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Dynamics of epithelial cell polarity in *Drosophila*: how to regulate the regulators?

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Apico-basal polarity is a hallmark of epithelial tissues. The integrated activity of several evolutionarily conserved protein complexes is essential to control epithelial polarity during development and homeostasis. Many components of these protein complexes were originally identified in genetic screens performed in *Drosophila* or *Caenorhabditis elegans* due to defects in cell polarity. With time, it became obvious that these protein complexes not only control various aspects of apico-basal polarity, but also perform a plethora of other functions, such as growth control, organization of endocytic activity, regulation of signaling and asymmetric cell division, to mention just a few. Here we summarize some results mostly obtained from studies in *Drosophila* to elucidate how variation in protein composition and modification of individual components contribute to make polarity complexes versatile platforms to fulfill a variety of functions.

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Regulation of polarity complexes by dynamic composition

The founding members of the three major polarity protein complexes Par3/Par6, Crb and Scrib (see Box 1 for definitions), were identified in genetic screens, performed in *Caenorhabditis elegans* and *Drosophila*, respectively. These proteins organize membrane-associated apical and lateral protein complexes, which led them to be called the Par-complex, composed of Par3/Par6/aPKC, the Crb-complex, comprising, besides Crb, the scaffolding proteins Sdt, *Drosophila* PATJ and *Drosophila* Lin-7, and the Scrib-complex, built from Scrib, Dlg and Lgl. Many of these proteins contain multiple protein-protein interaction motifs, such as PDZ-domain, SH3-domain or GUK-domain (Figure 1), thus enabling the recruitment of additional proteins into the complex (recently reviewed in [1]). More extensive studies have shown, however, that these protein complexes are not static, but highly dynamic entities, making it more appropriate to talk about Par-module, Crb-module and Scrib-module. In the case of the *Drosophila* Crb complex, the transmembrane protein Crb and the scaffolding proteins Sdt, *D*PATJ and *D*Lin-7 form the core complex, based on the observation that these four proteins co-localize whenever they are expressed in the same cell [2].

Various ways exist to modify the nature, and thus probably also the function, of these protein complexes. One of these consists in the expression of different isoforms of the same gene. More precise genome annotations predict that nearly all *Drosophila* polarity genes encode more than one isoform (see Flybase: http://flybase.bio.indiana.edu/). Based on these predictions, *Drosophila* may theoretically organize 96 different Par-complexes, 84 different Crb-complexes and 3.168 different Scrib-complexes. So far, however, very little is known about tissue-/cell type-specific expression of the different isoforms, nor how individual isoforms may affect the function of a given complex. At least two different Sdt isoforms are expressed in *Drosophila* photoreceptor cells. Upon overexpression, they exert opposite effects on the length of the stalk membrane, a portion of the apical membrane, where the Crb complex is localized [3]. Three different Crb isoforms exhibit stage-specific and tissue-specific expression (our unpublished results). Alternative splicing of the *crb* pre-mRNA in *Drosophila* embryos is controlled by the helicase Obelus. *obelus* mutant embryos are characterized by the aggregation of adhesion junction components and defects in centrosome positioning, and show upregulation of one *crb* RNA, the *crb-C* mRNA. The *obelus* phenotypes are mimicked by the overexpression of the *crb-C*, but not the *crb-A* isoform in otherwise wild-type embryos, pointing to specific functions of these two isoforms [4]. In mammals, isoform diversification of a given gene is often achieved by different genes, rather than by alternative splicing of one gene. Most of Par6A, for example, can be found at the TJs in MDCK cells and has no effect on TJs, while Par6B is mostly cytosolic and inhibits TJ formation. This difference goes along with a difference in the binding affinity to Pals1, the vertebrate orthologue of *Drosophila* Sdt [5]. Finally, the epithelial isoform of *Drosophila* Dlg lacks the L27-domain, while DlgS97, the isoform expressed in the neuromuscular junction, contains an L27-domain, thus enabling interaction with Lin7 [6,7] (Figure 2).

Box 1 Definitions and concepts

AJ: Adherens junction. Membrane-associated protein complex forming an adhesive junction between neighboring cells. Localized basal to the TJs in epithelial cells. They are composed of transmembrane proteins (E-cadherin), the extracellular domains of which are held together by homophilic interactions, and scaffolding proteins (e.g. α-catenin and β-catenin), which serve as anchors for the actin cytoskelelon.

Amnioserosa: A squamous transient extra-embryonic epithelial tissue that covers the dorsal part of the *Drosophila* embryo. It derives from the dorsal-most region of the cellular blastoderm.

AP-2: **A**dapter-**p**rotein 2-complex. A heteromeric protein complex, which is involved in the internalization of cargo from the cell membrane via clathrin-dependent endocytosis. The AP-2 complex comprises four subunits, the large α-adaptin and β2-adaptin, the medium μ2-adaptin and the small σ2-adaptin. The core region of AP-2 recognizes cargo proteins through specific recognition motifs in the cytoplasmic domain of these proteins.

Apico-basal polarity: Apicobasal polarity refers to asymmetry along the apical–basal cell axis and is a key feature of epithelial cells. Two functional and biochemical distinct membrane domains are distinguished: the apical membrane faces the external environment or an organ lumen, while the baso-lateral membrane contacts neighboring cells or the underlying extracellular matrix (ECM).

aPKC: **a**typical **p**rotein **k**inase C. First identified as component of the Par3/Par6 complex in *C. elegans* (PKC-3), required for asymmetric division in the early embryo [62].

Cellularization: Embryogenesis in *Drosophila* starts with 13 rounds of nuclear division without cytokinesis, resulting in a syncytium. During cellularization, membranes simultaneously invaginate between the nuclei, thus forming the cellular blastoderm, a single-layered epithelium of ~6000 cells enclosing the yolk.

Crb: Crumbs. Founding member of the Crb protein complex, originally identified in *Drosophila* [63]. Crb is a type I transmembrane protein, with a large extracellular domain and a short, highly conserved cytosplasmic domain [64]. Mammals contain three Crb genes, *Crb1*, *Crb2* and *Crb3*.

Dlg: Discs-large. Tumor suppressor protein, often part of the Scrib complex, localized at the lateral membrane of epithelial cells. Mutations in *dlg* have first been identified due to overgrowth of imaginal discs [65]. Dlg encodes a member of the MAGUK protein family.

Dorsal closure: A morphogenetic movement during *Drosophila* embryogenesis, during which the dorsal-most epithelium, the amnioserosa, becomes internalized into the embryo, while the lateral epidermis from both sides moves dorsally and eventually seals at the dorsal midline.

FBM: FERM domain-binding motif. Short stretch of amino acids in the cytoplasmic domain of transmembrane proteins that are recognized and bound by FERM domains.

FERM-domain: Named after the first four proteins containing this domain: protein **4**.1/**E**zrin/**R**adixin/**M**oesin [66]. FERM domains are often found in proteins that link integral membrane proteins to the actin cytoskeleton, whereby the FERM domain interact with a specific sequence in the cytoplasmic tail of the membrane proteins (reviewed in [67]).

Follicle epithelium: A single-layered epithelium that surrounds a 16-cell germline cyst (one oocyte and 15 nurse cells), thus forming egg chambers in the ovariole of the *Drosophila* ovary. Cells of the follicle epithelium form the eggshell (chorion, vitelline membrane) at late stages of oogenesis.

Germ band extension: The germ band is the region of the *Drosophila* embryo (and other insect embryos) that develops into the segmented part of the body (gnathal, thoracic and abdominal segments). During germ band extension/elongation, which takes about 100 min, the length of the germ band increases about two-fold, while its width decreases about two-fold. During this process, the germ band extends dorso-anteriorly.

GUK-domain: Guanylate kinase-like domain.

Imaginal disc: Tissues in the larvae of holometabolic insects that give rise to most of the external structures of the adult insect, such as the wings, the legs, the halters. They are ideal tissues to study growth and pattern formation in epithelia. (For more information, see: *The Interactive Fly* (http://www.sdbonline.org/sites/fly/aimain/1aahome.htm).)

L27 domain: **L**in**2**/Lin**7**-domain. Protein interaction domain first described in the *C. elegans* proteins Lin-2 and Lin-7. Most MAGUK proteins contain one or two L27 domains.

Lgl: Lethal(2)giant larvae. Scaffolding protein, often part of the Scrib complex, localized at the lateral membrane of epithelial cells. Mutations in lgl were first identified due to overgrowth of imaginal discs [68].

Lin-7: First identified in *C. elegans* as member of a tripartite complex containing, besides Lin7, the scaffolding proteins Lin2 and Lin10. The complex is required to anchor the LET-23 receptor at the baso-lateral membrane of vulval epithelial cells [69]. Called Veli (vertebrate homolog of Lin7 or Mals (= **ma**mmalian **LIN7** proteins)) in vertebrates/mammals.

MAGUK: **M**embrane-associated **gu**anylate **k**inase. Protein superfamily, characterized by the presence of one or several PDZ-domains, an SH3-domain and a guanylate kinase-domain, which is, however, catalytically inactive. Many MAGUKs additionally contain L27 domains. They act as scaffolding proteins at various junctions.

Par1/Par3/Par6: PARtitioning defective homologue-1, homologue-3, homologue-6. These and three more *par* genes were first identified in a genetic screen in *C. elegans* [70]. Mutations in these maternal effect genes show defects in asymmetric cell division in the early embryo and fail to partition P-granules asymmetrically. These proteins are highly conserved from *C. elegans* to human, and are also involved in the control of epithelial cell polarity. The posterior Par-1 protein encodes a Ser/Thr kinase, while Par3 and Par6 encode scaffolding proteins and localize, together with aPKC, at the anterior pole of *C. elegans* blastomeres and apically in epithelial cells (reviewed in [71,72]).

PATJ: **P**rotein **a**ssociated with **t**ight **j**unction/**P**als-1 **a**ssociated **t**ight **j**unction protein. Scaffolding protein, which contains several PDZ-domains and one L27 domain, first (wrongly) described as Discs Lost in *Drosophila* [73] and PATJ in vertebrates [74]. PATJ is recruited into the Crb complex by interaction of its L27 domain with the N-terminal L27-domain of Sdt.

PDZ-domain: Protein-protein interaction domain, named after the founding members, the postsynaptic protein **P**SD95, the septate junction protein of *Drosophila* **D**iscs large, and **Z**O-1, a protein of the *zonula occludens* (tight junction). PDZ domains are versatile motifs characterized by a hydrophobic pocket that can accommodate the PDZ-binding motif at the C-terminus of a transmembrane protein.

PBM: PDZ-binding motif. A short peptide, most often found at the very C-terminus of transmembrane proteins.

SAR: subapical region, also described as marginal zone, corresponds to the stalk membrane in *Drosophila* photoreceptor cells. A distinct region of the apical membrane, localized just apical to the ZA (zonula adherens), corresponding to the site where tight junctions in vertebrate epithelia are situated. The Crb proteins complex defines the SAR.

Scrib: Scribble. Founding member of the Scrib complex. Mutations in *Drosophila scrib* result in loss of the monolayered organization of many epithelia in the embryo. The protein localizes on the lateral membrane of epithelia [75].

Sdt: Stardust. First identified in a genetic screen performed in *Drosophila* [76]. Sdt encodes members of the MAGUK family [77,78]. Loss of *sdt* results in the loss of epithelial cell polarity in the embryo. Sdt binds via its PDZ domain to the C-terminus of Crb. The human homologue is MPP5 (**m**embrane associated **p**almitoylated **p**rotein-5), also called Pals1 (**P**rotein **a**ssociated with **L**in**7**).

SH3 domain: Src-homology 3 domain, often found in signaling molecules.

TJ: Tight junction. Also called *zonula occludens*. Membrane-associated protein complex, localized apically in epithelial cells of vertebrates, bringing neighboring membranes in close proximity. They are composed of transmembrane proteins (claudins, occludins), the extracellular domains of which being in close contact with each other, and scaffolding proteins (e.g. ZO-1), which serve as anchors for the actin cytoskelelon. TJs have two major functions: they act as barriers, to prevent the free diffusion of molecules and ions between neighboring cells, and serve as fence, which prevents the lateral diffusion of integral membrane proteins between the apical and lateral membrane domain. Thereby, they contribute to maintain polarity.

ZA: zonula adherens. An adhesion belt, which encircles the apex of epithelial cells. It mediates adhesion between neighboring cells through homophilic interaction of the extracellular domains of the adhesion molecule E-cadherin.

A second way to modify polarity complexes comes from the promiscuous behavior of some of their members. During cellularization, for example, Sdt interacts with Bazooka (Baz), the fly orthologue of Par3. Upon Baz phosphorylation by aPKC, Sdt is released and is now free to bind to Crb [8] (whether it is the same Sdt isoform that interacts with Baz and Crb, is an open question, since several Sdt isoforms are expressed in the embryo [9,10]). Crb, as another example, can interact via its C-terminus with the PDZ-domain of either Sdt or Par6 [11,12]. Specificity and binding affinities of PDZ-domains to their targets can be modulated by sequences adjacent to the PDZ-domain. Recent structural analysis revealed that the PDZ domain of Pals1 binds with much higher affinity to the PBM of Crb when it is linked with the SH3-domain and the GUK-domain [13]. Strikingly, the PDZ-binding motif of Crb can also interact with α-adaptin, a component of the AP-2 complex. While interaction with Sdt stabilizes Crb on the surface, Crb interaction with AP-2 results in its endocytosis, suggesting that competitive binding to either Sdt or α-adaptin controls the amount of Crb on the cell surface, a crucial parameter for proper apico-basal polarity [14]. How the proportion of Crb binding to either Sdt or α-adaptin is determined is an open question.

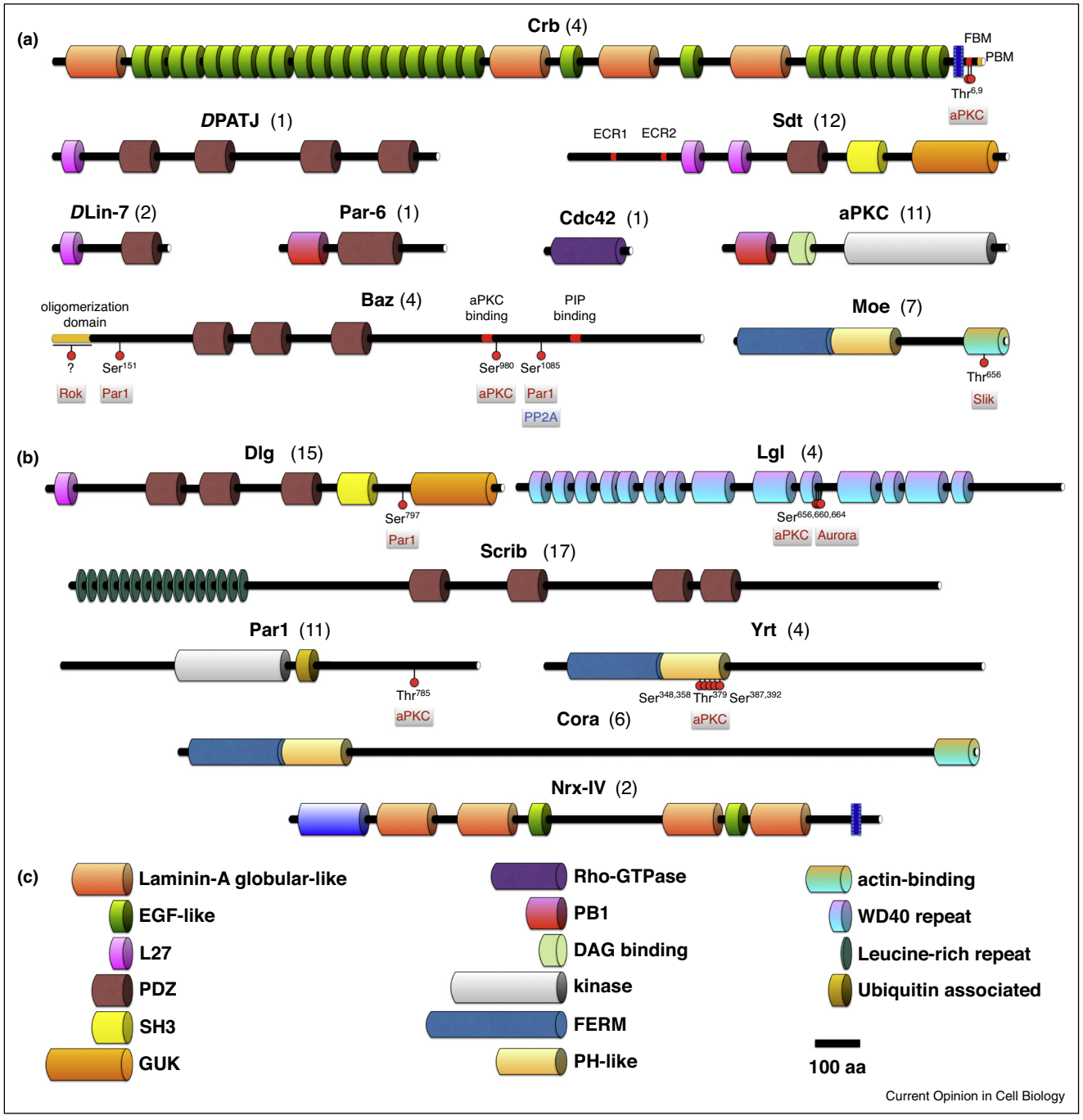
Finally, complexity can be increased by the (transient) recruitment of additional proteins into the complex in a tissue-specific and/or stage-specific manner. Beside the PBM, the Crb cytoplasmic tail contains a FBM. Three FERM proteins have been shown to bind to Crb, namely Yurt, Moesin and Expanded. Interaction of Crb with Expanded, an upstream component of the Hippo signaling pathway, keeps Expanded in an apical position, where it can suppress the activity of the Hippo pathway and thus overgrowth in imaginal discs [15–18]. Interaction between Crb and Yurt results in apical Yurt recruitment, where it negatively controls Crb activity, though the molecular mechanism is poorly understood [19]. Strikingly, a Crb protein carrying a mutation in the FBM rescues polarity of most epithelia of *crb* mutant embryos. Yet, these embryos fail to undergo dorsal closure due to an overactive actomyosin network, suggesting a specific interaction between Crb and probably Moesin in the amnioserosa to negatively regulate actomyosin dynamics [20•,21].

Regulation of polarity complexes by modification of their components

Modification of polarity proteins by phosphorylation can affect the localization, the assembly/composition, or the activity of protein complexes. Both the nature of the kinase involved as well as antagonistic activities of kinases and phosphatases contribute to the fine-tuning of polarity complex dynamics.

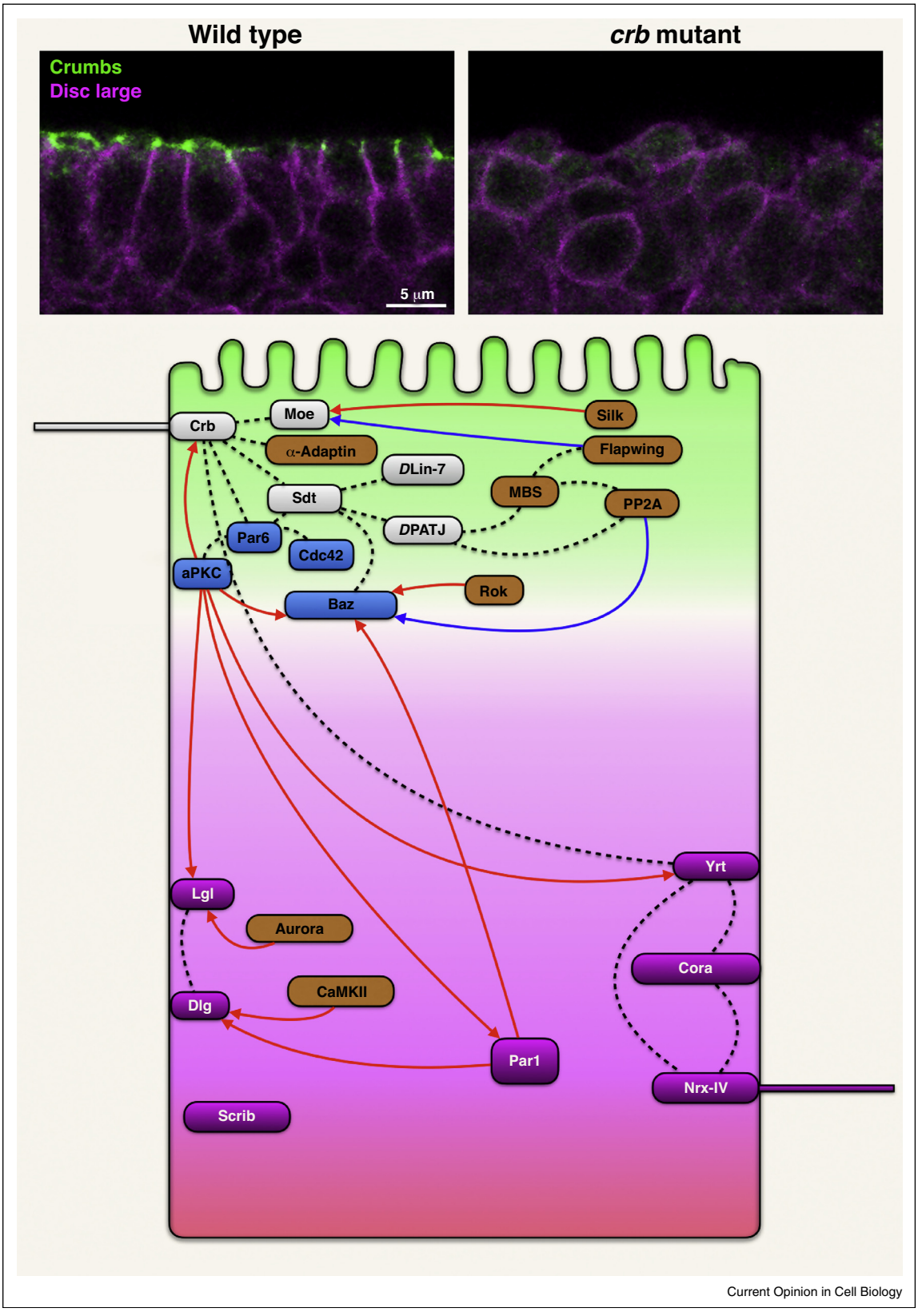
Mutual exclusion of polarity complexes from a given site of the cell through phosphorylation of one of its component is recurrently used to localize polarity complexes. Phosphorylation of Baz at Ser^{151} and Ser^{1085} by basally localized Par-1 excludes Baz from the basolateral membrane. Baz phosphorylation by Par-1 is counteracted by protein phosphatase 2A (PP2A), which interacts with *D*PATJ and dephosphorylates Baz at Ser^{1085} [22]. Refinement of Baz to an apico-lateral position, which overlaps with the SAR and the ZA, occurs through aPKC-mediated phosphorylation at Ser^{980}, during cellularization of the *Drosophila* embryo and in photoreceptor cells [23–25]. In the absence of aPKC, Baz accumulates, together with AJ components, at two foci, which are linked to centrosomes [26].

Figure 1



Graphic representation of *Drosophila* polarity proteins. Apical **(a)** and basolateral **(b)** protein domains, phosphorylation sites and functional motifs are shown. Below each phosphorylation site, the responsible kinase or phosphatase is indicated. The number in parenthesis next to each name indicates the number of predicted isoforms (number of unique polypeptides) according to FlyBase (flybase.org). **(c)** Shows the key for the different domains as well as the scale bar for the whole figure. EGF, epidermal growth factor; PDZ, PSD95/Dlg1/ZO-1 domain; L27, domain in receptor targeting proteins Lin-2 and Lin-7; FBM, FERM-domain binding motif; PBM, PDZ-domain binding motif; ECR, evolutionary conserved region; DAG, diacylglycerol; SH3, SRC Homology 3; PB1, Phox and Bem1p; PH, Pleckstrin homology. The canonical sequences used for the representation were obtained from UniProt (uniprot.org) and InterPro (ebi.ac.uk/interpro/) [79,80]. The accession numbers are given in parenthesis: Crb (P10040), *D*PATJ (Q9NB04), Sdt (Q0KHU9), *D*Lin-7 (Q8IMT8), Cdc42 (P40793), Par6 (O97111), aPKC (A1Z9X0), Baz (O96782), Moesin (P46150), Dlg (P31007), Lgl (P08111), Scrib (Q7KRY7), Par1 (Q9V8V8), Yurt (A0T1Z4), Cora (Q9V8R9), and Nrx-IV (Q94887).

Figure 2


Wild type
crb mutant
Crumbs
Disc large
5 μm
Crb
Moe
Silk
α-Adaptin
Flapwing
DLin-7
MBS
PP2A
Sdt
DPATJ
Par6
Cdc42
aPKC
Rok
Baz
Yrt
Lgl
Aurora
Cora
CaMKII
Dlg
Par1
Nrx-IV
Scrib
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In the same way as basal Par-1 excludes Baz from basal, apical aPKC activity restricts Lgl to the basolateral domain by phosphorylation of three conserved Ser residues (Ser^{656}, Ser^{660} and Ser^{664}) [27–31]. aPKC targets a polybasic region in Lgl that mediates plasma membrane-specific electrostatic interactions. Hence, phosphorylation of Lgl inhibits its membrane targeting by neutralizing positive charges of Arg and Lys residues in this region [32,33]. Whereas the kinase activity of aPKC ensures basolateral restriction of Lgl, phosphorylation of Lgl at Ser^{656} and Ser^{664} by Aurora kinases induces its relocation to the cytoplasm at early prophase in both epithelia and neuroblasts. Lgl relocation is essential to orient the mitotic spindle during symmetric division [34,35•].

Only recently, *in vitro* experiments showed that phosphorylation of at least one of the three conserved Ser residues in the mammalian homolog Lgl2 results in its binding to Dlg4 GUK domain [36•], showing for the first time that these two proteins can interact directly, though the relevance of such interactions *in vivo* remains to be analyzed. aPKC substrates contain a basic and hydrophobic motif (BHM) that interact directly with phospholipids. Phosphorylation of the BHM changes its electrostatic character and inhibits interaction with phospholipids [37••]. Thus, it will be interesting to analyze in the future whether aPKC inhibits Par-1 in *Drosophila*, as phosphorylation of hPar-1b (mammalian homologue) on the conserved Thr^{595} (Thr^{785} in *Drosophila*) negatively regulates its kinase activity and plasma membrane localization [38,39].

As noted above, phosphorylation of Baz by aPKC releases the Baz–Sdt interaction and allows Sdt to interact with Crb [8]. It has been suggested that aPKC also phosphorylates the FBM of Crb [40]. Although this phosphorylation was postulated to be important for Crb stabilization [41] and for the regulation of the Crb-Moesin interaction [42•], a *crb* allele carrying Ala substitutions for the four putative aPKC phosphorylation sites was reported to produce viable flies [43]. The FERM protein Yurt is another transient component of the Crb complex. Yrt directly binds to Crb FBM. Localized basolaterally in the early embryo, Yrt is later recruited apically by Crb, and negatively regulates Crb activity during late embryogenesis [19,44]. aPKC phosphorylates Yrt on several residues (Ser^{348}, Ser^{358}, Thr^{379}, Ser^{387}, and Ser^{392}), thus preventing its premature apico-lateral localization and integration into the Crb complex. Yrt, in turn, prevents apical accumulation of aPKC and thus ensures proper membrane domain formation [45].

Localization of polarity proteins can have a major impact on epithelial polarity and morphogenesis. As described above, Par-1 and aPKC are important to restrict Baz to the subapical region, where it directs assembly of AJs. In gastrulating wild-type *Drosophila* embryos, reduced phosphorylation of Baz by Par-1 is required for dorsal fold formation. Preceding dorsal fold formation, cells that initiate invagination show a basal shift of adherens junctions, while neighboring cells maintain apical ZA. Uniform expression of a Baz variant that cannot be phosphorylated by Par-1 or Par-1 downregulation by RNAi results in lateral localization of Baz, followed by a more lateral positioning of adherens junctions, which in turn abolishes the invagination of the epithelium during dorsal fold formation [46]. Par-1 not only inhibits spreading of Baz laterally, but also promotes a positive feedback loop between Baz and the centrosomes by phosphorylation of Baz as well as by its effect on centrosomal microtubules [47]. During germ band extension, Rho-kinase (Rok)-mediated phosphorylation of Baz oligomerization domain is important for planar polarized distribution of myosin II during cell intercalation [48]. Interestingly, in mammalian cells Rok also negatively regulates PAR-3 (Baz homolog), but the phosphorylation site (Thr^{833}) is different from the one in Baz [49]. Par-1 also regulates Dlg during synaptic development by Ser^{797}phosphorylation [50]. Concomitant overexpression of Dlg and Gliotactin in imaginal discs results in tissue overgrowth. Overgrowth was abolished when a mutant Dlg protein, Dlg^{S797A}, which could not longer be phosphorylated, was expressed along with Gliotactin [51].

In the Crb complex, direct binding of Moesin to the FBM of Crb mediates the interaction between plasma membrane proteins and the cytoskeleton [52,53]. Moesin is phosphorylated by Slik (Sterile20-like) kinase on Thr^{556}. This Thr residue is conserved in Merlin (Thr^{616}), another FERM protein that interacts with Crb via Expanded (reviewed in [54]). In wing imaginal disc epithelia, Slik simultaneously promotes Moesin function and inhibits Merlin, although there is no evidence of direct phosphorylation of Merlin by Slik [55–57]. Interestingly, Flapwing, a known myosin phosphatase [58], acts antagonistically to Slik [59]. Because *D*PATJ binds and inhibits the myosin-binding subunit of myosin phosphatases [60•,61], it will be of interest to analyze whether the Crb complex can regulate organization of the cytoskeleton and tissue growth through modulating the activity of these kinases and phosphatases.

(Figure 2 Legend) Apico-basal cell polarity and its regulation in *Drosophila*. Upper panel, left, displays immunofluorescence pictures showing the localization of Crb (green) and Dlg (magenta) in the epidermis of wild type *Drosophila* embryos. Upper right panel shows the loss of polarity (noticeable by the spread of Dlg along the plasma membrane) and the loss of tissue organization in the embryonic epidermis of *Drosophila* embryos mutant for *crb*. The scheme depicts the localization of the major polarity modules and their interactions. Phosphorylations are indicated by red arrows, dephosphorylations by blue arrows and interactions are depicted by dotted lines. Please note that the cartoon compiles interaction obtained from studies in various epithelia, that is, not all interactions will take place in every epithelium.

Conclusion

Several ways exist that allow polarity complexes to perform a multitude of functions in a cell type-specific and/or stage-specific manner. Here we concentrated on two major mechanisms, namely variation in protein composition of a given complex and modification of individual components and its possible consequences. We would like to point out, however, that additional mechanisms are put in place to increase the complexity of polarity modules. Therefore, we should be aware that results obtained in a given tissue or developmental stage may not be relevant in another tissue or at a different stage. This makes it challenging to define functions of these regulatory entities, which are essential for polarity regulation in all metazoa.

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- •• of outstanding interest

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Here, the authors identify a novel role of *Drosophila* Crb as a negative regulator of actomyosin dynamics during dorsal closure in the embryo. Embryos carrying a mutation in the Crb FBM die. While most epithelia develop normal polarity and undergo proper morphogenesis, the amnioserosa exhibits increased actomyosin activity, which is associated with disrupted adherens junctions. This function of Crb depends on *D*Moesin, the Rho1-GTPase, class-I p21-activated kinases and the Arp2/3 complex.

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