JCS ePress online publication date 21 May 2008

Multiple domains of Stardust differentially mediate localisation of the Crumbs-Stardust complex during photoreceptor development in *Drosophila*

Natalia A. Bulgakova[1], Özlem Kempkens[2] and Elisabeth Knust[1,*]

[1]Max-Planck Institute for Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, Dresden, Germany
[2]Institut für Genetik, Heinrich-Heine Universität Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany
*Author for correspondence (e-mail: knust@mpi-cbg.de)

Accepted 31 March 2008
Journal of Cell Science 121, 2018-2026 Published by The Company of Biologists 2008
doi:10.1242/jcs.031088

Summary

Drosophila **Stardust (Sdt), a member of the MAGUK family of scaffolding proteins, is a constituent of the evolutionarily conserved Crumbs-Stardust (Crb-Sdt) complex that controls epithelial cell polarity in the embryo and morphogenesis of photoreceptor cells. Although apical localisation is a hallmark of the complex in all cell types and in all organisms analysed, only little is known about how individual components are targeted to the apical membrane. We have performed a structure-function analysis of Sdt by constructing transgenic flies that express altered forms of Sdt to determine the roles of individual domains for localisation and function in photoreceptor cells. The results corroborate the observation that the organisation of the Crb-Sdt complex is differentially regulated in pupal and adult photoreceptors. In pupal photoreceptors, only the PDZ domain of Sdt – the binding site of Crb – is required for apical targeting. In adult photoreceptors, by contrast, targeting of Sdt to the stalk membrane, a distinct compartment of the apical membrane between the rhabdomere and the zonula adherens, depends on several domains, and seems to be a two-step process. The N-terminus, including the two ECR domains and a divergent N-terminal L27 domain that binds the multi-PDZ domain protein PATJ in vitro, is necessary for targeting the protein to the apical pole of the cell. The PDZ-, the SH3- and the GUK-domains are required to restrict the protein to the stalk membrane.** ***Drosophila*** **PATJ or** ***Drosophila*** **Lin-7 are stabilised whenever a Sdt variant that contains the respective binding site is present, independently of where the variant is localised. By contrast, only full-length Sdt, confined to the stalk membrane, stabilises and localises Crb, although only in reduced amounts. The amount of Crumbs recruited to the stalk membrane correlates with its length. Our results highlight the importance of the different Sdt domains and point to a more intricate regulation of the Crb-Sdt complex in adult photoreceptor cells.**

Key words: MAGUK, PDZ, L27 domain, Apical polarity

Introduction

Polarisation of cells largely depends on the asymmetric distribution of multiprotein complexes that define spatially and functionally distinct membrane-associated compartments within cells. Formation of these complexes brings proteins involved in the same process into close proximity, thereby facilitating their interaction and ensuring efficiency and specificity of function, such as adhesion or signalling. The core constituents of membrane-associated complexes are often scaffolding proteins and one or more transmembrane protein(s). The latter can be receptors or adhesion molecules and often serve to anchor the complex at the membrane. In several cases, multiprotein complexes provide the basis for formation of cell-type-specific junctions. Although precise localisation of these complexes is crucial for their function, little is known about the mechanisms that control targeting, assembly and maintenance of individual components at a particular site within a cell.

Central components of many membrane-associated protein complexes are scaffolding proteins, each of which possesses several protein-protein interaction domains that, together, allow a wide variety of interactions to occur. Membrane-associated guanylate kinases (MAGUKs) form a family of scaffolding proteins engaged in organising multiprotein complexes that are often associated with cellular junctions and signalling complexes, e.g. the vertebrate tight junction (TJ) and *Drosophila* septate junction (SJ) in epithelial cells, and the neuromuscular junction (NMJ). Their capacity to serve as a platform for recruiting larger protein assemblies results from the presence of multiple protein-protein interaction domains: one to three PSD-95/Discs large/zonula occludens 1 (PDZ) domains, a Src homology-3 (SH3)-domain and a guanylate kinase (GUK) domain. Some members additionally contain one or two Lin-2–Lin-7 (L27)-domains in their N-terminus and/or a Hook domain between the SH3- and the GUK-domain. This modular structure facilitates the recruitment of various components into supramolecular protein complexes, the composition of which often depends on the cell type and/or the developmental stage. Strikingly, genes that encode MAGUK-proteins often give rise to tissue- and stage-specific protein isoforms through alternative splicing, thus increasing their versatility and the possibility for cell-type-specific interactions, localisation and/or function. For example, *Drosophila discs large* (*dlg*) expresses an epithelia-specific isoform (Dlg-A) that lacks the L27 domain and is therefore unable to bind to *Drosophila* Lin-7 (also known as Veli), whereas an isoform containing the L27 domain (Dlg-S97) can associate with *Drosophila* Lin-7 in the neuromuscular junction (Bachmann et al., 2004; Mendoza et al., 2003). *Drosophila polychaetoid*, an orthologue of mammalian zonula occludens 1 (ZO-1, also known as TJP1), encodes two isoforms, one localising

apically and the other distributing more broadly along the lateral membrane of epithelial cells in the wing imaginal disc (Wei and Ellis, 2001).

Dissection of mechanisms that regulate protein complex localisation is complicated by the fact that protein complexes are highly dynamic structures whose composition can undergo rapid modifications. In addition, some proteins localise in several steps and mechanisms might differ depending on the type of tissue or the developmental stage. Localisation of *Drosophila* Dlg to the septate junctions in epithelial cells, for example, requires the combined action of its PDZ2 and Hook domain (Hough et al., 1997). Localisation of *Drosophila* Scribble (Scrib), a member of the LAP protein family, is a two-step process that first requires a region containing 16 leucine-rich repeats (LRR) to target the protein cortically, then relies on the four PDZ domains to restrict it to the baso-lateral membrane (Albertson et al., 2004).

One of the scaffolding proteins of the MAGUK family is *Drosophila* Stardust (Sdt), the PDZ-domain of which interacts with the four C-terminal amino acids of the transmembrane protein Crumbs (Crb). In addition to a SH3-, Hook- and GUK-domain, it contains evolutionary conserved region 1 and 2 (ECR1 and ECR2, respectively) in the N-terminus, which are required to interact with *Drosophila* Par-6 (Bachmann et al., 2001; Berger et al., 2007; Hong et al., 2001; Wang et al., 2004) and a canonical L27 domain that interacts with *Drosophila* Lin-7. *Drosophila sdt* encodes several protein isoforms that result from differential splicing and/or transcription initiation. Two of them, Sdt-A and Sdt-B2 (Fig. 1A), differ with respect to the presence or absence of a large exon (exon 3), which encodes an N-terminal 433 amino acid region that has no obvious domain structure. All proteins expressed in the retina lack this N-terminal extension (Berger et al., 2007).

Besides maintaining epithelial tissues in the *Drosophila* embryo (Bachmann et al., 2001; Hong et al., 2001; Tepass and Knust, 1993), Sdt (as well as its binding partners Crb and PATJ) is also required for morphogenesis and function of the photoreceptor cells (PRCs). During development, PRCs are specified from epithelial cells of the eye imaginal discs in third instar larvae. In pupae, PRCs undergo complex morphogenetic changes that are associated with a 90° shift of the apical membrane that finally adopts a lateral position. Subsequently, the apical membrane is subdivided into the stalk, a supporting membrane immediately apical to the zonula adherens (ZA), and the rhabdomere, a central region consisting of a highly pleated array of microvilli that harbours most of the phototransduction signalling cascade proteins. Conspicuous cell elongation accompanies rhabdomere formation (Longley and Ready, 1995). In contrast to embryonic epithelia that lack *sdt* or *crb*, mutations in *sdt*, *crb* or *PATJ* do not disrupt PRC apico-basal polarity. However, lack of any of these genes affects PRC morphogenesis. This is manifested by a failure to correctly expand the apical membrane, and by a reduction in the length of the stalk membrane (Berger et al., 2007; Hong et al., 2003; Izaddoost et al., 2002; Johnson et al., 2002; Nam and Choi, 2003; Nam and Choi, 2006; Pellikka et al., 2002; Richard et al., 2006a). In addition, mutations in all three genes lead to progressive light-induced retinal degeneration (Berger et al., 2007; Johnson et al., 2002; Richard et al., 2006a) (reviewed in Knust, 2007). During the first half of pupal development, members of the Crb complex are localised throughout the apical membrane, but are restricted to the stalk membrane at later stages and in PRCs of adult flies. The absence of any of these proteins in adult PRCs results in delocalisation of all the others (Berger et al., 2007; Richard et al., 2006a). Recently, *Drosophila*

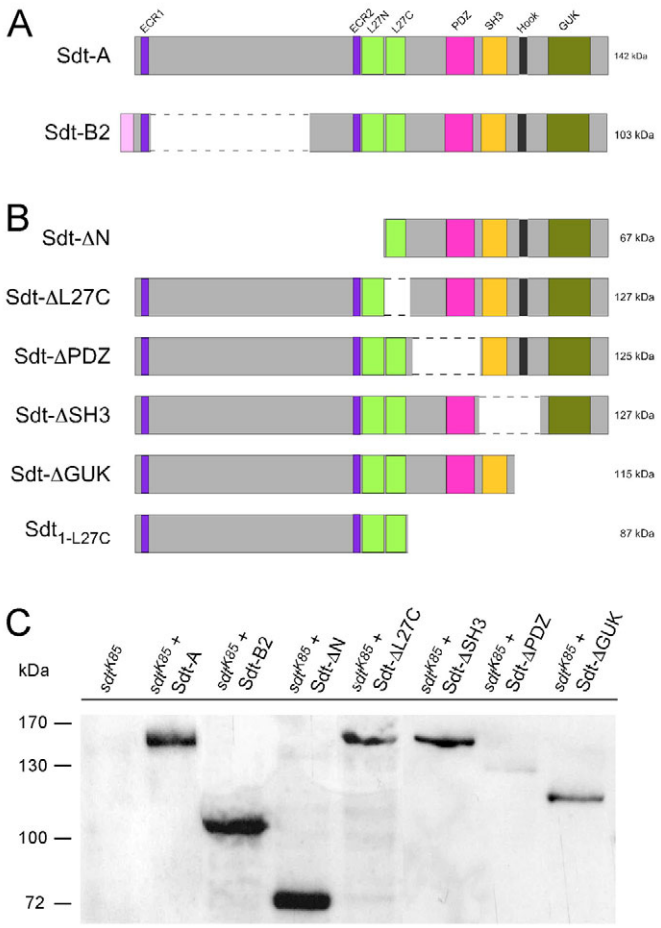

Fig. 1. Structure and expression of Sdt isoforms. (A) Two *sdt* transgenes encoding full-length Sdt proteins were used: SdtA [also known as Sdt-MAGUK1 (Bachmann et al., 2001)], and Sdt-B2 (Berger et al., 2007). (B) List of Sdt variants used in this study. All transgene-encoded proteins in A and B carry a FLAG-tag at their N-terminus. (C) Western blot analysis of Sdt-FLAG proteins from the eyes of flies that carry large clones mutant for sdt^{K85} and express the respective transgene using *Rh1*GAL4. Anti-FLAG antibody was used to detect Sdt-FLAG proteins. Eyes of flies with large clones mutant for sdt^{K85} but not expressing Sdt-transgenes were used as control.

Lin-7 was shown to be a component of the Crb complex in pupal and adult PRCs. In contrast to PRCs mutant for *sdt*, *crb* or *PATJ*, those mutant for *Lin-7* do not display morphogenetic defects and maintain Crb, Sdt and PATJ proteins at the stalk membrane. However, similar to the others, *Lin-7*-mutant PRCs degenerate in a light-dependent manner (Bachmann et al., 2008).

The core components of the *Drosophila* Crb-Sdt complex are conserved throughout evolution. In mammals, one orthologue of Sdt (MPP5, also known as Pals1), three of Crb (CRB1, CRB2 and CRB3) and two of *Drosophila* PATJ (INADL and MPDZ) were identified (reviewed in Assémat et al., 2008; Richard et al., 2006b). With the exception of CRB1, whose expression is restricted in mouse and human to retina and brain (den Hollander et al., 2002; den Hollander et al., 1999), all are expressed in multiple tissues throughout development. Here, they form apical membrane-associated protein complexes in association with cell-type-specific proteins. In cultured epithelial cells, these complexes are important for tight-junction stability (Michel et al., 2005; Shin et al., 2005;

Straight et al., 2004; Wang et al., 2004). In addition, mutations in human *CRB1* are associated with Retinitis pigmentosa 12 (RP12) and Leber congenital amaurosis (LCA) (reviewed in Assémat et al., 2008; Richard et al., 2006b). Mutation of *crb* and *sdt* orthologues in the zebrafish (*Danio rerio*) (*oko meduzy* and *nagie oko*, respectively) affects cellular patterning of the retina and apical membrane differentiation of some cell types, including the PRC (Malicki and Driever, 1999; Omori and Malicki, 2006; Wei and Malicki, 2002; Wei et al., 2006). Thus, the Crb-Sdt complex performs conserved functions during cell polarisation and retinal morphogenesis in different species.

Here, we describe a structure-function analysis of the Sdt protein(s) aimed at understanding the role of individual protein-protein interaction domains during PRC morphogenesis. We show that the function of individual domains in targeting Sdt to the apical or stalk membrane, and in recruiting and stabilising other complex members, exhibits striking differences in *Drosophila* pupal and adult stages. In addition, we provide evidence for the importance of specific domains in rescuing morphological defects caused by *sdt*-null mutation.

Results

Generation and characterisation of *sdt* transgenes

To study the function of Sdt and its different domains, we used two full-length transgenes that encode the two known isoforms Sdt-A and Sdt-B2 (Fig. 1A). These two isoforms differ in the presence or absence of exon 3, which encodes a 433 amino acid N-terminal region that has no obvious domain structure (Bachmann et al., 2001; Berger et al., 2007). Sdt-A was used as a template to produce multiple *sdt* transgenes carrying different deletions (Fig. 1B). Proteins encoded by the transgenes contain an N-terminal FLAG epitope tag, and were expressed using the UAS/GAL4 system (Brand and Perrimon, 1993). Since zygotic *sdt* mutants die during embryogenesis, we used a clonal approach to obtain *sdt* mutant photoreceptor cells (PRCs). We generated large eye clones (Newsome et al., 2000) mutant for sdt^{K85} and expressed the transgenes with *Rh1*-GAL4, which activates moderate levels of Gal4 in the outer photoreceptors, R1-R6, starting from the last third of pupal development. Alternatively, we used the mosaic analysis with a repressible cell marker (MARCM) technique that, additionally, allows mutant cells to be marked (Lee and Luo, 2001). sdt^{K85} was chosen because it is a complete null allele in PRCs, on the basis of protein expression and phenotype analysis (Berger et al., 2007). To make sure that the Sdt-FLAG proteins were produced, western blots of retinal extracts from *Rh1*GAL4/UAS-Sdt-Flag flies that contained no endogenous Sdt were probed with antibodies against FLAG or Sdt-PDZ (Fig. 1C, and data not shown).

The PDZ domain of Sdt is necessary for apical localisation at pupal stage

PRCs develop from epithelial cells of *Drosophila* larval eye imaginal discs. During the first half of wild-type pupal PRC development, endogenous Sdt localises, together with Crb, PATJ, Lin-7, Par-6 and F-actin, at the apical membrane (reviewed in Knust, 2007; Richard et al., 2006b). To determine the domain(s) of Sdt that are responsible for its apical targeting at this stage, we assayed the localisation of different transgene-encoded Sdt proteins in sdt^{K85}-mutant PRCs that do not express any endogenous Sdt protein (Fig. 2A). Of all Sdt protein domains tested, only the PDZ domain was necessary for apical localisation of Sdt, wheras Sdt variants that lack the PDZ domain (Sdt-ΔPDZ and $Sdt_{1\text{-}L27C}$) showed a low level

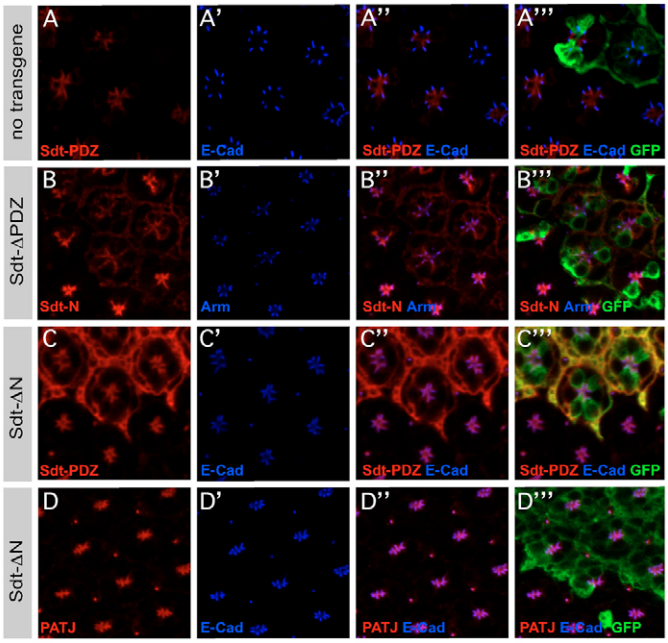

Fig. 2. (A-D) Localisation of Sdt and *Drosophila* PATJ in sdt^{K85} mutant pupal photoreceptor cells upon expression of different Sdt transgenes. Optical cross sections of pupal eyes at 40-45% p.d., before formation of the stalk membrane. MARCM clones mutant for sdt^{K85} (A) and those mutant for sdt^{K85} and expressing different Sdt-encoding transgenes (B-D) were stained with anti-Sdt-PDZ (A,C), anti-SdtN (B), or anti-PATJ (D) (red), and anti-E-cadherin (A,C,D) or anti-Arm (B) to stain the *zonula adherens* (blue). Mutant cells expressing a transgene are marked by GFP (green). Depending on the optical section level, the green staining is sometimes weaker. Cells not expressing GFP are wild type and serve as a control to demonstrate that Sdt and PATJ localise apically in PRCs at this stage.

of uniform cortical association (Fig. 2B-B''' and data not shown). All other proteins exhibit apical localisation (Fig. 2C-C''' and data not shown). In sdt^{K85}-mutant PRCs of the same stage, localisation of Crb and PATJ is known to be unaffected (Berger et al., 2007). Consistent with this, Crb and PATJ localisation was unaltered in the presence of any Sdt variant, independently of whether the transgene-encoded protein localised apically or not (Fig. 2D-D''' and data not shown). This result confirms that localisation of Crb and PATJ is independent of Sdt at this stage of *Drosophila* development (Berger et al., 2007).

ECR1 and ECR2 domains of Sdt are necessary to localise Par-6 apically at pupal stage

Drosophila Par-6, a member of the Par-protein network, is restricted to the apical pole in PRCs at 40-50% pupal development (p.d.) and requires *sdt* for its correct localisation (Berger et al., 2007; Hong et al., 2003). In the absence of *sdt*, apical Par-6 was strongly reduced and some Par-6 protein was detected basal to E-cadherin, a marker of the ZA (Fig. 3A-A'''). Par-6 has been shown to interact with Sdt in vitro, and this interaction depends on the presence of both ECR domains – ECR1 and ECR2 (Wang et al., 2004) (Ö.K. and E.K. unpublished data). To further analyse whether these Sdt domains are also required to recruit Par-6 in developing PRCs, we studied Par-6 localisation in sdt^{K85}-mutant pupal PRCs that expressed different *sdt*-transgenes. Sdt-ΔN, which lacks both ECR motifs, failed to restore apical *D*Par-6 protein localisation (data not shown), supporting the importance of these motifs for in vivo recruitment

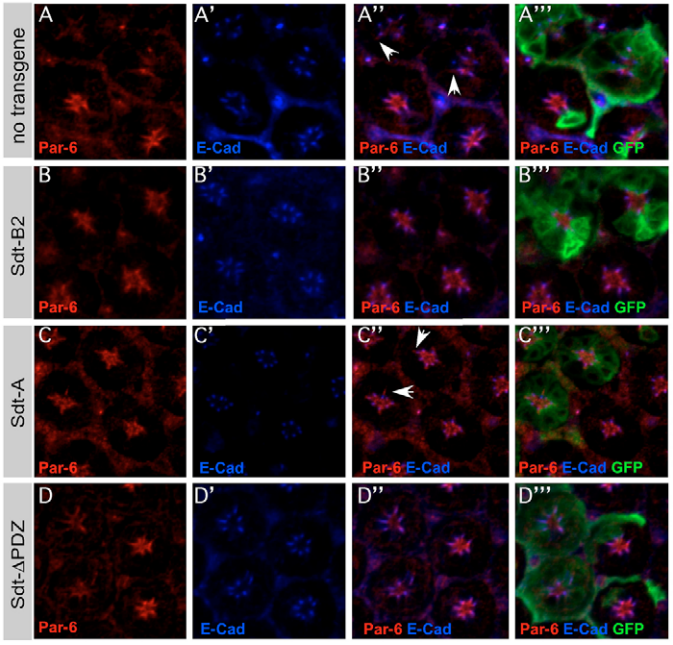

Fig. 3. (A-D''') Localisation of *Drosophila* Par-6 in sdt^{K85}-mutant pupal photoreceptor cells upon expression of different Sdt transgenes. Optical cross sections of pupal eyes at 40-45% p.d., before formation of the stalk membrane. sdt^{K85} mutant MARCM clones expressing different *sdt* transgenes, stained with anti-Par-6 (red) and anti-E-cadherin to stain the *zonula adherens* (blue). Mutant cells expressing a transgene are visualised using expression of GFP (green). Cells not expressing GFP are wild-type and serve as a control to demonstrate that Par-6 localises apically in PRCs at this stage. In sdt^{K85} mutant PRCs (A-A'''), Par-6 is reduced in amount and delocalised from the apical membrane (arrows). In sdt^{K85} mutant PRCs that express Sdt-B2 (B-B'''), Par-6 localises apically. In most sdt^{K85}-mutant PRCs that express Sdt-A (C-C'''), Par-6 localises apically but, occasionally, also basolateral (arrows). Expression of Sdt-ΔPDZ in sdt^{K85} mutant PRCs does not restore apical localisation of Par-6 (D-D''').

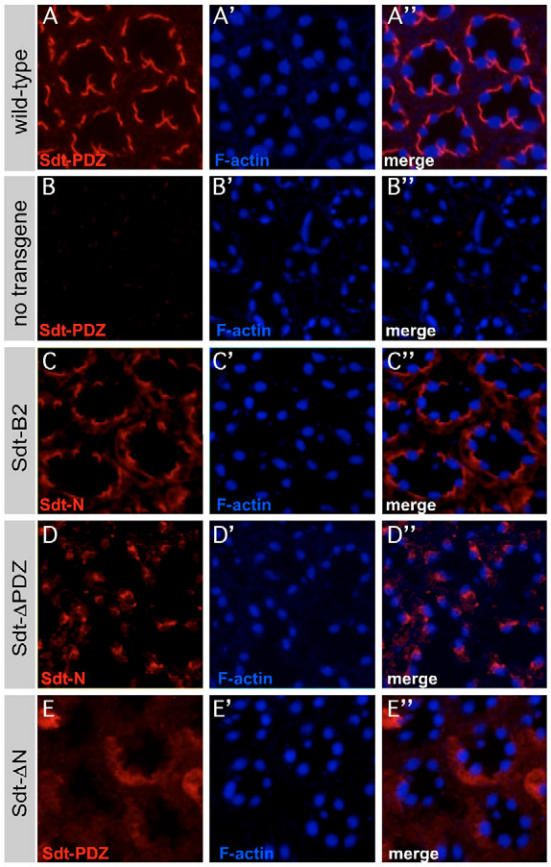

Fig. 4. Localisation of Sdt proteins encoded by transgenes in sdt^{K85}-mutant adult photoreceptor cells. Optical cross-sections of adult *Drosophila* eyes stained with antibodies against Sdt-PDZ (A,B,E) or with aSdt-N (C,D) (red), and phalloidin to highlight the F-actin-rich rhabdomeres (blue). (A) Sdt localises at the stalk membrane in wild-type PRCs. (B) sdt^{K85} eyes show morphological defects in the rhabdomeres and no Sdt protein can be detected. (C) Sdt-B2 localises at the stalk membrane when expressed in sdt^{K85}-mutant PRCs. (D) Sdt-ΔPDZ localises at the rhabdomere base when expressed in sdt^{K85}-mutant PRCs. (E) Sdt-ΔN is distributed throughout the cell when expressed in sdt^{K85}-mutant PRCs. Note that the stalk of R7 is not labelled by anti-Sdt antibody in C-E, because Rh1-Gal4 is only expressed in the six outer PRCs. This makes it sometimes more difficult to detect the rhabdomere of R7.

of Par-6. Of all tested proteins that contained ECR1 and ECR2, only Sdt-B2 completely rescued the apical localisation of Par-6 (Fig. 3B-B'''). Sdt-A and other variants, in which the distance between the two ECR domains is larger than in Sdt-B2, partially restored the apical localisation of Par-6 (Fig. 3C-C''' and data not shown), with the exception of Sdt-ΔPDZ (Fig. 3D-D'''). This is striking, because all of them, except Sdt-ΔPDZ, localise apically and contain both ECR motifs. We conclude from these results that apical localisation of *Drosophila* Par-6 is restored only by the expression of a Sdt protein in which the two ECR motifs are closely together, as SdtB2.

Multiple Sdt domains regulate its localisation at the stalk membrane in adult photoreceptor cells

In PRCs of adult wild-type eyes, Sdt is restricted to the stalk membrane, a defined region of the apical membrane between the rhabdomere and the ZA (Fig. 4A) (Berger et al., 2007; Hong et al., 2003). sdt^{K85}-mutant PRCs completely lack Sdt protein (Fig. 4B). To understand which Sdt domain(s) are required to target it to this restricted site, we expressed different *sdt*-transgenes in the absence of endogenous Sdt using *Rh1*GAL4. Transgenes encoding either full-length Sdt-A or Sdt-B2, or a protein that lacks the C-terminal L27 domain (Sdt-ΔL27C), completely restored normal Sdt localisation in otherwise *sdt* mutant PRCs (Fig. 4C-C'' and data not shown). The inner PRCs R7 and R8 that did not express Gal4, did also not express Sdt and served as an internal control. These data indicate that the C-terminal L27 domain, the binding site for *Drosophila* Lin-7, is dispensable for correct Sdt localisation at the stalk membrane. By contrast, Sdt proteins that lack the PDZ-, the SH3- or the GUK-domain were not detected at the stalk membrane, but found mainly to accumulate at the rhabdomere base (Fig. 4D-D'' and data not shown). Finally, in the absence of the N-terminus (Sdt-ΔN), the transgene-encoded protein was uniformly distributed throughout the cell in a sdt^{K85}-mutant background (Fig. 4E-E''). Taken together, these data demonstrate that, unlike in pupal PRCs, Sdt localisation to the stalk membrane in adult PRCs depends on the presence of several domains.

The L27N and L27C domains are required to stabilise and localise *Drosophila* PATJ and *Drosophila* Lin-7, respectively

Vertebrate Pals1 contains two L27 domains in its N-terminal part. The one located closer to the N-terminus, L27N, can interact with

the L27 domain of the multi-PDZ domain protein PATJ, whereas the other, L27C, located closer to the N-terminus, allows interaction with the single L27 domain of Lin-7 in vitro (Kamberov et al., 2000; Roh et al., 2002; Sheng and Sala, 2001). Unlike vertebrate Pals1, however, *Drosophila* Sdt contains only one well-conserved L27 domain [as predicted by the databases SMART or PROSITE (Bachmann et al., 2001; Hong et al., 2001)] that binds to *Drosophila* Lin-7 (Bachmann et al., 2004). However, the N-terminus of Sdt can also interact with PATJ in vitro (Roh et al., 2002). This poses the question of whether Lin-7 and PATJ compete to bind the same L27 domain on Sdt, or whether PATJ is recruited into the complex using a different interaction domain, similar to the vertebrate situation. As pointed out previously (Bachmann et al., 2004), a stretch of 70 amino acids with sequence homology to the PATJ-binding L27N domain of Pals1 precedes the canonical L27 domain of Sdt. Sequence comparison with other L27 domains (Doerks et al., 2000) revealed that this stretch carries several amino acid residues that are conserved at corresponding positions in related *Drosophila* species and in *Anopheles gambiense* (Fig. 5A). In yeast-two-hybrid interactions, the N-terminus of Sdt, including the cryptic L27N domain (amino acids 1-659 of Sdt-A), interacted with the N-terminal 87 amino acids of *Drosophila* PATJ, which contains an L27 domain. Fragments lacking this L27 domain ($Sdt_{1\text{-}566}$), or containing only part of it ($Sdt_{646\text{-}760}$), failed to interact with the N-terminus of *Drosophila* PATJ in yeast-two-hybrid assays (Fig. 5B). By contrast, a protein containing only L27N ($Sdt_{640\text{-}766}$) bound *Drosophila* PATJ. This suggests, that the divergent L27 domain of Sdt is functionally equivalent to L27N of Pals1 and can recruit *Drosophila* PATJ into the Crb-Sdt complex, whereas the canonical C-terminal L27 domain mediates interaction with *Drosophila* Lin-7 (Bachmann et al., 2004).

Drosophila PATJ and *Drosophila* Lin-7 are restricted to the stalk membrane in adult wild-type PRCs (Bachmann et al., 2008; Richard et al., 2006a), but are not localised in adult PRCs mutant for sdt^{K85} (Berger et al., 2007) (data not shown). Whereas the total amount of PATJ was strongly reduced in sdt^{K85} adult PRCs, the amount of Lin-7 was only slightly diminished (Fig. 6A). This discrepancy can be explained by the fact that Lin-7 is also localised in a Crb-Std-complex-independent way at the synapses between the PRCs and the first optic ganglion, the lamina (Bachmann et al., 2008). Thus, Sdt is crucial for PATJ and Lin-7 localisation at the stalk membrane, and their stabilisation in adult PRCs.

To further examine the relationship between these three proteins, and to confirm their interactions in vivo, we expressed different *sdt* transgenes and analysed the effects on PATJ and Lin-7 localisation and stability in the adult eye. Sdt-A and Sdt-B2 restored localisation of both PATJ and Lin-7 at the stalk membrane when expressed in sdt^{K85} adult PRCs (Fig. 7A-A'' and data not shown). Expression of these transgenes also restored the amount of both proteins to wild-type levels (Fig. 6A). Sdt proteins lacking the PDZ-, the SH3- or the GUK-domain, and thus localised at the rhabdomere base, recruited both PATJ and Lin-7 to this ectopic site (Fig. 7B-B'' and data not shown). They also rescued the amount of both proteins to wild-type levels (Fig. 6A).

Sdt protein lacking the L27C domain (the binding site for *Drosophila* Lin-7) localised at the stalk membrane. It restored PATJ localisation (data not shown) and wild-type amount (Fig. 6A). As expected, Lin-7 did not localise with Sdt (Fig. 7C-C''), and its levels remained reduced (Fig. 6A). This result is consistent with in-vitro interactions between Sdt and Lin-7 (Bachmann et al., 2004) and implies that such an interaction is essential for Lin-7 localisation and stability in PRCs of adult flies. Sdt-ΔN, which lacks the N-terminal L27-domain (and, hence, the binding site for PATJ) and is distributed throughout the cell (see Fig. 4D,D'), failed to localise

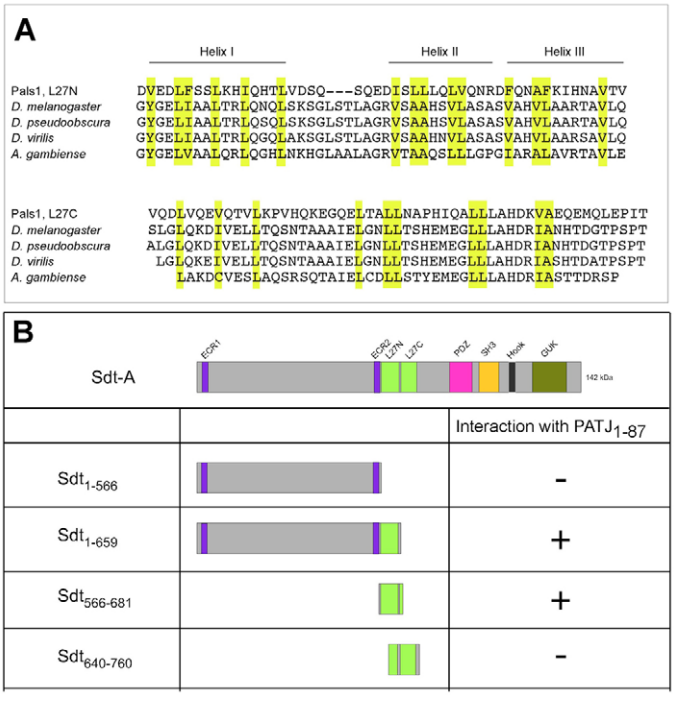

Fig. 5. The divergent L27 domain of Sdt interacts with the L27 domain of PATJ in yeast-two-hybrid assays. (A) Comparison of the two L27 domains of Pals1 with those of Sdt. The yellow boxes indicate conserved hydrophic amino acids required to form helices characteristic for L27 domains. (B) Only Sdt fragments containing the N-terminal L27 domain can bind a portion of PATJ that includes the L27 domain, in the yeast-two-hybrid system. The results of the interactions are tabulated on the right: +, interaction; –, no interaction.

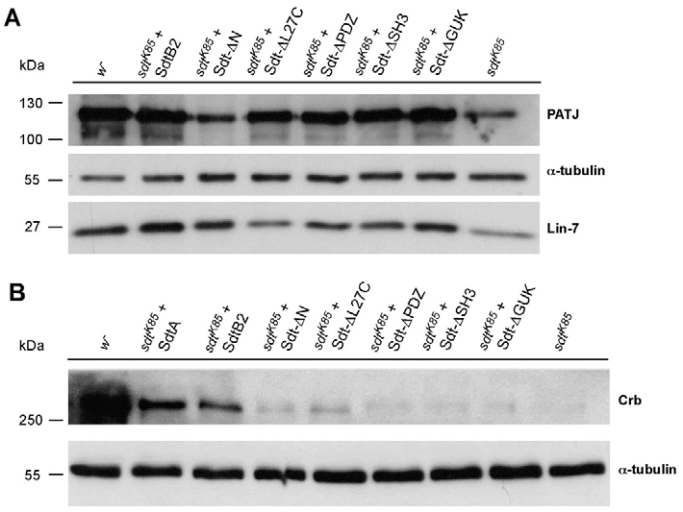

Fig. 6. Expression of PATJ, Lin-7 and Crb. Western blots of extracts from wild-type eyes (*w*), sdt^{K85}-mutant eyes, and sdt^{K85}-mutant eyes expressing different *sdt* transgenes (four eyes per lane), probed with anti-PATJ and anti-Lin-7 (A), and anti-Crb (B) antibodies. Eyes used for this experiment contained large mutant clones with hardly any wild-type ommatidia (~1%). antibody against α-tubulin was used as a loading control.

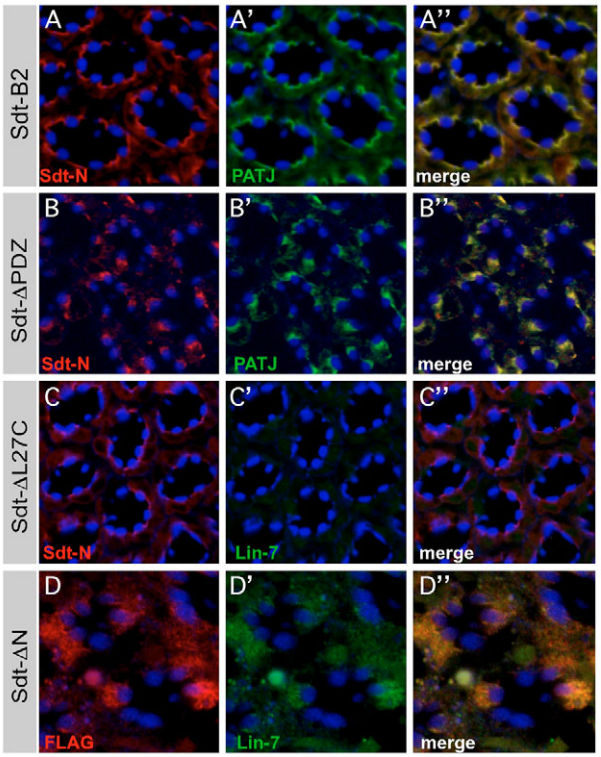

Fig. 7. Localisation of PATJ and Lin-7 proteins in sdt^{K85}-mutant adult photoreceptor cells upon expression of Sdt-encoding transgenes. Optical cross-sections of adult *Drosophila* eyes stained with anti-Sdt-N (A,B,C) or with anti-FLAG (D) (red), and with anti-PATJ (A,B) or anti-Lin-7 (C,D) (green). Phalloidin highlights the F-actin-rich rhabdomeres (blue). When Sdt-B2 is expressed in sdt^{K85} mutant PRCs (A-A''), both Sdt (A) and PATJ (A') localise at the stalk membrane. Upon Sdt-ΔPDZ expression in sdt^{K85} mutant PRCs (B-B''), Sdt (B) and PATJ (B') co-localise at the rhabdomere base. When Sdt-ΔL27C is expressed in sdt^{K85} mutant PRCs (C-C''), Sdt (C), but not Lin-7 (B') localises at the stalk membrane. When Sdt-ΔN is expressed in sdt^{K85} mutant PRCs (D-D''), Sdt (D) and Lin-7 (D') are distributed throughout the cell.

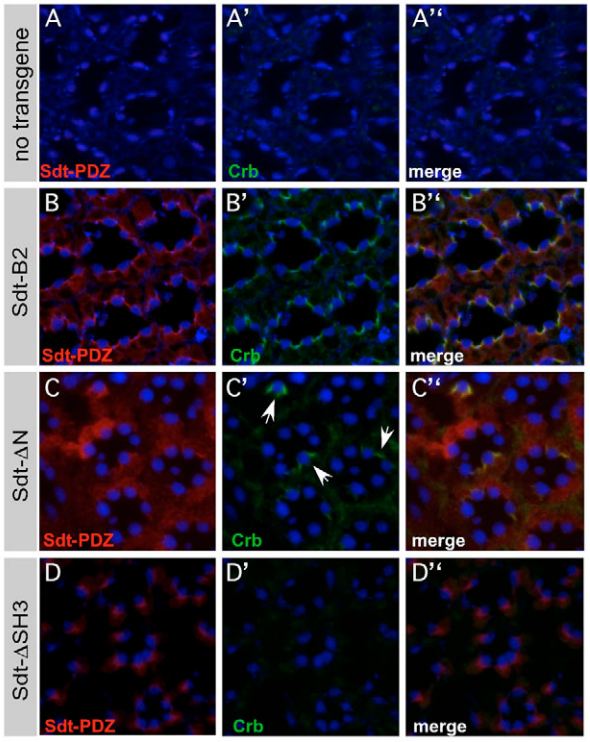

Fig. 8. Localisation of Crb in sdt^{K85} mutant adult photoreceptor cells upon Sdt-encoding transgene expression. Optical cross-sections of adult *Drosophila* eyes stained with anti-Sdt-PDZ (red) and anti-Crb (green). Phalloidin highlights the F-actin-rich rhabdomeres (blue). (A) In sdt^{K85}-mutant eyes, which develop rhabdomeres with morphological defects, no Sdt (A) or Crb (A') was detected. (B) Sdt-B2 localises at the stalk membrane when expressed in sdt^{K85}-mutant PRCs (B) and restores localisation of Crb at the stalk membrane (B'). (C) Sdt-ΔN expressed in sdt^{K85} mutant PRCs is spread throughout the cells and is only occasionally slightly enriched near the stalk membrane (C), where it then colocalises with Crb (C', arrows). (D) Sdt-ΔSH3 localises at the rhabdomere base when expressed in sdt^{K85} mutant PRCs (D), and no Crb is detected (D').

PATJ. Lin-7 was uniformly distributed throughout the cell, similar to the transgene-encoded protein (Fig. 7D-D'' and data not shown). The amount of Lin-7 in these PRCs equalled that of wild-type PRCs, whereas PATJ levels were the same as in sdt^{K85} mutant PRCs without transgene expression (Fig. 6A). These results suggest that, in adult PRCs (1) binding of PATJ and Lin-7 to Sdt requires the Sdt L27N- and L27C-domain, respectively; (2) localisation of both proteins depends on Sdt; (3) both proteins are stabilised upon interaction with Sdt. For stabilisation to occur, the proteins need not to be localised at the stalk membrane.

Sdt stabilises and localises Crb only when present at the stalk membrane

In adult eyes mutant for sdt^{K85}, no Crb was discovered at the stalk membrane (Fig. 8A-A'') and no Crb protein could be detected on western blots (Fig. 6B). Expression of Sdt-A, Sdt-B2 and Sdt-ΔL27C brought some Crb protein back to the stalk membrane (Fig. 8B-B'' and data not shown). In PRCs that express full-length Sdt proteins, Crb protein was detected by western blots, although at reduced amounts compared with wild type (Fig. 6B). Upon Sdt-ΔL27C expression, very low levels of Crb were detected (Fig. 6B). In response to Sdt-ΔN expression, Crb was detected at the stalk membrane in only those few cases where the transgene-encoded Sdt was enriched at the stalk membrane (Fig. 8C-C'', arrows), but the level of Crb detected by western blotting was very low (Fig. 6B). All other transgene-encoded proteins were unable to restore Crb protein accumulation in sdt^{K85} mutant PRCs, as revealed by immunofluorescence (Fig. 8D-D'' and data not shown) and western blot analysis (Fig. 6B).

In summary, these results demonstrate that the amount of Crb depends on the correct localisation, quantity and quality of Sdt protein. Sdt is not able to recruit Crb to ectopic positions within the cell, even if it contains the PDZ domain, the binding site for Crb. In addition, Crb is only stable when it is localised – together with Sdt – at the stalk membrane. In the assay used here, Sdt was not sufficient to recruit normal amounts of Crb to the stalk membrane. This suggests that additional, yet unknown factors contribute to Crb recruitment and/or stabilisation at the stalk membrane.

Rescuing stalk-membrane length of the *sdt* genetic null mutants

One intriguing phenotype caused by mutations in *sdt* is the reduction of stalk-membrane length in adult PRCs (Berger et al., 2007; Hong et al., 2003). Similarly, stalk membranes in PRCs mutant for *PATJ* or *crb* are reduced in length (Johnson et al., 2002; Nam and Choi, 2006; Pellikka et al., 2002; Richard et al., 2006a). At present, not

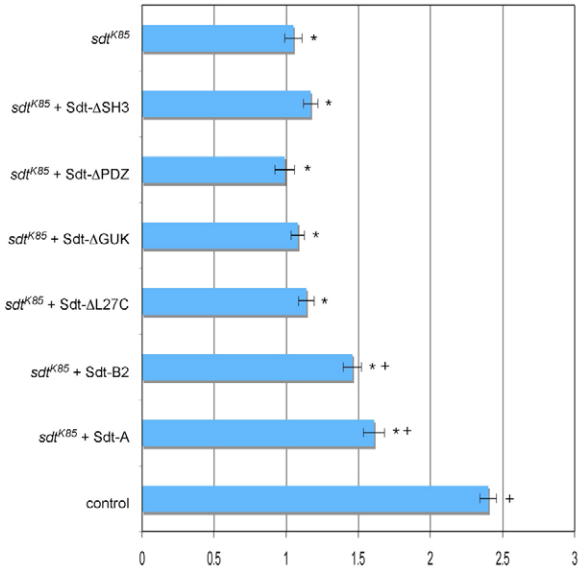

Fig. 9. Rescue of stalk-membrane length by Sdt-encoding transgenes. Stalk-membrane length (mean ± s.e.m.) in sdt^{K85} (n=115), sdt^{K85} + Sdt-ΔSH3 (n=123), sdt^{K85} + Sdt-ΔPDZ (n=107), sdt^{K85} + Sdt-ΔGUK (n=118), sdt^{K85} + Sdt-ΔL27C (n=127), sdt^{K85} + Sdt-B2 (n=118), sdt^{K85} + Sdt-A (n=124) and wild-type (n=117) PRCs. One unit represents 1 µm. *P<0.0001 or ^{+}P<0.0001 (two-tailed test), significantly different compared with wild type or sdt^{K85}, respectively.

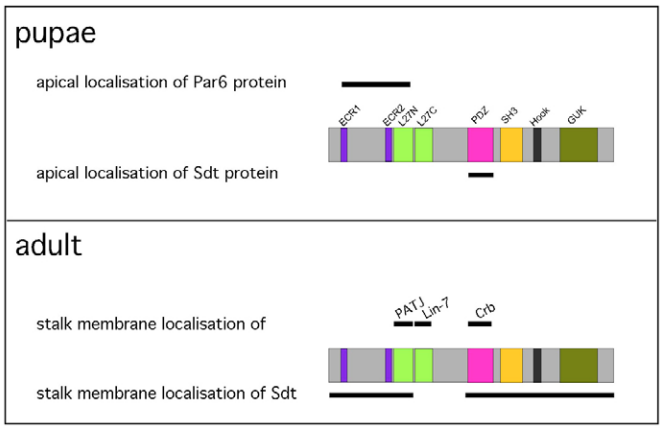

Fig. 10. Summary of results. Apical localisation of the Sdt protein in the pupae only depends on the PDZ domain, whereas its correct localisation to the stalk membrane in the adult depends on different domains. The various Sdt domains are required differentially to localise other proteins in the pupae and the adult.

much is known about the molecular basis of this phenotype. To address which part of Sdt is required for normal length, different *sdt* transgenes were expressed in sdt^{K85} mutant PRCs using *Rh1*-GAL4. The resulting eyes were analysed by electron microscopy. Around 20 ommatidia from two to four eyes of different individuals with the same genotype were photographed, and stalk-membrane length of R1-R6 was measured using ImageJ software (http://rsb.info.nih.gov/ij/). In PRCs mutant for sdt^{K85}, stalk-membrane length was reduced by more than 50% compared with wild type. Sdt-A or Sdt-B2 expression partially restored stalk-membrane length to a reduction of only 30% compared with wild type (Fig. 9). This stalk was significantly longer than that in PRCs mutant for sdt^{K85}, but significantly shorter than stalk-membrane length in control PRCs. All other *sdt* transgenes tested failed to rescue stalk-membrane length (Fig. 9). These data suggest that stalk-membrane length depends on the amount of Crb at this site, and is independent of PATJ or Lin-7 levels. High amounts of Crb (as in wild type) result in a long stalk membrane. No Crb (*crb*, *sdt*, *PATJ* mutants), or very low amounts of Crb (expression of Sdt-ΔL27C on a *sdt* mutant background, Fig. 6B), reduce stalk-membrane length. And, medium amounts of Crb at the stalk membrane (expression of Sdt-A and Sdt-B2 in a *sdt* mutant background, Fig. 6B, Fig. 8B-B") result in stalk membranes of medium length.

Discussion

Subcellular targeting of Sdt is differentially regulated in pupal and adult PRCs

Data presented here corroborate the view that distinct mechanisms control localisation of the Crb-Sdt complex in PRCs at different developmental stages (summarised in Fig. 10). This conclusion is further supported by the observation that a truncated PATJ protein, consisting of only L27 and the first PDZ domain, is localised correctly during the first half of pupal development, but is delocalised in adult PRCs (Nam and Choi, 2006; Richard et al., 2006a). The stability of the complex at pupal stages seems to depend only on Crb. In pupae, all core components of the complex are mislocalised in *crb*-mutant PRCs (Bachmann et al., 2008; Richard et al., 2006a) (and data not shown), whereas the absence of *sdt*, *PATJ* or *Lin-7* does not affect apical localisation of the others. Accordingly, Sdt localisation at this stage only depends on its PDZ domain that binds the cytoplasmic tail of Crb. Neither the non-canonical L27N domain of Sdt, which is responsible for binding PATJ as shown here, nor the other protein-protein interaction domains are required for Sdt localisation in pupal PRCs.

In the adult *Drosophila* eye, localisation of Crb-Sdt-complex core proteins to the stalk membrane is mutually dependent, with the exception of Lin-7, which is not required to localise other components (Bachmann et al., 2008; Berger et al., 2007; Richard et al., 2006a). Similarly, in zebrafish the levels of the Crb orthologous proteins require the function of the Sdt orthologue Nagie oko (Hsu et al., 2006). In the fly eye, changes observed at different developmental stages point to a transition in the mechanisms regulating the building and stability of the complex. As previously pointed out, this transition occurs gradually in the second half of pupal development (Richard et al., 2006a). At the same time, Bazooka, which is associated with the adherens junctions in the first half of pupal development, accumulates in the cytoplasm (Hong et al., 2003). The transition also correlates with the formation of stalk membrane, which initiates around 55% pupal development and ultimately separates the apical plasma domain into two distinct compartments (Longley and Ready, 1995). This process seems to require additional, more complex control mechanisms, as reflected by the fact that several Sdt domains are required for its proper localisation at later stages. It is very possible that other, yet unknown components contribute to the stability and/or restriction of Sdt at the stalk membrane.

Results presented here also suggest that in the adult *Drosophila* eye, localisation of Sdt occurs in several steps that rely on different domains. In the first step, Sdt is brought close to the apical membrane. This function is mediated by the N-terminus, including the two ECR domains and the N-terminal L27 domain. Since Par-6, a known binding partner of the ECR motifs, is localised basolaterally in adult PRCs (N.A.B., unpublished data), PATJ binding is more likely to be crucial for apical recruitment of Sdt. In

fact, no localised Sdt is detected in *PATJ*-mutant adult PRCs (Richard et al., 2006a). In the absence of all other domains besides the N-terminus (with the exception of L27C), Sdt proteins accumulate at the rhabdomere base, a specialised region that seems to have an important role in PRCs. Many proteins involved in morphogenesis, phototransduction or endocytosis, such as *Drosophila* moesin, TRPL (transient receptor potential-like) and Rab11 (Cronin et al., 2006; Karagiosis and Ready, 2004; Satoh et al., 2005), to mention just a few, are enriched there. The final step, recruitment of Sdt to the stalk membrane, requires the PDZ-, the SH3- and the GUK-domain. Whereas the PDZ-domain binds Crb, no binding partners for the SH3- and the GUK-domain are known. It was shown that these two domains can bind each other in vitro (Ö.K., unpublished data). Similar interactions between corresponding domains of the human MAGUK CASK were reported to occur either intramolecularly or intermolecularly between the GUK domain of human CASK and the SH3 domain of hDLG (Nix et al., 2000). In the MAGUK PSD-93, binding of a ligand to the PDZ domain releases intramolecular inhibition of the GUK domain by the SH3 domain (Brenman et al., 1998). This possible complexity currently does not distinguish whether the failure to recruit Sdt to the stalk membrane upon removal of one of these domains is due to either the lack of binding additional partner(s) or the lack of intramolecular interactions, or both.

Par-6 apical localisation in pupal PRCs requires the N-terminus of Sdt

Whereas Sdt is not required to restrict components of the Crb-Sdt complex to the apical membrane in pupal PRCs, the apical localisation of Par-6, a member of the Par-protein network, depends on Sdt at this developmental stage (Berger et al., 2007; Nam and Choi, 2003) (this work). Recently, several studies suggested a direct interaction between the Crb-Sdt and the PAR complex, but the proposed interactions differ with respect to the partners mediating the link. Results obtained from in vitro analysis have suggested a number of interactions: aPKC with both PATJ and the intracellular domain of Crb (Sotillos et al., 2004); the PDZ domain of Par-6 with either the N-terminus of Sdt and/or Pals1 or the C-terminus of CRB1 or CRB3 (Hurd et al., 2003; Kempkens et al., 2006; Lemmers et al., 2004; Wang et al., 2004); and the N-terminus of Par-6 with the third PDZ domain of PATJ (Nam and Choi, 2003). The observations that neither Crb nor PATJ localisation is affected in *sdt*-mutant pupal PRCs (Berger et al., 2007) (this work) and that expression of Sdt-B2 in *sdt*-mutant PRCs completely restores Par-6 apical localisation, strongly suggests that in pupal PRCs the interaction between the Crb complex and Par-6 is mediated by the ECR motifs of Sdt. Sdt-A, which carries an additional 433 amino-acid-long stretch between ECR1 and ECR2, only partially restored apical recruitment of Par-6, suggesting that separation of ECR1 from ECR2 interferes with efficient interactions between the two proteins.

The role of Sdt in organising and stabilising the Crb complex and stalk-membrane length in adult photoreceptor cells

Our results show that in adult PRCs, *sdt* controls localisation and stability of Crb, PATJ and Lin-7 but the mechanisms differ. Whenever a Sdt protein is expressed that contains binding domains for PATJ or Lin-7, the amount of the latter is, independently of localisation, restored to wild-type levels. By contrast, Crb protein is stabilised only when Sdt is associated with the stalk membrane (expression of Sdt-A, Sdt-B2, Sdt-ΔL27C and Sdt-ΔN). Interestingly, none of the constructs used, including the two full-length variants, rescued Crb protein to wild-type levels. One possible explanation is that other, yet uncharacterised Sdt isoforms are expressed in the eye, which, together with Sdt-B2 and/or unknown interaction partners of the Crb-Sdt complex, regulate the amount of Crb at the stalk membrane. Additional Sdt isoforms are predicted by Flybase (http://flybase.bio.indiana.edu) to exist. They mainly differ from the known forms in their N-termini, which suggests alternative interaction partners.

One striking phenotype observed in PRCs mutant for *crb*, *sdt* or *PATJ* is the reduction of stalk-membrane length (Berger et al., 2007; Johnson et al., 2002; Nam and Choi, 2003; Pellikka et al., 2002; Richard et al., 2006a). This raises questions about how the Crb-Sdt complex regulates the size of this distinct apical membrane compartment. Our results provide evidence that the amount of Crb protein is a crucial determinant of stalk-membrane length. This agrees with the observation that Crb overexpression increases stalk-membrane length (Pellikka et al., 2002). Interestingly, these authors showed that overexpression of a Crb protein that lacks the cytoplasmic domain and, hence, the binding site for Sdt, is sufficient to cause this increase. This suggests that either the transmembrane and/or extracellular domain of Crb regulates stalk-membrane growth. Sdt contributes to the stabilisation of Crb at the stalk and, hence, is indirectly involved in the control of stalk-membrane length. It will be interesting to explore the mechanism by which Crb regulates stalk-membrane length.

Materials and Methods

Fly strains and clonal analysis

Flies were kept at 25°C. sdt^{K85} allele was used in all assays as it gives a null phenotype in PRCs, and no protein is detected at pupal stage (Berger et al., 2007). Large sdt^{K85} clones were generated by crossing w^+ *GMR::hid cl FRT19A*/FM7; Rh1::Gal4; ey::FLP females [modified from (Newsome et al., 2000)] to *w* sdt^{K85} FRT19A/Y; *Tp(1;2)* sn^+ *72d/CyO*; UAS::Sdt-X males (where Sdt-X is any Sdt transgene). Mosaic analysis with a repressible cell marker (MARCM) clones, in which mutant cells are labelled using GFP (Lee and Luo, 2001) were induced by a 2-hour heat shock (37°C) at 48-72 hours and 72-96 hours of development in offspring of *hsFLP, tubG80 FRT19A/Y; Act-Gal4 UAS-CD8::GFP/CyO* females crossed to *w* sdt^{K85} FRT19A/Y; *Tp(1;2)* sn^+ *72d/CyO*; UAS::Sdt-X males (where Sdt-X is any Sdt transgene).

Generation of Sdt transgenes

sdt-full-length and -deletion constructs were generated through PCR amplification of specific *sdt* regions. Fragments were ligated and inserted in frame into pUAST-FLAG vector (kindly provided by Arno Müller (Division of Cell and Developmental Biology, University of Dundee, UK) to result in an N-terminal fusion of the FLAG epitope. Deletion constructs encode the following Sdt protein amino acids: Sdt-ΔN, 683-1289; Sdt-ΔL27C, 1-681 and 818-1289; Sdt-ΔPDZ, 1-760 and 928-1289; Sdt-ΔSH3, 1-924 and 1069-1289; Sdt-ΔGUK, 1-1035; $Sdt_{1\text{-}L27C}$, 1-791. Primer sequences for all transgenes are available upon request.

Generation of antibody against Sdt-N , western blot analysis and yeast-two-hybrid interactions

The N-terminal part of the Sdt-A isoform, corresponding to amino acids 7-566, was cloned into expression vector pGEX-4T-2 (details provided upon request). Antisera against GST-fusion protein were obtained by repeated immunisation of rats with affinity-purified protein (Eurogentech, Seraing, Belgium). Western blots were performed as described previously (Berger et al., 2007). For protein extraction from retinas, heads were first cut in halves with a scalpel, and the retinas were dissected using forceps. Although the brain tissue was carefully removed, we cannot exclude some remnants of the lamina, because it is tightly connected with the retina by the optic stalk. Membranes were stained using rabbit anti-Sdt-PDZ (1:10,000) (Berger et al., 2007), mouse anti-Crb-cq4 (1:100) (Tepass and Knust, 1993), rabbit anti-*Drosophila*-PATJ (1:5000) (Richard et al., 2006a), rabbit anti-*Drosophila*-Lin-7 (1:5000) (Bachmann et al., 2004), mouse anti-FLAG-M2 (1:1000, Sigma), and mouse anti-α-tubulin (1:2000, Developmental Studies Hybridoma Bank). Peroxidase-conjugated secondary antibodies (Dianova) were used 1:1000. Yeast-two-hybrid interactions were performed essentially as described (Kempkens et al., 2006).

Confocal and transmission electron microscopy

Immunohistochemistry on pupal eye discs and adult eyes (frozen sections) was done as described previously (Richard et al., 2006a). For immunofluorescence analyses, the following antibodies were used with Cy2-, Cy3- (Dianova) or Alexa-Fluor-647-

(Invitrogen) conjugated secondary antibody: rabbit anti-Sdt-PDZ (1:500) (Berger et al., 2007), rat anti-Sdt-N (1:200), mouse anti-FLAG-M2 (1:100, Sigma), rat anti-Crb (1:100), rabbit anti-*Drosophila*-PATJ (1:500) (Richard et al., 2006a), rabbit anti-*D*Lin-7 (1:500) (Bachmann et al., 2004), mouse anti-Arm (1:50), rat anti-*Drosophila* E-Cadherin (1:50) (Developmental Studies Hybridoma Bank), rabbit anti-GFP (1:500, Invitrogen), mouse anti-GFP (1:100, Invitrogen), and guinea pig anti-*D*Par-6 (1:1000, kindly provided by Andreas Wodarz, Department of Stem Cell Biology, Georg-August-University, Göttingen, Germany). Rhabdomeres were visualised by labelling F-actin with Alexa-Fluor-660-phalloidin (1:40; Molecular Probes). Section preparation for transmission electron microscopy and measurement of stalk-membrane length (R1-R6) was done as described previously (Richard et al., 2006a).

We thank Andreas Wodarz and the Developmental Studies Hybridoma Bank for antibodies, Michaela Rentsch for sections and Laurel Rohde for the critical reading of the manuscript. The work was supported by grants from the Deutsche Forschungsgemeinschaft (Kn250/21-1) and the EC (QLG3-CT-2002-01266).

Note added in proof

Similar to our findings, Bit-Avragim and colleagues have evidence that Nagie oko, the zebrafish Stardust ortholog, mediates the assembly of alternative multi-protein compositions of the Crumbs-Nagie oko and Par6-aPKC protein complexes in a highly tissue-specific manner (Bit-Avragim et al., 2008).

References

Albertson, R., Chabu, C., Sheehan, A. and Doe, C. Q. (2004). Scribble protein domain mapping reveals a multistep localisation mechanism and domains necessary for establishing cortical polarity. *J. Cell Sci.* **117**, 6061-6070.

Assémat, E., Bazellières, E., Pallesi-Pocachard, E., Le Bivic, A. and Massey-Harroche, D. (2008). Polarity complex proteins. *Biochem. Biophys. Acta* **1778**, 614-630.

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

Bachmann, A., Timmer, M., Sierralta, J., Pietrini, G., Gundelfinger, E. D., Knust, E. and Thomas, U. (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.

Bachmann, A., Grawe, F., Johnson, K. and Knust, E. (2008). *Drosophila* Lin-7 is a component of the Crumbs complex in epithelia and photoreceptor cells and prevents light-induced retinal degeneration. *Eur. J. Cell Biol.* **87**, 123-136.

Berger, S., Bulgakova, N. A., Grawe, F., Johnson, K. and Knust, E. (2007). Unravelling the genetic complexity of *Drosophila stardust* during photoreceptor morphogenesis and prevention of light-induced degeneration. *Genetics* **176**, 2189-2200.

Bit-Avragim, N., Hellwig, N., Rudolph, F., Munson, C., Stainier, D. Y.S. and Abdelilah-Seyfried, S. (2008).Divergent polarization mechanisms during vertebrate epithelial development mediated by the Crumbs complex protein Nagie oko. *J. Cell Sci.* (in press).

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

Brenman, J. E., Topinka, J. R., Cooper, E. C., McGee, A. W., Rosen, J., Milroy, T., Ralston, H. J. and Bredt, D. S. (1998). Localization of postsynaptic densitiy-93 dendritic microtubules and interaction with microtubule-associatd protein 1A. *J. Neurosci.* **18**, 8805-8813.

Cronin, M. A., Lieu, M. H. and Tsunoda, S. (2006). Two stages of light-dependent TRPL-channel translocation in *Drosophila* photoreceptors. *J. Cell Sci.* **119**, 2935-2944.

den Hollander, A. I., ten Brink, J. B., de Kok, Y. J., van Soest, S., van den Born, L. I., van Driel, M. A., van de Pol, D. J., Payne, A. M., Bhattacharya, S. S., Kellner, U. et al. (1999). Mutations in a human homologue of *Drosophila crumbs* cause retinitis pigmentosa (RP12). *Nat. Genet.* **23**, 217-221.

den Hollander, A. I., Ghiani, M., de Kok, Y. J., Wijnholds, J., Ballabio, A., Cremers, F. P. and Broccoli, V. (2002). Isolation of *Crb1*, a mouse homolog of *Drosophila crumbs*, and analysis of its expression pattern in eye and brain. *Mech. Dev.* **110**, 203-207.

Doerks, T., Bork, P., Kamberov, E., Makarova, O., Muecke, S. and Margolis, B. (2000). L27, a novel heterodimerization domain in receptor targeting proteins Lin-2 and Lin-7. *Trends Biochem. Sci.* **25**, 317-318.

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

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

Hough, C. D., Woods, D. F., Park, S. and Bryant, P. J. (1997). Organizing a functional junctional complex requires specific domains of the Drosophila MAGUK Discs large. *Genes Dev.* **11**, 3242-3253.

Hsu, Y.-C., Willoughby, J. J., Christensen, A. K. and Jensen, A. M. (2006). Mosaic eyes is a novel component of the Crumbs complex and negatively regulates photoreceptor apical membrane. *Development* **133**, 4849-4859.

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

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

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

Kamberov, E., Makarova, O., Roh, M., Liu, A., Karnak, D., Straight, S. and Margolis, B. (2000). Molecular cloning and characterization of Pals, proteins associated with mLin-7. *J. Biol. Chem.* **375**, 11425-11431.

Karagiosis, S. A. and Ready, D. F. (2004). Moesin contributes an essential structural role in *Drosophila* photoreceptor morphogenesis. *Development* **131**, 725-732.

Kempkens, Ö., Médina, E., Fernandez-Ballester, G., Özüyaman, S., Le Bivic, A., Serrano, L. and Knust, E. (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.

Knust, E. (2007). Photoreceptor morphogenesis and retinal degeneration: lessons from *Drosophila*. *Curr. Opin. Neurobiol.* **17**, 541-547.

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

Lemmers, C., Michel, D., Lane-Guermonprez, L., Delgrossi, M.-H., Médina, E., Arsanto, J.-P. and Le Bivic, A. (2004). CRB3 binds directly to Par6 and regulates the morphogenesis of the tight junctions in mammalian epithelial cells. *Mol. Biol. Cell* **15**, 1324-1333.

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

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

Mendoza, C., Olguín, P., Lafferte, G., Thomas, U., Ebitsch, S., Gundelfinger, E. D., Kukuljan, M. and Sierralta, J. (2003). Novel isoforms of Dlg are fundamental for neuronal development in *Drosophila*. *J. Neurosci.* **23**, 2073-2101.

Michel, D., Arsanto, J. P., Massey-Harroche, D., Beclin, C., Wijnholds, J. and Le Bivic, A. (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 Choi, K.-W. (2003). Interaction of Par-6 and Crumbs complexes is essential for photoreceptor morphogenesis in *Drosophila*. *Development* **130**, 4363-4372.

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

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

Nix, S. L., Chishti, A. H., Anderson, J. M. and Walther, Z. (2000). 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.

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

Pellikka, M., Tanentzapf, G., Pinto, M., Smith, C., McGlade, C. J., Ready, D. F. and Tepass, U. (2002). Crumbs, the *Drosophila* homologue of human CRB1/RP12, is essential for photoreceptor morphogenesis. *Nature* **416**, 143-149.

Richard, M., Grawe, F. and Knust, E. (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., Roepman, R., Aartsen, W. M., van Rossum, A. G., den Hollander, A. I., Knust, E., Wijnholds, J. and Cremers, F. P. (2006b). Towards understanding CRUMBS function in retinal dystrophies. *Hum. Mol. Genet.* **15**, R235-R243.

Roh, M. H., Makarova, O., Liu, C. J., Shin, K., Lee, S., Laurinec, S., Goyal, M., Wiggins, R. and Margolis, B. (2002). The Maguk protein, Pals1, functions as an adapter linking mammalian homologues of Crumbs and Discs Lost. *J. Cell Biol.* **157**, 161-172.

Satoh, A. K., O'Tousa, J., Ozaki, K. and Ready, D. F. (2005). Rab11 mediates post-Golgi trafficking of rhodopsin to the photosensitive apical membrane of *Drosophila* photoreceptors. *Development* **132**, 1487-1497.

Sheng, M. and Sala, C. (2001). PDZ domains and the organization of supramolecular complexes. *Annu. Rev. Neurosci.* **24**, 1-29.

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

Sotillos, S., Díaz-Meco, M. T., Caminero, E., Moscat, J. and Campuzano, S. (2004). *D*aPKC-dependent phosphorylation of Crumbs is required for epithelial cell polarity in *Drosophila*. *J. Cell Biol.* **166**, 549-557.

Straight, S. W., Shin, K., Fogg, V. C., Fan, S., Liu, C.-J., Roh, M. and Margolis, B. (2004). Loss of PALS1 expression leads to tight junction and polarity defects. *Mol. Biol. Cell* **15**, 1981-1990.

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

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

Wei, X. and Ellis, H. M. (2001). Localisation of the *Drosophila* MAGUK protein Polychaetoid is controlled by alternative splicing. *Mech. Dev.* **100**, 217-231.

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

Wei, X., Zou, J., Takechi, M., Kawamura, S. and Li, L. (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.