Front. Biol. 2012, 7(1): 73–82
DOI 10.1007/s11515-011-1160-4

REVIEW

Drosophila embryo syncytial blastoderm cellular architecture and morphogen gradient dynamics: Is there a correlation?

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Abstract **During embryo development in many metazoan animals, the first differentiated cell type to form is an epithelial cell. This epithelial layer is modified by developmental cues of body axes formation to give rise to various tissues. The cells that arise are mesenchymal in nature and are a source of other tissue types. This epithelial to mesenchymal transition is used for tissue type formation and also seen in diseases such as cancer. Here we discuss recent findings on the cellular architecture formation in the *Drosophila* embryo and how it affects the developmental program of body axes formation. In particular these studies suggest the presence of compartments around each nucleus in a common syncytium. Despite the absence of plasma membrane boundaries, each nucleus not only has its own endoplasmic reticulum and Golgi complex but also its own compartmentalized plasma membrane domain above it. This architecture is potentially essential for morphogen gradient restriction in the syncytial *Drosophila* embryo. We discuss various properties of the dorso-ventral and the antero-posterior morphogen gradients in the *Drosophila* syncytium, which are likely to depend on the syncytial architecture of the embryo.**

Keywords morphogen gradient, *Drosophila*, syncytium, embryo, cellular architecture

Introduction

Animal embryo development involves an elegant interplay of signal transduction coupled to transcription in a regulated manner in time and space. This determines organism body axes and tissue specification in a combinatorial manner. Higher metazoan animals start embryo development with the formation of an epithelial cell layer which morphs into other cell types to give rise to complex tissues. Morphogenetic movements during embryo development in this epithelial cell layer are dictated by developmental cues and lead to its invagination and transformation to give rise to new cell layers during gastrulation. This process of epithelial to mesenchymal transition, which results from cell morphogenesis is used repeatedly during organism development and is also a cause of diseases such as cancer when it occurs in an untimely manner in the adult life of an organism (Acloque et al., 2009).

Caenorhabditis (the nematode worm), *Drosophila* (the fruit fly), *Echinus* (the sea urchin), *Xenopus* (the toad) and *Mus musculus* (the mouse) embryos have provided much of the existing information about molecular mechanisms used for epithelial morphogenesis during development. In this review we will focus on the development of body axes during the formation of polarized plasma membrane in the syncytial blastoderm embryo of the fruit fly, *Drosophila melanogaster*. In insect embryos such as *Drosophila*, body axes are determined during the formation of oocyte during oogenesis (Roth and Lynch, 2009). The nurse cells and the follicle cells in the mother deposit the developmental cues for the formation of dorso-ventral and antero-posterior axes of the embryo during oogenesis. When the embryo is laid after fertilization, the first 13 nuclear divisions occur in the cytoplasm in the absence of cell division (Sonnenblick 1948; Bownes 1975). This syncytial organization invoked the hypothesis that, components in the cytoplasm are shared in the early embryo and absence of boundaries enabled establishment of concentration gradients of morphogens that further determine body axes and tissues of the embryo. However, despite the absence of plasma membrane boundaries, there is localized origin of body axes patterning gradients in the syncytial blastoderm. In this review, we summarize recent literature, which provides information about the cellular architecture of the syncytial blastoderm

Received May 10, 2011; accepted July 13, 2011
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embryo in *Drosophila* and discuss a possible relationship between this organization and the specific origin, spread and maintenance of morphogen gradients.

Organization of the *Drosophila* syncytial blastoderm embryo

Drosophila embryonic development starts as a syncytium. The first 13 nuclear division cycles in the embryo are synchronous, incomplete and the cytoplasm is not segregated by a plasma membrane boundary. Nuclear cycles 1–9 proceed within the interior of the embryo. Cytoskeletal rearrangements occurring during the syncytial cycles 6–9 promote spreading of the nuclei throughout the embryo and migration of the nuclei toward the periphery (Karr and Alberts, 1986). At the end of nuclear cycle 9, nuclei have reached the periphery of the embryo to form the syncytial blastoderm. At this stage the plasma membrane buds around each nucleus but does not encircle the nucleus leaving a possibility for exchange of molecular components between nuclei. By the end of cycle 13 there are 6000 nuclei at the cell periphery. In a prolonged interphase of cycle 14, cellularization takes place by extending the plasma membrane around each nucleus and giving rise to complete epithelial cells (Lecuit 2004). This is followed by gastrulation after which there is a resumption of cell divisions, which are asynchronous.

The nuclear cycles 10 to 13 occur at the embryo cell periphery. This is the time point at which morphogen gradient molecules such as Bicoid (Bcd) at the anterior pole and Dorsal (Dl) on the ventral side enter the nucleus and start acting on upstream regions of DNA binding elements and upregulating specific gene expression in a position specific manner. These gradients are generally believed to spread in the cytoplasm in the absence of plasma membrane boundaries. However, recent studies suggest that the syncytium has a distinct cellular architecture, which implies that there is limited sharing between adjacent nuclei despite the absence of complete plasma membrane boundaries (Frescas et al., 2006; Mavrakis et al., 2009).

Early studies carefully analyzing the lineage of each nucleus through the syncytial division cycle established that daughter nuclei remain in the vicinity of the mother nuclei (Minden and Agard, 1989). More recent studies have shown a similar feature for other cellular components and will be described in the next few sections.

Organelles in the syncytial blastoderm embryo

When the nuclei are dividing internally during the nuclear cycles 1–9, the endoplasmic reticulum (ER) and the Golgi complex are located at the periphery of the embryo. The ER exists as a continuous structure with some clusters and diffusion in the lumen occurs across relatively long distances. When the nuclei arrive at the periphery, the ER clusters unfold and redistribute around each nucleus. Most remarkably, diffusion of the lumenal KDEL-GFP at this point is restricted around one nucleus of the ER. The galactosyl transferase domain tagged with GFP which gets targeted to the Golgi complex also recycles from the ER within one nuclear domain. Together these studies show that each nucleus has its own secretory pathway in the absence of complete plasma membrane boundaries (Frescas et al., 2006) (Fig. 1). The Golgi complex mediated trafficking is also essential for the formation of the plasma membrane during cellularization (Sisson et al., 2000). Recent studies also suggest that Rab5 endosomes are compartmentalized and this is essential for giving rise to the Dl gradient (summarized in later sections) (Lund et al., 2010). These studies together suggest that the *Drosophila* embryo syncytium has individual cell-like compartments during nuclear cycles 10–13 and each 'pseudo-cell' is capable of synthesizing its own new proteins using the secretory pathway. These would presumably include important proteins, which target to the plasma membrane and function as receptors for activation of key signal transduction pathways responsible for giving rise to morphogen gradients.

The plasma membrane of the syncytial blastoderm embryo

The plasma membrane has distinct domains during nuclear cycles 10–13 in the syncytial blastoderm (Mavrakis et al., 2009). This can be readily seen during interphase of the syncytial nuclear cycles. The apical domain is rich in microvilli which were observed in early studies by scanning electron microscopy (Turner and Mahowald, 1977). They have also been observed recently by light microscopy in transgenic embryos containing a fluorescently tagged pleckstrin homology (PH) domain from phospholipase C (PLC) or PHPLCδ, which labels phosphatidylinositol diphosphate (PIP_2) in the plasma membrane and also using a fluorescently tagged palmitoyl group from Gap43, which gets anchored to the inner leaflet of the plasma membrane. This apical domain is distinct from a short lateral domain (approximately 2 μm) that contains junctional proteins and actin remodeling proteins. Thus, the plasma membrane displays epithelial-like characteristics in the absence of complete epithelial cells (Fig. 1) (Mavrakis et al., 2009). Whether this occurs in preparation for epithelial cell formation during cellularization in the interphase of cycle 14 or serves to give some differentiated character to the plasma membrane in the syncytium, can only be ascertained by further investigation. The polarized membrane may also serve an important function of restricting morphogen gradients while allowing the embryo to develop precisely and rapidly. Continuous photobleaching experiments of these fluorescently tagged

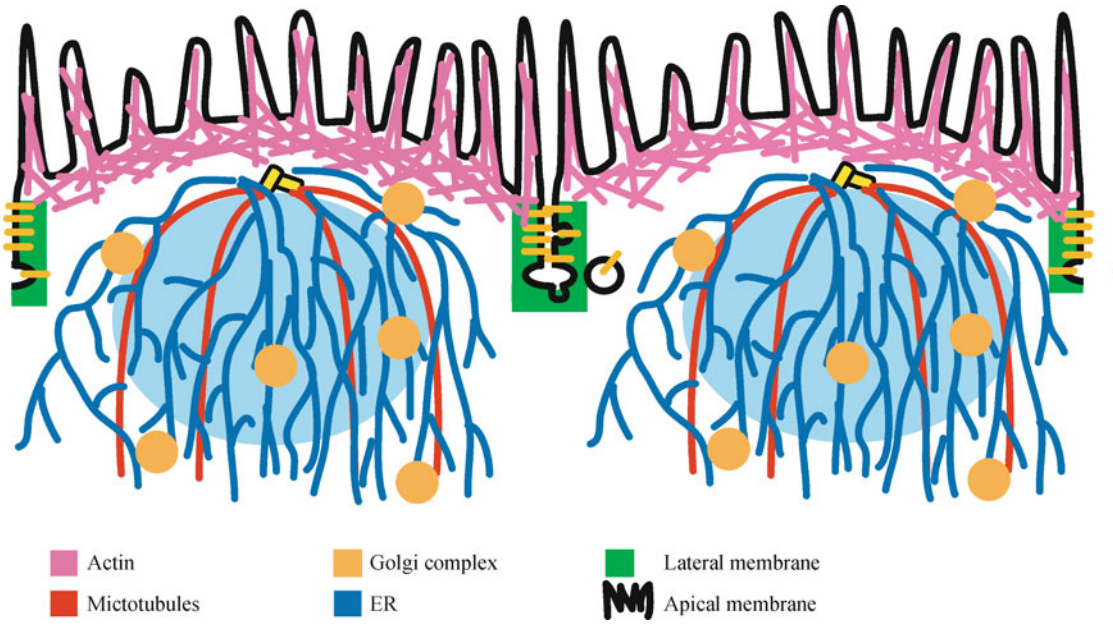

Figure 1 The cellular architecture in the syncytial *Drosophila* embryo during interphase of the nuclear cycles 11–13. The centrosomes are present apically. These form microtubules in the vertical direction. The endoplasmic reticulum and the Golgi complex are partitioned to each nucleus and show compartmentalization of diffusion around each nucleus. The actin cytoskeleton is present as caps beneath apical plasma membrane, which is rich in microvilli. The lateral plasma membrane, which surrounds each nucleus only partially during interphase of the syncytial cycle, contains specific transmembrane proteins such as Tl, junctional proteins such as Cadherin and Patj, cytoskeletal-remodeling proteins such as Anillin and Peanut and endocytosis proteins such as Dynamin and Clathrin (not drawn to scale).

plasma membrane markers targeting to the apical and the lateral domain showed that molecules move freely within the plasma membrane around one nucleus and not between neighboring nuclei. This implies that the compartmentalized secretory pathway can deliver components specifically to one domain and they would not diffuse across to neighboring domains. Most remarkably, this implies that activated receptors such as those in the Toll (Tl) signaling pathway (the transmembrane receptor that signals to activate Dl and thus is responsible for Dl gradient formation) in the membrane, would be maintained around one nucleus and would not diffuse to another nucleus (Mavrakis et al., 2009).

The lateral domain contains adherens junctional proteins such as Cadherin and sub apical complex proteins such as Patj (Mavrakis et al., 2009). It has cytoskeletal remodeling proteins such as Diaphanous, Anillin and Peanut and receptors such as Tl (Mavrakis et al., 2009) (Rikhy et al. unpublished observations). Activation of the Tl receptor pathway gives rise to the Dl protein gradient in the syncytial nuclei of the embryo. It is unclear whether Spätzle (Spz), the ligand for Tl, would be able to access Tl receptor in the compact lateral domains of the syncytial embryo. A recent study shows that endosomes may contain activated Tl bound to Spz, and these are also compartmentalized to one nucleus (Lund et al., 2010). Further, the transcription factor Dl is also compartmentalized (DeLotto et al., 2007). Disruption of actin results in greater diffusion in the plasma membrane, loss of recruitement of Septin and Patj to the membrane and increased spread of the Dl gradient toward the dorsal side of the embryo (Mavrakis et al., 2009). These studies together suggest that the plasma membrane organization is essential for restricting morphogens in the embryo. However, further studies perturbing the cellular organization in a specific way will be the key to establishing a role for the cellular architecture in influencing gradient dynamics.

The syncytial nuclear division cycles of the *Drosophila* embryo are one of the fastest known in the animal kingdom. The nuclear cycles 11, 12 and 13 occur in 10, 12 and 21 min respectively at 25°C (Foe and Alberts, 1983). The compartmentalization of the plasma membrane is maintained across the syncytial cycle from the mother nuclei to the daughter nuclei (Mavrakis et al., 2009). The plasma membrane around the parent nuclei is distributed to the daughter nuclei. The plasma membrane undergoes a significant amount of remodeling during the syncytial division cycle. The villi in the interphase plasma membrane appear to straighten out in metaphase to form metaphase furrows. The short 2 µm furrow in the interphase of the syncytial-blastoderm nuclear cycle extends upto approximately 8 µm in metaphase in nuclear division cycle 13 (Mavrakis et al., 2009). Earlier studies debated whether cytoskeletal remodeling was the primary mechanism of metaphase furrow formation and cellularization (Karr and Alberts, 1986). Recent studies that show that mutants in recycling endosome trafficking (*rab11*) result in loss of metaphase furrow formation establish that membrane trafficking is also responsible for bringing about the

formation of the metaphase furrow (Riggs et al., 2003).

Endocytosis at the plasma membrane is regulated during the syncytial division cycle and cellularization. Fluorescent dye uptake suggests that there is increased endocytosis during furrow extension from interphase to metaphase and in telophase during furrow regression. Localization of amphiphysin during furrow ingression suggests that this is Dynamin-mediated endocytosis. The molecular machinery, which is responsible for endocytosis during telophase, remains unknown. Dynamin and Clathrin are localized to the lateral plasma membrane indicating that endocytosis occurs preferentially from these membranes (Sokac and Wieschaus, 2008) (Rikhy, Mavrakis and Lippincott-Schwartz unpublished results). Nullo protein function is essential for onset of endocytosis in the syncytium and during cellularization. Nullo mutants show loss of endocytosis during furrow ingression. Nullo protein transcription and translation increases specifically during the syncytial blastoderm and cellularization and is responsible for inducing endocytosis during these stages (Simpsonand Wieschaus, 1990). This is the first example of a developmentally regulated protein, which directly regulates membrane trafficking activity in the form of endocytosis during the syncytial stage of development in the *Drosophila* embryo (Sokac and Wieschaus, 2008). The importance of endocytosis in regulating morphogen gradient activation such as Tl also corroborates the requirement of developmental regulation of the process during syncytial nuclear cycles (Lund et al., 2010).

Slam is another protein, which is developmentally regulated during blastoderm formation. Slam is expressed in the syncytial blastoderm and is seen at the furrow plasma membrane during nuclear cycle 11 (Postner and Wieschaus, 1994). Embryos mutant for Slam show a loss of polarized membrane growth during cellularization (Lecuit et al., 2002). This protein is also present in vesicles below the nuclei and is an important component regulating membrane trafficking of Tl and Neurotactin to the syncytial plasma membrane. Mutants in slam also show a defect in recruitment of key polarity proteins such as Myosin II, Armadillo and Patj during cellularization. There are possibly few defects in Slam and Nullo mutants during the syncytial nuclear division cycles. However since these proteins are present on the membrane during syncytial cycles, it remains to be assessed if they are necessary for membrane polarity and compartmentalization during these stages.

The cytoskeleton of the syncytial blastoderm embryo

The actin and tubulin cytoskeleton is highly dynamic during the syncytial nuclear division cycles. During the syncytial cycles 6–8 the non-muscle Myosin II undergoes cycles of cortical recruitment coincident with the nuclear divisions (Royou and Sullivan, 2002). This results in axial spreading of nuclei along the antero-posterior axis. However, *spaghetti squash (sqh)* mutant embryos that show complete depletion of Myosin II do not have defects until cellularization in the interphase of nuclear cycle 14. Therefore, Myosin II requirement during syncytial blastoderm formation is debatable. Actin disruption, on the other hand, severely impedes axial expansion of nuclei thus confirming a role for actin remodeling during this process. Nuclear migration toward the cortex to form the syncytial blastoderm is driven by the tubulin and actin cytoskeleton during nuclear cycles 8 and 9 (Baker et al., 1993; von Dassow and Schubiger, 1994).

Remodeling of the plasma membrane during the syncytial cycles requires dynamic remodeling of the cytoskeleton. Disruption of actin and tubulin dynamics by pharmacological agents results in loss of compartmentalization of the organelles and the plasma membrane (Frescas et al., 2006; Mavrakis et al., 2009). The microtubule cytoskeleton is spread vertically across each nucleus, emanating from apical centrosomes during interphase. As the syncytial nuclear division cycle proceeds to metaphase, the centrosomes migrate to the lateral side and this coincides with metaphase furrow formation (Fig. 1). Centrosome migration occurs beneath the plasma membrane independent of nuclei during cycle 10. The centrosome division cycles can be decoupled from those of the nuclear division by injecting aphidicolin in giant nuclei mutants (Freeman et al., 1986; Raff and Glover, 1989). Centrosomes can alone organize the actin and tubulin cytoskeleton and the plasma membrane in the absence of nuclei in nuclear cycle 10 (Raff and Glover, 1989).

Actin is present in caps in the apical region and this is transformed into rings, which help form the metaphase furrow (Fig. 1). Mutants in the Arp2/3 complex, which function to form branched actin and Diaphanous, which plays a role in bundled actin formation are defective in the formation of metaphase furrows (Stevenson et al., 2002; Afshar et al., 2000). The defect in metaphase furrow formation is also seen in RhoGEF2 mutants that deplete Diaphanous from metaphase furrow (Grosshans et al., 2005). All these mutations result in the presence of tripolar spindles, thus enforcing the fact that the metaphase furrows are important to keep adjacent spindles apart. Whether the metaphase furrow is also essential for keeping other components such as organelles or the cytoplasm of the syncytium apart remains to be investigated. It is worth noting that during metaphase, the nuclear contents are in the cytoplasm and mixing them will result in increased spreading of gradient molecules sequestered in the nucleus during interphase.

Anillin and Peanut are also present on the lateral membrane in the syncytium. Embryos mutant for these proteins are also defective in metaphase furrow formation (Field and Alberts, 1995; Field, 2005; Silverman-Gavrila et al., 2008). Anillin is a PH domain containing protein, which is essential for recruiting the GTPase septin, Peanut to the membrane. Anillin and Peanut are essential for stabilizing actin on the membrane. Septins are also important for forming a barrier in

the inner leaflet of the plasma membrane between the mother and daughter cells in yeast and where cilia attach to the body of the cell in mammalian cells (Takizawa et al., 2000; Hu et al., 2010; Kim S K et al., 2010). While the gross disruption of the actin cytoskeleton results in loss of polarity and compartmentalization in the syncytium; it remains to be seen which specific components of the actin cytoskeleton remodeling impart gradient restriction capability to body axes forming morphogens.

Potential role of syncytial compartmentalization on morphogen gradients

In the following sections, we discuss various features of each of the morphogen gradients in the syncytial-blastoderm embryo and allude to those, which may be sensitive to the compartment model of the syncytium. We also discuss recent literature on the mechanisms known to determine morphogen gradients in the syncytium.

Bicoid

Bicoid (Bcd) was one of the earliest identified morphogens involved in the patterning of the *Drosophila* embryo along the anterior-posterior axis (Fig. 2). Bcd is a homeodomain transcription factor that gives positional information to the embryo's anterior via differential expression of segmentation genes (gap genes and pair rule genes). The *bcd* gene is maternally transcribed and *bcd* mRNA is deposited at the anterior pole in the oocyte (Fig. 2A) (Driever and Nüsslein-Volhard 1988a; 1988b). The translation of the Bcd protein occurs during nuclear cycle 10 and Bcd protein is seen in nuclei in the anterior at that time. The Bcd gradient has been a unique system to understand the molecular mechanisms employed by morphogen gradients in the development and morphogen spread *in vivo*, and has been a popular subject for generating biophysical and computational models to define its spread.

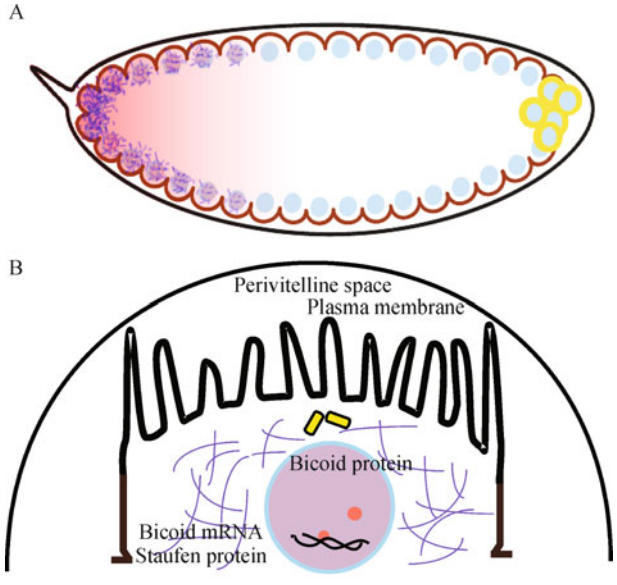

Figure 2 Bicoid (Bcd) gradient in the syncytium. The *bcd* mRNA and the Bcd protein form a gradient across the syncytial nuclei in the anterior (A). The Staufen protein is required for anchoring the *bcd* mRNA at the anterior of the embryo. The Bcd protein is formed from translation of the *bcd* mRNA and enters the nucleus where it activates specific downstream gene expression responsible for determining the anterior end of the embryo (B) (not drawn to scale).

Early attempts at understanding the formation of the Bcd gradient resulted in a model based on localized synthesis, degradation and spatially uniform distribution (SDD model). This model assumed that the *bcd* mRNA is anchored at the anterior end of the embryo and acts as a source of translated Bcd protein which diffuses freely across the length of the embryo. Diffusion was coupled with uniform degradation that followed first-order kinetics resulting in an exponential decay of Bcd protein concentration as one moves toward the posterior pole of the embryo. However, careful studies on assessing the Bcd diffusion coefficient using transgenic Bcd-GFP in the syncytial blastoderm (0.3 $\mu m^2/s$) found it to be 1–2 orders of magnitude lower than that predicted by the SDD model (10 $\mu m^2/s$) required to achieve the observed Bcd gradient within the developmental time frame (Grimm et al., 2010). These studies have raised questions about diffusion of the translated Bcd protein being the primary mechanism of spread of the gradient.

An attractive alternative to the SDD model was active RNA transport and synthesis model (ARTS) (Berleth et al., 1988; Lipshitz, 2009; Spirov et al., 2009). This is based upon the existence of a '*bcd* mRNA gradient' that overlaps almost perfectly with the Bcd protein gradient. Staufen (Stau) binds to the 3′UTR of Bcd and mediates its localization in the oocyte. Stau is a part of the messenger ribonucleoprotein (RNP) complex, which travels to the minus end of the microtubules via dynein dependent active transport (also dependent on Exuperantia (Exu)). At the minus end it binds to Swallow (Swa), which interacts with dynein and the microtubule organizing center (MTOC) (Weil et al., 2006). Once fertilization occurs, Swa is degraded and calcium signaling releases the mRNP complex (Weil et al., 2008). Also, the MTOCs at the anterior pole are disassembled and the cortical microtubular network does not seem to have overall polarity. During nuclear cycles 1–9, the mRNP complex is actively transported along this network driven by its concentration gradient. During the syncytial blastoderm stage, *bcd* mRNA is transported to the apical periplasm along astral microtubules (Spirov et al., 2009). The mRNA gradient becomes sharper. The nuclear concentration of Bcd remains approximately constant (with a small error of ~10%) at a specific spatial location along the anterior-posterior axis. The transport of Bcd to the apical end may be so that Bcd can

surround the entire nuclear surface and can rapidly enter the nuclei to maintain its concentration (Gregor et al., 2007).

Another model for the formation of the Bcd gradient assumes that bcd mRNA diffuses along the anterior-posterior axis and Bcd protein that is synthesized remains localized around each nucleus and does not diffuse (Dilão and Muraro, 2010). Directionless transport of mRNA on microtubules (Spirov et al., 2009) can be treated same as diffusion. The model also assumes that Bcd reaches steady-state, but leaves out nuclear effects of protein degradation and sequestration. This model provides a satisfactory description of the Bcd gradient using available experimental data, although it is constrained by the fact that several parameters like Bcd lifetime and extent of mRNA distribution are unknown.

Indeed, when the Bcd protein diffusion and the mRNA redistribution as well as nuclear degradation are together taken into account, stable nuclear concentrations of Bcd and scaling of the gradient along with embryo length can be explained (Deng et al., 2010). Bcd degradation might be mediated by Stau (Kim et al., 2005) based on the role of mammalian Stau protein in a process called Staufen mediated decay (SMD). Whether this impacts the degradation of Bcd in the syncytial blastoderm remains a point of contention. It is also not known whether post-translational modifications of Bcd might affect its gradient by changing its targeting to the nucleus or affecting its stability. For example, phosphorylation of Bcd by Torso (Tor) pathway refines the transcriptional activity of Bcd such that it is more pronounced at the anterior but is attenuated in the trunk of the embryo (Grimm et al., 2010). It will be interesting to assess the role of embryo architecture in maintaining the localization of the *bcd* mRNA, and therefore, the Bcd protein gradient.

The syncytial blastoderm embryo has compartments around each nucleus at a time when gradients spread across each nucleus. The Bcd gradient has been found to be stable across different syncytial division cycles (Coppey et al., 2007). A compartment model of Bcd has been described. This model treats the embryo's cortex as a series of identical compartments, each comprising a central nucleus and surrounding cytoplasm where transport within compartments occurs much faster than between adjacent compartments (Kavousanakis et al., 2010). The model also accounts for the fact that Bcd gradient scales with length of the embryo across species. However, the model rests on the assumption that Bcd has a finite lifetime on a short time scale and that the nucleus plays a crucial role both in the sequestration and degradation of Bcd. This nuclear-specific degradation is assumed to have a role in scaling the Bcd gradient and contributes to its robustness in eggs of different lengths. These models will allow an analysis of various parameters, which specifically affect different components of the Bcd gradient in future studies.

In contradiction to the above model, nuclei in the syncytial blastoderm have been recently shown not to play a significant role in shaping or scaling the Bcd gradient and nuclei are not a site for Bcd degradation (Coppey et al., 2008; Grimm and Wieschaus, 2010). A model was proposed that showed that the shape of the Bcd gradient is independent of the number of nuclei (Coppey et al., 2008). The model, however, considered a point source for Bcd and simple diffusion as the means for formation of the Bcd gradient. In a more recent study, a *bcd* mutant that lacked a nuclear localization signal (NLS) was employed to test whether targeting Bcd to the nucleus was important in gradient formation. Deletion of the NLS caused Bcd to be distributed uniformly throughout the nuclear-cytoplasmic space and reduced its nuclear lifetime ~2-fold. A linear relationship was observed between intensity profiles of wild type and mutant forms of Bcd in the cytoplasm (but not in the nuclei). Thus, failure to localize in the nucleus did not affect the scaling of Bcd with embryo length and also its anterior-posterior or center-to-surface distribution (Grimm and Wieschaus, 2010).

Torso–RTK signaling pathway

The Torso (Tor) receptor signaling gives rise to the terminal structures of the embryo. Tor is a transmembrane receptor tyrosine kinase (RTK) that is distributed evenly along the surface of the early *Drosophila* embryo (Sprenger et al., 1989). It is, however, selectively activated at the poles by the local activation of its ligand Trunk (Trk) probably by the protein Torso-like (Tsl) that is anchored to the vitelline membrane at the poles (Ventura et al., 2010). Tor signals via the mitosis activating protein kinase (MAPK) pathway and results in expression of the genes *tailless (tll)* and *huckebein (hkb)*, which are responsible for initiating development of the anterior and posterior regions of the embryo (Fig. 3).

Tor signaling results in the activation of terminal gap genes in the anterior and posterior terminals. The shape and the amplitude of the MAPK gradient formed due to Tor signaling changes during the syncytial division cycles (Coppey et al., 2008). In the posterior terminus, Tor signaling directly activates *gap* genes. However, in the anterior, both Tor signaling and Bcd interact to give rise to the head structures. Selective Tor signaling at the poles is important for the spatial control of Bcd protein expression at the anterior of the embryo. This is achieved by downregulation of the maternally deposited repressor Capicua (Cic). In the trunk of the embryo, Cic represses transcription of Bcd target genes by binding to sites in enhancers responsive to Bcd called Tor responsive elements (torREs). In the anterior region, however, the concentration of Bcd protein is high enough to overcome this repressive activity, allowing the formation of anterior structures. This is because Tor downregulates Cic and stabilizes Bcd at the anterior via phosphorylation by Rolled (Rl), the *Drosophila* homolog of MAP kinase (Löhr et al., 2009). Bcd and Cic may compete for the phosphorylation site of MAPK (Kim et al., 2010). This results in a negative feedback loop that would optimally control the concentration

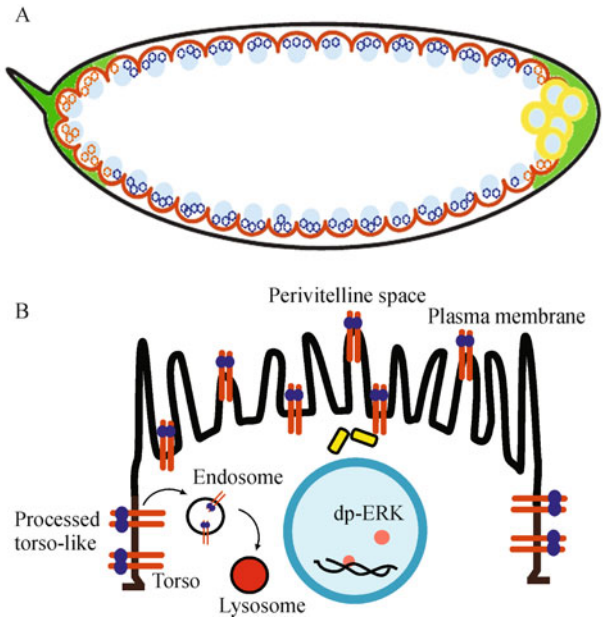

Figure 3 MAP kinase (MAPK) gradient in the syncytium. The MAPK gradient is formed on the anterior and the posterior by the activation of the Torso (Tor) receptor (A). The Tor receptor gets activated by processing of the Tor-like ligand in the perivitelline space. The Tor receptor is present all over the plasma membrane and is endocytosed and degraded in lysosomes after activation (B) (not drawn to scale).

of Bcd at the anterior. When the 3′UTR region of Bcd was deleted and Bcd was expressed uniformly across the anterior-posterior axis of the embryo, the result was an embryo with two heads (Löhr et al., 2009). Thus, Tor signaling at the anterior poles modulates the expression of Bcd by down-regulating Cic repression.

Tor also downregulates other repressors like grainyhead, female sterile, homeotic, tramtrack and groucho (Sprenger et al., 1989). Derepression causes the expression at the poles of several proteins that are otherwise not active. For example, Tor signaling selectively blocks the ability of Dl (Dl) to repress its target genes, *zerknüllt (zen)* and *decapentaplegic (dpp)*, while retaining its ability to activate *twist (twi)* and *snail (sna)* (Rusch and Levine, 1994). Tor, therefore, seems to be crucial for patterning the embryo both along the anterior-posterior and dorsal-ventral axes.

How Tor signaling is spatially restricted to the poles of the embryo and what molecules govern selective activation of Trk in the terminal region is a subject of intense investigation. It has recently been reported that three related proteins, Closca, Nasrat and Polehole maintain the integrity of the vitelline membrane while simultaneously stabilizing (via anchoring and protection) and activating secreted Tsl at its poles by crosslinking via non-disulphide bonds (Ventura et al., 2010). Tsl will thus be activated only in the poles and it may, in turn, spatially restrict the activation of Trk, although so far the exact mechanism for this interaction is unknown.

Endocytosis is important for the recycling and degradation of several membrane bound receptors including the RTK receptors. Hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs), an early endosomal protein responsible for the formation of multivescicular bodies (MVBs) and targeting to lysosomes, has been implicated in downregulation of two RTKs, epidermal growth factor receptor (EGFR) and Tor, via endocytosis (Lloyd et al., 2002). Maternal depletion of *hrs* shows spatial broadening and temporal elongation of Tor signaling at the blastoderm stage since the Tor receptor cannot be degraded in the lysosomes.

Downregulation of Tor signaling is especially important in the context of developing germ cells. The gene *polar granule component (pgc)* is required for germ-cell transcriptional repression of somatic genes such as *tll*, *zen* and *slam* that are important for cellularization and differentiation of somatic cells at the posterior pole of the embryo. In germ cells, RNA polymerase II is not phosphorylated at Ser2 in its C-terminal domain and hence there is transcriptional repression. Tor signaling inhibits the action of Pgc and lifts transcriptional repression in somatic cells (de Las Heras et al., 2009).

Dorsal

While Bcd specifies the anterior-posterior axis, another maternally deposited transcription factor Dorsal (Dl), specifies the dorsal-ventral axis in the embryo. It forms a ventral-to-dorsal gradient in the nucleus and a dorsal-to-ventral gradient in the cytoplasm (Fig. 4). Dl action is limited to the

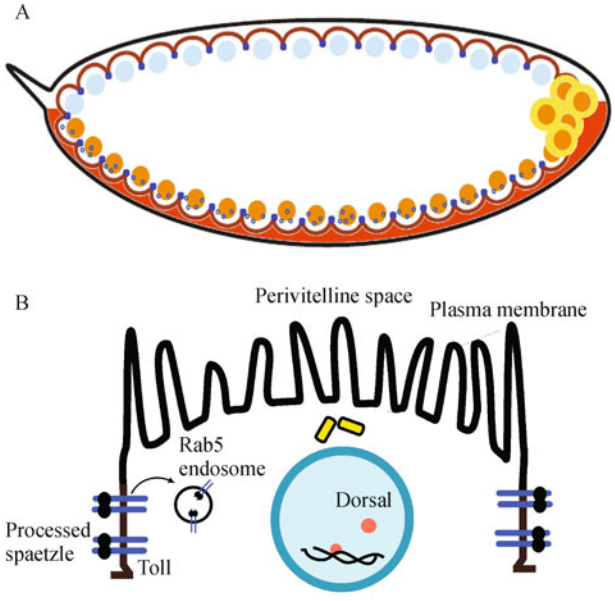

Figure 4 Dorsal (Dl) gradient in the syncytium. The Dl gradient in the syncytium is formed in response to activation of Toll (Tl) receptor by Späztle (Spz) (A). The Tl receptor is activated by cleaved Spz locally formed in the perivitelline space on the ventral side. The activated Tl receptor is endocytosed and has been shown to signal from Rab5 positive early endosomes (B) (not drawn to scale).

ventral half of the embryo in two major ways. The first is the fact that Toll (Tl) is itself activated only on the ventral side. This is because Tl is bound by its ligand Spätzle (Spz) which is cleaved and activated at the culmination of a cascade of serine proteases–Nudel (Ndl), Gastrulation-defective (Gd), Snake (Snk) and Easter (Ea) solely on the ventral side in the perivitelline space. However, the factors that trigger this localized activation of the serine protease cascade are unknown. Spz gradient is also modulated by the action of a serine protease inhibitor (Serpin) 27A on Ea. The second reason is that Pipe (Pip) is active only on the ventral side and *pipe* transcription is repressed in the dorsal half of the embryo by EGFR signaling in the dorsal follicle cells mediated by the ligand Gurken (Moussian and Roth, 2005).

The Tl receptor is present all over the plasma membrane of the embryo and has been shown to form a heterotetramer with processed Spz (Gillespie and Wasserman, 1994; Gangloff et al., 2008). Proteolysis of Spz causes a hydrophobic domain to be exposed, which binds to the Tl receptor and activates it (Weber et al., 2007; Arnot et al., 2010). Tl can mediate cell-cell adhesion in a heterotypic manner (upon activation) and this finding is consistent with the hypothesis that adhesion is caused by interaction of its extracellular domain composed of leucine-rich repeats (LRRs) (Keith and Gay, 1990). Further, Tl is localized on the small lateral membrane in interphase of the syncytial blastoderm along with junctional molecules (Mavrakis et al., 2009). This finding also supports the case for Tl mediating cell-cell adhesion in adjacent lateral membranes in the syncytium and during cellularization. Whether adhesive properties are important for Tl signaling or plasma membrane compartmentalization still remains to be assessed. Finally, as disclosed earlier, drug-induced depolymerization of actin that disrupts plasma membrane localization of junctional molecules and compartmentalization, was found to cause a spread in the Dl gradient toward the dorsal side, past the equator (Mavrakis et al., 2009).

Once Tl is activated, it signals via Weckle, MyD88, Tube and Pelle, also maternal factors. At high concentrations, Pelle undergoes autophosphorylation and subsequently phosphorylates Tl and Tube, thereby inactivating the signal and getting released form the complex in order to phosphorylate its final targets Cactus and possibly Dl which are initially a part of the Tl signaling complex (Edwards et al., 1997). Cactus is then targeted for ubiquitination and degradation. Dl actively shuttles between the cytoplasm and the nucleus; phosphorylation-dephosphorylation cycles of Dl dictate whether it is prominently localized in the nucleus (ventral side) or cytoplasm (dorsal side). The Dl gradient in the nucleus on the ventral side appears as soon as the nuclei arrive beneath the membrane during cycle 10 in the syncytial blastoderm and breaks down and reforms with every mitotic cycle (DeLotto et al., 2007).

An elegant model–strengthened by experimental data for the spread of the Dl gradient in the embryo–has been proposed to describe its dynamics in the syncytial blastoderm embryo. The model predicts that the amplitude of the Dl gradient increases as a function of the number of nuclei (the gradient is re-established after every nuclear cycle to a value higher than that in the previous cycle), while its shape remains constant (Kanodia et al., 2009). This depends primarily on the length of the interphase that increases with syncytial nuclear division number in the blastoderm embryo.

Dl plays dual roles as an activator and a repressor of transcription. It causes activation of *snail (sna)*, *twist (twi)*, *short gastrulation (sog)* and *brinker (brk)* and repression of *zerknült (zen)* and *tolloid (tld)* genes. Multiple combinations of *cis*-regulatory sequences occur to specify expression of genes downstream of Dl. These *cis*-regulatory sequences contain Dl binding sites of high or low affinity along with binding sites for other proteins like Twi and Groucho that interact with Dl; thus the activation and repression activities of Dl are not coupled to each other (Ratnaparkhi et al., 2006). It was previously thought that five distinct thresholds of Dl concentration gradient specify its gene activity (A. M. Huang et al., 1997); that number has now been revised to three (Papatsenko, 2005).

The role of Tor in modulating the Tl pathway has been described above. Another protein, Kurtz (Krz), a mammalian β-arrestin homolog, is a general RTK inhibitor that blocks Tor signaling. It is also responsible for shaping the Dl signal by restricting its expression domains. Krz limits the action of Tor to the poles so that Dl-mediated repression of *zen* is unhindered. Thus, Krz is a key player in mediating the crosstalk between the Tl and Tor pathways (Tipping et al., 2010).

A recent study using fluorescently tagged Tl receptor with photoactivatable green fluorescent proteins demonstrated that the Tl signaling complex is ensconced inside an endocytic compartment upon activation. Thus, activated Spz, which is freely diffusible in the perivitelline space, potentially gets trapped within endosomes in the ventral region; further localizing Tl signaling (Lund et al., 2010). This work supplements the finding that Tl colocalizes with components of the endocytic pathway Myopic (Mop) and Hrs (Huang et al., 2010).

Future perspectives

The *Drosophila* syncytial blastoderm embryo shows compartmentalization of organelles and the plasma membrane around each nucleus and a restricted source or origin of key morphogen gradients, which pattern the anterior-posterior and the dorsal-ventral axes. The properties of the cytoplasm, which give rise to differential gradient dynamics, remain to be assessed. The syncytial blastoderm embryo gives rise to very diverse kinetics of spread of each gradient. As the syncytial nuclear division cycles proceed, there is no change in the amplitude and shape of the Bcd gradient. However, in the same syncytium, there is conservation of shape of the Dl gradient with an increase in amplitude and change in shape

and amplitude of MAPK gradient at the termini. How different morphogen gradients make use of the pseudocellular properties of the syncytium, will give us an insight into their regulation as the syncytial cycles proceed toward gastrulation.

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