The Crumbs complex: from epithelial-cell polarity to retinal degeneration

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Summary

The evolutionarily conserved Crumbs protein complex is a key regulator of cell polarity and cell shape in both invertebrates and vertebrates. The important role of this complex in normal cell function is illustrated by the finding that mutations in one of its components, Crumbs, are associated with retinal degeneration in humans, mice and flies. Recent results suggest that the Crumbs complex plays a role in the development of other disease processes that are based on epithelial dysfunction, such as tumorigenesis or the formation of cystic kidneys. Localisation of the complex is restricted to a distinct region of the apical plasma membrane that abuts the zonula adherens in epithelia and photoreceptor cells of invertebrates and vertebrates, including humans. In addition to the core components, a variety of other proteins can be recruited to the complex, depending on the cell type and/or developmental stage. Together with diverse post-transcriptional and post-translational mechanisms that regulate the individual components, this provides an enormous functional diversity and flexibility of the complex. In this Commentary, we summarise findings concerning the organisation and modification of the Crumbs complex, and the conservation of its constituents from flies to mammals. In addition, we discuss recent results that suggest its participation in various human diseases, including blindness and tumour formation.

Key words: Polarity, Epithelia, Photoreceptor cell, Retinal degeneration, *Drosophila*

Introduction

Epithelia are polarised tissues that outline the cavities and surfaces of the body. They are specialised for secretion, absorption, protection or sensory functions. Polarisation of epithelial cells is manifested by distinct apical and basolateral membrane domains, which are separated by the zonula adherens (ZA), a belt-like structure encircling the apex of the cell. One key regulator of epithelial polarity is the highly conserved Crumbs protein complex, the central constituent of which, Crumbs (Crb), was initially discovered in *Drosophila melanogaster* as an essential protein for maintaining apicobasal polarity and integrity of embryonic epithelia (Tepass and Knust, 1990; Tepass et al., 1990). Since then, a plethora of data has accumulated showing that the composition, cellular localisation and function of the Crumbs complex is conserved from flies to humans. However, it remains to be uncovered how a single protein complex can regulate the shape, polarity and function of various cell types, including 'simple' secretory epithelial cells to highly specialised photoreceptor cells (PRCs) in the eye. Recent work indicates that, in addition to the core components (which are always found together), other proteins can associate with the complex, depending on the type and developmental stage of the cell, thus providing the Crumbs complex with functional diversity and flexibility. Here, we summarise current knowledge on the composition of the Crumbs complex, its function in flies and vertebrates, and its possible participation in the development of human diseases.

Core components of the Crumbs complex in *Drosophila*

Three conditions must be fulfilled for a protein to be considered a member of a given complex: the protein should, first, colocalise with and, second, co-immunoprecipitate with members of the complex and, third, the protein should directly interact with at least one other member of the complex. Although some proteins of the Crumbs complex meet all three of these criteria in all cell types in which they are expressed, others do so in only some cell types. Therefore, we have subdivided the members of the Crumbs complex into those that belong to the first group (core components) and those that belong to the second group (transient components).

On the basis of this definition, there are four proteins that can be classified as the core components of the *Drosophila* Crumbs complex: Crb, Stardust (Sdt), PATJ (protein associated with tight junctions or Pals1-associated tight junction protein) and Lin-7 (Fig. 1). Crb is a type-I transmembrane protein, the large extracellular domain of which is composed of 29 epidermal growth factor (EGF)-like repeats and four laminin-A globular-domain-like repeats. The small, 37-amino-acid cytoplasmic domain contains two highly conserved regions, the C-terminal PSD-95/Discs-large/ZO-1 (PDZ)-binding motif ERLI, and a 4.1/ezrin/radixin/moesin (FERM)-binding domain. *Drosophila sdt* encodes several protein isoforms that are scaffolding proteins of the membrane-associated guanylate kinase (MAGUK) family, members of which are characterised by the presence of two Lin-2/Lin-7 (L27) domains, a PDZ domain, a Src-homology 3 (SH3) domain and a guanylate kinase (GUK) domain (Bachmann et al., 2001; Berger et al., 2007; Bulgakova et al., 2008; Hong et al., 2001). PATJ, which was first described, erroneously, as Discs lost (Dlt) (Bhat et al., 1999), contains four PDZ domains and a single L27 domain at the N-terminus (Pielage et al., 2003). Lin-7 is a short protein of 195 amino acids that has an N-terminal L27 domain and a C-terminal PDZ domain (Bachmann et al., 2004). Both Crb and Sdt are essential for the maintenance of epithelial-cell polarity in the embryo (Bachmann et al., 2001; Hong et al., 2001; Tepass and Knust, 1990;
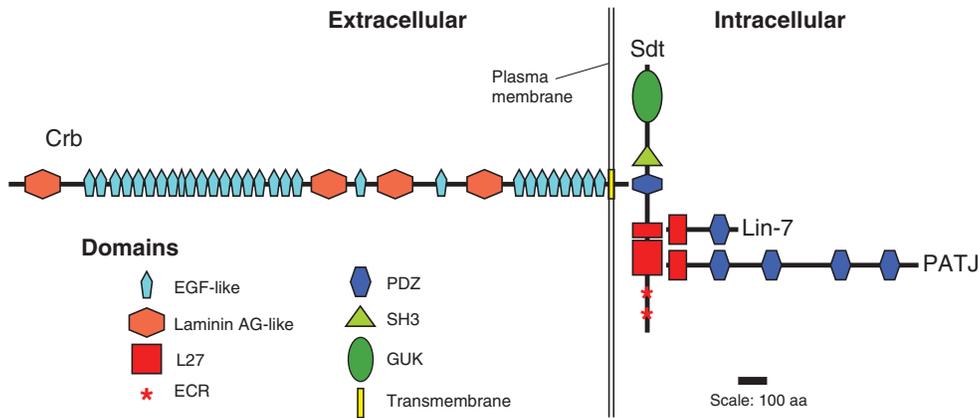
Fig. 1. Schematic diagram of the core proteins of the *Drosophila* Crumbs complex and the proposed structure of the complex. Four core components – Crb, Sdt, PATJ and Lin-7 – and their known domains are drawn to scale. The Sdt-B1 isoform is shown to represent the Sdt protein. AG, A-globulin.

Tepass and Knust, 1993). Expression of the membrane-bound cytoplasmic domain of Crb can suppress the *crb*-mutant embryonic phenotype to the same extent as expression of the full-length protein, which indicates that, in several embryonic epithelia, the cytoplasmic domain is of crucial importance for function. The rescuing function depends on an intact PDZ- and FERM-binding motif (Klebes and Knust, 2000; Wodarz et al., 1995).

The formation of the Crumbs complex is ensured by physical interactions between the different core components (Fig. 1). The central component, Sdt, acts as a 'hub' to organise a plasma-membrane-associated protein scaffold via an interaction between its PDZ domain and the C-terminal ERLI motif of Crb (Bachmann et al., 2001; Hong et al., 2001). The two L27 domains of Sdt bind to the L27 domains of PATJ and Lin-7 (Bachmann et al., 2004; Bulgakova et al., 2008; Roh et al., 2002). These core components always colocalise; this has been observed in embryonic epithelia derived from the ectoderm, in follicle epithelial cells (Bachmann et al., 2008; Horne-Badovinac and Bilder, 2008; Tanentzapf et al., 2000), in wing and eye imaginal discs (Bachmann et al., 2004), and in pupal and adult PRCs (Bachmann et al., 2001; Berger et al., 2007; Izaddoost et al., 2002; Johnson et al., 2002; Nam and Choi, 2003; Richard et al., 2006a). Lin-7 is the only core component known thus far that is also part of another protein complex in a non-epithelial tissue – in neuromuscular junctions. Here it associates with two other MAGUK proteins, the Discs large isoform DlgS97 and Metro (Bachmann et al., 2004) (André Bachmann, Ulrich Thomas and E.K., unpublished).

Strikingly, the Crumbs complex exhibits a highly asymmetric distribution within the cell, irrespective of species (see below) or cell type. It is concentrated in a distinct region of the apical plasma membrane that abuts the ZA; this region is called the subapical or marginal region in epithelial cells and the stalk in PRCs of *Drosophila* (Fig. 2) (Berger et al., 2007; Johnson et al., 2002; Pellikka et al., 2002; Richard et al., 2006a; Tepass, 1996). To date, the molecular mechanisms that define the localisation of the Crumbs complex to this distinct plasma-membrane region are not known. Studies that investigated the dependence of the core components of the Crumbs complex for their localisation in the cell suggest that there are both cell-type- and developmental-stage-specific regulatory mechanisms (Table 1). Loss of Crb function leads to the delocalisation and/or degradation of all other core proteins of the Crumbs complex in all tissues studied to date, including embryonic and follicle epithelia (Bachmann et al., 2001; Hong et al., 2001; Klebes and Knust, 2000; Li et al., 2008; Tanentzapf et al., 2000), and pupal and adult PRCs (Nam and Choi,

2003; Richard et al., 2006a). Loss of Sdt function has a similar outcome (Bachmann et al., 2001; Berger et al., 2007; Horne-Badovinac and Bilder, 2008), except for in pupal PRCs, in which Crb and PATJ remain correctly localised at the stalk membrane in the absence of Sdt (Berger et al., 2007). By contrast, mutations in *Lin-7* result in viable and fertile animals, and the other core components of the Crumbs complex localise as in wild-type animals (Bachmann et al., 2008). The complexity of the *Patj* chromosomal region and the fact that a considerable amount of PATJ gene product in the early embryo is provided by the female have so far prevented a detailed investigation of the phenotype of embryos deficient for this gene (Nam and Choi, 2006; Pielage et al., 2003). In *Patj*-mutant pupal PRCs, Crb and Sdt initially localise properly within cells, but
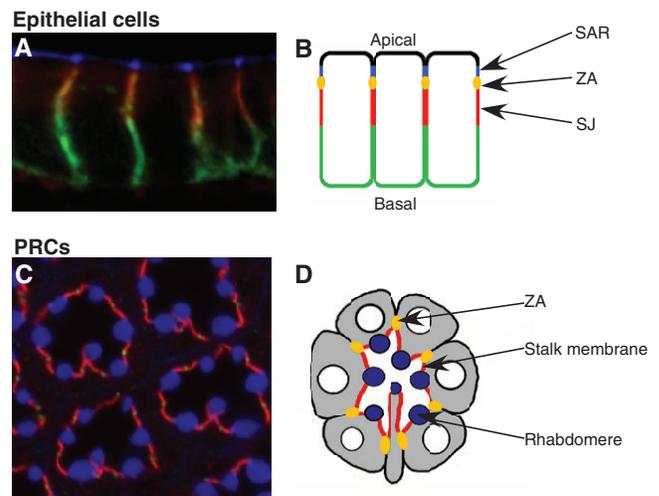

Fig. 2. Localisation of the Crumbs complex in *Drosophila* epithelial cells and PRCs. (A) Optical confocal sagittal section through embryonic epithelial cells stained with: Crb (blue), localised to the subapical region (SAR); Dlg (red), localised to septate junctions (SJ); and Lachesin (green), highlighting basolateral plasma membrane (images kindly provided by Nadine Muschalik). (B) Schematic representation of embryonic epithelial cells with different cellular domains. The colours correspond to those shown in A. (C) Optical confocal cross-section through the adult retina, stained with Sdt (red) to label the stalk membrane, E-cadherin (green) to label the zonula adherens (ZA) and F-actin (blue) to stain rhabdomeres. (D) Schematic representation of a cross-section of the PRCs of an ommatidium. Ommatidia are the units of the fly's compound eye and each contains eight PRCs, of which only seven can be detected in any focal plane. The surrounding pigment cells are not shown. The different membrane regions are indicated in the same colours as proteins at the corresponding site shown in C.

Table 1. Effects in different tissues of mutations in members of the Crumbs complex on the localisation of other core members

	Defect in			
Mutation in	Embryonic epithelia	Follicle epithelium	Pupal PRCs	Adult PRCs
crb	−	−*	−*	−
sdt	−*	−†	+*	−
PATJ	N.D.	N.D.	+*	−*
Lin-7	+	+	N.D.	+

−, all other core members of the Crumbs complex are delocalised; +, all other core members of the Crumbs complex are properly localised; N.D., not determined; *, Lin-7 was not analysed; †, PATJ and Lin-7 were not analysed.

become delocalised as development proceeds (Nam and Choi, 2006; Richard et al., 2006a).

Transient components of the Crumbs complex in *Drosophila*

Three of the four core components of the Crumbs complex are scaffolding proteins with multiple protein-protein interaction domains. Together, they serve as a versatile platform for the recruitment of other proteins and for bringing different proteins into close proximity, thereby facilitating their function and restricting their activity to a spatially distinct region of the cell. In addition, the various protein-protein interaction domains might enable the

organisation of diverse Crumbs complexes with cell-type-specific composition and/or function. As summarised below, transient components of the Crumbs complex can link it to other protein complexes (Fig. 3A), including phosphoinositide signalling networks and the actin cytoskeleton.

Members of the Par signalling network as transient components of the Crb complex

Studies in *Drosophila* (and vertebrates; see below) have shown that there are multiple interactions between members of the Crumbs complex and constituents of the apical Par signalling network, namely Par-6 and atypical protein kinase C (aPKC). Together with
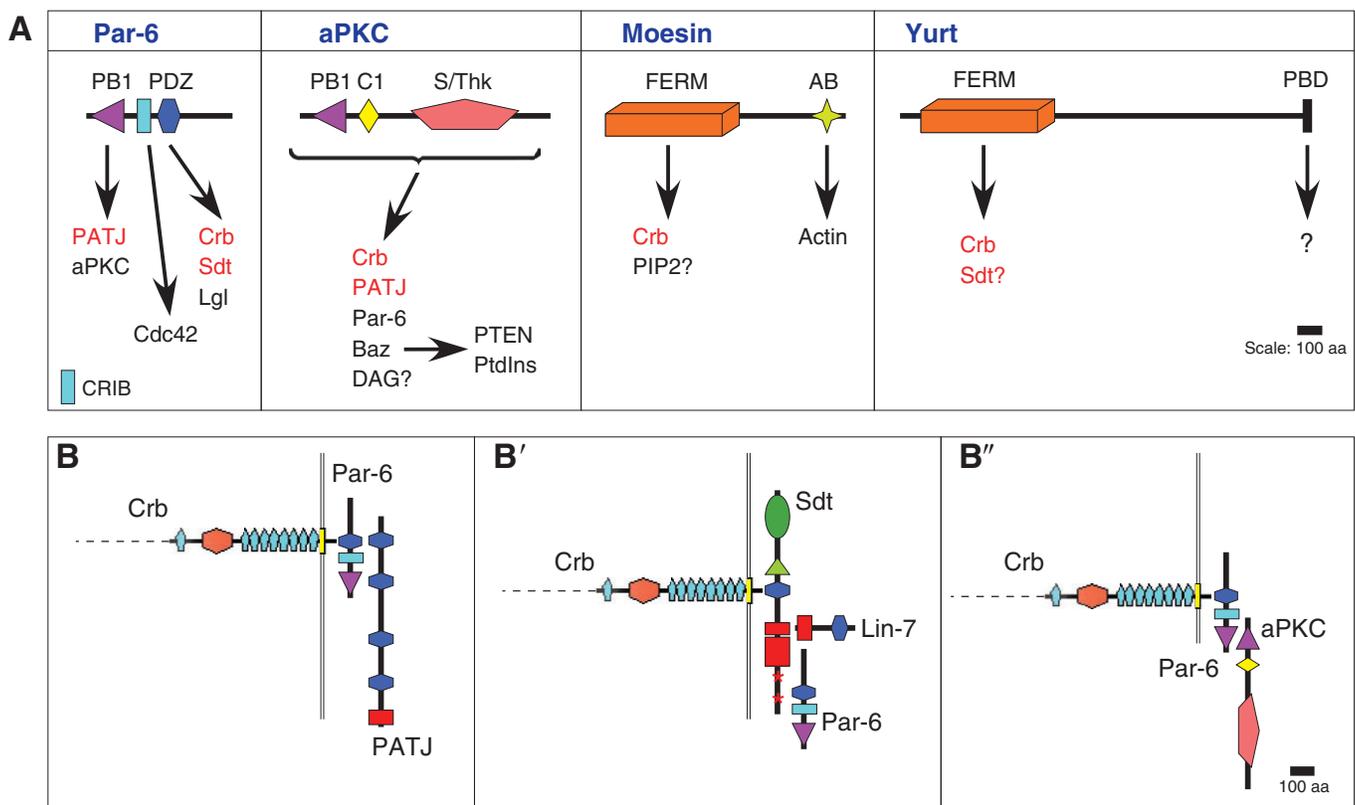

Fig. 3. Transient members of the Crumbs complex, their interactions and potential roles in modifying the organisation of the complex. (A) The most well-characterised transient components of the Crumbs complex are shown, as well as the interactions between their different domains and the Crumbs complex (red text) and other molecules (black text). Question marks denote interactions that have been demonstrated to occur between the vertebrate proteins but not in *Drosophila*. (B-B″) Potential modifications of the composition of the Crumbs complex upon Par-6 recruitment. Par-6 can connect Crb to PATJ without Sdt being recruited (B). Par-6 can also bind to Sdt, thereby preventing Sdt-PATJ binding (B′), or connect Crb to aPKC without involving other core components (B″). PATJ can additionally recruit Sdt and Lin-7 (B), and Par-6 can bring in PATJ or aPKC via its PB1 domain (B′). The symbols used for the protein domains are the same as in Fig. 1. The position of the CRIB domain of Par-6 (A) is shown as according to Betschinger et al. (Betschinger et al., 2003). Only part of the extracellular domain of Crb is depicted (represented by dashed line). Abbreviations not defined in the main text: AB, actin binding; C1, cysteine rich; S/Thk, serine/threonine kinase.

Bazooka (Par-3 in vertebrates), these proteins regulate apicobasal cell polarity in ectodermal epithelia of the *Drosophila* embryo as early as cellularisation, i.e. before Crb functions (reviewed by Assémat et al., 2008; Bilder et al., 2003; Macara, 2004). The PDZ domain of the scaffolding protein Par-6 can bind to the C-terminal ERLI motif of Crb (Kempkens et al., 2006). In addition, Par-6 binds to other members of the Crumbs complex in vitro: the Cdc42/Rac interactive binding (CRIB)-PDZ region of Par-6 binds to the evolutionarily conserved regions (ECRs) of Sdt, thereby preventing PATJ from binding to Sdt (Kempkens et al., 2006; Wang et al., 2004), and the PB1 (Phox and Bem 1) domain of Par-6 binds to the third PDZ domain of PATJ (Hutterer et al., 2004; Nam and Choi, 2003). These data suggest either that Par-6 can link Sdt to PATJ, that it can associate with the core Crumbs complex by binding to one of the scaffolding proteins or that Par-6 can directly interact with Crb (Fig. 3B-B″). However, it is still unclear which of these interactions occur in vivo. In some tissues – for example in pupal eye imaginal discs and embryonic epithelia – Par-6 colocalises with Crb and Sdt (Berger et al., 2007; Kempkens et al., 2006; Nam and Choi, 2003; Petronczki and Knoblich, 2001). By contrast, whereas the Crumbs complex can be found at the stalk membrane, preliminary data suggest that Par-6 localises basolaterally in PRCs of adult flies (N.A.B. and E.K., unpublished).

Par-6 can also recruit additional proteins into the complex. The CRIB domain of Par-6 binds to the small GTPase Cdc42 (Atwood et al., 2007; Hutterer et al., 2004; Joberty et al., 2000; Lin et al., 2000), thereby providing a link between the Crumbs complex and the actin cytoskeleton (Genova et al., 2000). Additionally, in vitro studies have revealed that the PDZ domain of Par-6 binds to Lethal (2) giant larvae (Lgl), a member of the basolateral Dlg-Scribble-Lgl protein network. This interaction results in the phosphorylation of Lgl, thereby preventing its apical localisation (Betschinger et al., 2003). The possibility that the Crumbs complex is linked to Lgl is particularly interesting because Lgl negatively regulates the function of the Crumbs complex both in embryonic epithelia and in PRCs (Bilder et al., 2003; Grzeschik et al., 2007; Tanentzapf and Tepass, 2003).

The serine/threonine protein kinase aPKC colocalises with members of the Crumbs complex in embryonic epithelial cells (Sotillos et al., 2004; Wodarz et al., 2000). By contrast, the cellular localisation of aPKC overlaps only partially with Crb in early pupal PRCs, and aPKC disappears from the stalk at later stages of PRC development (Hong et al., 2003; Nam and Choi, 2003). As revealed by in vitro data, aPKC might be recruited to the Crumbs complex either by PATJ, Crb or Par-6 (Betschinger et al., 2003; Sotillos et al., 2004). In the complex, aPKC might serve as a linker between the Crumbs complex and Bazooka. Bazooka directly binds to the phosphatase and tensin homolog (PTEN) with its second and third PDZ domains (von Stein et al., 2005), whereas its vertebrate orthologue, Par-3, binds phosphoinositides with high affinity through its second PDZ domain (Wu et al., 2007). Therefore, the Crb complex might be associated with the phosphoinositide signalling network. This association does not occur in pupal and adult PRCs, because Bazooka does not colocalise with Crb in these cells (Nam and Choi, 2003; Pinal et al., 2006). Together, the data discussed above suggest that there is more than one possible Crumbs complex, and that its components can vary depending on the cell type or function.

Linking the Crb complex to the cytoskeleton
The Crumbs complex might be linked to the actin cytoskeleton not only through Par-6 and Cdc42, but also via interactions with members of the FERM protein family, which can bind both to actin and to transmembrane proteins. The sole member of the *Drosophila* FERM protein family, Moesin, which has an N-terminal FERM domain and a C-terminal actin-binding domain (Polesello et al., 2002), immunoprecipitates with Crb, but a direct interaction between the two proteins has not yet been shown (Medina et al., 2002). Moesin is thought to act either by antagonising the activity of the small GTPase Rho (Speck et al., 2003) or by linking Crb to β$_H$-spectrin (Medina et al., 2002), thereby affecting these two regulators of the actin cytoskeleton (Schmitz et al., 2000; Thomas, 2001). Crb, Moesin and β$_H$-spectrin colocalise in embryonic epithelial cells in the subapical region (Speck et al., 2003), and the localisation of β$_H$-spectrin depends on Crb. Similarly, restriction of β$_H$-spectrin to the stalk of pupal and adult PRCs depends on Crb (Pellikka et al., 2002). However, the finding that Moesin, unlike the Crumbs complex, localises at the rhabdomere base of adult PRCs (Karagiosis and Ready, 2004) suggests that there is another connection between β$_H$-spectrin and the complex.

Another FERM-domain-containing protein, Yurt, which is important for morphogenesis of epithelial cells in *Drosophila* embryos (Hoover and Bryant, 2002), also transiently associates with the Crumbs complex in epithelia and in PRCs. Its FERM domain binds to the intracellular domain of Crb in vitro, and the two proteins co-immunoprecipitate (Laprise et al., 2006). Loss of Yurt function results in an expansion of the apical domain of epithelial cells and of the stalk membrane of PRCs. This phenotype is similar to that observed when Crb is overexpressed (Klebes and Knust, 2000; Laprise et al., 2006; Pellikka et al., 2002; Wodarz et al., 1995), which suggests that Yurt is a negative regulator of Crumbs-complex function.

Additional mechanisms that regulate the composition of the *Drosophila* Crumbs complex
It is most likely that the transient recruitment of proteins into the Crumbs complex has an important impact on its function. In addition, the activity of the complex can be further modulated by changing the activity of its components, either via the expression of different isoforms, by post-translational modifications or by altering the localisation of the messenger RNAs (mRNAs) that encode the different components.

Different isoforms obtained by alternative splicing
The expression of PDZ-domain-encoding genes is often regulated at the level of mRNA splicing, which gives rise to cell-type- and/or stage-specific isoforms that can differ in their capacity to interact with other proteins (Sierralta and Mendoza, 2004). For example, in *Drosophila*, alternative splicing of *dlg* transcripts gives rise to an epithelia-specific isoform, DlgA, which lacks the L27 domain and is therefore unable to bind to Lin-7, and another isoform, DlgS97, which contains the L27 domain and can associate with Lin-7 in the neuromuscular junction (Bachmann et al., 2004; Mendoza et al., 2003).

sdt transcripts are also alternatively spliced. To date, four different forms of Sdt have been characterised. Three of them, namely Sdt-A1 (also known as Sdt-MAGUK1), Sdt-B1 and Sdt-B2, are scaffolding proteins of the MAGUK protein family (Bachmann et al., 2001; Berger et al., 2007; Hong et al., 2001). Sdt-B1 and Sdt-B2 have slightly different N-termini, owing to different transcription and translation start sites. Sdt-A1 differs from Sdt-B1 and Sdt-B2 in that it has a large exon (exon 3) that encodes

an N-terminal 433-amino-acid region with no obvious domain structure. Sdt-A1 is expressed only in early embryos, whereas Sdt-B1 is expressed throughout embryogenesis. Sdt-B2, but not Sdt-A1 nor Sdt-B1, is expressed in the retina of adult flies (Berger et al., 2007; Horne-Badovinac and Bilder, 2008). On the basis of expressed sequenced tags (ESTs) (http://flybase.org), more Sdt isoforms are predicted to exist. They differ mainly in their N-termini and some exhibit tissue-specific expression (N.A.B. and E.K., unpublished).

Alternative splicing of mRNAs that encode other components provides further variability in the composition of the Crumbs complex. For example, two isoforms of Crb are predicted (http://flybase.org). Additionally, two Lin-7 isoforms are predicted (http://flybase.org), in which a difference in presence of one exon alters the distance between the L27 and PDZ domains. Furthermore, *yurt* encodes four differentially expressed protein isoforms, all of which maintain the PDZ-binding domain and the FERM domain (Laprise et al., 2006). Multiple protein isoforms are also envisaged for Moesin and aPKC (http://flybase.org). Taken together, we predict that the large number of isoforms of the core and transient components of the Crumbs complex can considerably expand its structural and/or functional diversity.

Regulation by mRNA localisation and post-translational modification

The subcellular localisation of specific mRNAs is a mechanism that concentrates proteins at a well-defined region within the cell. *crb* and *sdt* mRNAs are restricted to the apical pole of embryonic epithelia (Bachmann et al., 2001; Tepass et al., 1990) and to cells of the follicular epithelium. Localisation of *crb* mRNA depends on its 3′-untranslated region, whereas the localisation of *sdt* mRNA requires a sequence in the alternatively spliced exon 3. In the embryo, sequences in *sdt* exon 3 are sufficient for apical localisation of a reporter RNA. Both *crb* and *sdt* mRNAs require the activity of Dynein heavy chain 64C for their localisation (Horne-Badovinac and Bilder, 2008; Li et al., 2008).

To date, little is known about post-translational modification of the core components of the Crumbs complex. Preliminary evidence indicates that the intracellular domain of Crb can be phosphorylated in vivo in the embryo (Susann Özüyaman and E.K., unpublished). It is still unclear whether this is mediated by aPKC, as suggested by in vitro data (Sotillos et al., 2004), and whether this has any functional significance. The mammalian orthologues of Sdt belong to the MPP (membrane protein, palmitoylated) family of proteins, which are named according to a putative palmitoylation site between their SH3 and GUK domains. However, to date, an N-terminal palmitoylation signal has been shown to be necessary only for the proper membrane localisation and targeting to the synapse of PSD-95, a MAGUK that is localised at the postsynaptic density (Craven et al., 1999; El-Husseini et al., 2000). It is tempting to speculate that this targeting mechanism also applies to some Sdt isoforms, particularly for Sdt-A, in which a second palmitoylation signal is predicted in the extended N-terminus [using CSS-Palm 2.0 (http://csspalm.biocuckoo.org/)].

Core and transient components of the Crumbs complex are conserved in vertebrates

Since 1999, when the sequence of the vertebrate *crumbs homologue 1* (*CRB1*) was published (den Hollander et al., 1999), orthologues of all core components of the Crumbs complex have been identified, either by sequence similarities found in EST clones or by co-immunoprecipitation assays and/or interaction studies. Similar to many other proteins, several components of the Crumbs complex have more than one orthologue in vertebrates. There are three vertebrate CRB genes – *CRB1*, *CRB2* and *CRB3* [and five in zebrafish, owing to the partial duplication of the genome (Omori and Malicki, 2006)]. CRB3 has a completely different extracellular domain to the other CRB proteins. This domain contains only 56 amino acids and has no sequence similarity to that of the other CRB proteins; however, because the cytoplasmic domain is highly conserved, it is considered to be a member of the Crb protein family (reviewed by Gosens et al., 2008; Richard et al., 2006b). Vertebrate genomes encode three *Lin7* genes and two *PATJ* genes, *PATJ* and *MUPP1* (multi-PDZ-domain protein). *sdt* has several orthologues in the vertebrate genome that belong to the MPP family, of which MPP5 [also known as Pals1 (protein associated with Lin-7)] is most similar to the *Drosophila sdt* gene. The main features of the vertebrate Crumbs complex core components are summarised in Table 2.

Most of the proteins that interact transiently with the *Drosophila* Crumbs complex (described above) are also found in vertebrates, encoded by one or more copies per genome. This includes members of the apical Par proteins, namely Par-3 [formerly called ASIP (atypical PKC isotype-specific interacting protein) (Izumi et al., 1998)], Par-6α, Par-6β and Par-6γ, aPKCλ and aPKCζ (reviewed in Goldstein and Macara, 2007) and the Yurt orthologue YMO1 (EPB41L5; known as Mosaic eyes in zebrafish) (Gosens et al., 2007; Jensen and Westerfield, 2004). Most of these transiently interacting proteins are conserved not only in their domain organisation, but also in the nature of their interactions with other proteins in the Crumbs complex. We refer to recent reviews for extensive descriptions of additional links between the Crumbs complex and other protein networks (Assémat et al., 2008; Gosens et al., 2008; Macara, 2004). The presence of several orthologues of each fly protein in the complex greatly increases its variability in vertebrates, but at the same time it makes their analysis more difficult owing to possible functional redundancy.

Function of the Crumbs complex in vertebrate cells

In addition to the structural conservation of the core components of the Crumbs complex between flies and vertebrates, they also exhibit a similar subcellular localisation and, as shown in some cases, function. In vertebrates, the complex is confined to a distinct region of the apical plasma membrane that abuts the ZA; in epithelial cells, this region is known as the tight junction (TJ) and, in PRCs, as the inner segment. Knocking down the expression of PATJ, Pals1 or Lin-7 in Madin-Darby canine kidney (MDCK) cells results in delayed TJ formation, reduction of transepithelial resistance and defects in cell polarisation (Shin et al., 2005; Straight et al., 2004; Wang et al., 2007). In mice that carry homozygous mutations in *LIN-7C* [also known as *MALS-3* (mammalian LIN-7) or *VELI-3* (vertebrate homolog of LIN-7)], components of the Crumbs complex are either absent (Pals1 and PATJ) or strongly reduced (CRB) at the TJs of renal epithelia, whereas other TJ components, such as zona occludens 1 [ZO-1; also known as tight junction protein 1 (TJP1)], remain unaffected. As a consequence, kidney cells in mice that lack *LIN-7C* develop numerous cysts and fibrosis (Olsen et al., 2007). Similarly, morpholino-induced knockdown of zebrafish *crb2b* expression induces pronephric cysts (Omori and Malicki, 2006) and gross disorganisation of the architecture of podocytes, which are essential components of the filtration barrier of the zebrafish pronephros (Ebarasi et al., 2009).

CRB proteins, but probably not the other core components of the Crumbs complex, are also involved in the formation and stability of the primary cilium. Most vertebrate cells form a single, apical, often non-motile cilium, which defines a distinct apical membrane compartment. The ciliary membrane acts as an antenna that senses the external environment. It contains a variety of receptors and channels that transmit mechanical and chemical stimuli to intracellular transduction cascades, thereby regulating cellular differentiation and homeostasis. Morpholino-induced knockdown of zebrafish *crb2b* results in shorter and abnormally positioned cilia

Table 2. Core components of the vertebrate Crumbs complex

Genes	Features	References
crb orthologues		
Mammals		
Crb1	Expression in retina and brain; mutations in humans lead to retinitis pigmentosa 12 and Leber congenital amaurosis	(den Hollander et al., 1999)
	Required for PRC polarity and adhesion	(van de Pavert et al., 2004)
	Required for maintenance of microvilli in Müller glial cells	(van de Pavert et al., 2007)
Crb2	Mainly expressed in retina, brain and kidney	(van den Hurk et al., 2005)
Crb3	Widespread expression in many epithelia and skeletal muscles; alternatively spliced isoform Crb3b is expressed in several cell lines; Crb3b differs in its C-terminus, which terminates with -CLPI instead of -ERLI	(Lemmers et al., 2004; Makarova et al., 2003; Fan et al., 2007)
	Binds to Par-6 and regulates tight junctions	(Lemmers et al., 2004)
	Crb3-CLPI localises to the cilium; knockdown results in loss of cilia; interacts with importin β1	(Fan et al., 2007)
Zebrafish		
crb1	Morpholino-induced knockdown of *crb1* has no obvious mutant phenotype	(Omori and Malicki, 2006)
crb2a (*oko meduzy*, *ome*)	Neuronal patterning in the retina affected	(Malicki and Driever, 1999; Omori and Malicki, 2006)
crb2b	Morpholino-induced knockdown of *crb2b* results in shorter and abnormally positioned cilia in cells of the pronephric duct and shorter inner segment of PRCs	(Malicki and Driever, 1999; Omori and Malicki, 2006)
crb3a, *crb3b*	Morpholino-induced knockdown of *crb3a* results in length reduction of the auditory kinocilia in the inner ear	(Omori and Malicki, 2006)
sdt orthologues		
Mammals		
Pals1 (MPP5)	Identified as partner of Lin-7	(Kamberov et al., 2000)
	Required for localisation of Crb1 at the subapical region in Müller glial cells	(van Rossum et al., 2006; Roh et al., 2002a)
Zebrafish		
nagie oko	Mutation affects the patterning of the retina, heart morphogenesis and brain development	(Lowery and Sive, 2005; Rohr et al., 2006; Serluca, 2008; Wei and Malicki, 2002; Wei et al., 2006b)
PATJ orthologues		
Mammals		
PATJ	Identified by sequence similarity to *Drosophila* PATJ and by its association with Pals1	(Roh et al., 2002b)
	Contains eight PDZ domains and an N-terminal L27 domain; L27 domain interacts with the N-terminal L27 domain of Pals1 (MPP5)	(Lemmers et al., 2002)
	Interactions between PATJ and the tight-junction components ZO-3 and claudin and between MUPP1 and JAM (junctional adhesion molecule) have been suggested to link the Crumbs complex to the tight-junction complex	(Michel et al., 2005) (Roh et al., 2003)
MUPP1	Multi-PDZ-domain protein; 13 PDZ domains and an N-terminal L27 domain; initially recognised in a yeast two-hybrid screen as a binding partner of the serotonin 5-hydroxytryptamine type 2 (5-HT_{2c}) receptor; L27 domain interacts with the N-terminal L27 domain of Pals1 (MPP5)	(Ullmer et al., 1998)
Lin-7 orthologues		
Mammals		
MALS-1 (*VELI-1*), *MALS-2* (*VELI-2*), *MALS-3* (*VELI-3*)	Mice lacking all three genes die around birth without major defects in external tissue morphogenesis, but with deficiencies in breathing and in synaptic transmission	(Jo et al., 1999; Irie et al., 1999)
	MALS-3 (VELI-3) colocalises in the mouse retina with MPP5 in the outer limiting membrane, and with MPP4, another MAGUK protein, in the outer plexiform layer	(Stöhr et al., 2005) (Aartsen et al., 2006)
	Expressed in the kidney on the the basolateral membrane of cells of the collecting duct, and on the basolateral membrane and the tight junction in proximal tubule cells	(Olsen et al., 2005) (Straight et al., 2006)
	MALS-3 single-knockout mice exhibit regional defects in epithelial polarisation in the kidney, associated with disruption of tight junctions and development of cystic and fibrotic tissue	(Olsen et al., 2007)
	Knockdown of MALS-3 in epithelial cells in culture impairs tight-junction formation and destabilises Pals1 and PATJ	
Zebrafish		
lin7-1, *lin7-2*, *lin7-3*	Localisation apical to the adherens junctions in neuroepithelial cells depends on Nagie oko; expressed in various layers of the mature retina	(Wei et al., 2006a)

MALS, mammalian LIN-7; VELI, vertebrate homolog of LIN-7.

in cells in the pronephric duct, and knockdown of *crb3a* leads to length reduction of the auditory kinocilia in cells of the inner ear (Omori and Malicki, 2006). Accordingly, epithelial cells of the mouse kidney express CRB3 not only in the TJs, but also on primary cilia. Although CRB3 localisation at TJs depends on LIN-7C, ciliary localisation does not, and the structure and positioning of the cilia are not affected in the kidney of mice lacking LIN-7C (Olsen et al., 2007). These findings are similar to observations made in MDCK cells, in which the non-canonical CRB3B isoform that lacks the PDZ-binding motif ERLI (Fan et al., 2007; Fan et al., 2004) is localised on the ciliary membrane. Whether Par-3, Par-6 and aPKCζ, which colocalise with CRB3b on the cilium of MDCK cells, form a complex with CRB3b remains to be shown. Taken together, these data suggest that the Crumbs complex plays an important role in the apical differentiation of various cell types.

The Crumbs complex and human disease
Retinal degeneration
Mutations in human *CRB1* (OMIM 600105) are associated with a variety of autosomal-recessive retinal dystrophies (den Hollander et al., 2008). These include retinitis pigmentosa (RP) with preserved para-arteriolar retinal pigment epithelium (PPRPE), RP with Coats-like exudative vasculopathy, early onset RP without PPRPE, and Leber congenital amaurosis (LCA). The latter condition is one of the most severe inherited retinal dystrophies, with the earliest age of onset. So far, mutations in 14 genes are known to be associated with LCA, and they account for about 70% of all LCA cases that have been analysed. Of these, about 10% are associated with mutations in *CRB1* (den Hollander et al., 2008). In total, over 70 different sequence variants have been identified in more than 184 *CRB1* alleles of patients with retinal dystrophies. Most of the mutations have been mapped to the extracellular domain, which suggests that it plays an important role in PRCs, probably owing to its interactions with as-yet-unknown protein(s). No correlation between the form or severity of the disease and the type or location of the mutation have been found, probably owing to the fact that these conditions are also strongly influenced by the genetic background of an individual. *CRB2* is expressed in the retina and in several other tissues. To date, none of the identified *CRB2* variants found in RP and LCA patients have been determined to be the single causative mutation leading to disease (van den Hurk et al., 2005).

Fly, mouse and fish are important models in which to study the basis of human disease. In the case of Crb1, the similarity between the mutant phenotypes observed in flies and vertebrates is striking. Loss of *crb* in *Drosophila* PRCs results in the reduction of the stalk membrane and, in addition, the PRCs undergo light-dependent degeneration. Remarkably, morpholino-induced knockdown of zebrafish *crb2b* results in shortening of the inner segment of PRCs (Omori and Malicki, 2006), and patients that carry mutations in *CRB1* become blind (den Hollander et al., 1999). Loss of *Crb1* function in the mouse impairs the integrity of the outer limiting membrane (OLM), which is formed by the adherens junctions that connect PRCs to each other and to Müller glial cells. This defect results in the delamination of parts of the photoreceptor layer and neuronal cell death; following light exposure, the number of focal retinal lesions is increased in the mutant retina (van de Pavert et al., 2004). Substitution of a single amino acid in the sixth Ca^{2+}-binding EGF-like repeat of the extracellular domain (C249W), which mimics a mutation found in RP12 patients, resulted in a later onset of photoreceptor degeneration in comparison to the *Crb1*-knockout mouse. The retinal degeneration 8 (*rd8*) mouse carries a single base-pair deletion in *Crb1* that results in the expression of a truncated Crb1 protein that lacks the transmembrane and cytoplasmic domains. These mutant mice show fragmentation of the OLM, shortened photoreceptor inner and outer segments, and retinal degeneration in some areas. The severity of the phenotypes is strongly dependent on the genetic background, which might also explain the variation in the severity of the human disease. The less-severe phenotypes observed in *Crb1*-mutant mice compared with patients that have impaired *CRB1* function could also be due to the fact that the mouse retina contains only a few cones and does not have a fovea centralis.

Other components of the Crumbs complex also have a function in the vertebrate retina, as indicated by their expression patterns and the phenotypes that are induced upon mutations in the corresponding genes. Mutations in the zebrafish *sdt* orthologue *nagie oko* prevent the establishment and maintenance of the OLM, and disrupt the layering of the retina (Wei and Malicki, 2002; Wei et al., 2006a). In the mammalian retina, MPP5 colocalises with LIN-7C, MUPP1 [also known as multiple PDZ domain protein (MPD)] and PATJ in the subapical region, apical to the OLM (Stöhr et al., 2005; van de Pavert et al., 2004). The widespread expression of most members of the Crumbs complex suggests that they have a function in various cell types. Therefore, function of the genes encoding these proteins in the retina cannot easily be assessed, because their inactivation might lead to a severe syndromic phenotype from early in life, or even to early embryonic death.

Tumour progression
Epithelial-to-mesenchymal transition (EMT), which is implicated in metastasis, is often associated with the loss of expression of the cell-adhesion molecule E-cadherin and of TJ proteins. Although there is abundant evidence that the other polarity proteins are involved in the origin and/or progression of cancers (reviewed by Dow and Humbert, 2007; Gonzalez, 2007; Wodarz and Näthke, 2007), the role of the Crumbs complex in this process is poorly understood, despite the fact that it is expressed in many epithelia (Table 2). In *Drosophila*, the overexpression of Crb in the wing imaginal discs can induce overproliferation (Lu and Bilder, 2005). By contrast, some studies in vertebrates have reported that the loss of expression of components of the Crumbs complex was associated with tumour progression. MUPP1 and PATJ are subject to modifications by oncogenic proteins, such as tuberous sclerosis 2 (TSC2) and the adenovirus oncoprotein E4-ORF1 (Latorre et al., 2005; Massey-Harroche et al., 2007). E4-ORF1 disrupts TJ stability and apicobasal polarity by sequestering PATJ in the cytoplasm (Lee et al., 2000). Similarly, the E6 protein of the human papilloma virus HPV-18, one of the high-risk mucosal HPV types that is associated with cervical cancer, interacts with PATJ, thereby targeting it for degradation (Storrs and Silverstein, 2007). Specific downregulation of the expression of LIN-7C was observed in oral squamous-cell-carcinoma-derived cell lines. Re-expression of LIN-7C suppressed not only the invasive behaviour of these cell lines, but also the incidence of experimentally induced metastasis in immunodeficient mice (Onda et al., 2007).

In addition, both *CRB3* and *PATJ* are transcriptional targets of the E-cadherin repressor ZEB1 in the metastatic MDA-MB-231 breast-cancer cell line (Aigner et al., 2007). Similarly, *CRB3* expression was strongly repressed in immortal baby-mouse kidney epithelial cells that were selected for acquiring tumorigenicity (Karp et al., 2008). In both of these cell lines, re-expression of E-cadherin or CRB3, either by inactivation of the ZEB1 repressor or by inducing their expression with a transfected vector, partially or substantially restored TJ formation. These results suggest that there

is a link between members of the Crumbs complex, TJs and tumorigenesis. However, more detailed analyses are required to fully understand the relationship between the Crumbs complex and tumour progression.

Perspective: the Crumbs complex in apical differentiation

A common theme observed in fly and mammalian cells that lack components of the Crumbs complex is a reduction of the apical membrane, which, in turn, induces additional cell-type-specific defects. Among these are defects in adherens-junction or TJ stability, shortening of cilia, loss of cell polarity, defective cell morphogenesis or abnormal cellular homeostasis. However, it is unclear why some *Drosophila* epithelia are affected by the loss of Crumbs-complex functions, whereas others are not, despite the fact that the complex is expressed in both affected and unaffected tissues. Recent studies of the Malpighian tubules (the excretory organ) of the *Drosophila* embryo clearly showed that Crb is only required during major morphogenetic changes, such as convergent extension movements, when cells have to remodel intercellular junctions and apical surfaces. Inhibition of morphogenetic changes in *crb*-mutant embryos prevents the disintegration of the epithelium in the tubules and the epidermis (Campbell et al., 2009). Similarly, both ciliated epithelial cells and PRCs must constantly recycle their apical membrane. Strikingly, apical-membrane specialisations, such as cilia or rhabdomeres, are associated with transport vesicles (Satoh et al., 2005; Zhang et al., 2007). Future work will help to unravel the cell-biological basis that underlies the role of the evolutionarily conserved Crumbs protein complex in a variety of processes, such as epithelial integrity, ciliogenesis and the prevention of retinal degeneration.

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