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# Stepwise polarisation of the *Drosophila* follicular epithelium

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ABSTRACT

The function of epithelial tissues is dependent on their polarised architecture, and loss of cell polarity is a hallmark of various diseases. Here we analyse cell polarisation in the follicular epithelium of *Drosophila*, an epithelium that arises by a mesenchymal–epithelial transition. Although many epithelia are formed by mesenchymal precursors, it is unclear how they polarise. Here we show how lateral, apical, and adherens junction proteins act stepwise to establish polarity in the follicular epithelium. Polarisation starts with the formation of adherens junctions, whose positioning is controlled by combined activities of Par-3, β-catenin, and Discs large. Subsequently, Par-6 and aPKC localise to the apical membrane in a Par-3-dependent manner. Apical membrane specification continues by the accumulation of the Crumbs complex, which is controlled by Par-3, Par-6, and aPKC. Thus, our data elucidate the genetic mechanisms leading to the stepwise polarisation of an epithelium with a mesenchymal origin.



## Introduction

Epithelia are sheets of adherent cells that separate different compartments of the animal body, and their polarisation is essential for epithelial structure and function. Epithelial polarity is manifested in the formation of distinct membrane domains, a polarised cytoskeleton, polarised membrane transport and highly elaborated cell–cell junctions. Adherens junctions (AJs) are conserved junctions mediating homotypic cell adhesion between neighbouring epithelial cells. Typically, the AJs form an adhesive belt, called zonula adherens, close to the apical membrane of the epithelium facing the external environment. A main component of the AJs is the single-pass transmembrane protein E-cadherin, which mediates homophilic interactions with its extracellular part, while its intracellular part is associated with cytoskeletal and signalling proteins. Armadillo (Arm)/β-catenin is one of these intracellular binding proteins with a key role in AJ formation (see Gumbiner, 2005; Knust and Bossinger, 2002; Müller, 2000; Nelson, 2003; Tepass et al., 2001 for reviews).

The formation of AJs is an important hallmark for the establishment of polarised membrane domains as they subdivide the cell cortex into an apical and a basolateral region. Genetic screens in *Drosophila* and *Caenorhabditis elegans* identified several genes controlling the formation of AJs and polarised membrane domains. The identity of the different membrane domains is provided by the polarised localisation of distinct proteins. It has been shown for *Drosophila* that the lateral membrane is specified by the PDZ containing proteins Scribble and Discs large (Dlg) and by the WD-40 repeats containing protein Lethal (2) giant larvae (Lgl) (see Assemat et al., 2008 for review). The formation of the apical membrane requires a complex containing the transmembrane protein Crumbs (Crb) and the PDZ containing protein Stardust (Sdt). These two proteins are core components of this complex, and they have been shown to bind to several transient components, which contribute to cell polarisation (reviewed in Bulgakova and Knust, 2009). The PDZ domain containing protein Par-6 and the atypical protein kinase aPKC are two of these transient binding partners. Par-6 and aPKC can bind Par-3, another protein with PDZ domains whose fly homologue is called Bazooka (Baz). Depending on the cell type Baz, Par-6 and aPKC may localise to the apical cortex or to the AJs. Baz, Par-6, and aPKC are part of the apical Par signalling network, and their binding capacities appear to be regulated in a cell type specific manner. All of the above mentioned polarity proteins are highly conserved, and are thought to be required for the polarisation of most, if not all, types of epithelia (Assemat et al., 2008).

Functional analysis in *Drosophila* has led to a model of how these proteins interact to polarise the epithelium of the blastoderm embryo.

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In this model, apical and lateral membrane identity is provided by a regulatory hierarchy involving Baz, Crb, and the lateral proteins; Scribble, Dlg, and Lgl. The lateral proteins repress apical identity by antagonising Baz. At the apical membrane, Baz recruits Crb, which counteracts the activity of lateral proteins (Bilder et al., 2003; Tanentzapf and Tepass, 2003). Localisation of Baz to the apicolateral region of the epithelial cells seems to be a critical first step in the initiation of this regulatory mechanism. Baz protein uses the polarised cytoskeleton of the embryo for its localisation to the apicolateral region, where it controls the assembly of AJs (Harris and Peifer, 2004; Harris and Peifer, 2005). The mechanisms underlying epithelial polarisation have been discovered through investigation of the embryonic epithelium, which develops de novo from a syncytium by multiple invaginations and polarised growth of the plasma membrane. The follicular epithelium of the *Drosophila* ovary has a different developmental origin and arises, like many other epithelia, by a mesenchymal–epithelial transition (Tepass et al., 2001). In contrast to the embryonic epithelium, these cells arise from stem cell divisions, which generate mesenchymal precursors that migrate to and integrate into the newly formed epithelium. This raises the questions whether establishment of polarity varies depending on the genesis of the epithelium, and if different developmental programs are accompanied by distinct polarisation mechanisms.

The mesenchymal precursors of the follicular epithelium are generated by stem cells, which reside in the germarium, a structure located at the anterior tip of the ovaries. Within the germarium egg chambers (or follicles), the functional unit of oogenesis, are generated by the encapsulation of a germ line cyst by a monolayer of epithelial cells (see scheme in Fig. 1a). The somatic epithelial stem cells are located in the middle region of the germarium (Margolis and Spradling, 1995) and give rise to follicle cell precursors, which subsequently migrate posteriorly towards the germ line cyst. In the posterior part of the germarium the follicle cells make contact with the cyst, and form an epithelial sheet surrounding the germ line cells. After encapsulation of the cyst, newly formed egg chambers bud off from the germarium. The budding egg chamber represents stage 1 of oogenesis and is enclosed by a cuboidal epithelium (Horne-Badovinac and Bilder, 2005, and references therein). Initially low, the frequency of epithelial cell divisions in the germarium increases in response to the growth of the germ line cyst until ceasing at stage 6 of oogenesis (Wang and Riechmann, 2007).

In comparison to the epithelium of the early embryo, we know relatively little about the mechanisms polarising the follicular epithelium. Analysis of agametic ovaries revealed that follicle cell contact to the germ line is essential for correct epithelial formation (Margolis and Spradling, 1995; Goode et al., 1996). The finding that expression of proteins Egghead and Brainiac is required within the germ line cyst for formation and polarisation of the epithelium led to the idea that germ line signalling is instructive for correct epithelial formation (Goode et al., 1996). Although the exact nature of this signal is unknown, the identification of Egghead and Brainiac as glycosyltransferases suggests that it is modified by lipid-linked oligosaccharide chains (Wandall et al. 2005). Further analysis of agametic ovaries has shown that the presence of the basement membrane surrounding the germarium is sufficient to specify the basal membrane of the follicle cells. The correct localisation of apical and lateral markers is dependent on the germ line cyst indicating that the putative signal from the germ line is important for the polarisation of the apical and lateral membrane domains. The finding that localisation of the apical protein, Crb, is completely abolished in agametic ovaries provides further support for a central role of the germ line in epithelial polarisation (Tanentzapf et al., 2000).

Crb accumulation at the apical membrane is also dependent on AJs and the microtubule motor Dynein. Recent data show that mRNAs encoding Crb and Sdt are localised to the apical membrane in a Dynein-dependent manner, suggesting that they are transported along microtubules (Horne-Badovinac and Bilder, 2008; Li et al., 2008). Additionally, the apical localisation of Crb is dependent on *arm*, indicating a critical role for AJs in follicle cell polarisation (Tanentzapf et al., 2000). The polarisation of the lateral membrane involves the lateral exclusion of Baz by Par-1. Par-1 kinase localises to the lateral membrane, where it inhibits the formation of Baz–aPKC complexes by Baz phosphorylation (Benton and St Johnston, 2003).

Although several reports demonstrate important roles for many of the known polarity regulators in the follicular epithelium, it remains largely unknown how these proteins interact during epithelial polarisation (Manfruelli et al., 1996; Goode and Perrimon, 1997; Bilder et al., 2000; Tanentzapf et al., 2000; Abdelilah-Seyfried et al., 2003; Benton and St Johnston, 2003). Elucidation of these interactions requires a careful analysis of the localisation of known polarity proteins during epithelial formation, based on which a functional analysis can be performed. Here, we examine the spatial distribution and functions of polarity regulators in the follicular epithelium, and present a model for its stepwise polarisation. In the first step, the lateral membrane and the AJs are specified by combined activities of Baz, Arm, and Dlg. In the second step, apical identity is specified by Baz, Par-6, and aPKC, which regulate the apical localisation of the Crb complex in the third step of epithelial polarisation.

## Materials and methods

### *Fly strains*

We used the following alleles and FRTs for our study: $arm^{YD35}$ FRT9-2 (Peifer and Wieschaus, 1990), $baz^{XI106}$ FRT9-2 (Wieschaus et al., 1984), FRT82B $crb^{11A22}$ (Tepass and Knust, 1990), FRT42B $apkc^{l(2)k406403}$ (Wodarz et al., 2000), $par\text{-}6^{226}$ FRT9-2 (Petronczki and Knoblich, 2001), $sdt^{XP96}$ FRT19A (Wieschaus et al., 1984), and $dlg^{M52}$ $^{FRT}$ 101 (Woods and Bryant, 1991).

### *Antibodies*

Primary antibodies: rabbit anti-GFP preabsorbed (1:400), goat anti-GFP FITC-conjugated (1:200, Biozol), rabbit anti-Baz preabsorbed (1:500, A. Wodarz), mouse anti-Arm (1:400, Hybridoma Bank), rat anti-DE-cadherin (1:50, Hybridoma Bank), rabbit anti-Par-6 preabsorbed (1:500), rabbit anti-aPKC (1:200, Santa Cruz), rat anti-Crb (1:250, E. Knust), rabbit anti-Sdt (1:250, E. Knust), mouse anti-Dlg (1:50, Hybridoma Bank).

Secondary antibodies (Invitrogen) with different fluorescent dyes (Alexa 488/568/633) were used in a 1:400 dilution.

### *Induction of homozygous follicle cells clones using the FRT/FLP system*

Flies carrying the mutant allele on a chromosome with an FRT site were crossed to males carrying the respective wild type allele and a GFP reporter gene with the corresponding FRT site and an FLP recombinase under the control of a heat shock promoter on another chromosome (Xu and Rubin, 1993). Flies were allowed to lay eggs over night and eggs developed for 24 hours. During the following 4 days, eggs and larvae were treated with a heat shock at 37 °C for 1 hour, respectively. After heat shock procedure larvae were kept at 25 °C until hatching. Freshly enclosed females were collected and supplied with some males. After 24 hours at 25 °C, females were dissected and ovaries prepared according to the staining protocol.

### *Immunohistology*

Ovaries were fixed in 8% formaldehyde in PBS (phosphate-buffered saline) for 10 minutes followed by two washing steps in 0.1% PBT (PBS + 0.1% Triton X-100). Ovaries were blocked in 0.5% bovine serum albumin (BSA) diluted in 0.1% PBT before they were