the head lack exon 3. Strikingly, the mammalian Sdt ortholog Pals1/MPP5 does not contain this stretch, but rather resembles Sdt-B variants.

RT–PCR analysis performed on RNA preparations of embryos showed that transcripts for Sdt-A isoforms are detectable only in 0- to 4-hr-old embryos. Therefore one may speculate that this protein functions in processes specific to early Drosophila embryogenesis, such as early gastrulation. Since sdt^{M120} and sdt^{EH681} mutant embryos develop a strong mutant phenotype, we conclude that expression of Sdt-A is essential for embryogenesis. Embryos derived from sdt^{EH681} germ-line clones do not show an enhanced mutant cuticle phenotype in comparison to zygotic sdt^{EH681} embryos (TEPASS and KNUST 1993), suggesting that this *sdt* transcript is transcribed very early during embryogenesis, and/or that the maternal germ line with only half the *sdt* gene dose is insufficient for the maternal provision of this isoform in a sdt^{EH681} embryo. Previous experimental data using a different readout pointed to a maternal contribution of *sdt*. When crb^{11A22} homozygous mutant embryos carried an additional copy of the sdt^{+} gene (*i.e.*, crb^{11A22}/crb^{11A22}; $Tp(1;2)sn^{+72b}/+$) the *crb* mutant phenotype was strongly suppressed. However, when these embryos were derived from females that were heterozygous for *sdt* ($sdt^{EH681}/+$; $crb^{11A22}/+$), the *crb* mutant phenotype was only very weakly suppressed (TEPASS and KNUST 1993). This suggests that maternal expression of *sdt* provides some function in the embryo. Additional experiments are required to test whether Sdt-A performs a specific function in the embryo and if so, how the region encoded by exon 3 contributes to this function. This region does not affect *in vitro* binding to the N terminus to *D*Par-6, since both variants interact with the PDZ domain of *D*Par-6 (WANG *et al.* 2004; KEMPKENS *et al.* 2006).

Our data further allow the conclusion that the complete absence of Sdt protein expression in pupal and adult PRCs is associated with a complete loss of

function, characterized by morphogenetic defects and light-dependent retinal degeneration. This is the case for sdt^{K85} and sdt^{E195}. Mutations in these alleles truncate Sdt proteins in the first and second L27 domain, respectively. Although sdt^{N5} carries a stop codon in the N-terminal L27 domain (Figure 1A) (Hong *et al.* 2001 and our results), Sdt protein of wild-type size can be detected with an antibody directed against the PDZ domain in the mutant retina of adult flies. The protein is produced in reduced amounts, but is properly localized in pupal and adult PRCs of this allele. This observation can be explained by assuming a readthrough of the stop codon during translation. But why should the stop codon in sdt^{N5} be read through, but those in sdt^{K85} and sdt^{E195} located close by terminate translation? Different factors may facilitate readthrough of a stop codon, such as the nucleotides following the stop codon or the nature of the stop codon itself (reviewed in Namy *et al.* 2004). It has been predicted for yeast and Drosophila that the sequence C-A-A immediately 3′ of a stop codon enhances the chance for a readthrough (Sato *et al.* 2003; Williams *et al.* 2004). In mammals, a UGA followed by a C or a U has a three times higher chance of being suppressed during translation in comparison to a UGA followed by an A or G (Mccaughan *et al.* 1995). In the three *sdt* alleles under discussion, the sequence 3′ to the stop codon in sdt^{K85} and sdt^{E195} is A-A-C and U-C-G, respectively, while that in sdt^{N5} is C-G-A. The latter thus carries a C immediately 3′ to the stop codon and is more similar to the consensus sequence C-A-A, making a readthrough more likely. The efficiency of readthrough can also be influenced by the stop codon itself. For example, the Drosophila *kelch* gene encodes two proteins translated from a single mRNA, in which the formation of the longer form is the result of a stop codon suppression. Formation of a full-length protein was abolished upon mutating the existing UGA stop codon into a UAA codon (Robinson and Cooley 1997). In fact, the three *sdt* alleles under discussion here differ with respect to their premature stop codon, which is UGA in sdt^{N5}, but UAA in sdt^{K85} and sdt^{E195}. This, again, makes a readthrough in sdt^{N5} more likely than in the other two alleles. Stop codon suppression can also be regulated in time and space, as shown for the *kelch* RNA, for which stop codon suppression was most efficient in imaginal discs (Robinson and Cooley 1997). Similarly, the proposed stop codon suppression in sdt^{N5} seems to be developmentally regulated. While full-length Sdt protein can be detected in pupal and adult eyes of sdt^{N5}, no Sdt protein was found in embryos mutant for this allele (data not shown). This suggests that termination of translation at this premature stop codon in sdt^{N5} is more tightly controlled in the embryo than in PRCs.

Although sdt^{N5} mutant PRCs still produce full-length Sdt protein, albeit in reduced amounts, they have a shorter stalk membrane and undergo light-dependent retinal degeneration. This observation led us to believe that a strong decrease in protein expression levels may account for the difference in phenotype between sdt^{N5} and the class I alleles, which have wild-type levels of Sdt protein. However, we cannot rule out the possibility that additional Sdt isoforms expressed in the head may be differentially affected by the mutation.

The mutation in sdt^{XP96} changes the first nucleotide (G) of the intron that follows exon 6 into an A (Hong *et al.* 2001). As suggested by the authors, the loss of the splice site will lead to a stop codon shortly after and thus to a truncated protein that lacks the GUK domain. sdt^{XP96} pupal PRCs express strongly reduced amounts of Sdt proteins at early stages, which are normally localized, while no localized protein is detected in later pupae and adult mutant PRCs, indicating that a truncated protein is unstable in later stages. This is consistent with our observation, that proper localization of transgene-encoded Sdt variants in pupal PRCs does not require the GUK domain, while this domain is necessary for stalk membrane localization in the adult eye (N. Bulgakova and E. Knust, unpublished results). Although we cannot completely rule out pupal expression of a Sdt variant that is not affected by the mutation, the results indicate for the first time a functional relevance of the GUK domain *in vivo*. In addition, the data support the model recently derived from the analysis of *DPATJ*, which suggests different mechanisms regulating the localization of the Crb/Sdt/*D*PATJ complex in the pupae and the adult eye. A truncated *D*PATJ protein, containing the L27 domain and two of the four PDZ domains, is apically localized in the pupae, but fails to be stabilized at the stalk membrane in adult PRCs (Richard *et al.* 2006a).

The presence of different isoforms as a result of stage- and tissue-specific alternative splicing is quite common for members of the MAGUK-protein family. The Drosophila tumor suppressor *discs large* (*dlg*), for example, encodes an isoform, called Dlg-S97, which is specifically expressed in the neuromuscular junction (Mendoza *et al.* 2003). It differs from the form expressed in epithelial cells by the presence of an L27 domain in its N terminus, which allows for interaction with *D*Lin-7 in the neuromuscular junction (Bachmann *et al.* 2004). Tissue-specific expression of different isoforms together with the presence of multiple protein–protein interaction domains make members of the MAGUK-protein family ideally suited for modulating the composition of membrane-associated protein complexes. Interestingly, no differential splicing of the *sdt* ortholog Pals1/MPP5 has been reported so far. However, the mammalian Crb-complex localized at the outer limiting membrane in the mammalian retina contains, besides MPP5/Pals1, additional members of the membrane palmitoylated protein-family (MPP), such as MPP3 and MPP4, which might have partially redundant functions. MPP4 was shown to recruit MPP5/Pals1 into the CRB1-complex through direct interaction between its SH3 domain and

the GUK domain of MPP5/Pals1, while MPP5, in turn, recruits MPP3 (Kantardzhieva *et al.* 2005, 2006; Aartsen *et al.* 2006). *In vitro* interactions found between the SH3 and the GUK domain of Sdt point to a similar behavior of these domains in the Drosophila protein (Ö. Kempkens and E. Knust, unpublished results) and strengthen the functional importance of the GUK domain. It is tempting to speculate that in vertebrates, different functions are mediated by similar proteins, which are encoded by several genes, while in Drosophila a single gene, *sdt*, fulfills all these functions. This is achieved by the expression of multiple isoforms, produced by differential splicing.

The data presented here now provide an ideal basis for further studies to unravel the function of the Crb/Sdt complex in different cellular contexts, in particular in the PRCs. In the mouse, the Sdt ortholog Pals1/Mpp5 colocalizes with Crb1/Crb2/Crb3 at the outer limiting membrane of PRCs, and Crb1 prevents retinal disorganization and light-dependent dystrophy (Mehalow *et al.* 2003; van De Pavert *et al.* 2004). The high degree of evolutionary conservation implied by these findings suggests that further elucidation of the tissue-specific functions of the different Sdt isoforms in Drosophila may also provide important contributions to our understanding of the function of this protein complex in mammalian epithelial and photoreceptor cells.

We are particularly indebted to Thomas Hummel and Christian Klämbt for allowing us to use the five *sdt* alleles, sdt^{XH21}, sdt^{M120}, sdt^{K70}, sdt^{K85}, and sdt^{E195}, which were extremely helpful for our analysis. We thank Andreas Wodarz and the Developmental Studies Hybridoma Bank at the University of Iowa for antibodies and Mélisande Richard and André Bachmann for discussions and critical reading of the manuscript. This work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB 590, Kn250/21-1, and GRK 320) and the European Consortium (QLG3-CT-2002-01266).

LITERATURE CITED

Aartsen, W. M., A. Kantardzhieva, J. Klooster, A. G. van Rossum, S. A. van de Pavert *et al.*, 2006 Mpp4 recruits Psd95 and Veli3 towards the photoreceptor synapse. Hum. Mol. Genet. **15:** 1291–1302.

Alloway, P. G., L. Howard and P. J. Dolph, 2000 The formation of stable rhodopsin-arrestin complexes induces apoptosis and photoreceptor cell degeneration. Neuron **28:** 129–138.

Bachmann, A., M. Schneider, F. Grawe, E. Theilenberg and E. Knust, 2001 *Drosophila* Stardust is a partner of Crumbs in the control of epithelial cell polarity. Nature **414:** 638–643.

Bachmann, A., M. Timmer, J. Sierralta, G. Pietrini, E. D. Gundelfinger *et al.*, 2004 Cell type-specific recruitment of *Drosophila* Lin-7 to distinct MAGUK-based protein complexes defines novel roles for Sdt and Dlg-S97. J. Cell Sci. **117:** 1899–1909.

Bilder, D., M. Schober and N. Perrimon, 2003 Integrated activity of PDZ protein complexes regulates epithelial polarity. Nat. Cell Biol. **5:** 53–58.

den Hollander, A. I., J. B. ten Brink, Y. J. de Kok, S. van Soest, L. I. van den Born *et al.*, 1999 Mutations in a human homologue of Drosophila crumbs cause retinitis pigmentosa (RP12). Nat. Genet. **23:** 217–221.

den Hollander, A. I., J. R. Heckenlively, L. I. van den Born, Y. J. de Kok, S. D. van der Velde-Visser *et al.*, 2001 Leber congenital amaurosis and retinitis pigmentosa with Coats-like exudative vasculopathy are associated with mutations in the crumbs homologue 1 (CRB1) gene. Am. J. Hum. Genet. **69:** 198–203.

Eberl, D. F., and A. J. Hilliker, 1988 Characterization of X-linked recessive lethal mutations affecting embryonic morphogenesis in *Drosophila melanogaster*. Genetics **118:** 109–120.

Grawe, F., A. Wodarz, B. Lee, E. Knust and H. Skaer, 1996 The *Drosophila* genes *crumbs* and *stardust* are involved in the biogenesis of adherens junctions. Development **122:** 951–959.

Harris, T. J. C., and M. Peifer, 2004 Adherens junction-dependent and -independent steps in the establishment of epithelial cell polarity in *Drosophila*. J. Cell Biol. **167:** 135–147.

Harris, T. J. C., and M. Peifer, 2005 The positioning and segregation of apical cues during epithelia polarity establishment in *Drosophila*. J. Cell Biol. **170:** 813–823.

Hong, Y., B. Stronach, N. Perrimon, L. Y. Jan and Y.-N. Jan, 2001 Drosophila Stardust interacts with Crumbs to control polarity of epithelia but not neuroblasts. Nature **414:** 634–638.

Hong, Y., L. Ackerman, L. Y. Jan and Y.-N. Jan, 2003 Distinct roles of Bazooka and Stardust in the specification of *Drosophila* photoreceptor membrane architecture. Proc. Natl. Acad. Sci. USA **100:** 12712–12717.

Hurd, T. W., L. Gao, M. H. Roh, I. G. Macara and B. Margolis, 2003 Direct interaction of two polarity complexes implicated in epithelial tight junction assembly. Nat. Cell Biol. **5:** 137–142.

Izaddoost, S., S.-C. Nam, M. A. Bhat, H. J. Bellen and K.-W. Choi, 2002 *Drosophila* Crumbs is a positional cue in photoreceptor adherens junctions and rhabdomeres. Nature **416:** 178–183.

Johnson, K., F. Grawe, N. Grzeschik and E. Knust, 2002 *Drosophila* Crumbs is required to inhibit light-induced photoreceptor degeneration. Curr. Biol. **12:** 1675–1680.

Kantardzhieva, A., I. Gosens, S. Alexeeva, I. M. Punte, I. Versteeg *et al.*, 2005 MPP5 recruits MPP4 to the CRB1 complex in photoreceptors. Invest. Ophtalmol. Vis. Sci. **46:** 2192–2201.

Kantardzhieva, A., S. Alexeeva, I. Versteeg and J. Wijnholds, 2006 MPP3 is recruited to the MPP5 protein scaffold at the retinal outer limiting membrane. FEBS J. **273:** 1152–1165.

Kempkens, Ö., E. Médina, G. Fernandez-Ballester, S. Özüyaman, A. Le Bivic *et al.*, 2006 Computer modelling in combination with in vitro studies reveals similar binding affinities of *Drosophila* Crumbs for the PDZ domains of Stardust and *Dm*Par-6. Eur. J. Cell Biol. **85:** 753–767.

Kiselev, A., M. Socolich, J. Vinós, R. W. Hardy, C. S. Zuker *et al.*, 2000 A molecular pathway for light-dependent photoreceptor apoptosis in *Drosophila*. Neuron **28:** 139–152.

Klebes, A., and E. Knust, 2000 A conserved motif in Crumbs is required for E-cadherin localisation and zonula adherens formation in *Drosophila*. Curr. Biol. **10:** 76–85.

Lee, T., and L. Luo, 2001 Mosaic analysis with a repressible cell marker (MARCM) for *Drosophila* neural development. Trends Neurosci. **24:** 251–254.

Lemmers, C., E. Medina, M.-H. Delgrossi, M. Didier, J.-P. Arsanto *et al.*, 2002 hINAD1/PATJ, a homolog of Discs Lost, interacts with Crumbs and localizes to tight junctions in human epithelial cells. J. Biol. Chem. **277:** 25408–25415.

Longley, R. L. J., and D. F. Ready, 1995 Integrins and the development of three-dimensional structure in the *Drosophila* compound eye. Dev. Biol. **171:** 415–433.

Lotery, A. J., S. G. Jacobson, G. A. Fishman, R. G. Weleber, A. B. Fulton *et al.*, 2001a Mutations in the CRB1 gene cause Leber congenital amaurosis. Arch. Ophthalmol. **119:** 415–420.

Lotery, A. J., A. Malik, S. A. Shami, M. Sindhi, B. Chohan *et al.*, 2001b CRB1 mutations may result in retinitis pigmentosa without para-arteriolar RPE preservation. Ophthalmic Genet. **22:** 163–169.

Malicki, J., and W. Driever, 1999 *oko meduzy* mutations affect neuronal patterning in the zebrafish retina and reveal cell-cell interactions of the retinal neuroepithelium. Development **126:** 1235–1246.

McCaughan, K., C. Brown, M. Dalphin, M. Berry and W. Tate, 1995 Translational termination efficiency in mammals is influenced by the base following the stop codon. Proc. Natl. Acad. Sci. USA **92:** 5431–5435.

Mehalow, A. K., S. Kameya, R. S. Smith, N. L. Hawes, J. M. Denegre *et al.*, 2003 CRB1 is essential for external limiting membrane integrity and photoreceptor morphogenesis in the mammalian retina. Hum. Mol. Genet. **12:** 2179–2189.

Mendoza, C., P. Olguin, G. Lafferte, U. Thomas, S. Ebitsch *et al.*, 2003 Novel isoforms of Dlg are fundamental for neuronal development in *Drosophila*. J. Neurosci. **23:** 2093–2101.

Michel, D., J. P. Arsanto, D. Massey-Harroche, C. Beclin, J. Wijnholds *et al.*, 2005 PATJ connects and stabilizes apical and lateral components of tight junctions in human intestinal cells. J. Cell Sci. **118:** 4283–4293.

Nam, S.-C., and K.-W. Choi, 2003 Interaction of Par-6 and Crumbs complexes is essential for photoreceptor morphogenesis in *Drosophila*. Development **130:** 4363–4372.

Nam, S.-C., and K.-W. Choi, 2006 Domain-specific early and late function of Dpatj in Drosophila photoreceptor cells. Dev. Dyn. **235:** 1501–1507.

Namy, O., J.-P. Rousset, S. Naphtine and I. Brierley, 2004 Reprogrammed genetic decoding in cellular gene expression. Mol. Cell **13:** 157–168.

Newsome, T. P., B. Asling and B. J. Dickson, 2000 Analysis of *Drosophila* photoreceptor axon guidance in eye-specific mosaics. Development **127:** 851–860.

Nichols, R., and W. L. Pak, 1985 Characterization of *Drosophila melanogaster* rhodopsin. J. Biol. Chem. **260:** 12670–12674.

Omori, Y., and J. Malicki, 2006 *oko meduzy* and related *crumbs* genes are determinants of apical cell features in the vertebrate embryos. Curr. Biol. **16:** 945–957.

Pellikka, M., G. Tanentzapf, M. Pinto, C. Smith, C. J. McGlade *et al.*, 2002 Crumbs, the *Drosophila* homologue of human CRB1/RP12, is essential for photoreceptor morphogenesis. Nature **416:** 143–149.

Richard, M., F. Grawe and E. Knust, 2006a *D*PATJ plays a role in retinal morphogenesis and protects against light-dependent degeneration of photoreceptor cells in the *Drosophila* eye. Dev. Dyn. **235:** 895–907.

Richard, M., R. Roepman, W. M. Aartsen, A. G. van Rossum, A. I. den Hollander *et al.*, 2006b Towards understanding CRUMBS function in retinal dystrophies. Hum. Mol. Genet. **15:** R235–R243.

Robinson, D. N., and L. Cooley, 1997 Examination of the function of two kelch proteins generated by stop codon suppression. Development **124:** 1405–1417.

Roh, M. H., O. Makarova, C. J. Liu, K. Shin, S. Lee *et al.*, 2002 The Maguk protein, Pals1, functions as an adapter linking mammalian homologues of Crumbs and Discs Lost. J. Cell Biol. **157:** 161–172.

Sato, M., H. Umeki, R. Saito, A. Kanai and M. Tomita, 2003 Computational analysis of stop codon readthrough in *D. melanogaster*. Bioinformatics **19:** 1371–1380.

Shin, K., S. Straight and B. Margolis, 2005 PATJ regulates tight junction formation and polarity in mammalian epithelial cells. J. Cell Biol. **168:** 705–711.

Stohr, H., L. L. Molday, R. S. Molday, B. H. Weber, B. Biedermann *et al.*, 2005 Membrane-associated guanylate kinase proteins MPP4 and MPP5 associate with Veli3 at distinct intercellular junctions of the neurosensory retina. J. Comp. Neurol. **481:** 31–41.

Straight, S. W., K. Shin, V. C. Fogg, S. Fan, C.-J. Liu *et al.*, 2004 Loss of PALS1 expression leads to tight junction and polarity defects. Mol. Biol. Cell **15:** 1981–1990.

Tanentzapf, G., and U. Tepass, 2003 Interactions between Crumbs, Lethal giant larvae and Bazooka pathways in epithelial polarization. Nat. Cell Biol. **5:** 46–52.

Tepass, U., 1996 Crumbs, a component of the apical membrane, is required for zonula adherens formation in primary epithelia of *Drosophila*. Dev. Biol. **177:** 217–225.

Tepass, U., and E. Knust, 1993 Crumbs and stardust act in a genetic pathway that controls the organization of epithelia in Drosophila melanogaster. Dev. Biol. **159:** 311–326.

van de Pavert, S. A., A. Kantardzhieva, A. Malysheva, J. Meuleman, I. Versteek *et al.*, 2004 Crumbs homologue 1 is required for maintenance of photoreceptor cell polarization and adhesion during light exposure. J. Cell Sci. **117:** 4169–4177.

van Rossum, A. G., W. M. Aartsen, J. Meuleman, J. Klooster, A. Malysheva *et al.*, 2006 Pals1/Mpp5 is required for correct localization of Crb1 at the subapical region in polarized Muller glia cells. Hum. Mol. Genet. **15:** 2659–2672.

Wang, Q., T. W. Hurd and B. Margolis, 2004 Tight junction protein Par6 interacts with an evolutionarily conserved region in the amino terminus of PALS1/Stardust. J. Biol. Chem. **279:** 30715–30721.

Wang, Q., X.-W. Chen and B. Margolis, 2006 PALS1 regulates E-cadherin trafficking in mammalina epithelial cells. Mol. Biol. Cell **18:** 874–885.

Wei, X., and J. Malicki, 2002 *nagie oko*, encoding a MAGUK-family protein, is essential for cellular patterning of the retina. Nat. Genet. **31:** 150–157.

Wei, X., J. Zou, M. Takechi, S. Kawamura and L. Li, 2006 Nok plays an essential role in maintaining the integrity of the outer nuclear layer in the zebrafish retina. Exp. Eye Res. **83:** 31–44.

Wieschaus, E., C. Nüsslein-Volhard and G. Jürgens, 1984 Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. III. Zygotic loci on the X chromosome and fourth chromosome. Rouxs Arch. Dev. Biol. **193:** 296–307.

Williams, I., J. Richardson, A. Starkey and I. Stansfield, 2004 Genome-wide prediction of stop codon readthrough during translation in the yeast Saccharomyces cerevisiae. Nucleic Acids Res. **32:** 6605–6616.

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

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