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REVIEW

Dynamics of cortical domains in early *Drosophila* development

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ABSTRACT

Underlying the plasma membrane of eukaryotic cells is an actin cortex that includes actin filaments and associated proteins. A special feature of all polarized and epithelial cells are cortical domains, each of which is characterized by specific sets of proteins. Typically, an epithelial cell contains apical, subapical, lateral and basal domains. The domain-specific protein sets contain evolutionarily conserved proteins, as well as cell-type-specific factors. Among the conserved proteins are, the Par proteins, Crumbs complex and the lateral proteins Scribbled and Discs large 1. Organization of the plasma membrane into cortical domains is dynamic and depends on cell type, differentiation and developmental stage. The dynamics of cortical organization is strikingly visible in early *Drosophila* embryos, which increase the number of distinct cortical domains from one, during the pre-blastoderm stage, to two in syncytial blastoderm embryos, before finally acquiring the four domains that are typical for epithelial cells during cellularization. In this Review, we will describe the dynamics of cortical organization in early *Drosophila* embryos and discuss the processes and mechanisms underlying cortical remodeling.

KEY WORDS: *Drosophila*, F-actin, Cortical compartment, Cortical domain, Epithelia

Introduction

Below the plasma membrane in eukaryotic cells, an actin cortex containing a meshwork of actin filaments and associated proteins can be found. In all polarized and epithelial cells, cortical domains exist, which are characterized by specific sets of proteins, and, typically, these are the apical, subapical, lateral and basal domains. These sets of domain-specific proteins contain cell-type-specific proteins, as well as proteins that are conserved throughout evolution, among which are the Par proteins, which had originally been identified in *Caenorhabditis elegans* based on their function in establishing zygotic anterior-posterior polarity (reviewed in Lang and Munro, 2017), the adherens junctions complex of the zonula adherens and markers for the lateral domain, Scribbled, Discs large 1 (Dlg1) and Lethal giant larvae (Lgl) (reviewed in Campanale et al., 2017).

Among the proteins in the cortex are cortical proteins and actin-associated proteins, such as nucleators, crosslinkers and motors, as well as integral membrane proteins (Fig. 1A) (reviewed in Honigmann and Pralle, 2016). The cortex is able to react to external and internal signals and has important functions in cell division, motility, cell shape changes, cell rearrangement and mechanical stability. Cortical domain organization is linked to cell polarity and is important for cell behavior, and, consequent with this, tissue morphogenesis and embryonic development, in a variety of species (reviewed in Munjal and Lecuit, 2014). Cortical domains are set up by the differential localization of proteins that confer identity to cortical domains and are maintained, for example, by lateral diffusion barriers (Fig. 1A) (reviewed in Honigmann and Pralle, 2016). Besides their function in epithelial cells, Par proteins also define anterior-posterior polarity in the *C. elegans* zygote and *Drosophila* oocyte (reviewed in Nance and Zallen, 2011), as well as separating the inner and outer cells in early mouse embryos, which give rise to the first cell lineages (Korotkevich et al., 2017) (Fig. 1B). Further functions of cortical domains in non-epithelial cells include axon specification and polarization of neurons, for example, with Par-3 [Bazooka (Baz) in flies] and Par-6 proteins being restricted to the apical tip growth cone of axons (reviewed in Insolera et al., 2011), and directed migration of astrocytes, where localization of the Par complex to the leading edge is seen (reviewed in Suzuki and Ohno, 2006) (Fig. 1B).

Several mechanisms for the establishment and maintenance of cortical domains and the molecular factors involved have been delineated. These include mutual exclusion, as the lateral proteins Scribbled, Lgl, Dlg1 and Par-1, exclude apical proteins and adherens junctions from the lateral domain (Bilder et al., 2000; McKinley and Harris, 2012; Tanentzapf and Tepass, 2003; Yamanaka et al., 2006). Directional transport and vesicle trafficking is also assumed to have an important role in the establishment and maintenance of cortical domains. As the generic mechanisms for the establishment and maintenance of typical cortical domains in epithelial cells have been covered in several excellent reviews (Goldstein and Macara, 2007; Krämer, 2000; Laprise and Tepass, 2011; Lecuit, 2004; Mazumdar and Mazumdar, 2002), we will focus here on the dynamic nature of cortical domains and emphasize the relevance of their remodeling in early development of *Drosophila*.

Dynamics of cortical domains in early *Drosophila* embryos

Cortical organization and remodeling is tightly linked to embryonic development (Fig. 2). From the single uniform cortical domain during the pre-blastoderm stage, the first cortical differentiation takes place during syncytial blastoderm development, where two cortical domains are seen during the interphase and three domains during mitosis (Foe et al., 1993). With mid-blastula transition and the switch to cellularization in interphase 14, a subapical domain is added to give rise to the typical epithelial organization with four cortical domains (reviewed in Harris, 2012).

Uniform cortex in pre-blastoderm embryos

Following fertilization, the nuclei and their associated centrosomes reside deep within the yolk where they undergo the first nine nuclear cycles. During this stage, the embryonic surface is covered by microprojections of the plasma membrane that are comparable to microvilli (Turner and Mahowald, 1976). The cortex is uniformly organized with an even distribution of F-actin and Myosin II (Table 1) (Karr and Alberts, 1986; Warn et al., 1984, 1980; Young et al., 1991). Cortical Myosin II localization occurs in cycles linked to embryonic mitotic cycles and, along with this, cortical

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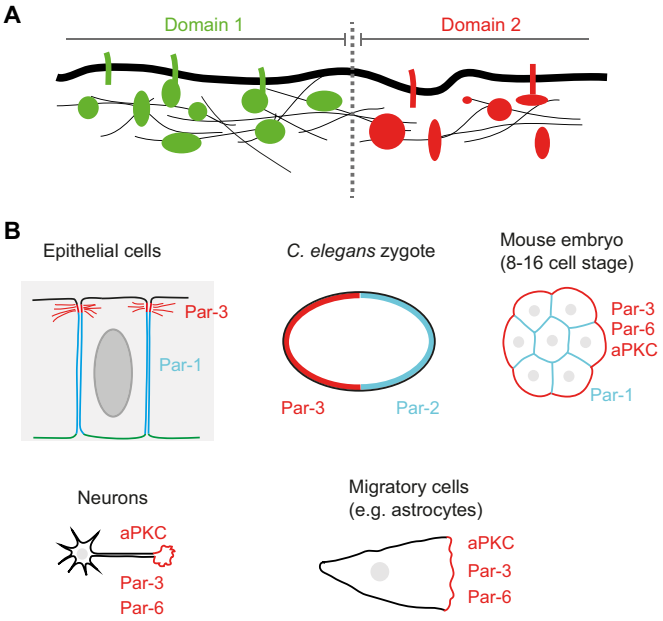

Fig. 1. Cortex and cortical domains. (A) Schematic representation of the cortex. A thin layer of F-actin lies below the plasma membrane, which includes myosin motors, regulators, nucleators and crosslinkers of F-actin. Embedded in the cortex are specific components that are associated with or integrated in the plasma membrane. Different cortical domains are generated by domain-specific sets of cortical components. Lateral diffusion of cortical domain components across the domain boundary is inhibited as shown by the dashed line. (B) Examples of cortical domains. Epithelilal cells show a typical distribution of Par proteins with Par-1 localizing to the lateral domain (light blue) and Par-3 to the zonula adherens with adherens junctions (red) (Harris, 2012). In the *C. elegans* zygote, anterior-posterior polarity is defined by two cortical domains, with Par-3 localizing to the anterior cortical domain (red) and Par-2 defining the posterior half of the zygote (light blue) (Nance and Zallen, 2011). In eight-cell stage mouse embryos, apical-basal polarity becomes defined by the localization of Par-3, Par-6 proteins and aPKC to apical domains (red), whereas Par-1 localizes to the baso-lateral cortex (light blue) (Korotkevich et al., 2017; Vinot et al., 2005). Neurons show polarized cortical domains with aPKC, Par-3 and Par-6 proteins localizing to the apical tip (red) (Insolera et al., 2011). Migratory cells exhibit localization of aPKC, Par-3 and Par-6 to the leading edge (Suzuki and Ohno, 2006).

contractions and elongation of the anterior-posterior axis take place (Royou et al., 2002). Staining for endoplasmic reticulum (ER) markers has shown that the cortex is associated with endoplasmic ER (Frescas et al., 2006) that appears to be organized in a continuous and interconnected membrane system. Fluorescence loss in photobleaching (FLIP) of a cortical ER marker indicates that it is mobile and its diffusion is not delimited by diffusion barriers (Frescas et al., 2006). ER morphology generally depends on microtubules (Terasaki et al., 1986; Waterman-Storer and Salmon, 1998), and, consistent with this, microtubules were detected at or close to the cortex (Table 1) (Frescas et al., 2006; Karr and Alberts, 1986). Unpolymerized tubulin and short microtubules that surround small particles, likely yolk granules, can be detected (Karr and Alberts, 1986; Frescas et al., 2006) despite the absence of an obvious microtubule-organizing center (Karr and Alberts, 1986). These microtubules are important for ER localization, as nocodazole treatment leads to a loss of the cortical association of the ER (Frescas et al., 2006).

Although the pre-blastoderm cortex has this simple and unstructured organization, it exhibits plasticity and can actively respond to signals like wounding (Abreu-Blanco et al., 2011, 2014).

Caps and intercap regions in syncytial blastoderm embryos

During nuclear cycles 7–9, the nuclei together with their associated centrosomes and cytoplasm migrate from the interior of the yolk towards the cortex (Foe et al., 1993). As soon as the nuclei appear at the cortex, cytoplasmic buds are formed above the nuclei and its associated pair of centrosomes (Fig. 2). Although they are most prominent at the anterior pole, the cytoplasmic buds uniformly cover the entire embryonic surface (Foe and Alberts, 1983). This represents the first morphological and molecular differentiation of the embryonic cortex into distinct domains, designated here as caps and intercaps. Within the buds or caps, the plasma membrane forms extended microvilli-like membrane folds (Turner and Mahowald, 1976). Consistent with this, caps are strongly enriched for F-actin (Karr and Alberts, 1986; Kellogg et al., 1988; Warn et al., 1984, 1987), actin-binding proteins such as Arp2/3, suppressor of cAMP receptor (SCAR) (Stevenson et al., 2002; Zallen et al., 2002) and Moesin (Rikhy et al., 2015), as well as proteins functionally related to the actin cytoskeleton, such as spectrins (Thomas and Williams, 1999) and the unconventional guanine nucleotide exchange factor (GEF) complex of ELMO (also named Ced-12 in flies) and Sponge (Schmidt et al., 2018) (see Table 1). Despite the high F-actin content of caps, Myosin II is not specifically enriched in caps but in intercaps instead (Royou et al., 2002; Warn et al., 1980).

The plasma membrane in the region between the caps (intercaps) appears relatively smooth with only occasional bulbous projections (Turner and Mahowald, 1976) and forms a fold, which becomes more prominent during cycles 12 and 13. In addition to membrane morphology and F-actin content, the separation into two cortical domains is indicated by segregation of marker proteins. GAP43, which attaches to the membrane through a palmitoylated residue (Zacharias et al., 2002), is uniformly distributed over caps and intercaps, whereas Toll (Tl) and Slow as molasses (Slam) segregate to the intercap region (Table 1) (Mavrakis et al., 2009; Schmidt et al., 2018).

The centrosomes are responsible for the segregation of the cortex into caps and intercaps, as there is a strict correlation between emergence of centrosomes with nuclei at the cortex and bud formation (Foe and Alberts, 1983; Karr and Alberts, 1986; Warn et al., 1987). In addition, embryos with ‘lonesome’ centrosomes (i.e. not associated with a nucleus) are sufficient to induce caps (Peel et al., 2007; Raff and Glover, 1989; Yasuda et al., 1991).

The link between centrosomes and the cortex is unclear. The increase in F-actin within the caps depends on Arp2/3, which is activated by SCAR (Zallen et al., 2002). SCAR and Arp2/3 activity and, subsequently, actin polymerization in the caps might be controlled through activation of Rac1 by the unconventional GEF complex ELMO–Sponge (Fig. 3A). ELMO and Sponge are required for cap formation, as the plasma membrane remains flat without any cytoplasmic buds and a uniformly distributed cortical F-actin in *ELMO* and *sponge* mutant embryos (Postner et al., 1992; Schmidt et al., 2018; Winkler et al., 2015). The function of centrosomes at the cortex may involve microtubule-based transport or anchoring, as Kinesin-1 and the Dynein complex are enriched at the caps (Cytrynbaum et al., 2005; Winkler et al., 2015) (Fig. 3A). Alternatively, a microtubule-independent mechanism is supported by the observation that the actin caps form even in embryos where the microtubules are depolymerized through treatment with colchicine (Stevenson et al., 2001).

The separation into two cortical domains may be linked to the compartmentalization of the plasma membrane, that is, the generation of boundaries that limit the movement and spreading of membrane and cortical components between adjacent caps. This

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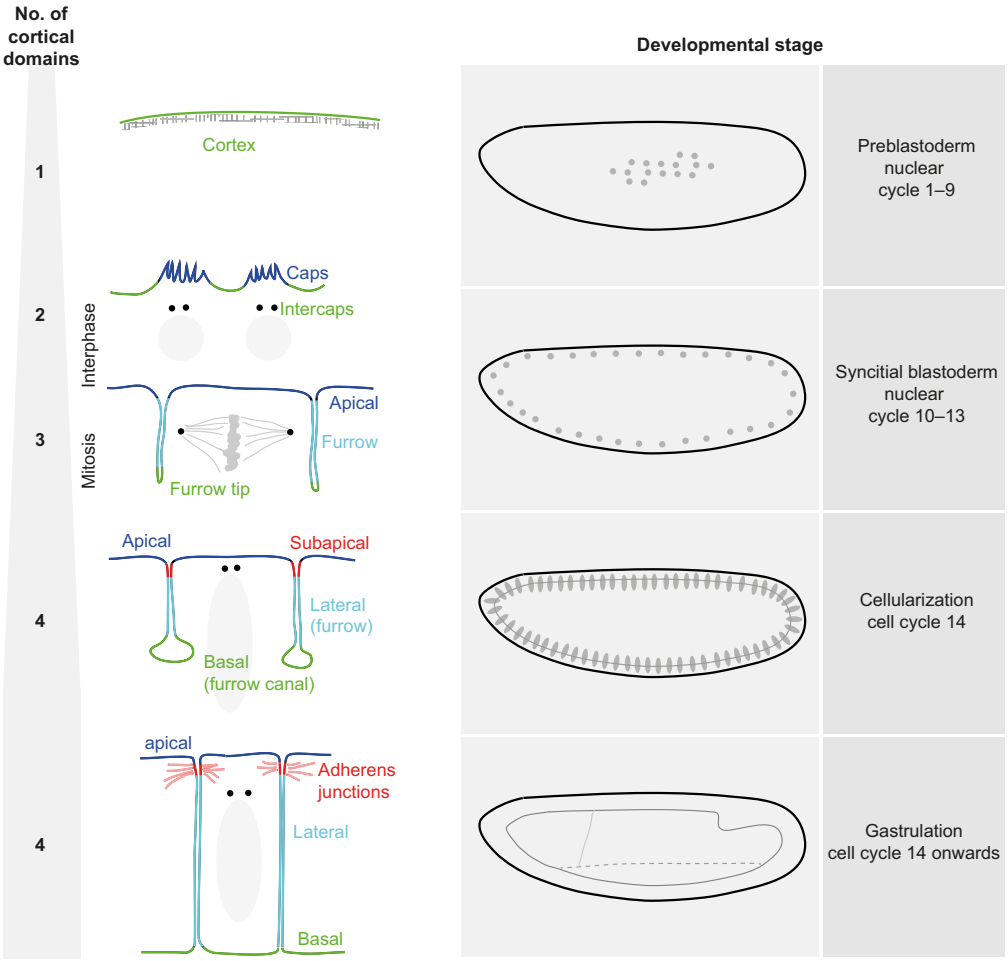

Fig. 2. Dynamics of cortical domains in *Drosophila* embryos. Schematic representation of cortical domains in early *Drosophila* embryos in relation to the developmental stages and nuclear cycles. During embryonic development, the number of cortical domains increase from one during pre-blastoderm stage to two (caps and intercaps) in the syncytial blastoderm stage during the interphase and three (apical, lateral and basal) during mitosis. During early cellularization, the new subapical domain emerges between apical and lateral domain. The subapical region matures and contains adherens junctions, which are introduced during gastrulation.

has been shown by photobleaching experiments in syncytial blastoderm embryos, in which a cap and its connected intercap region do not exchange cortical and membrane components with the neighboring domains (Mavrakis et al., 2009). The restricted mobility of cortical components depends on the F-actin network, as treatment with latranculin A, which prevents F-actin assembly, alleviated the mobility of cortical markers (Mavrakis et al., 2009).

The differentiation into cortical domains may also be linked to a segregated distribution of phospholipids, as has been observed in generic epithelial cells (reviewed in Gassama-Diagne and Payrastre, 2009; Shewan et al., 2011). However, no such polarized distribution has so far been reported for the syncytial embryo.

Cortical domains in the metaphase furrow

During mitosis 10 to 13, the individual spindles and their associated chromosomes are separated by transient invaginations of the plasma membrane, termed metaphase or pseudo-cleavage furrows (Foe et al., 1993; Karr and Alberts, 1986) (Fig. 2). These transient and dynamic furrows reach a maximum extension of ∼10 µm during metaphase 13, and form and retract within a short time frame of ∼5 min (Cao et al., 2008; Karr and Alberts, 1986; Sherlekar and Rikhy, 2016). The metaphase furrows are important for proper chromosome segregation, as mutant embryos that lack the metaphase furrows [e.g. *diaphanous* (*dia*) mutants] show a mis-segregation of chromosomes with low frequency (Afshar et al., 2000; reviewed in Sullivan and Theurkauf, 1995).

During mitosis, three cortical domains are present, an apical, lateral and basal domain, as visualized by segregation of respective marker proteins. F-actin and the cortical proteins Amphiphysin, Anilin, Dia, Syndapin, Myosin II and Patj are strongly enriched at the tip of metaphase furrows (basal domain) (Afshar et al., 2000; Field and Alberts, 1995; Mavrakis et al., 2009; Sherlekar and Rikhy, 2016). Toll and Dlg1 are found at the lateral furrow and are excluded from the apical side and the basal tip (Cao et al., 2008; Lee et al., 2003; Mavrakis et al., 2009), whereas Canoe, Peanut and Scrambled all localize to lateral and apical domains and are excluded from the basal tip (Harris and Peifer, 2004; Mavrakis et al., 2009; Sawyer et al., 2009; Stevenson et al., 2001) (see also Table 1). In contrast, the markers GAP43 and the pleckstrin homology domain of phospholipase C-δ1 ($PLC\delta_1$), which binds with high affinity to phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$], are evenly distributed throughout the plasma membrane (Gay and Keith, 1992; Mavrakis et al., 2009; Rikhy et al., 2015). However, the metaphase furrows are highly dynamic, and the reports for protein localization might be incomplete as most of the reports are based on fixed specimens.

Membrane trafficking might be important for the differentiation of cortical domains in the metaphase furrow (Fig. 3B). The localization and activity of Dynamin during the syncytial embryo divisions plays an important role in maintaining early embryonic compartmentalization as its inhibition leads to an impaired metaphase furrow and perturbed compartmentalization during interphase (Rikhy et al., 2015). Further evidence for a role of membrane trafficking comes from the observation that the F-BAR protein Syndapin is involved in the maintenance and organization of

Journal of Cell Science (2018) 131, jcs212795. doi:10.1242/jcs.212795

Table 1. Marker proteins of cortical domains in early *Drosophila* embryos

Position	Protein	Comment	Vertebrate homolog	References
Pre-blastoderm cortex				
Cortex	F-actin	Cortical filaments		Karr and Alberts (1986)
	Tubulin	Short polymerized forms at cortex		Karr and Alberts (1986)
	Myosin II	Uniform distribution; also called *zipper* in *Drosophila*	Non-muscle myosin II	Abreu-Blanco et al. (2011)
Syncytial blastoderm – interphase				
Cap	ELMO	Forms a complex with Sponge	Ced-12	Schmidt et al. (2018)
	F-actin			Karr and Alberts (1986)
	Sponge	Forms a complex with ELMO	DOCK3	Schmidt et al. (2018)
	Moesin	Binds to F-actin		Rikhy et al. (2015)
	α-Spectrin			Pesacreta et al. (1989)
	Arp2/3	Enriched at the rims of caps		Stevenson et al. (2002)
Intercap	Slam	Membrane associated		Schmidt et al. (2018)
	Toll	Transmembrane protein	Toll-like proteins	Mavrakis et al. (2009)
	Peanut	Member of the septin family	SEPT7	Fares et al. (1995); Field and Alberts (1995)
	Anillin	Scraps (Scra) in *Drosophila*, septin		Fares et al. (1995); Field and Alberts (1995)
	Myosin II		Non-muscle myosin II	Royou et al. (2004); Warn et al. (1980)
	β-Spectrin		SPTBN1	Thomas and Williams (1999)
	β_{Heavy}-Spectrin	Isoform of β-Spectrin		Thomas and Williams (1999)
	E-Cadherin	Enriched in intercap		Mavrakis et al. (2009)
Syncytial blastoderm – mitosis				
Lateral	Discs-large 1		DLG1	Harris and Peifer (2004)
	Toll	Transmembrane protein		Mavrakis et al. (2009)
Apicolateral	E-Cadherin			Mavrakis et al. (2009)
	Canoe		Afadin	Sawyer et al. (2009)
	Scrambled			Stevenson et al. (2001)
	Bazooka		Par-3	Harris and Peifer (2004)
Basal	Amphiphysin	F-BAR protein		Sokac and Wieschaus (2008a)
	Anillin	Scraps (Scra) in *Drosophila*, septin		Field and Alberts (1995)
	Patj			Mavrakis et al. (2009)
Cellularization				
Apical	Par-6		PARD6G	Hutterer et al. (2004); Harris and Peifer (2005)
	Crumbs	Forms a complex with Std and Patj, transmembrane protein	Crumbs 1–3	Tepass et al. (1990)
	Cdc42			Hutterer et al. (2004)
	Stardust (Std)	Forms a complex with Crumbs and Patj	Pals1	Horne-Badovinac and Bilder (2008)
	aPKC			Harris and Peifer (2005)
	Moesin			McCartney and Fehon (1996)
Subapical	Canoe		Afadin	Sawyer et al. (2009)
	Bazooka	PDZ-domain protein	Par3	Harris and Peifer (2004)
	Amadillo		β-Catenin	Harris and Peifer (2004)
	Sponge		DOCK3	Schmidt et al. (2018)
	E-Cadherin			Harris and Peifer (2004)
Lateral	Discs-large 1	Acts with Scrib and Lgl	DLG1	Woods and Bryant (1991)
	Scribbled	Acts with Lgl and Dlg1		Bilder and Perrimon (2000)
	Lgl	Acts with Scrib and Dlg1		Bilder and Perrimon (2000)
	E-Cadherin			Harris and Peifer (2004)
	β-Spectrin		SPTBN1	Thomas and Williams (1999)
	Neurotactin	Transmembrane protein		Lecuit and Wieschaus (2000)
	Toll	Transmembrane protein		Lecuit et al. (2002)
Basal	F-actin			Karr and Alberts (1986)
	Slam	Membrane-associated		Stein et al. (2002); Wenzl et al. (2010)
	Patj		ZO-1 (TJP1)	Bhat et al. (1999); Pielage et al. (2003)
	RhoGEF2	Rho1 GEF		Großhans et al. (2005)
	Dia	Member of the formin family	DIAPH1	Afshar et al. (2000); Großhans et al. (2005)
	Nullo			Hunter et al. (2002)
	Bottleneck	Binds to $PI(3,4,5)P_3$ and $PI(4,5)P_2$		Reversi et al. (2014); Schejter and Wieschaus (1993)
	Amphiphysin	F-BAR protein		Sokac and Wieschaus (2008a)
	Serendipity-α			Postner and Wieschaus (1994); Schweisguth et al. (1990)
	$PI(3,4,5)P_3$	Phospholipid detected with the GFP::Akt-PH sensor		Reversi et al. (2014)
	Myosin II		Non-muscle myosin II	Royou et al. (2004); Warn et al. (1980)
	Peanut	Septin	SEPT7	Adam et al. (2000)
	Dunk			He et al. (2016)
	Rho1		RhoA	Padash Barmchi et al. (2005)
	Cip4	F-BAR protein	Toca-1	Yan et al. (2013)
	Anillin	Scraps in *Drosophila*		Field and Alberts (1995)
	β_{Heavy}-Spectrin	Isoform of β-Spectrin		Thomas and Williams (1999)

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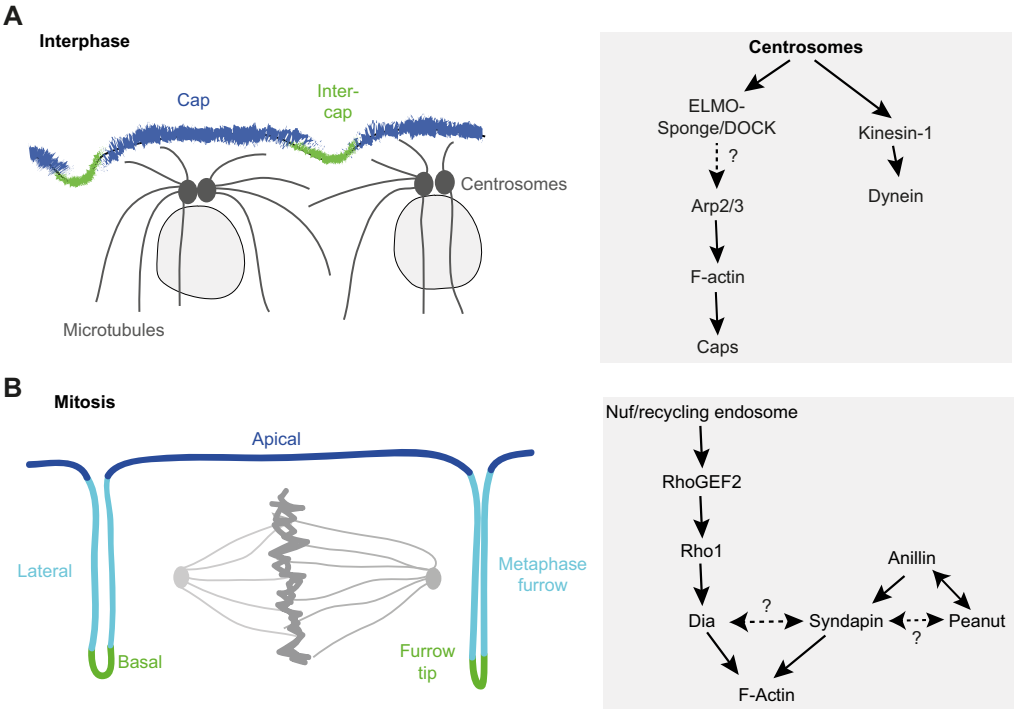

Fig. 3. Cortical domains during syncytial blastoderm. (A) Two cortical domains are present during interphase, named the cap (blue) and intercap (green). The genetic pathways linking centrosomes to domain formation are depicted on the right. (B) Three cortical domains are observed during mitosis, the apical domain (dark blue), lateral domain (metaphase furrow, light blue) and basal domain (furrow tip, green). The genetic pathways responsible for formation of the metaphase furrow are depicted on the right.

the metaphase furrow, as *syndapin* mutants have short metaphase furrows with mislocalized Peanut, Dia and Amphiphysin, leading to a misorganized F-actin network (Sherlekar and Rikhy, 2017).

The lack of metaphase furrows in embryos mutant for *dia* could be explained by the function of this formin in nucleating and elongating F-actin (Yan et al., 2013). Indeed, several studies show that proper F-actin polymerization is required for the elongation of the metaphase furrow (Cao et al., 2008; Webb et al., 2009), which is mediated by Dia and its activator RhoGEF2 (Grosshans et al., 2005; Padash Barmchi et al., 2005). Interestingly, the correct localization of RhoGEF2 to the furrow has been found to be mediated by recycling endosome (RE)-derived vesicles, whose transport is dependent on the RE-associated proteins Nuclear fallout (Nuf) and Rab11 (Cao et al., 2008), further pointing to the importance of membrane trafficking in establishing the metaphase furrow (Fig. 3B).

Cortical organization during cellularization

Cellularization during interphase 14 is a special stage in fly embryonic development. It mediates the transition from syncytial to cellular development and from a maternal to zygotic control of gene expression (reviewed in Blythe and Wieschaus, 2015; Liu and Grosshans, 2017). This stage is generally referred to as the mid-blastula transition (reviewed in Farrell and O'Farrell, 2014; Yuan et al., 2016).

In contrast to the preceding nuclear cycles, the plasma membrane forms a stable furrow between adjacent nuclei at the onset of interphase 14 (termed the cellularization furrow). Over the following hour, the furrow slowly ingresses to its final length of ~35 µm, which encloses each of the cortical nuclei into the resulting individual cells, thereby giving rise to a polarized and single-layered columnar epithelium surrounding the yolk (Foe et al., 1993) (Fig. 2 and Box 1).

During initial cellularization, two types of furrows are observed, newly emerging furrows between corresponding daughter nuclei of mitosis 13 and 'old' furrows. The old furrows are derived from metaphase furrows that retract to ~3 µm in length (He et al., 2016) before they transform into a cellularization furrow and then ingress in synchrony with the 'new' furrows. For correct positioning of the new furrows, a flow of Myosin II towards the new furrow is required

Box 1. Cellularization at a glance

A characteristic feature of the mid-blastula transition is the switch from syncytial to cellular development. Associated with remodeling of the cell cycle from fast nuclear divisions to an embryonic cycle (Liu and Grosshans, 2017), the cytoskeletal, membrane and cortical organization fundamentally changes and cellularization starts (Foe et al., 1993; Schejter et al., 1992). The most obvious morphological feature is the ingression of furrows. Ingression starts slowly (~0.1–0.3 µm/min) and gradually accelerates to a final speed of 0.8–1.2 µm/min (Lecuit et al., 2002, Acharya et al., 2014).

F-actin plays a central role in membrane ingression and formation of the furrow and the cortical domains during cellularization. In addition to the uniform cortical F-actin, enrichment of F-actin is initially observed at apical actin caps and then detected at the basal tip, the furrow canal, thereby forming a regular hexagonal network enclosing the nuclei (Warn and Magrath, 1983). F-actin dynamics and organization is controlled by several proteins whose function is related to F-actin, including RhoGEF2, Dia, Nullo, Sry-α, Abl tyrosine kinase, Discontinuous actin hexagons and Death-associated protein kinase related (Afshar et al., 2000; Chougule et al., 2016; Grevengoed et al., 2003; Grosshans et al., 2005; Rothwell et al., 1999; Schweisguth et al., 1990; Sokac and Wieschaus, 2008a,b).

During the second half of cellularization, when the furrow has passed the basal side of the nuclei, the furrow canals widen in a process termed 'basal closure'. This leads to a gradual separation of the blastoderm cells from the yolk cell. Final disconnection involves membrane fusion and occurs only towards the end of cellularization (Foe et al., 1993). The driving force for basal closure is provided by Myosin II (Royou et al., 2004) and involves an inhibitory mechanism by Bottleneck and basally enriched $PI(4,5,6)P_3$, which counteract $PI(4,5)P_2$-mediated actomyosin contractility (Schejter and Wieschaus, 1993; Reversi et al., 2014). Furthermore, the Arf-GEF Steppke restrains the basal actomyosin network during early cellularization by promoting local endocytosis that leads to local reduction in the levels of Rho1, preventing premature contraction (Lee and Harris, 2013).

Journal of Cell Science (2018) 131, jcs212795. doi:10.1242/jcs.212795

during the first minutes of cellularization, which is mediated by the zygotic gene *dunk* in an unknown manner (He et al., 2016). Following Dunk-dependent flow, Myosin II is recruited by Slam, which then drives further ingression of the cellularization furrow independently of Dunk (He et al., 2016). As no pre-patterning is present at these sites, *de novo* polarization of the cortex and the emergence of cortical domains occurs at the new furrows, and a segregation of cortical markers can be observed at the onset of cellularization. Importantly, the difference between old and new furrows vanishes as soon as ingression starts (Acharya et al., 2014).

The cellularization furrow is distinct from the metaphase furrow in several aspects. The metaphase furrow is linked to the mitosis, whereas the cellularization furrow forms in interphase (Foe et al., 1993). Beside a clear difference in the kinetics of elongation and retraction of the furrows (see Box 1), a striking difference is the emergence of a subapical domain, which is introduced as a region between apical and lateral domains.

Similar to the cortical differentiation in syncytial blastoderm embryos into caps and intercaps, the centrosomes also trigger the cortical polarization during cellularization (Acharya et al., 2014). Indeed, lonesome centrosomes are sufficient to induce and organize cellularization furrows, as observed by the segregation of the lateral and basal cortical markers, Dlg1 and Slam. Accordingly, centrosome ablation inhibits marker segregation, and the basal marker Slam remains distributed along the entire membrane (Acharya et al., 2014), suggesting that centrosomes provide the initial information for cortical differentiation and the restriction of cortical markers to their respective domain (Fig. 4).

In the following sections, we will discuss the signaling pathways that contribute to the establishment of the cortical domains that emerge during cellularization in more detail. However, we will not discuss the apical domain as the function of this domain during cellularization has not been studied in detail and no specific marker proteins have been reported yet.

Establishment of the basal domain

The basal domain forms a specific morphological structure. The furrow canal leads the ingressing furrow and will broaden during the second half of cellularization before it finally encloses the adjacent cells (Foe et al., 1993) (Fig. 4). In this way, the basal domain of the cellularization furrow is different from the basal tip of the metaphase furrow and also distinct from the generic basal domain of epithelial cells that arises later.

The basal domain is specified by two redundant signaling pathways (Fig. 4). The first pathway is triggered by a complex between Slam and its mRNA, which localizes to the basal domain throughout cellularization (Table 1) (Acharya et al., 2014; Wenzl et al., 2010; Yan et al., 2017). The restriction of Slam to the prospective basal domain depends on recycling endosomes and the arfophilin Nuf, which is necessary for cycling of Rab11 (Riggs et al., 2003). The requirement for Nuf and/or REs for the exclusion from Slam from the apical and lateral domains is demonstrated by what is seen in *nuf* mutants, which have impaired and disorganized REs, and in which Slam is uniformly distributed over the plasma membrane (Acharya et al., 2014). In hypomorphic *nuf* situations, when a furrow forms, Slam is detected at the lateral and basal domain instead of being restricted to the basal domain, indicating that domain segregation is impaired (Acharya et al., 2014). Slam activates Rho signaling by recruiting RhoGEF2 to the prospective basal domain through a physical interaction that involves the PDZ domain of RhoGEF2 and an unconventional PDZ-binding motif within the C-terminal part of Slam (Wenzl et al., 2010) (Fig. 4). Downstream of Rho1, actin polymerization is mediated by Dia and actomyosin contractility induced by Myosin II (Afshar et al., 2000; Grosshans et al., 2005; Padash Barmchi et al., 2005; Wenzl et al., 2010), resulting in furrow invagination. Furthermore, other polarity markers such as Patj become basally restricted in a manner that depends on their direct or indirect interaction with Slam (Table 1; Wenzl et al., 2010).

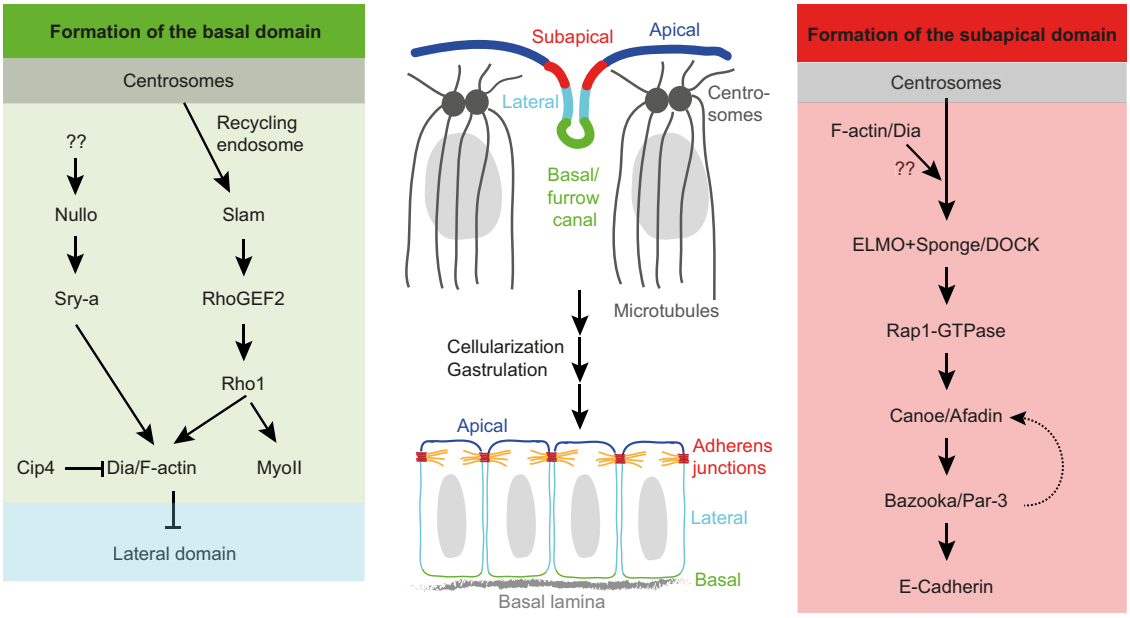

Fig. 4. Cortical domains during cellular blastoderm/cellularization. Schematic illustration of a furrow during early cellularization with apical (dark blue), subapical (red), lateral (light blue) and basal (green, furrow canal) domains depicted. Centrosomes are apical to the nuclei and constitute the anchor for the microtubule basket as depicted. Cellularization results in an epithelium with apical, lateral and basal domains, which is later connected to the basal lamina (gray) and contains adherens junctions (red). The genetic pathways for formation of the basal domain and separation from the lateral domain, and those involved in the formation of the subapical domain are shown on the left and right, respectively.

In parallel, a second signaling pathway is established by Nullo, which accumulates in the basal domain, depending on N-terminal myristoylation and an N-terminal cluster of positively charged amino acids (Table 1) (Hunter and Wieschaus, 2000; Postner and Wieschaus, 1994) (Fig. 4). Depending on Nullo, Serendipity-α (Sry-α) becomes restricted to the prospective basal domain (Schweisguth et al., 1990; Postner and Wieschaus, 1994). Nullo and Sry-α control F-actin, possibly also through the formin Dia, as *RhoGEF2 nullo* and *slam nullo* double mutants exhibit a stronger phenotype than single mutants, with a uniform distribution of Dia and loss of the basal domain (Grosshans et al., 2005, Acharya et al., 2014). Importantly, both *nullo* and *slam* are zygotic genes that are expressed early in cellularization (Lecuit et al., 2002; Postner and Wieschaus, 1994), which distinguishes this stage from the preceding nuclear cycles. Therefore, their expression may confer the timing information that controls the new cortical organization in cellularization.

Both, the Slam and Nullo pathways contribute to specification of the basal domain, as loss of a single pathway, for instance in the single mutants of *nullo* or *RhoGEF2*, leads to a dispersed pattern of F-actin with regions that have a proper cortical organization and regions without any specified cortical domains (Acharya et al., 2014; Wenzl et al., 2010). This, in turn, leads to a disrupted furrow array and, ultimately, to the formation of multinuclear cells (Hunter and Wieschaus, 2000; Wenzl et al., 2010). Therefore, the specification of the basal domain appears to be essential for furrow formation and ingression of the plasma membrane.

The basal domain also has a peculiar morphological structure. During the onset of cellularization, dynamic transient tubular extensions in the micrometer range arise from the basal membrane spanning into the cytoplasm as observed by staining with the N-BAR protein Amphiphysin or other markers of the basal domain such as Slam (Sokac and Wieschaus, 2008a; Yan et al., 2013). With progression of cellularization, these tubular extensions disappear. In embryos, in which F-actin is reduced, such as owing to cytochalasin treatment or in *dia* or *nullo* mutants, the tubular extensions persist throughout cellularization (Bogdan et al., 2013; Sokac and Wieschaus, 2008a; Yan et al., 2013). Three not mutually exclusive models have been proposed with regard to the dynamics of the tubular extensions. First, that tubules act as membrane reservoirs that regulate furrow ingression rates, given that absence of tubules in *amphiphysin* mutants leads to increased ingression rates (Su et al., 2013). Second, according to the so-called endocytosis model, that tubular extensions give rise to endocytic vesicles and so promote the turnover of the basal domain, thereby restraining the endocytosis of proteins, such as Peanut, Patj and Myosin II (Sokac and Wieschaus, 2008a). Third, the cortex model suggests that the tubular extensions reflect a weak cortex that allows the infolding of the plasma membrane (Yan et al., 2013). With the formation of the basal domain and progression of cellularization, cortical F-actin builds up and suppresses tubulation in the region of the basal domain (Simpson and Wieschaus, 1990; Sokac and Wieschaus, 2008b). In support of the cortex model, the ultrastructure of the furrow canal in *dia* mutants shows extensive blebbing and folding, in contrast to the wild-type membrane, which is straight and flat (Grosshans et al., 2005). These reports indicate that F-actin produced and organized by *dia* is needed to suppress tubulation. Further studies are needed to distinguish between the models, which are not mutually exclusive, in order to delineate the function of the tubular extensions and to reveal mechanistic insights.

The ability to segregate the basal markers from the lateral factors appears to be linked to the micrometer-sized tubular extensions within the basal domain. In all situations, in which these tubular extensions persist throughout cellularization, lateral markers were found to invade the basal domain, while the basal domain remained intact, as judged by the restriction of basal markers (Sokac and Wieschaus, 2008a; Yan et al., 2013). Physical barriers within the membrane such as cell–cell junctions are unlikely to contribute to the exclusion of lateral markers, as embryos with impaired E-Cadherin-based junctions are still able to exclude lateral markers (Sokac and Wieschaus, 2008b).

Alternatively, the interaction of the actin cortex with the plasma membrane might mediate the segregation of the basal and lateral domains. Indeed, the F-BAR protein Cip4, which links the plasma membrane to the actin cytoskeleton (Fricke et al., 2009), antagonizes basal-lateral domain segregation, as overexpression of Cip4 resulted in the spreading of lateral markers into the basal domain, similar to what is seen for *dia* mutants (Yan et al., 2013) (Fig. 4). Mechanistically, Cip4 has been shown to directly bind to Dia and to inhibit Dia-mediated F-actin nucleation and elongation *in vitro* (Yan et al., 2013; reviewed in Bogdan et al., 2013). However, it remains unclear how the inhibition of Dia by Cip4 would lead to an exclusion of lateral markers. Dia may promote a stable actin cortex with corresponding lower turnover of the plasma membrane, whereas Cip4 may counteract this. Owing to the large amounts of Dia and F-actin at the basal domain, this model would predict a softer cortex and higher rates of membrane turnover in the lateral domain than in the basal domain, as well as a uniformly soft cortex with high membrane turnover in *dia* or *nullo* mutants. However, further research is needed to test whether this hypothesis is indeed true.

Taken together, these findings suggest that two pathways triggered by the zygotically expressed proteins Slam and Nullo specify the basal domain and also help to establish a stable actomyosin network that is needed to stabilize the basal furrow and to execute the contractions that eventually close the adjacent cells.

The lateral domain

The lateral domain, which is located above the basal domain already contains lateral marker proteins (e.g. Scribbled), in metaphase furrows during syncytial nuclear cycles as discussed above. With the formation of the cellularization furrow at the position between the daughter nuclei of the last nuclear division, a new lateral domain will arise (Schmidt et al., 2018). Similar to their localization in the metaphase furrow, Dlg1, Scribbled and Lgl mark the region apical to the basal domain (Bilder et al., 2000). However, they do not function in furrow ingression or the formation of the subapical domain during early cellularization, as the localization of the subapical marker Canoe is not affected in *scrib* mutants (Schmidt et al., 2018). In fact, the function for the Dlg1–Scrib–Lgl complex in cortical organization emerges later (Bilder and Perrimon, 2000; Bilder et al., 2000). Par-1 is uniformly distributed at the cortex during early cellularization, but by mid-to-late cellularization, Par-1 decreases apically and basally and thus becomes restricted to the lateral domain (McKinley and Harris, 2012). The role of Par-1 for cortical domains and cellularization is complex. *Par-1*-depleted embryos lack some of the cellularization furrows, indicating an early function in furrow ingression. Par-1 also functions in clearing Baz) from the lateral domain as Baz spreads into the lateral furrow in *Par*-1-depleted embryos (McKinley and Harris, 2012). As Par-1 is excluded from the subapical domain only late in cellularization, it is likely that clearing of Baz from the lateral domain by Par-1 is a gradual process that functions in addition to Canoe-dependent subapical recruitment of Baz.

Taken together, cortical domains appear to be formed largely independently of each other in early cellularization. During the course of cellularization, however, they mutually interact, which then leads to lateral exclusion of Baz from the lateral domain, for example.

Emergence of the subapical domain

The lateral, basal and apical domains all have predecessors in the cap and intercap regions, or the metaphase furrow during the syncytial blastoderm. However, the subapical domain is special in that it emerges between the apical and lateral domains as a new feature during cellularization.

The subapical domain is specified by a signaling pathway that involves the small GTPase Rap1 and the actin-binding protein Canoe (Afadin in mammals) (Fig. 4). It controls the subapical localization of the conserved markers Baz (Par-3 in mammals), Par-6 and atypical protein kinase C (aPKC), as well as accumulation of the complex between E-Cadherin, Arm and α-Catenin during the course of cellularization through an unknown mechanism (Choi et al., 2013; reviewed in Harris, 2012) (Fig. 4; see also Table 1). Whereas Canoe is restricted to the subapical domain from the onset of cellularization (Schmidt et al., 2018), Baz and E-Cadherin only gradually accumulate there (Harris and Peifer, 2004). A prominent subapical localization of Baz and the E-Cadherin complex is only apparent by the end of cellularization. The subapical accumulation of Baz depends on cytoskeletal cues, such as binding to an actin scaffold and Dynein-mediated transport in basal to apical direction (Harris and Peifer, 2005).

Although it is known that Canoe is needed for proper subapical localization of Baz (Choi et al., 2013), it is still unclear how Baz is recruited by Canoe (Fig. 4). Canoe might recruit Baz to the subapical domain by direct binding, as supported by a protein recruitment assay performed in S2 cells (Choi et al., 2013). Alternatively, Canoe might act indirectly and/or transiently with Baz, as they do not show obligatory colocalization during cellularization (Choi et al., 2013). For instance, Canoe could control the microtubule-dependent apical transport of Baz through an unknown mechanism (McKinley and Harris, 2012). Another model has been proposed based on the recruitment of Baz by membrane lipids, as Baz contains a PH domain in its C-terminus (Krahn et al., 2010a,b). However, as no specific subapical enrichment of phosphatidylinositols (PIPs) has been detected (Reversi et al., 2014), it is unlikely that these phospholipids are involved in the subapical restriction of Baz during cellularization.

The source of the information that initially positions the subapical domain between the apical and lateral domains is unknown. The GTPase Rap1 is known to act upstream of subapical protein Canoe as restricted Canoe localization during cellularization is lost in *Rap1* mutant embryos (Sawyer et al., 2009). Rap1 requires an initial signal for positioning Canoe to the newly emerging subapical domain, as it is uniformly distributed over the entire membrane (Sawyer et al., 2009). Rap1 activation is most likely spatially restricted, which subsequently leads to the subapical restriction of Canoe (Fig. 4). Indeed, the expression of a constitutively active form of Rap1 leads to mislocalization of Canoe, as well as of Baz and Arm, to the lateral domain (Bonello et al., 2018).

The dynamics and function of the upstream regulators of Rap1 may provide clues to the origin of the positional information, and multiple GEFs and GTPase-activating proteins have been described for Rap1. A promising candidate is the GEF Dizzy, which has been shown to be required for Rap1 activity in the assembly of apical adherens junction in the mesoderm anlage (Spahn et al., 2012). During late cellularization, Dizzy is involved in the localization of Canoe to tricellular junctions (Bonello et al., 2018), but not in its subapical restriction (Schmidt et al., 2018). This latter function appears to be fulfilled by the ELMO–Sponge complex, which is an unconventional GEF, as subapical restriction of Canoe is perturbed in *ELMO* and *sponge* mutant embryos (Schmidt et al., 2018). It is assumed that ELMO (Ced-12) provides the PH domain for membrane association and Sponge (the homolog of mammalian Dock180) entails the enzymatic activity and confers specificity for Rap1 and also Rac (Biersmith et al., 2011; Komander et al., 2008; Yajnik et al., 2003). The ELMO–Sponge complex is enriched at the prospective subapical domain during the onset of cellularization (Schmidt et al., 2018), so the complex could provide local activation of Rap1 and, through this, spatial information for the introduction of the subapical domain (Fig. 4).

Although a signaling pathway involving ELMO–Sponge has been defined to act through Rap1 to restrict Canoe, and consequently Baz and E-Cadherin, to the subapical domain, the mechanism that changes the distribution of the ELMO–Sponge complex during onset of cellularization is much less clear. Strikingly, the localization of ELMO–Sponge changes from a disc-like pattern at the caps in the syncytial blastoderm to a ring-like pattern during onset of cellularization. The molecular basis for this is unknown, but is likely to be linked to the structure and dynamics of actin caps and to the mid-blastula transition and possibly newly transcribed zygotic factors (Schmidt et al., 2018).

An important open question is the role of the cytoskeleton. The subapical restriction of Baz and Canoe requires F-actin assembly, as drug-induced F-actin depolymerization results in the dispersion of Canoe and Baz (Choi et al., 2013; Harris and Peifer, 2005). However, a direct function of F-actin in the positioning of subapical cues is unlikely, as F-actin is not visibly enriched at the subapical domain during early cellularization, but instead accumulates only later in development at adherens junctions (Choi et al., 2013).

Transition to epithelial organization during gastrulation

The make-up of the cortex in generic epithelia includes the apical, lateral and basal domains. The region between the apical and lateral domain is further differentiated into the extreme apical region or marginal zone as marked by Crumbs and the region containing the adherens junctions (Harris and Peifer, 2004; reviewed in Tepass, 2012). However, this organization is partially independent of the cortical organization that is set up during cellularization. Mutants that exhibit an impaired subapical domain during cellularization such as *canoe* or *Dynein heavy chain 64C*, recover during gastrulation with a clearly subapically restricted localization of Baz and E-Cadherin (Choi et al., 2013; Harris and Peifer, 2005). It appears that upon transition from cellularization to gastrulation, the conserved components for epithelial and cortical organization, such as the Par proteins, take over control from the cellularization-specific mechanism based on Rap1 and Canoe.

Such a transition is also obvious in the dynamics of the subapical marker proteins. Baz and E-cadherin localize to a more apical position where they form the zonula adherens (reviewed in Harris, 2012), and the localization of Baz becomes independent of Canoe (Choi et al., 2013). During gastrulation, Baz localization is mainly governed by the mutual exclusion of factors between the different cortical domains, in that, Baz is excluded from the lateral domain by the presence of Par-1 (McKinley and Harris, 2012), and from the apical domain by Par-6 and Crumbs (Bilder and P,errimon, 2000; Hutterer et al., 2004; Krahn et al., 2010a,b; Morais-de-Sá et al., 2010). Similarly, the lateral proteins Dlg1, Scribbled and Lgl are

important for the lateral exclusion of subapical and apical proteins (Bilder and Perrimon, 2000; Bilder et al., 2003; Hutterer et al., 2004; Tanentzapf and Tepass, 2003). We will not cover this aspect here in more detail, as several excellent reviews have recently been published addressing epithelial organization (e.g. Coopman and Djiane, 2016; Harris, 2012; Laprise and Tepass, 2011; Nance, 2014).

Conclusions and future perspectives

Here, we reviewed the specification of cortical domains in early *Drosophila* embryos. Starting from a uniform cortex of the zygote, the number of distinct cortical domains typically increases to two, three and finally four domains during the first 3 h of development. The dynamics of cortical domain organization is strictly linked to the developmental program and thus provides an excellent assay to investigate the underlying genetic and molecular mechanisms. This notion holds especially true for the *de novo* formation of the subapical domain during the onset of cellularization. During this developmental stage, several morphological features of the embryo change as part of the mid-blastula transition. It is likely that the formation of the subapical domain depends on the activation of the zygotic genome in a similar manner to what is required for cell cycle remodeling and membrane invagination.

As cellularization is a special process during which an epithelium is formed, the dynamics of *de novo* formation of cortical domains can be directly visualized. With the genetic tractability of *Drosophila*, key aspects of domain formation, such as how centrosomes can induce formation of different domains within the cortex, or how sorting of cortical determinants is achieved can be investigated.

The simple cortical organization and dynamics of early *Drosophila* embryos may serve as a model for cortical dynamics in generic epithelia. Obtaining further insights into these processes will allow us to dissect the respective roles of vesicle trafficking, membrane turnover, lateral diffusion and physical barriers in domain separation and sorting of cortical components. An understanding of the formation, dynamics and maintenance of cortical domains in *Drosophila* could also help us to understand the partly conserved processes in higher organism as epithelial dynamics also have a role in morphogenesis and associated diseases.

Competing interests

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