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mRNA Localization and Translational Control in *Drosophila* Oogenesis

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Localization of an mRNA species to a particular subcellular region can complement translational control mechanisms to produce a restricted spatial distribution of the protein it encodes. mRNA localization has been studied most in asymmetric cells such as budding yeast, early embryos, and neurons, but the process is likely to be more widespread. This article reviews the current state of knowledge about the mechanisms of mRNA localization and its functions in early embryonic development, focusing on *Drosophila* where the relevant knowledge is most advanced. Links between mRNA localization and translational control mechanisms also are examined.

Cell polarization requires proteins to be asymmetrically localized, which can be achieved by localizing specific mRNAs to particular regions of the cytoplasm so that their translation occurs only there mRNA localization is often inefficient, thus it is usually coupled to translational control mechanisms that repress translation of unlocalized mRNA while allowing translation of the localized mRNA to proceed. Genome-wide analysis of mRNA localization in early *Drosophila* embryos showed that the majority of mRNAs are asymmetrically distributed (Lécuyer et al. 2007; Tomancak et al. 2007).

The *Drosophila* oocyte is a valuable model system to study mRNA localization and translational control. In organisms such as *Drosophila* in which zygotic transcription does not commence until many nuclear or cellular divisions have occurred, translational control of maternally encoded mRNAs necessarily has a widespread role in regulating gene expression so that the initial stages of development can proceed. *Drosophila* oocytes develop within multicellular entities called egg chambers (King 1970). Each egg chamber contains a syncytium of 16 germ line cells (called cystocytes), which are connected by cytoplasmic bridges (ring canals). Only one cystocyte adopts an oocyte fate and completes meiosis while its siblings develop into polyploid nurse cells. The nurse cells are highly active in transcription and translation, and mRNAs and proteins expressed in those cells are transferred to the oocyte through the ring canals to the oocyte, whereas the oocyte nucleus is largely quiescent. Toward the end of oogenesis, the nurse cells expel their cytoplasm into the oocyte and afterward undergo apoptosis. The germ line cyst is surrounded by a single layer of follicle cells (the follicular epithelium), which not only secrete the eggshell but also play pivotal roles in signaling pathways that help establish oocyte polarity.

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FOUR mRNAs ESSENTIAL FOR EMBRYONIC PATTERN SPECIFICATION ARE LOCALIZED TO THREE CYTOPLASMIC REGIONS OF THE *DROSOPHILA* OOCYTE: ANTERIOR, POSTERIOR, AND ANTERODORSAL

The future embryonic body axes are specified during oogenesis, and mRNA localization and translational control are crucial for this (Bastock and St Johnston 2008; Kugler and Lasko 2009; Becalska and Gavis 2009). Four localized mRNAs, *oskar* (*osk*), *nanos* (*nos*), *bicoid* (*bcd*), and *gurken* (*grk*), are the key players in embryonic axis specification (Fig. 1), and for this reason their regulation has been especially well studied. The anterior–posterior axis is elaborated through localization of *bcd* mRNA to the anterior of the oocyte, and localization of *osk* and *nos* mRNAs to the posterior of the oocyte. In late-stage oocytes, *bcd* and *nos* are translationally repressed. This repression is relieved after fertilization, and the corresponding proteins are produced in opposing gradients that initiates a cascade of zygotic gene expression that directs anterior–posterior patterning. As will be discussed in more detail below, formation of the anterior-to-posterior Bcd gradient is primarily achieved through localization of its mRNA at the anterior pole, whereas formation of the posterior-to-anterior Nos gradient is achieved through translational repression of its mRNA by Bcd, and enrichment of its mRNA at the posterior where it is translationally active.

osk mRNA begins to be translated during mid-oogenesis to nucleate the formation of the pole plasm, a specialized cytoplasm at the

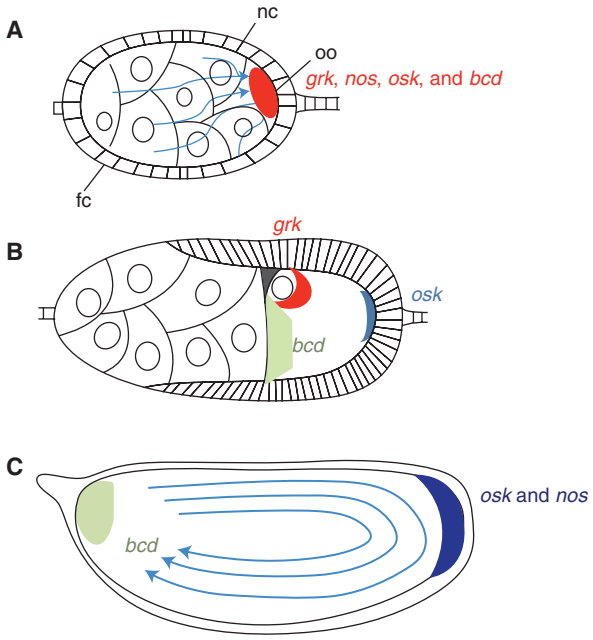

Figure 1. Localization of patterning mRNAs in *Drosophila* oogenesis. (*A*) In early oogenesis, several mRNAs, including *grk*, *nos*, *osk*, and *bcd*, are transported from the nurse cells through cytoplasmic bridges called ring canals into the oocyte. This involves minus-end directed transport along microtubules (blue arrows) mediated by the dynein motor complex. Abbreviations: nc, nurse cells, fc, follicle cells, oo, oocyte. (*B*) In mid-oogenesis, *osk* mRNA localizes to the posterior of the oocyte, *grk* mRNA localizes to the anterodorsal corner in close association with the oocyte nucleus, and *bcd* mRNA localizes to the anterior pole. (*C*) In late oogenesis, centrifugal cytoplasmic streaming (delineated by arrows) coupled with posterior anchoring brings about a further posterior enrichment of *osk* mRNA as well as posterior enrichment of *nos* mRNA. The distribution of *bcd* mRNA at the anterior pole is further refined.

posterior of the oocyte that contains large RNP complexes called polar granules that include posterior and germ cell determinants such as Nos, and which is therefore required in the embryo for posterior patterning and primordial germ cell specification. Like *nos*, *osk* mRNA localizes to the posterior pole where it is active, and is translationally silenced elsewhere. Grk, an epidermal growth factor receptor (EGFR) ligand, is crucial for the establishment of both the anterior–posterior and dorsal–ventral axes during oogenesis (González-Reyes et al. 1995; Roth et al. 1995). Grk is secreted from the oocyte to locally activate EGFR in adjacent follicle cells, and restricting its deployment enables it to specify spatial information. During early oogenesis, EGFR activation by Grk assigns posterior fate to a subpopulation of follicle cells that is essential for polarizing the oocyte and establishing anterior–posterior polarity. Later, Grk produced from localized mRNA at the anterodorsal corner of the oocyte specifies the dorsal–ventral axis by inducing dorsal fate in the follicle cells immediately adjacent.

CIS-ACTING ELEMENTS THAT ARE ESSENTIAL FOR mRNA LOCALIZATION USUALLY INCLUDE STEM-LOOP STRUCTURES

Transport of many mRNAs, including *grk*, *bcd,* and *osk*, from the nurse cells to the oocyte occurs prior to overall cytoplasmic transfer, and proceeds via minus end-directed transport on the microtubule cytoskeleton that is driven by the dynein motor complex (Clark et al. 2007). Two proteins, Egalitarian (Egl) and Bicaudal-D (Bic-D), working in concert are directly responsible for linking mRNAs to dynein and to microtubules (Fig. 2) (Navarro et al. 2004; Dienstbier et al. 2009). Although associating with the localization element of the mRNA to be transported, the amino-terminal region of Egl directly binds to the carboxyl-terminal domain (CTD) of Bic-D (Dienstbier et al. 2009), which in turn interacts with dynein through dynactin (Hoogenraad et al. 2003). Egl also interacts with dynein light chain. A structural study using NMR spectroscopy indicates that a stem-loop with two double-stranded RNA

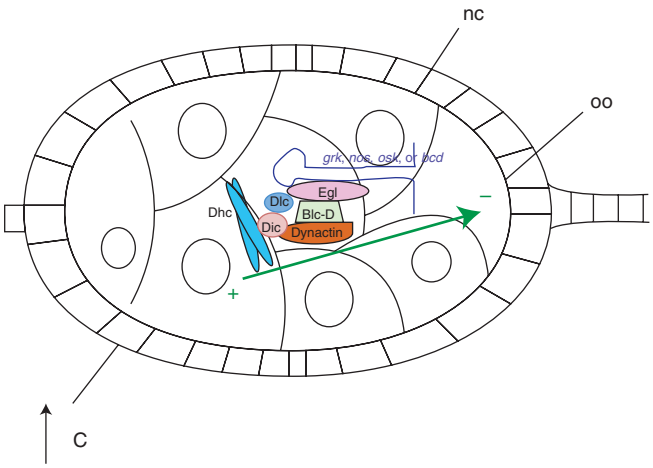

Figure 2. Model for linking mRNAs to the microtubule cytoskeleton for minus-end directed transport. Egalitarian (Egl) interacts directly with localization signals on mRNAs, with the carboxy-terminal end of Bicaudal-D (Bic-D), and with dynein light chain (Dlc). Bic-D interacts directly with dynactin, which in turn binds to dynein through its intermediate chain (Dic). Dynein heavy chain (Dhc) interacts with microtubules (green arrow) and catalyzes movement toward the minus-end. Although both in vivo and in vitro evidence exists to support this model for some instances of dynein-directed minus-end transport, and Egl and Bic-D are required for accumulation of *grk*, *nos*, *osk*, and *bcd* mRNAs into the oocyte, it has not yet been directly shown that this mechanism governs this particular localization event.

helices in an unusual A′-form conformation (Arnott et al. 1972) is a crucial recognition site for this complex (Bullock et al. 2010).

ANTERIOR TARGETING, ANCHORING, AND TRANSLATIONAL REGULATION OF *bcd*

Localization of *bcd* mRNA proceeds through several steps (St Johnston et al. 1989). The initial phase of *bcd* localization to the anterior cortex of the oocyte requires Exuperantia (Exu) protein (Berleth et al. 1988; Cha et al. 2001; Mische et al. 2007). Exu-containing ribonucleoprotein particles (RNPs) display dynamic movements that are very similar to those displayed by injected fluorescent *bcd* mRNA, and GFP-Exu is recruited to injected *bcd* mRNA (Theurkauf and Hazelrigg 1998; Wilhelm et al. 2000). Exu is phosphorylated by the Par-1 kinase, and this post-translational modification of Exu is important for anterior Exu and *bcd* mRNA localization (Riechmann and Ephrussi 2004).

Genetic evidence has implicated several additional proteins as involved in anchoring of *bcd* at the anterior cortex, including Staufen (Stau), an RNA binding protein, Swallow (Swa), the γ-tubulin ring complex components γTub37C, dGrip75 and dGrip128, and the microtubule-associated protein Mini Spindles (Msps) (Ferrandon et al. 1994; Schnorrer et al. 2002; Moon and Hazelrigg 2004; Vogt et al. 2006; Weil et al. 2006). However, the roles of some of the molecules may be indirect. Super-resolution microscopy has shown that Swa, which was once thought to link *bcd* mRNA containing RNPs to the dynein motor complex, actually does not precisely colocalize in the same particles as *bcd* (Weil et al. 2010). Rather, Swa appears to regulate the actin cytoskeleton, which in turn could be essential for anchoring *bcd* mRNA. Unlike Swa, Stau is found in the same particles as *bcd* and appears to be directly involved in recruiting it to the dynein motor (Weil et al. 2010). *bcd* mRNA anchoring also requires the ESCRT-II complex. The three conserved ESCRT complexes (ESCRT-I, -II, and -III) collaborate to mediate endosomal sorting; only ESCRT-II is required for anterior *bcd* mRNA localization, however, suggesting that a different mechanism is involved (Irion and St Johnston 2007). One subunit of ESCRT-II, VPS36, binds directly to sequences in the *bcd* 3′UTR and localizes to the anterior of the oocyte in a *bcd* mRNA-dependent but *stau*-independent manner, indicating that ESCRT-II acts upstream of Stau in the *bcd* localization pathway. A recent genetic screen has identified *short stop*, which encodes a spectroplakin protein that binds both actin and microtubules, and *Su(Mir)2*, whose identity is unknown, as encoding other potential factors involved in *bcd* anchoring (Chang et al. 2011).

FORMATION OF THE ANTERIOR–POSTERIOR BICOID PROTEIN GRADIENT FROM THE LOCALIZED *bcd* mRNA

As mentioned above, in the early *Drosophila* embryo, an anterior-to-posterior gradient of Bcd protein is established from its anteriorly localized mRNA. Bcd is a transcription factor, acting as a graded morphogen that influences developmental decisions in a concentration-dependent manner (Driever and Nüsslein-Volhard 1988). As nuclei migrate during syncytial divisions into different regions of the embryo, they activate expression of various sets of patterning genes based on the concentration of Bcd they encounter, and thus on their position along the anterior–posterior axis.

Despite indications that this classic model of Bcd function is insufficient to explain results observed when the Bcd gradient is physically perturbed, flattened, or abolished (Lucchetta et al. 2008; Löhr et al. 2009; Ochoa-Espinosa et al. 2009), it remains clear that Bcd is an important morphogen and the characteristics of its graded distribution need to be carefully controlled. Substantial attention has therefore been given in recent years as to how exactly the Bcd gradient is generated, taking into account the physical properties of the embryonic cytoplasm and the diffusion characteristics of *bcd* mRNA and Bcd protein. Initial attempts at modeling the Bcd gradient considered the mRNA as a point source and postulated that protein diffusion was the dominant means in which the gradient was produced (Houchmandzadeh et al. 2002; Gregor et al. 2007a,b), along with a constant

amount of degradation. This model, however, does not fully agree with measured diffusion constants that predict a shorter length scale for the Bcd gradient than observed (Gregor et al. 2007b). Subsequently, it was realized that formation of the *bcd* mRNA gradient that presages the protein gradient is critical for establishing the latter (Spirov et al. 2009). Quantitative measurement of *bcd* mRNA and Bcd-GFP protein in real time indicates that the mRNA distribution is more tightly restricted to the anterior than the protein, implying that protein movement from the graded mRNA distribution makes an essential contribution to producing the protein gradient (Little et al. 2011). The recent discovery that Fates-shifted, a ubiquitin ligase substrate specificity receptor that targets Bcd for degradation, is required for formation of a normal Bcd gradient and for correct anterior–posterior patterning, makes it evident that regulation of Bcd protein stability is an important aspect of how the gradient is produced (Liu and Ma 2011).

Translational control appears not to be involved in establishing the Bcd gradient, but it is involved in temporal regulation because localized *bcd* mRNA is apparent from mid-oogenesis when Bcd protein is not detectable. Mutations in *pumilio* (*pum*), which encodes an RNA binding protein, or deletion of a consensus Pum binding site in the *bcd* 3′ UTR leads to increased Bcd expression during embryogenesis (Gamberi et al. 2002), but it is unknown whether this mechanism mediates translational repression during oogenesis.

TARGETING *osk* AND *nos* mRNAs TO THE POSTERIOR POLE PLASM

Both *osk* and *nos* are enriched at the posterior pole of the oocyte in a region termed the pole plasm, and their translation within the oocyte and syncytial embryo is restricted to that region.

osk Localization Is Microtubule-Dependent but Anchoring Requires F-Actin

As discussed earlier for *bcd*, initial loading of *osk* into the oocyte also proceeds through microtubule-dependent motor driven transport, and like *bcd*, *osk* is initially transported via a minus-end directed dynein-mediated process. Beginning in mid-oogenesis, *osk* begins to accumulate in the posterior of the oocyte, and this localization is an essential first step for pole plasm assembly.

Localization of *osk* mRNA to the pole plasm requires *cis*-acting elements in its 3′ UTR and nuclear imprinting of unspliced *osk* with exon junction complex components (Mago Nashi, Y14, eIF4AIII) and Hrp48 (Hachet and Ephrussi 2004; Huynh et al. 2004; Palacios et al. 2004; Yano et al. 2004). Although splicing of the first intron of *osk* pre-mRNA is essential for its localization, reporter mRNAs lacking introns but containing *osk* 3′ UTR elements can localize via RNA:RNA dimerization with imprinted endogenous *osk*, even if that endogenous *osk* cannot be translated (Jambor et al. 2011). *osk* localization also requires a specific association with Stau, a RNA binding protein that interacts with certain stem-loop structures in the 3′ UTR (Micklem et al. 2000). Posterior localization of *osk* is microtubule dependent, but unlike the earlier phase, it is driven by the plus-end directed motor kinesin. Real-time analysis of the movements of individual *osk*-containing particles shows they are not highly directed, and that posterior enrichment is accomplished through a collection of random walks that is slightly biased toward the posterior, reflecting a similar weak enrichment of microtubule plus-ends at the oocyte posterior (Zimyanin et al. 2008).

A later stage of *osk* localization takes advantage of rapid movements of the oocyte cytoplasm that occur in later oogenesis and involves anchoring of the mRNA in the posterior pole plasm (Sinsimer et al. 2011). Anchoring *osk*-containing mRNPs at the posterior requires specifically the longer of two Osk protein isoforms, the endocytic pathway, and rearrangements of the F-actin cytoskeleton (Vanzo et al. 2007; Tanaka et al. 2011). Actomyosin-based transport is implicated in short-range movements that sharpen the polarization of *osk* mRNA distribution at the posterior pole (Krauss et al. 2009). Osk itself induces the formation of long F-actin projections from the posterior cortex into the pole plasm, corroborating the link

between *osk* anchoring and the actin cytoskeleton (Babu et al. 2004).

Posterior Accumulation of *nos* Is Inefficient and Proceeds as a Consequence of Cytoplasmic Streaming

nos mRNA also specifically accumulates in the posterior pole plasm, but its localization is inefficient, with only an approximate 4% enrichment in the posterior half of early embryos (Bergsten and Gavis 1999). Translational repression is therefore the primary mechanism for excluding Nos outside the posterior. In fact, localization of *nos* mRNA is dispensable for somatic patterning, although it is required for germ cell development (Gavis et al. 2008). *nos* mRNA moves throughout the oocyte during a period of rapid cytoplasmic streaming that commences in mid-oogenesis, and gradually accumulates in the pole plasm through an anchoring mechanism (Forrest and Gavis 2003; Weil et al. 2006). Rumpelstiltskin (Rump), an hnRNP M homolog, binds to one of several 3′ UTR elements involved in *nos* mRNA localization and acts directly in its localization (Jain and Gavis 2008). Recently, mutations in *aubergine* (*aub*) were shown to affect *nos* localization, and Aub protein can be copurified with the *nos* 3′ UTR and with Rump (Becalska et al. 2011). Although Aub has been implicated in silencing of retrotransposons in the germline, its function in *nos* localization appears unrelated to this, as mutations in other genes involved in retrotransposon silencing do not have a similar effect on *nos*.

grk mRNA LOCALIZATION IS A MICROTUBULE-DEPENDENT PROCESS

grk mRNA, though mostly transcribed in the nurse cells, accumulates in the oocyte and colocalizes with the oocyte nucleus throughout much of oogenesis. In early oogenesis the oocyte nucleus is located at the posterior, and *grk* mRNA accumulates there. Later, when the oocyte nucleus moves to an anterodorsal position, *grk* mRNA forms a crescent between the apical surface of the nucleus and the neighboring region of the cortex. Transcription of *grk* from the oocyte nucleus is not essential for this, because a similar distribution is observed in mosaic egg chambers in which the oocyte nucleus is homozygous for an RNA-null *grk* allele (Caceres and Nilson 2005). Further, *grk* transcription from the oocyte nucleus is not required for patterning, as dorsal follicle cell fates and the dorsal-ventral embryonic axis are specified in these mosaics.

Initial transport of *grk* mRNA from the nurse cells to the oocyte uses the dynein and Bic-D/Egl dependent pathway described above for *bcd* and *osk*. *grk* first accumulates along the anterior cortex, then it is transported laterally toward the oocyte nucleus (MacDougall et al. 2003; Jaramillo et al. 2008). This second phase of *grk* transport also depends on dynein and the microtubule cytoskeleton, and the oocyte nucleus appears to nucleate a distinct population of microtubules, which are thought to mediate lateral displacement (MacDougall et al. 2003; Januschke et al. 2006; Delanoue et al. 2007). There is some controversy about the nature of the *cis*-acting elements that are essential for *grk* localization. Studies of injected fluorescently-tagged *grk* mRNA implicated an element within the protein-coding region, termed the *grk* localization signal (GLS) as essential for both oocyte targeting and anterodorsal localization (Van De Bor et al. 2005). However, an analysis of localization of RNA produced from a series of modified *grk* transgenes indicates that the GLS is not sufficient for anterodorsal accumulation and that another element must be involved (Lan et al. 2010).

grk mRNA Anchoring also Requires Microtubules and Dynein

Microtubules and Dhc are required not only for *grk* mRNA transport but also for *grk* anchoring (Delanoue et al. 2007). How dynein switches from a dynamic to a static mode is not fully understood, but it clearly involves the activity of *squid* (*sqd*), which encodes an hnRNP, and perhaps *K10*, as mutations in either of those two genes abrogate stable *grk* accumulation at the anterodorsal corner (Jaramillo et al. 2008; Lan et al. 2010).

PROTECTION FROM MATERNAL DEGRADATION CAN RESULT IN GERM CELL ACCUMULATION OF SPECIFIC mRNAs

Another mechanism that can lead to asymmetric distribution of mRNAs in the early embryo involves protection from RNA degradation. This was first established as a mechanism for enrichment of *Hsp83* mRNA in the primordial germ cells (Bashirullah et al. 1999). Many maternally-expressed mRNAs are degraded at the maternal-to-zygotic transition, through the mediation of Smaug (Smg), a sequence-specific RNA binding protein that recruits the CCR4 deadenylase complex whose translation is drastically up-regulated on egg activation (Semotok et al. 2005; Tadros et al. 2007). Smg is also required for zygotic expression of the *miR-309* cluster microRNAs, that mediate destabilization of a large set of maternal mRNAs (Bushati et al. 2008; Benoit et al. 2009). Degradation of unlocalized *nos* mRNA by Smg also involves recruitment of two transposon-encoded piwi-associated RNAs (piRNAs) that are complementary to sequences in the *nos* 3′ UTR (Rouget et al. 2010). Pumilio, another RNA-binding protein that can recruit the CCR4 deadenylase complex, has also been implicated in maternal transcript destabilization (Gerber et al. 2006 and see below). As primordial germ cells remain transcriptionally silent throughout early embryogenesis, maternal mRNAs whose degradation involves the action of zygotically transcribed molecules such as the *miR-309* cluster may be preferentially stabilized in those cells (Walser and Lipshitz 2011).

TRANSLATIONAL CONTROL OF *osk* IS ELABORATE

osk mRNA is translated into two different isoforms, called Long Osk and Short Osk, that are expressed from different initiation codons in the *osk* mRNA (Markussen et al. 1995). Short Osk is sufficient to induce the accumulation of all other pole plasm components and to rescue the functions of *osk* in posterior patterning and germ cell specification, whereas Long Osk induces F-actin projections that are required for anchoring its mRNA at the posterior pole (Vanzo et al. 2007; Tanaka and Nakamura 2008).

In early oogenesis *osk* translation is repressed by RNA interference (RNAi), as mutations in several genes (including *armitage, aubergine, cutoff, maelstrom, spindle-E, zucchini*, and *squash*) involved in piRNA processes cause precocious *osk* translation in early oocytes (Findley et al. 2003; Cook et al. 2004; Tomari et al. 2004; Chen et al. 2007; Lim and Kai 2007; Pane et al. 2007). However, the axis patterning defects also observed in these mutants appear not to result directly from *osk* overexpression, but rather from defects in microtubule organization resulting from inappropriate activation of DNA damage signaling (Klattenhoff et al. 2007). As *osk*-containing mRNPs begin to localize to the oocyte posterior, translation is blocked through a different mechanism, operating at the level of ribosome recruitment, by Cup, an eIF4E-binding protein that can interfere with the eIF4E-eIF4G interaction (Nakamura et al. 2004). Cup is recruited to *osk* by Bruno (Bru), an RNA binding protein with three RNA recognition motifs (RRMs). Through all three RRMs, Bru interacts directly with specific sequences (Bru-response elements, or BREs) in the *osk* 3′ UTR, and represses its translation (Snee et al. 2008). Surprisingly, however, recent evidence indicates that, although Cup indeed induces translational repression, this does not require its eIF4E-binding activity and thus does not involve competition for eIF4G (Igreja and Izaurralde 2011; Jeske et al. 2011). Rather, Cup recruits the CCR4 deadenylase complex to its target mRNAs and reduces *osk* poly(A) tail length. Cup-associated mRNAs are not subsequently degraded, however, as they are protected by an amino-terminal regulatory domain of Cup through a mechanism that prevents decapping and requires one of its two eIF4E binding motifs.

Bru also represses translation in another manner, by packaging *osk* mRNA into heavy particles that render it inaccessible to the translational machinery (Chekulaeva et al. 2006). Further insight into the nature of silencing complexes came from a study of polypyrimidine tract binding protein (PTB), which is required for translational repression of *osk* during early

oogenesis (Besse et al. 2009). PTB binds with high affinity and cooperativity to the *osk* 3′ UTR, at several pyrimidine-rich sites, and catalyzes oligomerization of multiple *osk* mRNA molecules through bridging interactions.

Although mutation of BREs generally results in precocious *osk* translation, when endogenous *osk* mRNA is totally absent, translation from an *osk* transgene that lacks the distal pair of BREs but is otherwise complete (*osk* C^-) is reduced. This was surprising as the opposite result would be expected from removing the BREs which were believed to be strictly repressor elements. In a genetic background in which *osk* mRNA with an intact 3′ UTR but an early stop codon is also expressed, *osk* C^- is translated at a higher level. This implies that the distal pair of BREs is bifunctional, operating in different contexts as a repressor or an activator element. Further, these results indicate that the presence of *osk* mRNA with an intact 3′ UTR in *osk* mRNPs can facilitate activation of *osk* C^- translation in trans, illustrating that mRNA molecules in the same RNP are coordinately regulated (Reveal et al. 2010).

Hrp48, an abundant RNA-binding protein that interacts with elements in both the 5′ and 3′ UTRs of *osk*, is essential for *osk* localization and also contributes to its translational regulation (Huynh et al. 2004; Yano et al. 2004; Norvell et al. 2005). Live imaging of *osk* in *hrp48* mutant ovaries implicate *hrp48* in assembling *osk* into cytoplasmic particles (Mhlanga et al. 2009) and for its subsequent association with Staufen, a translational activator of *osk* (Kim-Ha et al. 1995; Micklem et al. 2000; Braat et al. 2004; Mhlanga et al. 2009). Glorund, an hnRNP F/H family member, also associates with Hrp48 and may be another component of these particles (Kalifa et al. 2009). Another RNA binding protein, Bicaudal-C (Bic-C), has been implicated genetically as a negative regulator of *osk* translation (Saffman et al. 1998). Bic-C directly recruits the CCR4 deadenylase complex to target mRNAs through an association with its NOT3/5 subunit (Chicoine et al. 2007). These targets could potentially include *osk*.

Later in oogenesis, *osk* repression is alleviated, and translation activated, for the small proportion of *osk* RNA that is localized to the pole plasm. A key activator of *osk* translation is Orb, the *Drosophila* homolog of *Xenopus* cytoplasmic polyadenylation element binding protein (CPEB). Orb directly associates with two poly(A) polymerases, PAP and Wispy (Wisp). PAP is required during mid-oogenesis to promote Osk expression, whereas Wisp functions only during late oogenesis and in the early embryo (Benoit et al. 2009). An RNA binding protein that promotes CCR4-mediated deadenylation, Bicaudal-C, interacts with Orb, PAP, and Wisp, and possibly inhibits their association with target mRNAs (Castagnetti and Ephrussi 2003; Chicoine et al. 2007; Cui et al. 2008; Benoit et al. 2009).

nos TRANSLATION IS ALSO HIGHLY REGULATED

Nos protein is restricted to the posterior germ plasm by RNA localization and by translational repression of *nos* mRNA outside that region. *nos* regulation is mediated by a 90 nt region of the 3′ UTR, termed the translational control element (TCE) (Crucs et al. 2000; Forrest et al. 2004). The TCE forms a complex secondary structure, and mutations that disrupt any portion of this structure prevent the binding of repressors of *nos* and render the entire element inactive (Forrest et al. 2004). Different parts of the TCE interact with different trans-acting factors at different developmental stages to ensure translational repression of unlocalized *nos* mRNA. During late oogenesis, repression is mediated by Glorund (Glo), an hnRNP F/H ortholog that binds to the stem of stem-loop III of the TCE (Kalifa et al. 2006). Another part of the TCE, the loop of stem-loop II, contains a Smaug Recognition Element (SRE), the binding site for Smg, which represses *nos* in early embryogenesis outside the pole plasm. Smg interacts with Cup, an eIF4E-binding protein that was discussed above in the context of *osk* regulation. The Cup-Smg interaction is required for Smg-mediated repression of SRE-containing mRNAs in embryo extracts (Nelson et al. 2004). Smg also interacts directly with the POP2 subunit of the CCR4 deadenylase complex, recruiting it to a

large set of maternal mRNAs in the early embryo, including *nos*, and targeting them for decay (Semotok et al. 2005; Zaessinger et al. 2006; Tadros et al. 2007). Thus, *nos* mRNA is repressed in two distinct ways by Smg: by cap-dependent translational repression and by deadenylation of the silenced transcript (Fig. 3). Osk relieves Smg/CCR4-dependent deadenylation of *nos*, thus enabling its translation in the pole plasm (Zaessinger et al. 2006). Consistent with this, both the 5′ cap structure and the presence of a poly(A) tail are required for TCE-mediated repression of a reporter construct in cell-free extracts prepared from ovaries, although the poly(A) tail does not affect repression in similar extracts prepared from early embryos (Andrews et al. 2011). Mutational analysis suggests that Glo is required for both the cap-dependent and poly(A)-dependent types of repression, although the mechanisms for its function remain unclear.

Translational regulation of ten other mRNAs that localize to the pole plasm at a similar developmental stage as *nos* was compared with the regulation of *nos* itself (Rangan et al. 2009). In all cases, the 3′ UTRs were sufficient to drive posterior localization and temporally restricted patterns of translation of the mRNAs. Often translational activation correlated with an increase in poly(A) tail length, but surprisingly for at least two of the mRNAs (*pgc* and *gcl*), reduction of *orb* activity had little effect on translation. Consistent with other results, this may indicate that it is more critical to regulate

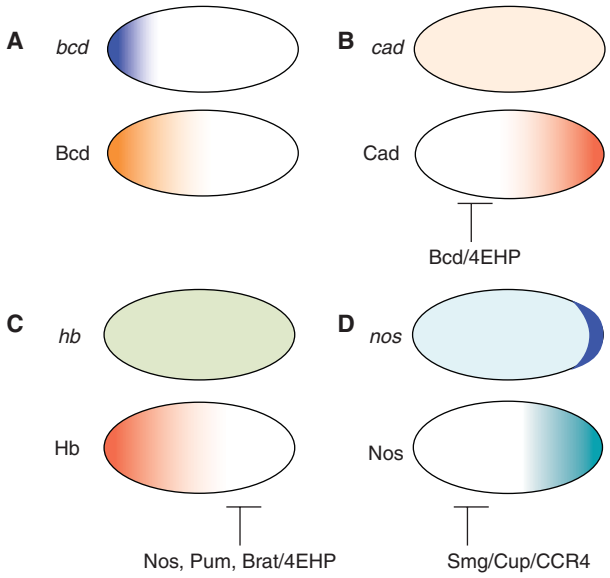

Figure 3. Mechanisms of establishing protein gradients in the early embryo prior to the onset of zygotic transcription. (*A*) Maternally expressed *bcd* mRNA (*top* panel) is localized in a steep gradient at the anterior pole. Bcd protein (*lower* panel) is translated from that localized mRNA and diffuses toward the posterior. (*B*) Maternally-expressed *cad* mRNA (*top* panel) is uniformly distributed. Translation of *cad* mRNA is, however, repressed by Bcd-mediated recruitment of 4EHP, resulting in a posterior-to-anterior gradient of Cad protein (*lower* panel) that is a mirror image of the Bcd gradient. (*C*) Maternally expressed *hb* mRNA (*top* panel) is uniformly distributed. Translation of *hb* mRNA is repressed by a complex of Nos, Pum, and Brat, that recruits 4EHP and probably other negative regulators to restrict Hb protein (*lower* panel) to the anterior half of the embryo. (*D*) Maternally expressed *nos* mRNA (*top* panel) is enriched at the posterior pole but present elsewhere. Translation of *nos* outside the posterior is repressed by Smg, which can recruit the repressor protein Cup and also the CCR4 deadenylase complex. Unlocalized *nos* is also targeted by piRNAs (not shown). Nos protein (lower panel) is translated from posteriorly-localized *nos* that is protected from degradation and repression.

deadenylation rather than polyadenylation in regulating translation.

4EHP, AN ALTERNATE CAP-BINDING PROTEIN, REPRESSES TRANSLATION OF *CAUDAL* AND *HUNCHBACK* mRNAs

Caudal (Cad), a transcription factor, is a master regulator of posterior patterning in many insects (Olesnicky et al. 2006). In *Drosophila* maternally expressed Cad forms a posterior-to-anterior gradient in the early embryo, and although its function is somewhat redundant with other regulatory factors it is nevertheless involved in activating posterior-specific zygotic genes (Schulz and Tautz 1995). In a role different from its function as a transcription factor, Bcd represses translation of *cad* mRNA, thus producing a gradient of Cad protein that is the mirror image of the Bcd gradient (Fig. 3) (Dubnau and Struhl 1996, Rivera-Pomar et al. 1996). Bcd binds to the 3′ UTR and recruits 4E homology protein (4EHP), an eIF4E-related cap binding protein that cannot bind eIF4G (Hernández et al. 2005), and thus cannot nucleate assembly of an active cap-binding complex (Cho et al. 2005). Females homozygous for a *4EHP* allele produce embryos with anterior defects, like those produced by *bcd* mutants, that fail to repress *cad* translation in the anterior. These phenotypes could be rescued by *4EHP* transgenic constructs, but not by constructs producing mutant forms of 4EHP that were abrogated for binding to the structure or to Bcd. Similarly, transgenically produced forms of Bcd that were abrogated for 4EHP binding could not repress *cad* translation. These results showed that a complex of 4EHP and Bcd, interacting with the cap structure and the 3′ UTR, respectively, circularizes *cad* mRNA and renders it translationally inactive.

4EHP was later shown to be involved in repression of *hb* mRNA in the posterior of the embryo (Fig. 3) (Cho et al. 2006), which had long been known to involve formation of a complex including Nos, Pum, Brain Tumor (Brat, an NHL-domain containing protein), and an element in the *hb* 3′ UTR called the Nanos-response element (NRE) (Sonoda and Wharton 2001). In the case of *hb*, 4EHP is recruited to the 5′ cap structure through an interaction with the NHL domain of Brat. As the binding sites for 4EHP on Bcd and Brat are not similar in sequence, and the latter does not resemble an eIF4E binding motif, it is possible that the interaction between 4EHP and Brat is indirect.

The translational repressor Pum is involved in many cellular and developmental processes in *Drosophila* other than embryonic patterning, including restriction of Cyclin B expression to the germline (Kadyrova et al. 2007), regulation of sodium current in motoneurons (Muraro et al. 2008), regulation of presynaptic morphology (Menon et al. 2004), regulation of dendrite morphogenesis in peripheral neurons (Ye et al. 2004), and maintenance of germline stem cell self-renewal (Gilboa and Lehmann 2004; Wang and Lin 2004; Szakmary et al. 2005; Li et al. 2009; Kim et al. 2010). Pum binds to a consensus sequence UGUANAUA (Gerber et al. 2006) and frequently operates in a complex with Nos. Often, Pum repression is independent of the cap structure and 4EHP, involving instead CCR4-mediated deadenylation of its target mRNAs (Wreden et al. 1997; Gamberi et al. 2002; Goldstrohm et al. 2006). A recent study provides evidence that Nos is not always required for Pum activity, and that regions outside the Pum C-terminal domain that binds Nos and Brat possess substantial translational repressor activity (Weidmann and Goldstrohm 2012).

VASA (VAS) IS A TRANSLATIONAL ACTIVATOR OF SPECIFIC GERM-LINE mRNAs

Activators of translation of specific mRNAs have not been identified as frequently as repressors, and less is known about their function. One translational activator that is involved in *Drosophila* embryonic development is Vas, a DEAD-box type RNA helicase. Complete loss of Vas blocks oogenesis and results in female sterility, whereas females homozygous for weaker *vas* alleles produce embryos that lack a germ line and posterior somatic segments. Vas binds to eIF5B, a translation factor that functions in ribosomal subunit joining, and mutations in *vas* and *eIF5B*

genetically interact (Carrera et al. 2000). Severe *vas* mutations strongly reduce Grk accumulation in the oocyte (Styhler et al. 1998; Tomancak et al. 1998). A mutant form of Vas (VasΔ617) was generated whose ability to bind eIF5B was reduced 10-fold in yeast two-hybrid assays (Johnstone and Lasko 2004). Oocytes that express only VasΔ617 fail to accumulate normal levels of Grk, suggesting that Vas activates *grk* translation through an interaction with eIF5B. Vas has also been shown to bind specifically to a U-rich motif present in the 3′ UTR of another mRNA, *mei-P26*, and to positively regulate its translation in germ cells through that interaction (Liu et al. 2009). Again, the VasΔ617 mutation abrogates the effect.

Like many of the RNA binding proteins discussed in this review, Vas appears to have more than one function. It is a component of nuage, organelles that are involved in piRNA-mediated transposon silencing, and it has recently been implicated in regulating mitotic chromosome condensation in the *Drosophila* germline (Pek and Kai 2011). Dependent on the activities of *aub* and *spn-E*, two piRNA pathway genes, Vas accumulates in perichromosomal foci during mitosis, and facilitates the recruitment of Barren, which in turn is required for correct chromosome condensation and segregation. This function of Vas appears to be independent of translation, as the VasΔ617 mutant form operates normally in this regard.

FUTURE DIRECTIONS

In this field, some simple models have given way to more complicated ones over the past several years, and this trend will likely continue, because more extensive analysis has in many cases revealed novel unexpected activities for proteins and regulatory elements that were thought to be fully understood. Cup, the BREs, and perhaps Pum are just some examples of this. On first glance it seems perhaps illogical that many molecules involved in mRNA localization and translational control cannot be assigned a unitary function. What must be remembered, however, is that mRNAs and the proteins that regulate them are contained within a heterogeneous and highly dynamic set of RNPs, and that a given mRNA or protein might be a component of many different species of RNP at different developmental times or in different cellular or spatial positions. If we consider that the RNPs, not individual molecules, are the real functional units in translational regulation, manipulation of a single gene that encodes a single RNP component might actually disrupt many different species of RNP, and thus lead to multiple effects. Advances in quantitative imaging and proteomics (see, for instance, Slobodin and Gerst 2011) will likely make it possible to more fully characterize the panoply of RNPs that are involved in mRNA localization and translational control in *Drosophila* oocytes in upcoming years, which will be a critical step forward in developing a deeper understanding of these processes.

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