Chapter 279

Translational Control in Invertebrate Development

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INTRODUCTION

Extensive study of invertebrate model organisms, most notably *Drosophila*, has shown that translational regulation allows for precise control of spatial and temporal gene expression patterns. Expression from specific mRNAs is controlled by mechanisms that target multiple components of the translational machinery, promote the degradation of silenced transcripts, or sequester them into large RNPs. This chapter will address several models of translational control that are presently emerging from this field.

TRANSLATIONAL CONTROL TARGETS OSKAR TO THE POLE PLASM

Positional information established in the developing *Drosophila* oocyte defines the spatial axes of the embryo and of the adult fly. Many proteins required for posterior patterning and germ cell specification accumulate as mRNAs in a specialized cytoplasm, called pole plasm, at the posterior pole of the egg. Their translation is active within the pole plasm, but repressed throughout the rest of the oocyte and embryo [1–3].

oskar (*osk*) is required for specification of posterior soma and germ line, and its mRNA is under complex translational control (Figure 279.1). *osk* mRNA localizes to the pole plasm where it is translationally active. Bruno (Bru), an RRM-type RNA binding protein, represses translation of unlocalized *osk* by binding to *cis*-elements called Bruno response elements (BREs), of which three pairs are present in the *osk* 3'UTR. Bru negatively regulates *osk* translation in the oocyte through two distinct mechanisms: first by blocking recognition of the 5' cap structure by recruiting Cup, an eIF4E binding protein that competitively inhibits recruitment of eIF4G, and, second, in a cap independent manner, by packaging *osk* RNA into heavy particles that render it inaccessible to the translational machinery [4, 5].

Several proteins involved in repression of unlocalized *osk* RNA translation, such as Hrp48 and Squid, are directly implicated in *osk* RNA localization to the posterior. Others interact biochemically and genetically with RNA localization factors [6–10]. The existence of multiple *osk* containing complexes suggest that *osk* RNA may be present in RNPs whose components are dedicated to the dual task of preventing Osk misexpression while targeting *osk* RNA to the site of pole plasm assembly. An *osk* RNA null mutant is more phenotypically severe than *osk* protein null mutants, and expression of an *osk* transgene with a mutated start codon is able to rescue the more severe aspects of the RNA null phenotype, indicating that *trans* factors bind to the *osk* 3'UTR in a very sensitive stoichiometric relationship [11].

Me31B, a DEAD box helicase and conserved processing body (P-body) protein related to yeast Dhh1p, is frequently associated with *osk* mRNA. Me31B and *Drosophila* decapping proteins 1 and 2 (dDcp1 and dDcp2) form cytoplasmic particles, similar to P-bodies, which may mediate *osk* mRNA degradation [12]. Me31B containing complexes are implicated in translational repression of *osk* in mid-stage egg chambers [8]. Finally, Me31B coimmunoprecipitates with Bru in an RNA dependent manner, suggesting that Me31B, Cup, Bru, and eIF4E may also associate in RNPs [5].

Activation of *osk* translation in the pole plasm requires multiple factors, including Staufen (Stau), a dsRBD-type RNA binding protein also required for *osk* localization [13]. *osk* translation also is enhanced by cytoplasmic polyadenylation mediated by Orb, the *Drosophila* homolog of vertebrate cytoplasmic polyadenylation element binding protein (CPEB). Orb interacts physically with Bru, yet

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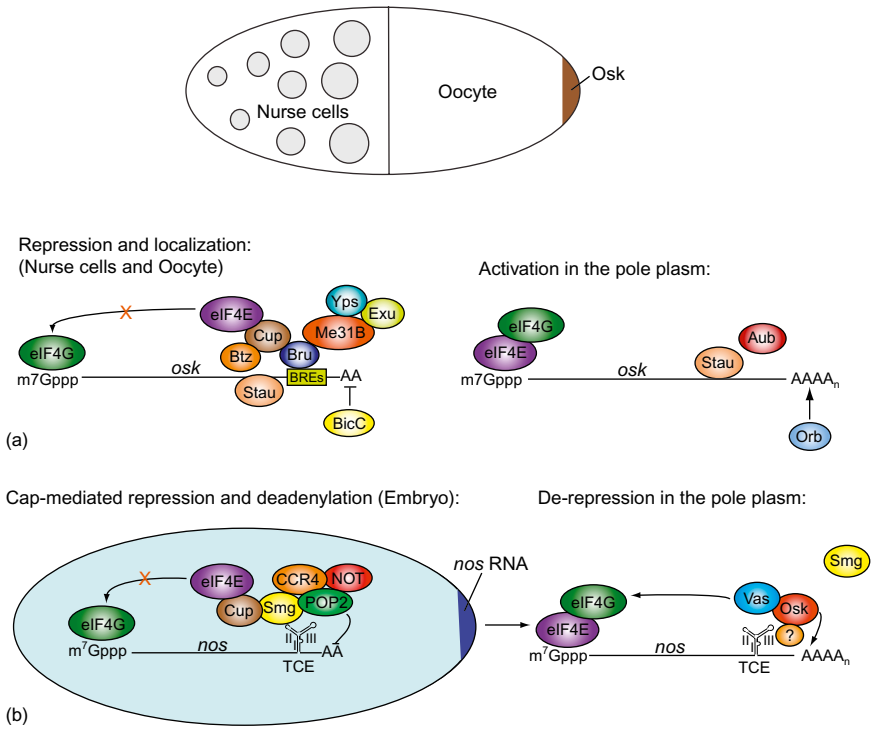

FIGURE 279.1 Regulation of osk and nos RNAs is highly complex.
(a) RNA localization and translational control work in concert to restrict the distribution of Oskar (Osk) protein to the pole plasm. *osk* RNA is transcribed in the nurse cells, and transported through the ring canals into the early oocyte. At this stage *osk* RNA is present in RNPs dedicated to the dual task of preventing Osk misexpression while targeting *osk* RNA to the posterior of the oocyte. Once localized to the pole plasm, *osk* RNA is translationally active. (b) *nos* RNA accumulates in the pole plasm, but is present throughout the bulk cytoplasm at low levels. Smg represses the expression of unlocalized *nos* RNA both by preventing initiation of translation and by promoting deadenylation of the repressed transcript. Translation at the posterior requires the activity of multiple pole plasm components.

the addition of a long poly(A) tail to a chimeric *osk-lacZ* mRNA does not overcome Bru mediated repression *in vitro* [14]. In contrast, Bicaudal-C (Bic-C), interacts physically with Orb, but negatively regulates germline mRNAs by recruiting the CCR4/NOT deadenylase complex through binding its NOT3/5 subunit. Bic-C and Orb likely act antagonistically to regulate gene expression through poly(A) tail length [15].

Translationally repressed *osk* mRNA sediments in sucrose gradients in polysome containing fractions, indicating that *osk* must be subject to a form of translational repression that does not prevent association with ribosomes [16]. Consistently, mutations affecting the rasiRNA pathway such as *aubergine* (*aub*) cause premature Osk expression during early oogenesis [17, 18]. However, Aub activates *osk* translation in the pole plasm [19]; the basis for these contrasting roles at different developmental stages remains unexplained. Like Stau, Aub does not simply alleviate Bru mediated repression in the pole plasm, as translation of *oskBRE*$^{-}$ remains sensitive to *aub* function. Pole cell formation requires Dicer-1 and dFMRP, but not Dicer-2, suggesting that the miRNA, and not the siRNA, pathway is involved in promoting Osk translation during embryogenesis [20].

TRANSLATIONAL CONTROL TARGETS NANOS TO THE POLE PLASM

Restriction of Nos protein to the pole plasm, by RNA localization and by repression of *nos* mRNA in the bulk cytoplasm, is established in late oogenesis and maintained in early embryogenesis [1–3]. Translational control of *nos* is mediated by a 90 nt region of the 3'UTR, termed the translational control element (TCE) [21] (Figure 279.1). The TCE forms a complex secondary structure that is critical to its function; mutations that disrupt any portion of this structure prevent the binding of repressors of *nos* and render the entire element inactive [22]. Repression during oogenesis is mediated by Glorund (Glo), an hnRNP F/H homolog that binds to the stem of stem-loop III of the TCE. *nos* mRNA is translated upon localization at the posterior of the oocyte and either loss of Glo binding, or loss of *glo* function leads to misexpression of unlocalized *nos* mRNA [23].

The loop of stem-loop II contains a Smaug recognition element (SRE), the binding site for another translational repressor, Smaug (Smg) [24]. Smg interacts with Cup, and this interaction is required for Smg mediated repression of SRE containing mRNAs in embryo extracts [25]. Smg also interacts directly with POP2 and CCR4 of the CCR4/POP2/NOT

deadenylase complex, recruiting it to a large set of maternal mRNAs in the early embryo, including *nos*, and targeting them for decay [26, 27]. Thus, *nos* mRNA is repressed in two distinct ways by Smg: by cap dependent translational repression and deadenylation of the silenced transcript. Osk relieves Smg/CCR4 dependent deadenylation of *nos*, thus enabling its translation in the pole plasm [27]. As for *osk*, translationally repressed *nos* co-sediments with polysomes [28] suggesting even more layers of translational control.

Vasa (Vas) has been implicated in activating translation of *nos* RNA at the posterior of the embryo [29]. Vas binds to eIF5B, an initiation factor that promotes ribosomal subunit joining. Embryos containing only a mutant form of maternal Vas that is severely reduced for eIF5B binding always fail to form pole cells but are sometimes patterned normally, suggesting that the requirement of Vas for *nos* translation may not be absolute [30]. When the *nos* TCE is deleted, translation no longer depends on *vas*, implying that Vas acts to overcome Smg mediated repression [29]. Since Smg and Osk interact directly [31], as do Vas and Osk [32], a complex of pole plasm components may deactivate Smg on *nos* transcripts, which enter the posterior region, preventing deadenylation and allowing normal translation to occur.

THE HUNCHBACK GRADIENT IS ESTABLISHED BY TRANSLATIONAL CONTROL

Nos generates an anterior to posterior gradient of the Hunchback (Hb) transcription factor by repressing translation of the maternal *hb* transcript in the posterior part of the embryo. To do this, Nos forms a complex with Pumilio (Pum), Brain Tumor (Brat) and Nos response element (NRE) sequences within the *hb* 3'UTR [33]. Nos mediated translational repression of *hb* involves multiple mechanisms, one affecting polyadenylation [34], and another that is poly(A) independent [35]. Poly(A) independent repression of *hb* is mediated through an interaction between Brat and 4EHP, a cap binding protein that cannot recruit eIF4G and thereby represses translation [36]. The Hb gradient is expanded toward the posterior of embryos with reduced maternal *4EHP* or *brat*. The normal Hb expression pattern cannot be fully restored by either a mutant form of 4EHP that is unable to bind the 5' cap structure, or by a form of Brat that is unable to bind 4EHP [36]. 4EHP dependent regulation also establishes the posterior–anterior embryonic gradient of Caudal (Cad), a transcription factor required for posterior somatic development. In this case, 4EHP is recruited to uniformly distributed *cad* mRNA by Bicoid, which is present in an opposing anterior–posterior gradient [37].

Nos and Pum also repress *CyclinB* translation in pole cells [38]. Brat is not required for *CyclinB* repression, demonstrating that the ternary complex of Nos, Pum, and NRE sequences can interact with different cofactors [33, 39]. Nos mediated regulation of *CyclinB* involves the CCR4 deadenylase [39], indicating that like Smg and Bic-C, Nos may destabilize target mRNAs.

TRANSLATIONAL CONTROL IN THE *DROSOPHILA* NERVOUS SYSTEM

The developing oocyte is not the only tissue in which translational control makes an important contribution to genetic regulation. In fact, a recent large scale study has shown that subcellular localization of specific mRNAs is widespread, implying as well that the proportion of translationally controlled mRNAs may be large [40]. Translational control has been found to be involved in many aspects of development and function of the *Drosophila* nervous system. Interestingly, many mechanisms of translational control that are being uncovered in the nervous system involve the same regulatory factors that have been implicated in embryonic patterning and germ line development.

NOS AND PUM REGULATE DENDRITE MORPHOGENESIS AND NEURONAL PLASTICITY

Aside from their germ-line phenotype, *pum* mutants exhibit decreased long term memory [41], defects in eye morphology and optic nerve development [42, 43], and aberrant locomotor activity [44]. A more recent study examined the consequences of overexpressing, and specifically inactivating, Nos and Pum in larval dendritic arborization (da) neurons [45]. This work showed a requirement for Nos and Pum in the morphogenesis of higher order dendritic branches. The overexpression phenotype was much more severe from a *nos* transgene lacking the TCE than from one possessing it, suggesting that, as in the germ line, TCE dependent translational control mechanisms regulate *nos* in da neurons. The level of *paralytic* (*para*) mRNA, which encodes a specific voltage-gated sodium ion channel, is substantially higher in a loss-of-function *pum* allele, a phenotype that is rescued by a wild-type *pum* transgene [46]. The effects of Pum on *para* and potentially other mRNAs encoding ion channels may explain its role in regulating neuronal excitability [47].

THE *DROSOPHILA* FRAGILE X GENE ENCODES A TRANSLATIONAL REPRESSOR THAT FUNCTIONS IN NEURON AND SYNAPSE DEVELOPMENT, AND ALSO IN THE GERM LINE

Fragile X syndrome is the most common type of hereditary mental retardation in humans, and *Drosophila* has become

a valuable model system for its study, in part because only a single fragile X gene (*fmr1*) exists in the fly [48]. *fmr1* mutants exhibit overgrown neuromuscular junctions while *fmr1* overexpression reduces their growth [49, 50]. Fmr1 protein binds to, and represses translation of, *futsch* mRNA, which encodes microtubule associated protein 1B [51]. The mechanism for this repression is unknown, but could involve microRNAs, as the *futsch* 3'UTR contains predicted microRNA binding sites [48], and Armitage, a component of the miRNA machinery, is required for synaptic protein synthesis and long term memory [52]. Fmr1 has also been implicated in translational control in developing oocytes, as a negative regulator of Orb [53]. Interestingly, in both oocytes and neurons Fmr1 is present in RNPs, related to P-bodies, that share many common components [54].

TRANSLATIONAL REPRESSION THROUGH MICRORNAS

microRNAs (miRNAs) are estimated to control the translation and/or stability of approximately one-quarter of all cellular mRNAs [55]. Genome-wide approaches have identified numerous miRNAs and their potential targets. However, *Caenorhabditis elegans* miRNAs *let-7* and *lin-4*, the first to be identified [56–58], remain the best understood miRNAs by far. *lin-4* and *let-7* are 22{ts}nt RNAs that are complimentary to sequences in the 3'UTRs of their target mRNAs, enabling RNA{ts}:{ts}RNA interactions. *let-7* forms an imperfect duplex with the *lin-41* 3'UTR, in which unpaired bases form a bulged structure that is required for *let-7* function *in vivo* [59, 60]. Formation of an imperfect duplex may be the critical feature of miRNA regulation that leads to translational repression, without inducing siRNA mediated RNA degradation [55]. Although *lin-4* and *let-7* target regulation involves RNA degradation [61], these miRNAs primarily target translation at the initiation step. Depletion of eIF6, an inhibitor of 80S ribosome assembly, diminishes *lin-4* mediated repression of endogenous target protein and mRNA levels *in vivo* [62]. *let-7* inhibits cap dependent translation in human cell lines by directly targeting 5' cap recognition by eIF4F [63, 64]. This is believed to occur through the activity of an associated Argonaute protein, Ago2, which interacts with the 5' cap structure through a domain similar to the cap binding domain of eIF4E [65]. Additional evidence suggests that both *lin-4* and *let-7* can also repress translation at post-initiation steps [66, 67].

Localization of RNA induced silencing complexes (RISC) and their targets to cytoplasmic foci implicates P-bodies in the establishment or maintenance of miRNA mediated translational control in several species [64]. For example, *C. elegans* AIN-1, an miRISC component, associates with *lin-4* and other miRNAs, and targets ALG-1 containing miRISCs to such foci [68].

ROLE OF TRANSLATIONAL CONTROL IN REGULATING GROWTH

In *Drosophila*, two major signaling pathways operate antagonistically to regulate organismal growth [69]. Insulin signaling promotes growth, while signaling mediated by the steroid 20-hydroxyecdysone (20E) inhibits growth. These hormone pathways regulate the expression and activity of eIF4E binding protein (4E-BP), which, like Cup, inhibits translation by competing with eIF4G for binding to the cap binding protein eIF4E. The absence of insulin activates transcription of the transcriptional regulator FOXO, which in turn activates transcription of *4E-BP* [70–72]. 20E signaling promotes this effect by increasing nuclear concentration of FOXO [69]. FOXO also activates transcription of the insulin receptor gene (*INR*), which is then translated at a high level through an internal ribosome entry site that evades 4E-BP regulation [73]. Thus growth is inhibited, but the organism is sensitized to further nutrient dependent signals. Insulin signaling relieves FOXO mediated repression, activates ribosomal protein S6 kinase (S6K), and inactivates 4E-BP by phosphorylation, thus stimulating translation and growth [74, 75].

4E-BP MEDIATED TRANSLATION REPRESSION IS CRITICAL UNDER STRESS CONDITIONS

Thor mutants (*Thor* encodes 4E-BP) are viable under laboratory conditions but exhibit increased sensitivity to hypoxia and starvation [76, 77], indicating that 4E-BP mediated growth control is especially important under stress. Supporting this, *4E-BP* transcription is upregulated upon infection with bacteria [78], and *Thor* mutants exhibit increased sensitivity to infection with *Candida albicans* [79].

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