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Establishing and maintaining cell polarity with mRNA localization in *Drosophila*

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Abstract

How cell polarity is established and maintained is an important question in diverse biological contexts. Molecular mechanisms used to localize polarity proteins to distinct domains are likely context-dependent and provide a feedback loop in order to maintain polarity. One such mechanism is the localized translation of mRNAs encoding polarity proteins, which will be the focus of this review and may play a more important role in the establishment and maintenance of polarity than is currently known. Localized translation of mRNAs encoding polarity proteins can be used to establish polarity in response to an external signal, and to maintain polarity by local production of polarity determinants. The importance of this mechanism is illustrated by recent findings, including *orb2*-dependent localized translation of *aPKC* mRNA at the apical end of elongating spermatid tails in the *Drosophila* testis, and the apical localization of *stardust A* mRNA in *Drosophila* follicle and embryonic epithelia.

Keywords

aPKC; mRNA localization; orb2; Par proteins; polarity; spermatogenesis; stardust

Introduction

mRNA localization coupled with on-site translation is a conserved mechanism across eukaryotes (reviewed in [1–4]). This mechanism is used for local protein production at the yeast bud during reproduction, at the dendrites of neurons in flies and mammals, and at the leading edge of migrating mammalian cells [5–7]. Localization of transcripts and their on-site translation is an efficient way to generate protein asymmetry, because one transcript can produce multiple protein molecules. Localization of mRNAs prior to their translation also prevents protein expression in an inappropriate region of the cytoplasm, which is especially

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important for mRNAs encoding proteins with local function, such as patterning determinants that specify cell fates, or polarity proteins that specify discrete subcellular domains.

mRNA localization depends on a pre-existing cell polarity, a polarity that distinguishes distinct subcellular domains and is critical for properly targeting mRNAs to their appropriate destinations within the cell. Polarity is a characteristic feature of eukaryotic cells, enabling them to compartmentalize specific activities in distinct subcellular domains. Proteins that function to establish and maintain cell polarity are typically associated with the cell cortex and are localize asymmetrically within the cell to define different subcellular domains. For instance, in *Caenorhabditis elegans* the partitioning defective, or *Par* genes, are required for polarization of the zygote [8, 9]. The asymmetric distribution of Par proteins along the cortex, a process that defines the anterior and posterior cortical domains, is triggered by an external signal, the sperm-donated pronucleus and centrosome that specify the posterior. The distinct Par domains orchestrate the asymmetric division of the *C. elegans* zygote, and the partitioning of cell fate determinants. The establishment and maintenance of polarity requires antagonistic cross-regulatory interactions between the anterior and posterior Par proteins, which are conserved among diverse species and cell types to define opposing polarity domains. Among these diverse contexts, cross-regulatory interactions between opposing complexes are critical for polarity establishment and maintenance.

The idea that cell polarity is upstream of mRNA localization has long been a paradigm in the field; however, recent studies in *Drosophila* have raised the possibility that this relationship is in some instances turned on its head so that the establishment and/or maintenance of cell polarity depends upon mRNA localization. This review will discuss two examples in which the localization of mRNAs encoding polarity proteins appears to play a critical role in establishing and maintaining polarity. The first is *orb2*-dependent translational regulation of *aPKC* mRNA in the context of the *Drosophila* spermatid cyst. The second is the localization of *stardust* and *crumbs* mRNAs to the apical region of *Drosophila* follicle and embryonic epithelia. These studies suggest that mRNA localization is not always downstream of a previously established cell polarity, but instead might participate directly in the process of establishing and maintaining polarity.

Polarity proteins define distinct subcellular domains

Cell polarity depends upon a special group of proteins which function to establish and then maintain distinct domains within the cell. In *C. elegans*, proteins encoded by six PAR genes, PAR-1-6, and two other proteins, atypical protein kinase C (aPKC), and cdc42, are required to instruct the first division of the zygote. The first division is asymmetric: the two daughter cells are unequal in volume and the posterior daughter cell inherits P granules, membrane-less organelles of RNAs, and proteins that specify the germline [10]. Asymmetric division depends upon the proper distribution of the PAR proteins in the anterior and posterior cortex of the zygote. aPKC, PAR-3, and PAR-6 form an anterior complex, PAR-1 and PAR-2 form a posterior complex, and PAR-4 and PAR-5 remain symmetrically localized around the cortex [9, 11–14].

Following the discovery of the PAR proteins in *C. elegans*, it was realized that these proteins are polarity regulators that are conserved from worms to flies to mammals [15–17]. Both nematodes and fruit flies are used as model systems to understand how Par polarity is established and maintained [18]. In *Drosophila*, Par proteins are required in epithelia to define apical and basolateral domains and position junctions between these domains (for review see [19, 20]. For example aPKC, Par-3 (known as Bazooka in flies), and Par-6 form a complex to promote apical identity, and Par-1 localizes to the basolateral domain. Genetic screens in *Drosophila* identified additional proteins that are required to define the apical and basolateral domains of epithelia – the Crumbs/Stardust/Patj complex localizes to the apical domain [21–24], and proteins of the Scribble/Discs Large complex and Lethal Giant Larvae (Lgl) localize to the basolateral domain [25].

Establishing and maintaining polarity requires feedback mechanisms

Polarization of the *C. elegans* zygote is initiated by an external cue; the sperm-donated centrosome specifies the posterior of the zygote [26]. The PAR domains are formed during a polarity establishment phase due to differential actomyosin contractility between the anterior and posterior domains of the zygote [27]. After the domains are established, positional information provided by the sperm is no longer required to maintain polarity [26]. Instead, self-sustaining feedback loops stabilize the anterior and posterior domains to maintain the polarity decision.

The feedback mechanism thought to be most critical for establishing and maintaining polarization is mutual antagonism between polarity complexes. In the *C. elegans* embryo, aPKC, PAR-1, and PAR-4 are kinases, and mutual exclusion by phosphorylation is key regulatory mechanism in polarity establishment and maintenance. PAR-3 and PAR-6 are PDZ domain containing proteins, PAR-5 is a 14-3-3 protein and PAR-2 is not conserved, but functions redundantly with a conserved polarity regulator, Lgl [28, 29]. Antagonistic interactions between the anterior and posterior PAR proteins are required to execute both polarity establishment and maintenance. For example phosphorylation of PAR-1 and PAR-2 by aPKC keeps the posterior complex from associating with the anterior cortex [30, 31]. Conversely, the posterior complex functions to exclude PAR-3 from the posterior cortex of the zygote [28, 29, 32, 33]. Cross-regulatory interactions between the Par protein kinases are conserved. In *Drosophila*, Par-1 phosphorylation of Baz promotes the disassembly of the apical Par complex in epithelial cells [34]. In turn, aPKC inhibits Lgl and Par-1 localization in the apical domain by phosphorylation of both of these proteins [31, 35, 36]. Other sorts of cross-regulatory interactions between the apical and basal Par proteins also contribute to the polarization process. For example aPKC activity is limited to the apical domain through the action Supernumerary Limbs (Slmb), a substrate adaptor for an E3 ubiquitin ligase, while from the basolateral domain [37–39].

mRNA localization coupled with on-site translation is a widespread mechanism for generating protein asymmetry

Before discussing possible roles for mRNA localization in the establishment and maintenance of cell polarity, we will briefly review what is known about this mechanism for

expressing proteins at subcellular locations where they are needed [40, 41]. mRNA localization depends upon special *cis*-acting sequences that are recognized by factors which participate in different steps in the localization pathway. These factors can associate with mRNAs when they are transcribed, during nuclear export and during cytoplasmic transport to the appropriate subcellular domain (for review of mRNA localization, see [1, 2, 42–44]). The resulting mRNP complexes are important for ensuring mRNA stability, for mediating the localization process and for controlling the translational activity of the mRNA. Mechanisms used to localize mRNAs include active transport [45], diffusion coupled with entrapment [46], and selective degradation [47, 48]. Active transport relies on linking mRNAs to cytoskeletal motors that move them in a directed manner along a polarized microtubule network toward the site of localization. Diffusion can be entirely passive or facilitated by an underlying directional cytoplasmic flow. The latter is the case for *nanos* mRNAs that are localized to the posterior pole of the *Drosophila* oocyte [46].

It is important that mRNAs remain translationally silent while in transit. For this reason, specific RNA binding proteins that function to inhibit translation are found associated with the mRNAs prior to their localization. For example in the *Drosophila* oocyte the Bruno protein is a translational repressor of localized mRNAs. It binds to Bruno-Response Elements (BREs) in the 3′untranslated region (UTR) of target mRNAs and is thought to be responsible for preventing the translation of BRE containing mRNAs while they are in transit from the site of synthesis to their final destination [49–53].

mRNA localization in *Drosophila* embryos and egg chambers

A model system that highlights the importance of mRNA localization is the *Drosophila* blastoderm embryo and the oocyte. In the *Drosophila* blastoderm embryo, the localization of mRNAs to the apical surface helps prevent signaling molecules and pair rule gene products from diffusing away from their sites of function in the syncytial embryo prior to cellularization [54]. Transcripts are localized apically, and include the signaling molecules *wingless*. Wingless is a secreted Wnt protein, and the apical localization of wingless transcripts is important for targeting the secreted protein to the apical membrane [55]. Other apically localized transcripts include pair-rule gene transcripts including even *skipped* and *hairy* [54–56].

In the fly oocyte, patterning determinants that specify both the anterior-posterior (A-P) and dorsal-ventral (D-V) body axes are localized as mRNAs [4, 57–60]. During mid-stages of oogenesis a reorganization of oocyte microtubule polarity requires the function of Par proteins [17, 61–63]. This repolarization event instructs the localization of maternal transcripts such as *bicoid* to the anterior, *oskar* to the posterior and *gurken* to the dorsal-anterior corner. MacDonald and Struhl [64] identified *cis*-acting sequences within the *bicoid* 3′UTR that are necessary for localizing *bicoid* mRNA to the anterior of the oocyte. After fertilization, *bicoid* mRNA is translated resulting in formation of an anterior to posterior protein gradient of Bicoid, a transcription factor and translational repressor, which controls head and thorax development [65]. Subsequent studies showed that 3′UTR sequences are necessary and sufficient for the localization of other maternal mRNAs, such as *nanos* and *orb* [66, 67]. *nanos* mRNAs are captured at the posterior pole of the oocyte during late stages

of oogenesis [46]. *nanos* mRNAs are translated in the embryo, and specify abdomen formation [66]. Orb is an RNA-binding protein and a founding member of the conserved cytoplasmic polyadenylation element binding protein (CPEB) family [68]. Orb localizes around the cortex of the oocyte and functions as a translational activator by promoting polyadenylation of localized mRNAs. Orb expression is regulated by a positive autoregulatory feedback loop; Orb protein binds to the 3′UTR of *orb* mRNA, and Orb further influences translation within the oocyte by regulating expression of other transcripts, including those important for A-P and D-V axis formation [69–74].

Sequences in the 3′UTR of transcripts are not sufficient for all maternal mRNAs important for polarity axes establishment in the fly oocyte. For example *gurken* mRNA localization depends on sequences in the 5′UTR, 3′UTR, and coding region of the transcript [75–77]. Gurken is a transforming growth factor-α-like protein that signals from the germline to neighboring follicle cells [58, 59]. Local expression of Gurken protein at the dorsal anterior corner of the oocyte is required to specify dorsal follicle cell fate.

Stabilizing the Par-1 domain with localized translation of *oskar* mRNA

Microtubule organization of the *Drosophila* oocyte depends on the function of Par proteins, and during mid-stages of oogenesis allows for the trafficking of mRNA patterning determinants to their site of localization (described above). Par-1 localizes to the posterior cortex of the oocyte and is required for the enrichment of microtubule plus-ends at the posterior [63, 78]. This microtubule organization is critical for the localization of *oskar* mRNA to the posterior, and *bicoid* and *gurken* mRNA to the anterior of the oocyte at this stage. Translation of *oskar* mRNA at the posterior is important for the recruitment of determinants which specify the germline and abdomen in the embryo [79]. Interestingly, however, Par-1 accumulation at the posterior pole also depends on *oskar* mRNA localization. Overexpression of *oskar* mRNA results in the formation of ectopic Oskar patches and the recruitment of Par-1 and microtubule plus-ends to these ectopic sites [80]. Consistent with the idea that *oskar* mRNA translation is important for maintaining polarity, mutants of *oskar* that result in reduced Oskar protein levels also display reduced levels of microtubule plus-ends at the posterior [80]. This example illustrates the idea that mRNA localization is not only a downstream consequence of polarization, but localized translation of proteins that influence polarity is required for maintaining polarity.

mRNA localization as a mechanism for subcellular targeting of polarity proteins

The use of mRNA localization to promote the local accumulation of polarity proteins could complement the system of inhibitory cross-regulatory interactions between apical and basal factors that define cell polarity. Moreover, since the polarity proteins that localize to the same subcellular domain often assemble into complexes, the localized translation of mRNAs encoding constituents of the complex would promote complex assembly by generating high local concentrations of the polarity proteins. This would tend to reinforce the cross-regulatory interactions between the different polarity proteins and promote the establishment

and maintenance of polarity. Consistent with this possibility, there are a number of examples in which mRNAs encoding polarity proteins are targeted to the relevant subcellular domain.

Localization of transcripts encoding polarity proteins has been observed in multiple contexts. For instance, *bazooka* (Par-3) transcripts are localized apically in *Drosophila* embryonic epithelia [15]. In embryonic rat neurons, Par-3 mRNA is locally translated in axons in response to nerve growth factor signaling, implicating local translation of Par-3 in axonal outgrowth [81, 82]. mRNA localization is also important for the expression of Coracle protein, a basolateral polarity protein, at the *Drosophila* neuromuscular junction [83]. Par-6 mRNA is localized to the neuromuscular junction and is transported as a component of a large RNP particle [83, 84]. Here we will discuss examples in which the localization of mRNAs encoding polarity proteins – including *aPKC*, *stardust*, and *crumbs* mRNA – contributes to polarity establishment. However, since there is evidence suggesting that mRNAs encoding several other polarity proteins are also localized, it possible that involvement of mRNA localization in the establishment and/or maintenance of polarity is in fact common place.

aPKC mRNA is locally translated at the apical end of the elongating spermatid cyst in *Drosophila*

In the *Drosophila* germline, a germline stem cell divides asymmetrically producing one daughter cell that self-renews as a germline stem cell and the other daughter cell is positioned away from the stem cell niche. The latter undergoes four rounds of cell division without cytokinesis to give rise to a 16-cell cyst (reviewed in [85]). In males, each cell of the cyst completes meiosis resulting in 64 interconnected spermatid cells. The cells within a spermatid cyst rearrange shortly after meiosis, so that all 64 haploid nuclei cluster at the basal end of the cyst relative to the apical-basal polarity of the testis itself. Flagellar axonemes localize apically and flagellar tails of each cell in the cyst elongate apically, directed toward the germline stem cells and mitotic cysts at the apical end of the organ [86].

A differentiated sperm cell is highly polarized – the sperm head orients basally and the flagellar axoneme tail extends apically. The first of two important steps in spermatid differentiation is the clustering of all of the nuclei on the same side of the cyst. The other critical step is orienting cyst polarity so that flagellar axoneme tails extend in the apical direction, while nuclei cluster toward the basal end of testis. Xu et al. [87] showed that aPKC activity is required for this second step of polarization. When *aPKC* is partially compromised in hypomorphic alleles, or in RNAi knockdowns, the orientation of cyst polarization with respect to the apical-basal axis of the testis appears to be randomized. In some *aPKC* mutant cysts, polarization is in the correct orientation so that the nuclei cluster on the basal side and the flagellar axonemes extend apically. In other *aPKC* mutant cysts, polarization is reversed; the spermatid nuclei are clustered on the apical side, while the flagellar axonemes extend toward the basal end of the testis. These observations argue that one function for aPKC in spermatid differentiation is in properly orienting the spermatid cysts within the context of the testes itself. Consistent with a role in defining the apical domain, aPKC protein is localized to the apical side of wild-type spermatid cysts during the

initial polarization, and subsequently accumulates in a sharp band close to the apical tip of the elongating flagellar axonemes.

Intriguingly, this polarization context seems to be unique because other Par polarity proteins, such as Bazooka and Par-1, are not localized in the spermatid cyst [87]. This suggests that other mechanisms besides mutual antagonism between polarity complexes may orchestrate cyst polarization and the apical localization of aPKC protein. Previous studies on a small group of mRNA species that are transcribed postmeiotically have shown that many of these mRNAs accumulate in a "comet" pattern in the elongating spermatid tails [88]. The "comet head" is localized at the apical end of the elongating spermatid tails and has the highest concentration of the mRNAs. Extending back toward the spermatid nuclei is the comet tail, which contains progressively lower levels of mRNA. This same comet pattern is observed for a special *aPKC* mRNA species, *aPKC-RA*, which is only expressed postmeiotically. This mRNA has an unusually long 3′UTR that is not found in other *aPKC* mRNAs. Consistent with the idea that *aPKC-RA* is used specifically to target aPKC protein to the apical end of the spermatid tails, antibody staining shows that aPKC accumulates in a sharp band at one edge of the mRNA comet head. The long 3′UTR in *aPKC-RA* mRNA has multiple cytoplasmic polyadenylation elements (CPEs) that serve as recognition elements for CPEB proteins. While the CPEB protein Orb is required in the female germline for proper oogenesis, it is largely dispensable in the male germline [68]. Instead, a second fly CPEB, Orb2, plays a critical role in spermatogenesis [89]. Consistent with the idea that one of these functions is in controlling the localization and/or translation of *aPKC-RA mRNA*, Orb2 is found to bind to the *aPKC-RA* 3′UTR both in vitro and in vivo. Moreover, during spermatid cyst elongation Orb2 protein accumulates in a comet-like pattern that coincides with the comet pattern of the *aPKC-RA* mRNA.

A further connection between *aPKC-RA* and Orb2 comes from the defects in cyst polarization evident in a hypomorphic mutant allele of *orb2*, $orb2^{\Delta Q}$. As observed when *aPKC* activity is partially compromised, the orientation of cyst polarization within the testes is disrupted in the $orb2^{\Delta Q}$ mutant. A subset of the cysts elongates in the wrong direction, and in these cysts *aPKC-RA* and aPKC protein are not properly localized at the tip of the elongating flagellar axonemes.

It is likely that the functioning of *orb2* in cyst polarization is not limited to properly orienting polarization with respect to the main axis of the testes. In *orb2* null mutant alleles, spermatid cysts fail to undergo polarization and a range of defects are observed in mutant testes. In some cysts, the nuclei remain in the middle, instead of clustering to one side. In other cysts, the nuclei are scattered throughout. Although these defects point to a more intimate connection to cyst polarization, there is a complication. Because *orb2* is required for the progression of meiosis, the severe polarization defects observed in the null allele could be a downstream effect from earlier abnormalities [89]. Since aPKC activity was only partially compromised, a role for *aPKC* in cyst polarization beyond that in orienting the direction of polarization of the cyst within the testes is also possible. Other genes required for initial steps of spermatid cysts polarization include the exocyst complex [90]. The exocyst complex is a conserved protein complex that directs membrane addition and has been shown in many contexts to be required for cell polarity and growth [91]. Testes

compromised for exocyst function contain bipolar cysts with nuclei at both the apical and basal end of the cyst [90].

Orb2 mediated translational regulation of *aPKC-RA* provides a positive feedback mechanism for polarity establishment

Establishing polarity of the germline cysts requires orienting cyst polarization with respect to the apical-basal axis of the testis. One plausible idea is that an orientation signal emanates from either the apical or the basal somatic cystocyte cell that encapsulates the spermatid cyst. These two somatic cells are derived from a population of somatic stem cells at the apical tip of the testes and they surround the germline daughter cell that ultimately gives rise to the 64-cell spermatid cyst [92]. The two somatic cells grow without division, encapsulating the germ cells as they go through mitosis and meiosis. The apical “tail” cystocyte and basal “head” cystocyte cells are clearly distinct from each other as they express different markers [93]. Thus, one of these somatic cells could provide an external signal that would serve to trigger polarization and orient the germline cyst with respect to the testis.

To establish polarity within the cyst, an external signal from the apical somatic cell could function to activate the translation of *aPKC* mRNA (Fig. 1). In this model, this signal would not be produced by the basal somatic cell and *aPKC* mRNAs on the basal side of the cyst would remain translationally repressed. The target for the apical signal would be Orb2 protein associated *aPKC-RA* mRNAs. Since the activity of CPEB proteins is regulated phosphorylation [69, 94–96], the external apical signal would be expected to trigger the Orb2 phosphorylation. This would in turn lead to the polyadenylation of the *aPKC-RA* mRNA and its translational activation.

This model predicts that multiple feedback loops are important to execute cyst polarization. Like *aPKC-RA*, *orb2* mRNAs have multiple CPE motifs in their 3′UTRs and *orb2* is thought to autoregulate its own expression. Orb2 protein and mRNA co-localize at the apical end of wildtype spermatid cysts. However, in *orb2* or *aPKC* mutant cysts that are not correctly oriented, *orb2* mRNA and protein do not accumulate at high levels at the end of spermatid tails [87]. If the orientation signal also prompted the phosphorylation of Orb2 bound to *orb2* mRNAs, this positive feedback loop would increase the local concentration of Orb2, potentially leading to a further stimulation of *aPKC-RA* mRNA translation. Accumulation of aPKC protein at the apical side of the cyst may be used to positively feedback on *aPKC-RA* mRNA localization. Another possible positive feedback mechanism for locally upregulating Orb2 activity in response to the external polarization signal would be direct phosphorylation of Orb2 by aPKC. Two observations are consistent with this possibility. First, the Orb2 protein comet in elongating spermatid tails is lost when *aPKC* activity is compromised. Second, Orb2 protein has consensus aPKC phosphorylation motifs. Taken together, these observations suggest that *aPKC* might play a direct role in the *orb2* positive autoregulatory feedback loop.

mRNA localization of *stardust* and *crumbs* in *Drosophila* follicle cell and embryonic epithelia

During *Drosophila* oogenesis, a somatic epithelium of follicle cells surrounds a germline cyst to form an egg chamber. The follicle cell surface that contacts the germline cells defines the apical domain, and follicle cells secrete an extracellular matrix basement membrane basally. Stardust and Crumbs are required to define the apical domain in the follicle epithelia, as well as in embryonic epithelia. Stardust is a PDZ domain containing protein that binds to the cytoplasmic tail of the transmembrane protein Crumbs. Usually, Stardust and Crumbs reciprocally recruit the other to the apical domain of the cell. While studying polarity in the follicle epithelium, Horne-Badovinac and Bilder [97] discovered that the apical localization of Stardust is only partially dependent on Crumbs during the early stages of follicle cell development. Therefore, another mechanism is important for apical targeting of Stardust independent of Crumbs localization.

To understand how Stardust is apically localized in these cells, the authors examined mutants recovered in a screen for follicle cell clones that fail to establish apical-basal polarity. Multiple alleles of *dynein heavy chain*, *dhc*, a subunit of the microtubule minus-end directed motor, were recovered in the screen and these mutants display a similar multilayering epithelia phenotype to that of *stardust* and *crumbs* mutants. The apical localization of Crumbs and Stardust is abolished in dhc mutant follicle cell clones; however, the localized accumulation of other apical-basal polarity regulators, such as Bazooka, aPKC, and Par-6, is only moderately affected.

Because *stardust* and *crumbs* mRNAs are apically localized in embryonic epithelia [21, 22], the authors tested whether dynein transports *stardust* transcripts apically in the follicle cells. This is the case. *stardust* mRNA is localized to the apical region of follicle cells in early stages of development, stages 2–4 of oogenesis. During these early stages, follicle cells are actively dividing, suggesting that mRNA localization of polarity regulators may be important in proliferating epithelia. The follicle cells are in contact with the germline cells, so it is possible that a signal specifying the apical surface of the follicle cells originates from contact with the germline. In later stages when follicle cells are no longer dividing, stardust mRNA is not localized. The authors observed similar developmental regulation of *stardust* mRNA localization in embryos. Transcripts are apically localized during the first half of embryogenesis and become uniformly distributed by embryonic stage 15.

Transcripts of *crumbs* are also localized apically and in a dynein-dependent manner, although at later stages of follicle development. *Crumbs* mRNA is uniformly distributed in the follicle cells during stages 2–4 when *stardust* mRNA is localized; however, *crumbs* transcripts are apically localized at stage 10 when *stardust* mRNA is not localized [97]. A complementary study by Li et al. [98] found that the *crumbs* 3′UTR is necessary and sufficient for apical localization of *crumbs* transcripts in both the follicle cells and embryonic epithelia. This reciprocal manner of mRNA localization of *stardust* and *crumbs* guarantees a high concentration of apical polarity protein throughout follicle cell development, which can function to recruit the other polarity protein in the complex. This

local production of Stardust or Crumbs and recruitment of other complex members could act to limit the Crumbs complex and maintain a discrete apical domain.

Localization of *stardust* and *crumbs* mRNA functions in polarity establishment

Horne-Badovinac and Bilder found that the Stardust A isoform is expressed early during follicle cell development and accounts for apically localized transcripts (Fig. 2). In contrast, while the Stardust B isoform is also expressed early, its transcripts are not apically enriched. During later stages of oogenesis only the Stardust B isoform is expressed and *stardust* transcripts are not localized. To determine the role of *stardust A* mRNA localization in polarity establishment, an allele of *stardust* that only produces functional Stardust B protein was tested. If *stardust* mRNA localization is important for polarity establishment, then the expression of only the *stardust B* mRNA in this mutant should not be sufficient for the establishment of epithelial polarity. This was found to be the case in embryonic epithelia, but not follicle cell epithelia. In early embryonic epithelia, the *stardust* mutant that only expresses the *stardust B* mRNA displays severe defects in epithelial development. While this mutant also fails to properly localize Stardust and Crumbs proteins to the apical surface in follicle cells, the polarity defects are not sufficient to disrupt the functioning of the follicle cells or the development of the egg chamber. This would argue that *stardust A* mRNA localization is not required for follicle cell polarization. One plausible idea is that there may be other mechanisms in early follicle cells for that help promote polarization. In this case it is possible that under conditions that partially compromise these activities, the localization of *stardust* mRNA in the follicle epithelia would be critical to ensure proper polarization.

Experiments by Li et al. suggest that a combination of both mRNA localization and protein recruitment is responsible for ensuring that Crumbs accumulates apically. Indicating that the *crumbs* 3′UTR is critical for polarity establishment, protein expressed from a transgene that expresses a *crumbs* mRNA that contains the 3′UTR is able to rescue *crumbs* mutant follicle cell polarity defects. In contrast, an identical transgene encoding a transcript that lack the *crumbs* 3′UTR does not localize Crumbs protein to the apical domain and consequently fails to rescue the *crumbs* mutant defects [98]. Interestingly, while a tagged-Crumbs protein expressed from the transgene lacking the *crumbs* 3′UTR is not properly localized in a *crumbs* mutant background, it is localized to the apical domain in wild-type cells [98]. This finding argues that Crumbs protein localization can be mediated by a pathway that does not depend directly on the localization of the *crumbs* mRNA.

Alternative splicing as a mechanism for developmentally regulating mRNA localization

In the follicle epithelia, localization of *stardust* mRNA does not depend upon 3′UTR. Instead, Horne-Badovinac and Bilder [97] found that exon 3 of the *stardust* transcript is necessary and sufficient for apical localization of the spliced mRNA in the follicle epithelia. This exon is spliced into the mRNA encoding the Stardust A isoform but is excluded from the mRNA encoding the Stardust B isoform. This observation would suggest that Stardust A

has a unique role in polarity establishment in the embryonic epithelia, and that the splicing of the *stardust* pre-mRNA may be regulated to ensure that *stardust A* mRNA is expressed during polarity establishment. In this context, it is interesting to note that the unique 3′UTR of *aPKC-RA* mRNA is also generated by alternative splicing [87]. Expression of this *aPKC* mRNA species is specifically upregulated following the completion of meiosis.

There are precedents for a connection between mRNA processing in the nucleus and mRNA localization in the cytoplasm. Many *trans*-acting factors bind nascent transcripts in the nucleus but have important roles once mRNA is transported to the cytoplasm. A well-studied example in which splicing is required for the binding of localization factors is the *oskar* mRNA. The 3′UTR by itself is not sufficient for localization of *oskar* mRNA to the posterior of oocyte. In addition, splicing of the first intron of the *oskar* pre-mRNA is required [99]. The mRNA expressed from an *oskar* transgene lacking the first intron, with exons 1 and 2 already joined, is not properly localized [99]. This suggests that the exon-junction complex (EJC), which binds to premRNAs at exon junctions once they are spliced, is needed for posterior pole localization [99]. It is possible that there is a similar sort of coupling between splicing in the nucleus and proper localization in the cytoplasm that regulates the localizations of both *stardust A* and *aPKC-RA* mRNAs.

Conclusion and outlook

While cross-regulatory interactions between polarity proteins is a normal mechanism for establishing polarity, there are now several examples in which a noncanonical mechanism that depends primarily on mRNA localization is deployed. As is typically the case for the canonical pathway, an external signal appears to be used to orient and initiate polarization. However, rather than promoting cross-regulatory interactions, this signal instead functions to activate the translation of mRNAs encoding polarity proteins. The local accumulation of polarity proteins would promote the assembly of polarity complexes and this in turn would initiate the canonical cross-regulatory pathway. In addition, there could also be positive feedback mechanisms involving the polarity proteins that instruct further mRNA localization to help maintain the initial polarity decision. The existence of such a positive feedback loop is suggested by studies on the polarity protein aPKC and the CPEB protein Orb2. Orb2 is responsible for the localized translation of *aPKC* mRNA; in turn, Orb2 accumulation depends upon *aPKC* activity.

One way to explore the functional significance of mRNA localization in polarity establishment would be to use the CRISPR/Cas9 system to make mutants specifically defective for mRNA localization, but not protein function. This is possible if the 3′UTR is necessary and sufficient for mRNA localization, such as in the case of *crumbs*. To directly test the requirement of *crumbs* mRNA localization in polarity establishment, the 3′UTR of *crumbs* could be deleted from the genome and replaced with 3′UTR sequences that are not localized. If mRNA localization is required for polarity establishment, then it would be expected that this mutant displays the typical polarity defects of *crumbs* mutants. In the case of mRNAs encoding polarity proteins that are translationally regulated, engineering a mutant 3′UTR lacking translational regulation motifs could be used to directly test the requirement for regulated translation of polarity proteins.

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Abbreviations

aPKC	atypical protein kinase C
CPE	cytoplasmic polyadenylation element
CPEB	cytoplasmic polyadenylation element binding protein

References

1. St Johnston D. Moving messages: the intracellular localization of mRNAs. Nat Rev Mol Cell Biol. 2005; 6:363–75. [PubMed: 15852043]
2. Martin KC, Ephrussi A. MRNA localization: gene expression in the spatial dimension. Cell. 2009; 136:719–30. [PubMed: 19239891]
3. Besse F, Ephrussi A. Translational control of localized mRNAs: restricting protein synthesis in space and time. Nat Rev Mol Cell Biol. 2008; 9:971–80. [PubMed: 19023284]
4. Lasko P. MRNA localization and translational control in *Drosophila* oogenesis. Cold Spring Harb Perspect Biol. 2012; 4:a012294. [PubMed: 22865893]
5. Long RM, Singer RH, Meng X, Gonzalez I, et al. Mating type switching in yeast controlled by asymmetric localization of ASH1 mRNA. Science. 1997; 277:383–7. [PubMed: 9219698]
6. Sutton MA, Schuman EM. Dendritic protein synthesis, synaptic plasticity, and memory. Cell. 2006; 127:49–58. [PubMed: 17018276]
7. Mili S, Moissoglu K, Macara IG. Genome-wide screen reveals APC-associated RNAs enriched in cell protrusions. Nature. 2008; 453:115–9. [PubMed: 18451862]
8. Kemphues KJ, Priess JR, Morton DG, Cheng NS. Identification of genes required for cytoplasmic localization in early C. elegans embryos. Cell. 1988; 52:311–20. [PubMed: 3345562]
9. Kemphues K. PARsing embryonic polarity. Cell. 2000; 101:345–8. [PubMed: 10830161]
10. Munro E, Bowerman B. Cellular symmetry breaking during Caenorhabditis elegans development. Cold Spring Harb Perspect Biol. 2009; 1:a003400. [PubMed: 20066102]
11. Goldstein B, Macara IG. The PAR proteins: fundamental players in animal cell polarization. Dev Cell. 2007; 13:609–22. [PubMed: 17981131]
12. Etemad-Moghadam B, Guo S, Kemphues KJ. Asymmetrically distributed PAR-3 protein contributes to cell polarity and spindle alignment in early C. elegans embryos. Cell. 1995; 83:743–52. [PubMed: 8521491]
13. Watts JL, Etemad-Moghadam B, Guo S, Boyd L, et al. Par-6, a gene involved in the establishment of asymmetry in early C. elegans embryos, mediates the asymmetric localization of PAR-3. Development. 1996; 122:3133–40. [PubMed: 8898226]
14. Tabuse Y, Izumi Y, Piano F, Kemphues KJ, et al. Atypical protein kinase C cooperates with PAR-3 to establish embryonic polarity in Caenorhabditis elegans. Development. 1998; 125:3607–14. [PubMed: 9716526]
15. Kuchinke U, Grawe F, Knust E. Control of spindle orientation in *Drosophila* by the Par-3-related PDZ-domain protein Bazooka. Curr Biol. 1998; 8:1357–65. [PubMed: 9889099]
16. Hung TJ, Kemphues KJ. PAR-6 is a conserved PDZ domain-containing protein that colocalizes with PAR-3 in Caenorhabditis elegans embryos. Development. 1999; 126:127–35. [PubMed: 9834192]

17. Shulman JM, Benton R, St Johnston D. The *Drosophila* homolog of C. elegans PAR-1 organizes the oocyte cytoskeleton and directs oskar mRNA localization to the posterior pole. Cell. 2000; 101:377–88. [PubMed: 10830165]
18. Nance J, Zallen JA. Elaborating polarity: PAR proteins and the cytoskeleton. Development. 2011; 138:799–809. [PubMed: 21303844]
19. Rodriguez-Boulan E, Macara IG. Organization and execution of the epithelial polarity programme. Nat Rev Mol Cell Biol. 2014; 15:225–42. [PubMed: 24651541]
20. Tepass U, Tanentzapf G, Ward R, Fehon R. Epithelial cell polarity and cell junctions in *Drosophila*. Annu Rev Genet. 2001; 35:747–84. [PubMed: 11700298]
21. Tepass U, Theres C, Knust E. Crumbs encodes an EGF-like protein expressed on apical membranes of *Drosophila* epithelial cells and required for organization of epithelia. Cell. 1990; 61:787–99. [PubMed: 2344615]
22. Bachmann A, Schneider M, Theilenberg E, Grawe F, et al. *Drosophila* Stardust is a partner of Crumbs in the control of epithelial cell polarity. Nature. 2001; 414:638–43. [PubMed: 11740560]
23. Hong Y, Stronach B, Perrimon N, Jan LY, et al. *Drosophila* Stardust interacts with Crumbs to control polarity of epithelia but not neuroblasts. Nature. 2001; 414:634–8. [PubMed: 11740559]
24. Bhat MA, Izaddoost S, Lu Y, Cho KO, et al. Discs lost, a novel multi-PDZ domain protein, establishes and maintains epithelial polarity. Cell. 1999; 96:833–45. [PubMed: 10102271]
25. Bilder D, Li M, Perrimon N. Cooperative regulation of cell polarity and growth by *Drosophila* tumor suppressors. Science. 2000; 289:113–6. [PubMed: 10884224]
26. Cowan CR, Hyman AA. Centrosomes direct cell polarity independently of microtubule assembly in C. elegans embryos. Nature. 2004; 431:92–6. [PubMed: 15343338]
27. Hird SN, White JG. Cortical and cytoplasmic flow polarity in early embryonic cells of Caenorhabditis elegans. J Cell Biol. 1993; 121:1343–55. [PubMed: 8509454]
28. Beatty A, Morton D, Kemphues K. The C. elegans homolog of *Drosophila* lethal giant larvae functions redundantly with PAR-2 to maintain polarity in the early embryo. Development. 2010; 137:3995–4004. [PubMed: 21041363]
29. Hoege C, Constantinescu AT, Schwager A, Goehring NW, et al. LGL can partition the cortex of one-cell Caenorhabditis elegans embryos into two domains. Curr Biol. 2010; 20:1296–303. [PubMed: 20579886]
30. Hao Y, Boyd L, Seydoux G. Stabilization of cell polarity by the C. elegans RING protein PAR-2. Dev Cell. 2006; 10:199–208. [PubMed: 16459299]
31. Hurov JB, Watkins JL, Piwnica-Worms H. Atypical PKC phosphorylates PAR-1 kinases to regulate localization and activity. Curr Biol. 2004; 14:736–41. [PubMed: 15084291]
32. Boyd L, Guo S, Levitan D, Stinchcomb DT, et al. PAR-2 is asymmetrically distributed and promotes association of P granules and PAR-1 with the cortex in C. elegans embryos. Development. 1996; 122:3075–84. [PubMed: 8898221]
33. Cuenca AA, Schetter A, Aceto D, Kemphues K, et al. Polarization of the C. elegans zygote proceeds via distinct establishment and maintenance phases. Development. 2003; 130:1255–65. [PubMed: 12588843]
34. Benton R, St Johnston D. *Drosophila* PAR-1 and 14-3-3 inhibit Bazooka/PAR-3 to establish complementary cortical domains in polarized cells. Cell. 2003; 115:691–704. [PubMed: 14675534]
35. Betschinger J, Mechtler K, Knoblich JA. The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl. Nature. 2003; 422:326–30. [PubMed: 12629552]
36. Tian AG, Deng WM. Lgl and its phosphorylation by aPKC regulate oocyte polarity formation in *Drosophila*. Development. 2008; 135:463–71. [PubMed: 18094021]
37. Morais-de-Sa E, Mukherjee A, Lowe N, St Johnston D. Slmb antagonises the aPKC/Par-6 complex to control oocyte and epithelial polarity. Development. 2014; 141:2984–92. [PubMed: 25053432]
38. Skwarek LC, Windler SL, de Vreede G, Rogers GC, et al. The F-box protein Slmb restricts the activity of aPKC to polarize epithelial cells. Development. 2014; 141:2978–83. [PubMed: 25053431]

39. Fletcher GC, Lucas EP, Brain R, Tournier A, et al. Positive feedback and mutual antagonism combine to polarize Crumbs in the *Drosophila* follicle cell epithelium. Curr Biol. 2012; 22:1116–22. [PubMed: 22658591]
40. McCaffrey LM, Macara IG. Widely conserved signaling pathways in the establishment of cell polarity. Cold Spring Harb Perspect Biol. 2009; 1:a001370. [PubMed: 20066082]
41. Nelson WJ. Adaptation of core mechanisms to generate cell polarity. Nature. 2003; 422:766–74. [PubMed: 12700771]
42. Jansen RP. MRNA localization: message on the move. Nat Rev Mol Cell Biol. 2001; 2:247–56. [PubMed: 11283722]
43. Mili S, Macara IG. RNA localization and polarity: from A(PC) to Z(BP). Trends Cell Biol. 2009; 19:156–64. [PubMed: 19251418]
44. Holt CE, Bullock SL. Subcellular mRNA localization in animal cells and why it matters. Science. 2009; 326:1212–6. [PubMed: 19965463]
45. Weil TT, Forrest KM, Gavis ER. Localization of bicoid mRNA in late oocytes is maintained by continual active transport. Dev Cell. 2006; 11:251–62. [PubMed: 16890164]
46. Forrest KM, Gavis ER. Live imaging of endogenous RNA reveals a diffusion and entrapment mechanism for nanos mRNA localization in *Drosophila*. Curr Biol. 2003; 13:1159–68. [PubMed: 12867026]
47. Ding D, Parkhurst SM, Halsell SR, Lipshitz HD. Dynamic Hsp83 RNA localization during *Drosophila* oogenesis and embryogenesis. Mol Cell Biol. 1993; 13:3773–81. [PubMed: 7684502]
48. Bashirullah A, Halsell SR, Cooperstock RL, Kloc M, et al. Joint action of two RNA degradation pathways controls the timing of maternal transcript elimination at the midblastula transition in *Drosophila* melanogaster. EMBO J. 1999; 18:2610–20. [PubMed: 10228172]
49. Kim-Ha J, Kerr K, Macdonald PM. Translational regulation of oskar mRNA by bruno, an ovarian RNA-binding protein, is essential. Cell. 1995; 81:403–12. [PubMed: 7736592]
50. Snee M, Benz D, Jen J, Macdonald PM. Two distinct domains of Bruno bind specifically to the oskar mRNA. RNA Biol. 2008; 5:1–9. [PubMed: 18388491]
51. Webster PJ, Liang L, Berg CA, Lasko P, et al. Translational repressor bruno plays multiple roles in development and is widely conserved. Genes Dev. 1997; 11:2510–21. [PubMed: 9334316]
52. Filardo P, Ephrussi A. Bruno regulates gurken during *Drosophila* oogenesis. Mech Dev. 2003; 120:289–97. [PubMed: 12591598]
53. Norvell A, Kelley RL, Wehr K, Schupbach T. Specific isoforms of squid, a *Drosophila* hnRNP, perform distinct roles in Gurken localization during oogenesis. Genes Dev. 1999; 13:864–76. [PubMed: 10197986]
54. Davis I, Ish-Horowicz D. Apical localization of pair-rule transcripts requires 3′ sequences and limits protein diffusion in the *Drosophila* blastoderm embryo. Cell. 1991; 67:927–40. [PubMed: 1959136]
55. Simmonds AJ, dosSantos G, Livne-Bar I, Krause HM. Apical localization of wingless transcripts is required for wingless signaling. Cell. 2001; 105:197–207. [PubMed: 11336670]
56. Wilkie GS, Davis I. *Drosophila* wingless and pair-rule transcripts localize apically by dynein-mediated transport of RNA particles. Cell. 2001; 105:209–19. [PubMed: 11336671]
57. Theurkauf WE, Smiley S, Wong ML, Alberts BM. Reorganization of the cytoskeleton during *Drosophila* oogenesis: implications for axis specification and intercellular transport. Development. 1992; 115:923–36. [PubMed: 1451668]
58. Roth S, Neuman-Silberberg FS, Barcelo G, Schupbach T. Cornichon and the EGF receptor signaling process are necessary for both anterior-posterior and dorsal-ventral pattern formation in *Drosophila*. Cell. 1995; 81:967–78. [PubMed: 7540118]
59. Gonzalez-Reyes A, Elliott H, St Johnston D. Polarization of both major body axes in *Drosophila* by gurken-torpedo signalling. Nature. 1995; 375:654–8. [PubMed: 7791898]
60. Neuman-Silberberg FS, Schupbach T. The *Drosophila* dorsoventral patterning gene gurken produces a dorsally localized RNA and encodes a TGF alpha-like protein. Cell. 1993; 75:165–74. [PubMed: 7691414]

61. Becalska AN, Gavis ER. Bazooka regulates microtubule organization and spatial restriction of germ plasm assembly in the *Drosophila* oocyte. Dev Biol. 2010; 340:528–38. [PubMed: 20152826]
62. Doerflinger H, Vogt N, Torres IL, Mirouse V, et al. Bazooka is required for polarisation of the *Drosophila* anterior-posterior axis. Development. 2010; 137:1765–73. [PubMed: 20430751]
63. Doerflinger H, Benton R, Torres IL, Zwart MF, et al. *Drosophila* anterior-posterior polarity requires actin-dependent PAR-1 recruitment to the oocyte posterior. Curr Biol. 2006; 16:1090–5. [PubMed: 16753562]
64. Macdonald PM, Struhl G. Cis-acting sequences responsible for anterior localization of bicoid mRNA in *Drosophila* embryos. Nature. 1988; 336:595–8. [PubMed: 3143913]
65. Driever W, Nusslein-Volhard C. The bicoid protein determines position in the *Drosophila* embryo in a concentration-dependent manner. Cell. 1988; 54:95–104. [PubMed: 3383245]
66. Gavis ER, Lehmann R. Localization of nanos RNA controls embryonic polarity. Cell. 1992; 71:301–13. [PubMed: 1423595]
67. Lantz V, Schedl P. Multiple cis-acting targeting sequences are required for orb mRNA localization during *Drosophila* oogenesis. Mol Cell Biol. 1994; 14:2235–42. [PubMed: 8139529]
68. Lantz V, Ambrosio L, Schedl P. The *Drosophila* orb gene is predicted to encode sex-specific germline RNA-binding proteins and has localized transcripts in ovaries and early embryos. Development. 1992; 115:75–88. [PubMed: 1638994]
69. Tan L, Chang JS, Costa A, Schedl P. An autoregulatory feedback loop directs the localized expression of the *Drosophila* CPEB protein Orb in the developing oocyte. Development. 2001; 128:1159–69. [PubMed: 11245581]
70. Lantz V, Chang JS, Horabin JI, Bopp D, et al. The *Drosophila* orb RNA-binding protein is required for the formation of the egg chamber and establishment of polarity. Genes Dev. 1994; 8:598–613. [PubMed: 7523244]
71. Christerson LB, McKearin DM. Orb is required for anteroposterior and dorsoventral patterning during *Drosophila* oogenesis. Genes Dev. 1994; 8:614–28. [PubMed: 7926753]
72. Chang JS, Tan L, Schedl P. The *Drosophila* CPEB homolog, orb, is required for oskar protein expression in oocytes. Dev Biol. 1999; 215:91–106. [PubMed: 10525352]
73. Castagnetti S, Ephrussi A. Orb and a long poly(A) tail are required for efficient oskar translation at the posterior pole of the *Drosophila* oocyte. Development. 2003; 130:835–43. [PubMed: 12538512]
74. Chang JS, Tan L, Wolf MR, Schedl P. Functioning of the *Drosophila* orb gene in gurken mRNA localization and translation. Development. 2001; 128:3169–77. [PubMed: 11688565]
75. Thio GL, Ray RP, Barcelo G, Schupbach T. Localization of gurken RNA in *Drosophila* oogenesis requires elements in the 5′ and 3′ regions of the transcript. Dev Biol. 2000; 221:435–46. [PubMed: 10790337]
76. Saunders C, Cohen RS. The role of oocyte transcription, the 5′UTR, and translation repression and derepression in *Drosophila* gurken mRNA and protein localization. Mol Cell. 1999; 3:43–54. [PubMed: 10024878]
77. Van De Bor V, Hartswood E, Jones C, Finnegan D, et al. Gurken and the I factor retrotransposon RNAs share common localization signals and machinery. Dev Cell. 2005; 9:51–62. [PubMed: 15992540]
78. Cox DN, Lu B, Sun TQ, Williams LT, et al. *Drosophila* par-1 is required for oocyte differentiation and microtubule organization. Curr Biol. 2001; 11:75–87. [PubMed: 11231123]
79. Ephrussi A, Dickinson LK, Lehmann R. Oskar organizes the germ plasm and directs localization of the posterior determinant nanos. Cell. 1991; 66:37–50. [PubMed: 2070417]
80. Zimyanin V, Lowe N, St Johnston D. An oskar-dependent positive feedback loop maintains the polarity of the *Drosophila* oocyte. Curr Biol. 2007; 17:353–9. [PubMed: 17275299]
81. Hengst U, Deglincerti A, Kim HJ, Jeon NL, et al. Axonal elongation triggered by stimulus-induced local translation of a polarity complex protein. Nat Cell Biol. 2009; 11:1024–30. [PubMed: 19620967]
82. Macara IG, Iioka H, Mili S. Axon growth-stimulus package includes local translation. Nature Cell Biol. 2009; 11:919–21. [PubMed: 19648976]

83. Gardiol A, St Johnston D. Staufen targets coracle mRNA to *Drosophila* neuromuscular junctions and regulates GluRIIA synaptic accumulation and bouton number. Dev Biol. 2014; 392:153–67. [PubMed: 24951879]
84. Speese SD, Ashley J, Jokhi V, Nunnari J, et al. Nuclear envelope budding enables large ribonucleoprotein particle export during synaptic Wnt signaling. Cell. 2012; 149:832–46. [PubMed: 22579286]
85. de Cuevas M, Lilly MA, Spradling AC. Germline cyst formation in *Drosophila*. Annu Rev Genet. 1997; 31:405–28. [PubMed: 9442902]
86. Fabian L, Brill JA. *Drosophila* spermiogenesis: big things come from little packages. Spermatogenesis. 2012; 2:197–212. [PubMed: 23087837]
87. Xu S, Tyagi S, Schedl P. Spermatid cyst polarization in *Drosophila* depends upon apkc and the CPEB family translational regulator orb2. PLoS Genet. 2014; 10:e1004380. [PubMed: 24830287]
88. Barreau C, Benson E, Gudmannsdottir E, Newton F, et al. Postmeiotic transcription in *Drosophila* testes. Development. 2008; 135:1897–902. [PubMed: 18434411]
89. Xu S, Hafer N, Agunwamba B, Schedl P. The CPEB protein Orb2 has multiple functions during spermatogenesis in *Drosophila* melanogaster. PLoS Genet. 2012; 8:e1003079. [PubMed: 23209437]
90. Fabian L, Wei HC, Rollins J, Noguchi T, et al. Phosphatidylinositol 4,5-bisphosphate directs spermatid cell polarity and exocyst localization in *Drosophila*. Mol Biol Cell. 2010; 21:1546–55. [PubMed: 20237161]
91. Somers WG, Chia W. Recycling polarity. Dev Cell. 2005; 9:312–3. [PubMed: 16139221]
92. Gonczy P, DiNardo S. The germ line regulates somatic cyst cell proliferation and fate during *Drosophila* spermatogenesis. Development. 1996; 122:2437–47. [PubMed: 8756289]
93. Gonczy P, Viswanathan S, DiNardo S. Probing spermatogenesis in *Drosophila* with P-element enhancer detectors. Development. 1992; 114:89–98. [PubMed: 1576968]
94. Sarkissian M, Mendez R, Richter JD. Progesterone and insulin stimulation of CPEB-dependent polyadenylation is regulated by Aurora A and glycogen synthase kinase-3. Genes Dev. 2004; 18:48–61. [PubMed: 14724178]
95. Tay J, Hodgman R, Sarkissian M, Richter JD. Regulated CPEB phosphorylation during meiotic progression suggests a mechanism for temporal control of maternal mRNA translation. Genes Dev. 2003; 17:1457–62. [PubMed: 12815066]
96. Wong LC, Costa A, McLeod I, Sarkeshik A, et al. The functioning of the *Drosophila* CPEB protein Orb is regulated by phosphorylation and requires casein kinase 2 activity. PLoS One. 2011; 6:e24355. [PubMed: 21949709]
97. Horne-Badovinac S, Bilder D. Dynein regulates epithelial polarity and the apical localization of stardust A mRNA. PLoS Genet. 2008; 4:e8. [PubMed: 18208331]
98. Li Z, Wang L, Hays TS, Cai Y. Dynein-mediated apical localization of crumbs transcripts is required for Crumbs activity in epithelial polarity. J Cell Biol. 2008; 180:31–8. [PubMed: 18195099]
99. Hachet O, Ephrussi A. Splicing of oskar RNA in the nucleus is coupled to its cytoplasmic localization. Nature. 2004; 428:959–63. [PubMed: 15118729]

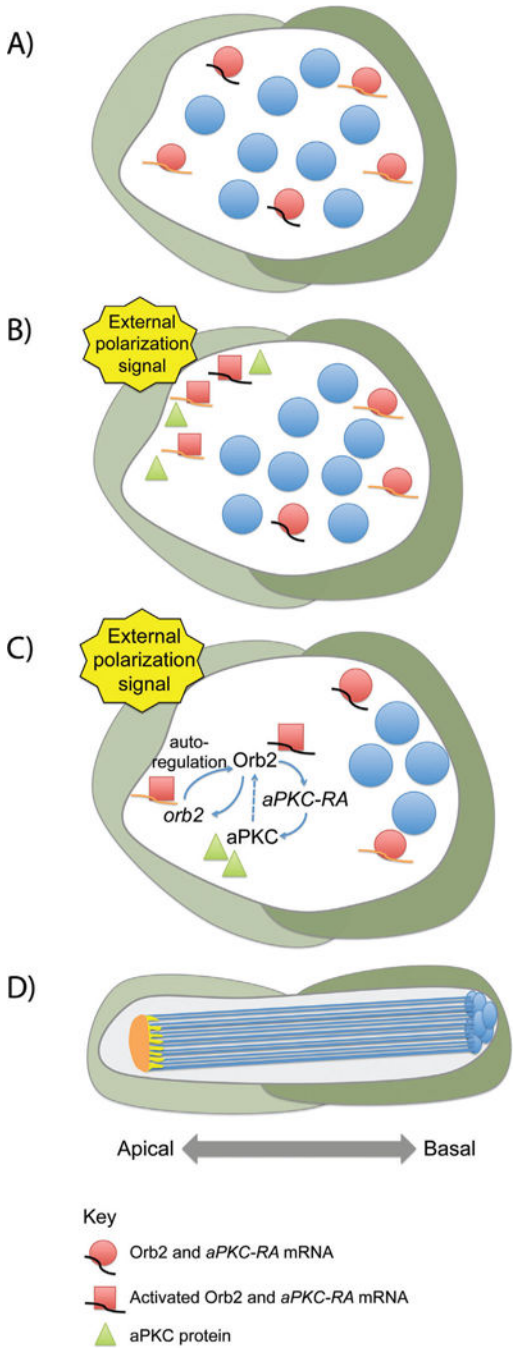

Figure 1.

The role of Orb2 dependent *aPKC-RA* translation in establishing spermatid cyst polarity. **A:** After meiosis is complete, 64 haploid nuclei (blue circles), Orb2 protein (red circles), *aPKC-RA*, and *orb2* mRNA (curvy lines) are equally distributed throughout the germline cyst. Orb2 protein binds to CPE sites in the 3′UTR of target transcripts, but activation of translation does not occur until Orb2 is activated phosphorylation. The somatic apical “tail” cystocyte (light green) is oriented apically, and the basal “head” cystocyte (dark green) is oriented basally. **B:** A nonautonomous signal, possibly originating from a somatic cystocyte

cell, initiates polarization of the germline cyst. The external signal converts Orb2 to an active state (red squares) on the apical side of the cyst, allowing for translation of *orb2* and *aPKC-RA* mRNAs. Activation of Orb2 initiates polarity establishment by local translation of *aPKC-RA* and accumulation of aPKC protein (green triangles) on the apical side of the cyst. **C:** The polarity decision is maintained by positive feedback loops. Orb2 autoregulates its own expression by activating translation of *orb2* mRNAs, and also promotes accumulation of aPKC protein on the apical side of the cyst. aPKC protein may directly phosphorylate Orb2 (dotted arrow) to keep Orb2 in an active state. Accumulation of aPKC on the apical side of the cyst may be instructive for apical localization of *aPKC-RA* and *orb2* mRNAs. **D:** The elongating spermatid cyst positions the haploid nuclei at the basal side of the cyst and the "comet" at the apical side of the cyst. The "comet head" (orange) is at the apical end of the elongating tails and contains localized mRNAs such as *aPKC-RA* and *orb2*. The "comet tails" (yellow) contain apically localized proteins such as Orb2 and aPKC.

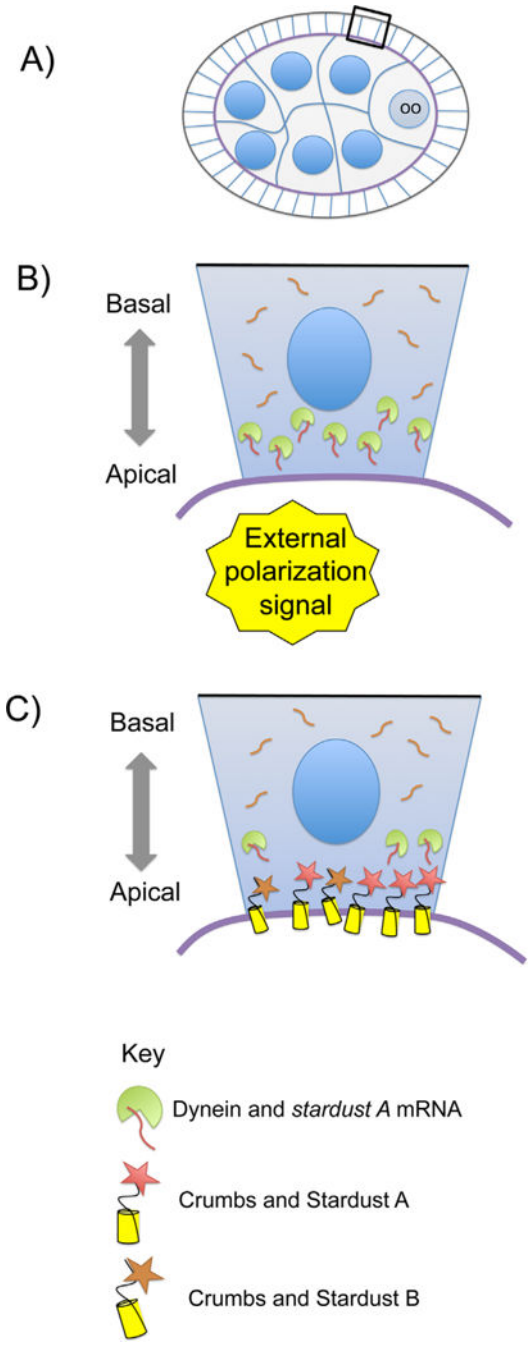

Figure 2.

The role of *stardust A* mRNA localization in follicle cell polarity establishment. **A:** In the *Drosophila* ovary, an egg chamber consists of 16 germline cells surrounded by a follicle epithelium. The oocyte (labeled “oo”) is positioned at the posterior of the germline cyst and contacts somatic follicle cells. The other 15 germline cells are nurse cells that support development of the oocyte. The apical surface of the follicle cells (purple) are in contact with the germline cyst cells. A follicle cell is outlined by the black box, and is the subject of **B** and **C. B:** In the young follicle cells a non-autonomous signal, possibly originating from

the germline, initiates polarization and *stardust A* mRNA (red) are transported by dynein (green) to the apical subregion. *stardust B* mRNA (orange) is not enriched apically. **C:** To maintain polarity, both Stardust A and Stardust B protein isoforms stabilize Crumbs (yellow) apically. This polarization may positively feedback on mRNA localization to further accumulate Stardust protein apically.