Apicobasal polarity complexes

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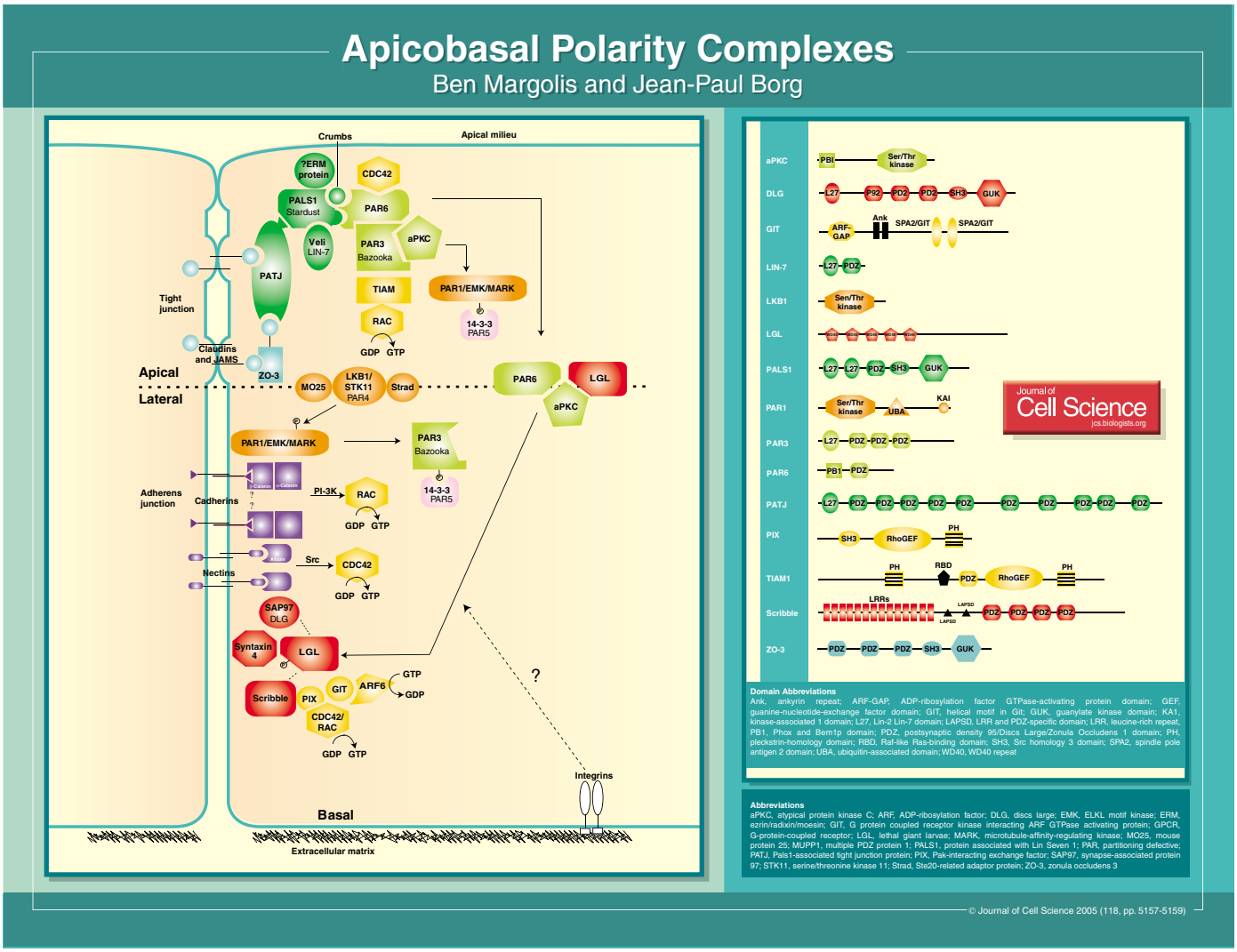
Journal of Cell Science 118, 5157-5159
Published by The Company of Biologists 2005
doi:10.1242/jcs.02597

Asymmetric distribution of proteins and other molecules within cells leads to cell polarization. One of the most studied examples occurs in epithelial cells that polarize to form apical and basolateral surfaces (Nelson, 2003). Although we know much about directed protein trafficking after cells are polarized, less is known about the initial mechanisms that lead to polarization. Genetic and biochemical studies in mammalian systems and lower organisms have begun to reveal the pathways that control this process. The current model is that apical and basolateral protein complexes are mutually antagonistic, which leads to the distribution of proteins in a polarized fashion (Bilder et al., 2003; Tanentzapf and Tepass, 2003). In this model, distinct protein kinases become localized in a polarized fashion and, through phosphorylation, control the localization of other proteins (see below).

The poster indicates the major proteins thought to play a role in the initiation of apicobasal polarity and is based on studies in mammalian and *Drosophila* cells. The antagonistic apical and basolateral protein complexes are separated by a dotted line. Currently, the apical complexes are better understood owing to conservation in structure and function between the mammalian and *Drosophila* systems. By contrast, some of the basolateral protein complexes are less well understood in mammalian cells.

Studies of the apical domain have focused on two major complexes, the Crumbs-PALS1(Stardust)-PATJ complex and the PAR3(Bazooka)-PAR6-aPKC complex (Macara, 2004). (Names in parenthesis indicate *Drosophila* nomenclature if different from the mammalian name.) In mammalian cells, these complexes localize to the tight junction seal, a fence of tight junction complexes that separates apical and basolateral domains. In *Drosophila* the proteins concentrate in the



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subapical complex (also known as the marginal zone), which is also located at the border between apical and basolateral membranes, but no junctional seal is formed (Knust and Bossinger, 2002).

Crumbs is an apical transmembrane protein first identified in *Drosophila* that can directly bind through its C-terminal tail to a PDZ domain in PALS1 (Stardust) (Bachmann et al., 2001; Hong et al., 2001). Crumbs also has a region in its intracellular domain that can bind to members of the ezrin-radixin-moesin (ERM) family of proteins but the exact role of this domain in polarity is uncertain. PALS1 (Stardust) is a scaffold that has multiple protein-protein interaction domains and is a member of the membrane-associated guanylate kinase (MAGUK) family of proteins. It can interact with the small PDZ domain protein Lin-7 through one L27 domain and with PATJ (formerly known as Discs Lost in *Drosophila*) through a second L27 domain. PATJ is a multi-PDZ-domain scaffold protein highly related to MUPP1 that can bind to tight junction proteins such as claudins and zonula occludens 3 (ZO-3) through its PDZ domains (Roh and Margolis, 2003).

The second protein complex localized to the tight junction is the PAR6-PAR3(Bazooka)-aPKC complex. The role of this protein complex in polarity was first described in the *C. elegans* zygote and since then its role in polarity has been confirmed in many cell systems (Macara, 2004). PAR3/Bazooka and PAR6, like PALS1(Stardust) and PATJ, are PDZ domain scaffold proteins involved in multiple protein-protein interactions. The key effector of the complex is aPKC, a kinase that can directly interact with Par6 through PB1 domains. aPKC plays a pivotal role in polarity signaling by phosphorylating proteins and altering their localization along the polarity gradient. For example, one substrate of aPKC is a lateral protein, lethal giant larvae (LGL), which forms a separate complex with aPKC and PAR6 that excludes PAR3 (Betschinger et al., 2003; Plant et al., 2003; Yamanaka et al., 2003). Phosphorylation of LGL is thought to exclude it from the apical membrane and facilitate targeting to the lateral membrane.

Small G proteins of the Rho family, especially CDC42, also appear to play an important role in control of the apical complex (Macara, 2004). The role of CDC42 in cell polarity appears to be highly conserved from yeast to man (Etienne-Manneville, 2004). In mammalian and *Drosophila* systems, CDC42 binds to PAR6 and probably increases the activity of aPKC within the PAR6-PAR3(Bazooka)-aPKC complex. It might also play a role in localization and modulation of other protein complexes (Macara, 2004). Small G protein guanine-nucleotide-exchange factors (GEFs) such as Tiam1 appear to concentrate at apical complexes and are important for tight junction formation and possibly polarization (Chen and Macara, 2005; Liu et al., 2004).

The Crumbs-PALS1(Stardust)-PATJ and PAR3(Bazooka)-PAR6-aPKC complexes directly interact. PALS1 can bind to PAR6, and Crumbs can bind directly to PAR6 in addition to PALS1 (Hurd et al., 2003b; Lemmers et al., 2004). A current concept is that the PAR3(Bazooka)-PAR6-aPKC core complex is a universal effector of polarity and that Crumbs-PALS1(Stardust)-PATJ is a specific adaptor targeting this effector in epithelial polarity. Still unclear is how this complex initially localizes to mark the apical-basolateral boundary.

Polarity complexes are also found at the lateral surface. First among these are the proteins that mediate cell-cell adhesion, including the transmembrane cadherin and nectin proteins (Nelson, 2003; Sakisaka and Takai, 2004). Both of these adhesion protein families connect to multiple signaling pathways, including small G proteins of the Rho family. The initiation of cell-cell adhesion appears to be an important step in early polarization by specifying the lateral membrane. However the exact mechanisms involved in localization of the polarity effectors by these adhesion receptors are still under investigation. Like the apical complexes, serine/threonine kinases are thought to be important effectors of the polarization signal. The PAR1 kinase (also known as ELKL motif kinase, EMK) has been shown in *Drosophila* and mammalian cells to localize to the lateral membrane of epithelia and control polarization (Cohen et al., 2004; Doerflinger et al., 2003). PAR1 has also been identified in mammalian cells as microtubule-affinity-regulating kinase (MARK), which suggests that regulation of microtubules might be important in the polarization process (Biernat et al., 2002). Indeed, PAR1 has important control over microtubule organization in *Drosophila* and mammalian polarity models. In addition, PAR1 regulates the localization of polarity proteins. For example, it can phosphorylate PAR3, leading to the binding of 14-3-3 protein to phosphorylated PAR3 (Benton and St Johnston, 2003; Hurd et al., 2003a). In turn, PAR1 can be phosphorylated by aPKC, which prevents its membrane targeting (Hurov et al., 2004; Suzuki et al., 2004). PAR1 is also a substrate of another kinase, LKB1 (also known as STK11 or PAR4), which regulates apical membrane formation (Baas et al., 2004). LKB1 appears to require two cofactors for cytosolic activity, MO25 and Strad, but understanding its role in polarity is confounded by the existence of multiple substrates (Lizcano et al., 2004).

Like the PDZ-domain-based complexes in the apical domain, there might also be a similar lateral complex, including the PDZ domain proteins Scribble and Discs Large (DLG), as well as the WD40 protein, LGL (Bilder, 2004). In *Drosophila*, impaired activity of these basolateral proteins promotes a compromised localization of apical markers that expand the lateral membranes and lead to epithelial overgrowth (Bilder, 2004). The molecular basis of this defect remains unclear but the Scribble-DLG-LGL pathway is known to antagonize the apical Crumbs and PAR complexes (Bilder et al., 2003; Tanentzapf and Tepass, 2003). However, it is not clear whether Scribble, DLG and LGL actually form a protein complex. These proteins might also control a signalling cascade, whose disruption in mutant flies leads to a tumorigenic process (Zeitler et al., 2004). Scribble, DLG and LGL have highly conserved roles in mammals in terms of protein organization and subcellular localization, and their roles as neoplastic tumor suppressors in flies have boosted studies in vertebrates (Bilder, 2004). Nevertheless, there is no evidence to date that supports a role of the mammalian proteins in apical-basal

polarity, despite overlapping functions demonstrated by rescue experiments in flies using human proteins (Bilder, 2004). Redundancy or functional divergence during evolution might explain the inability to demonstrate an effect. At the molecular level, LGL and Scribble are connected to trafficking machinery. LGL associates with syntaxin 4, a component of the basolateral exocytotic machinery (Musch et al., 2002) whereas Scribble binds to PIX and GIT, two regulators of the ARF6 and CDC42/RAC small GTPases (Audebert et al., 2004). More studies are needed to establish whether this Scribble-PIX-GIT-ARF6 pathway has a role in apicobasal polarity. In addition, the function of basal proteins such as integrins in epithelial polarization requires further investigation.

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