mRNA localization in the *Drosophila* germline

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Localization and the associated translational control of mRNA is a well established mechanism for segregating cellular protein expression. *Drosophila* has been instrumental in deciphering the prevailing mechanisms of mRNA localization and regulation. This review will discuss the diverse roles of mRNA localization in the *Drosophila* germline, the cis-elements and cellular components regulating localization and the superimposition of translational regulatory mechanisms. Despite a history of discovery, there are still many fundamental questions regarding mRNA localization that remain unanswered. Take home messages, outstanding questions and future approaches that will likely lead to resolving these unknowns in the future are summarized at the end.

Roles for mRNA Localization

To ensure intracellular proteins act at the right time and place, cells exhibit a variety of mechanisms. Unlike in *S. cerevisiae* where many bud tip localizing proteins do not require localization of their transcripts, the overwhelming majority of mRNA localization in the *Drosophila* germline is coupled with protein function. Many animals, including *Drosophila*, rely on maternal transcripts to coordinate early development in processes that involve assorted post-transcriptional regulatory events.[1] Growing evidence suggests that post-transcriptional regulation of gene expression is more prevalent than transcriptional control.[2,3] *Drosophila*, with its heavy reliance on maternal mRNAs and its numerous experimental advantages, has emerged as a top model for addressing fundamental questions underpinning both mRNA localization and translational regulation.[4,5]

Somatic tissues also employ mRNA localization, but show integral differences to oocytes and eggs. Many somatic tissue transcripts appear more enriched on subcellular structures than tightly localized and exhibit a greater diversity of patterns suggestive of a less concerted process.[6] Moreover, somatic tissue transcripts have significantly shorter half-lives than maternal mRNA

which is relevant when considering localization mechanisms.[7] mRNA localization in somatic tissues has been well discussed in other reviews and will not be addressed further in this article.[1,8,9] In the developing *Drosophila* embryo, spatial regulation of gene expression by mRNA localization results in molecular asymmetries that coordinate early patterning events and leads to the segregation of fate determinants that are important in building the germ plasm. It is now clear that the sources of these asymmetries arise from highly orchestrated mRNA localization events that occur prior to fertilization, during oogenesis.

Two Key Roles for mRNA Localization in Early and Mid Oogenesis

Drosophila oogenesis is composed of 14 morphologically defined stages (**Fig. 1**). The oocyte develops in an egg chamber composed of somatically derived follicular epithelial cells encapsulating an interconnected set of 16 germline cells.[10] One of these germline cells becomes the future oocyte and the remaining 15 develop as nurse cells that provide a supporting role, producing maternal mRNAs and cytoplasmic components required for oocyte and ultimately embryonic development.[10] Nurse cells passively and actively exchange cytoplasm through actin-rich ring canals in early and mid-oogenesis until these supporting cells apoptose at stage 10b, initiating the late phase of oogenesis. Before the regulated demise of the nurse cells, the oocyte has already achieved two key objectives through mRNA localization: 1) events essential for establishing the anterior-posterior (A/P) and dorsal-ventral (D/V) axis have been initiated by two spatially and temporally distinct rounds of *gurken* (*grk*) mRNA translation[11]; 2) local translation of *oskar* (*osk*) mRNA sets in motion the establishment of the future germline.[12-15] *grk* mRNA is first localized in early oogenesis (stage 2–6) to the posterior pole of the oocyte. Following translation, Grk protein, a TGF-α homolog, signals to the surrounding follicle cells causing them to adopt posterior fates.[16,17] These follicle cells then signal back to the oocyte resulting in a rearrangement of the oocyte microtubule (MT) cytoskeleton[18] and the pushing of the oocyte nucleus to the anterior margin.[19] The signaling with follicle cells is a pivotal moment in *Drosophila* development as axis patterning and germline establishment depend on the new cytoskeletal configuration.[16,17,20]

A second round of *grk* mRNA localization occurs at mid-oogenesis (stage 7–10a) and results in the formation of a tight RNA cap over the oocyte nucleus, which is now anteriorly located.[16] *grk* mRNA translation again results in a signaling cascade with the surrounding follicle cells, this time defining the dorsal-ventral axis.[16,21,22] Both rounds of *grk* expressions require
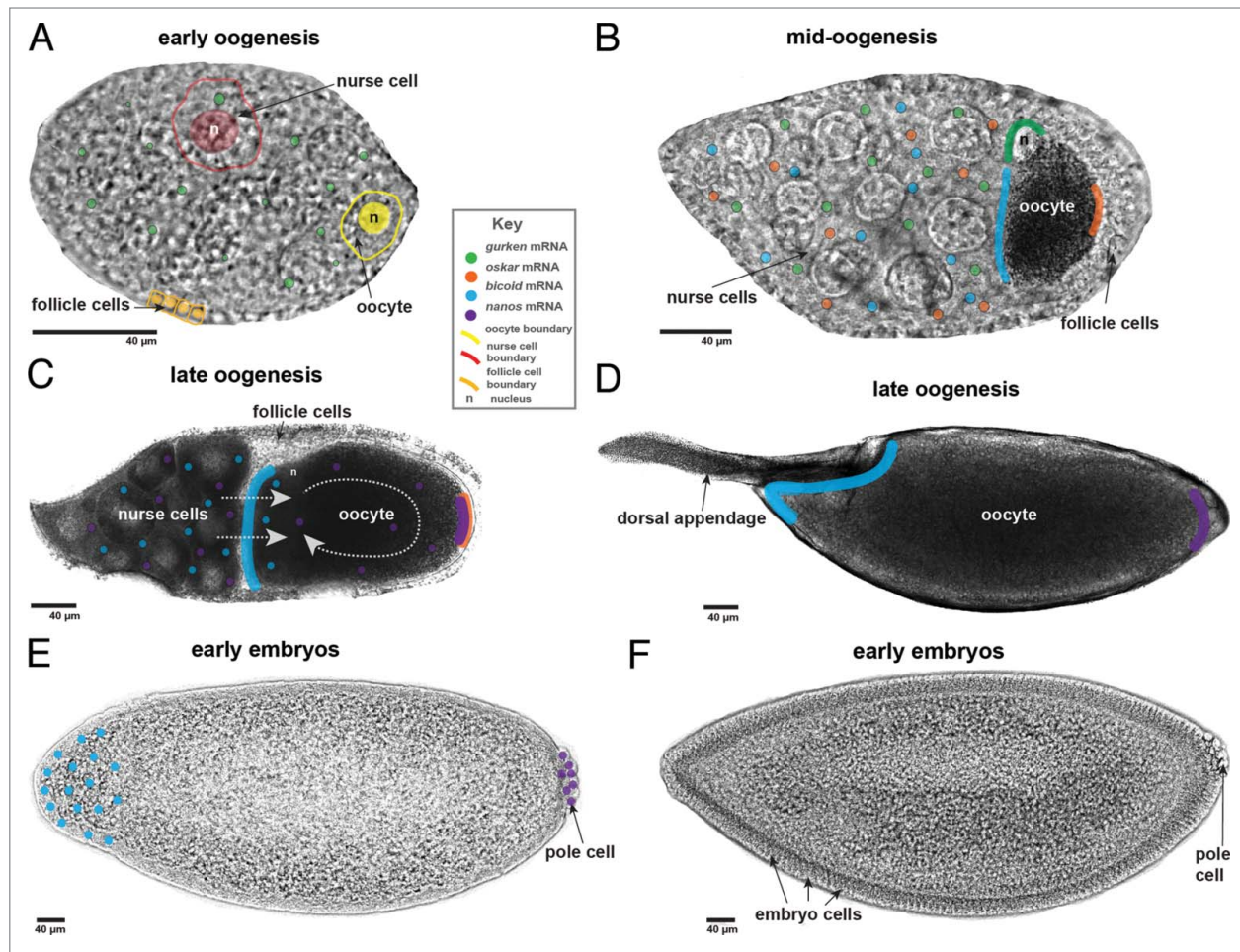
Figure 1. Stages of *Drosophila* oogenesis and early embryogenesis. (**A**) Early oogenesis- stage 6 egg chamber. *grk* mRNA, synthesized in the nurse cells, localizes at the posterior of the oocyte where it is translated and signals to the overlying follicle cells. (**B**) Mid- oogenesis- stage 8 egg chamber. *bcd*, *grk*, and *osk* mRNA, synthesized in the nurse cells, localize to specific regions of the oocyte. (**C**) Late oogenesis -stage 10b/11 egg chamber. Nurse cells dump their cytoplasm into the oocyte while ooplasmic streaming drives a unidirectional mixing of the ooplasm. *bcd* and *nos* mRNA localize to the anterior and posterior poles, respectively. (**D**) Late oogenesis-stage 14 egg chamber. Oogenesis is complete and the mature egg is ready for laying, a process that can be delayed for days or even weeks. (**E**) Early embryogenesis-stage 4 embryo. In the syncytial blastoderm, translated *bcd* and *nos* mRNA form opposing protein gradients that pattern the A/P axis. (**F**) Early embryogenesis- stage 5 embryo. The blastoderm is cellularized.

an intact cytoskeleton, molecular motors, a cis-acting localization sequence and trans-acting factors that are described below.[5]

Accumulation of *osk* mRNA at the posterior pole takes place over the course of mid-oogenesis.[23] Prior to being deposited in the oocyte for localization, *osk* mRNA undergoes a multitude of interactions in the nucleus, nurse cell cytoplasm and ooplasm essential for its localization.[24] These events are well described in other reviews.[25,26] At the posterior pole, where Grk protein first signaled, Osk protein sets the foundation on which the future germline is built.

mRNA Localization in Late Oogenesis Establishes the A/P Body Axis

The start of late oogenesis (stage 10b-14) is marked by a second major cytoskeletal re-arrangement initiated by changes in cortical actin[27] that result in two dynamic phenomenon: 1) rapid extrusion of nurse cell cytoplasm into the oocyte (nurse cell dumping)[10]; 2) unidirectional churning or mixing of the ooplasm (ooplasmic streaming).[28] The reorganization of the oocyte MTs into the bundles at the cortex is important not only because of their requirement for ooplasmic streaming but it means that there are no longer MTs that could transport mRNAs to the posterior. During these late stages of oogenesis, *nanos* (*nos*) and *bicoid* (*bcd*) mRNA are localized, to the posterior and anterior poles respectively, by two different mechanisms.[29,30] At its destination, *nos* mRNA is translated while *bcd* mRNA remains translationally repressed until after fertilization.[31,32] Egg laying can be delayed for days or even weeks by physical or environmental strategies. Importantly, the localized mRNAs remain intact and properly positioned such that normal development can ensue once the eggs are fertilized. This raises many intriguing questions about the extended stability of mRNA in late stage oocytes.

Following the events of egg activation, fertilization and egg deposition, opposing protein gradients of Bcd and Nos form that pattern the A/P axis of the developing embryo.[32-35] Live imaging has shown *nos* mRNA actively segregates with Vasa (Vas) protein into the germ cell progenitors, the pole cells, that bud from the posterior of the embryo following fertilization.[36] In addition to its patterning role, *nos* is also required for the transcriptional and mitotic quiescence of the germ cells and their migration to the gonad. Numerous mRNAs, in addition to *nos*, are localized to the germ plasm and inherited by the germ cells. These transcripts presumably encode proteins that specify germ cell fate and provide a maternal supply for the early development of the germ cells, before becoming transcriptionally active.

The generation of the anterior to posterior Bcd protein gradient has been extensively studied.[37-39] It has become clear that *bcd* mRNA is not a point source, but rather both the mRNA and protein form gradients.[40] However, detection of single *bcd* mRNA particles shows that mRNA distribution alone is not sufficient to explain the observed Bcd protein gradient and it is hypothesized that active or passive protein movement is required.[41] There is also evidence that Grk protein can be localized in the egg chamber independently of the mRNA. In germline clones of Hephaestus, the ortholog of polypyrimidine tract-binding protein (PTB), *grk* mRNA localization is wild-type with Grk protein failing to be restricted to the D/A corner.[42]

Mechanisms of mRNA Localization

Genetic screens, mutant analysis, genome-wide approaches and biochemistry experiments have all helped to establish the variety of unique and conserved trans-acting proteins and cis-acting sequences that are required for accumulating mRNAs in discrete destination. Regardless of mechanism, cis-acting elements in the mRNA are required for trans-acting proteins to recognize and bind. These interactions build a ribonucleoprotein (RNP) complex with many protein factors and mRNAs of the same or even different species. Cis-acting elements are typically in the 3′ untranslated region (UTR), where hindrance with translation is unlikely, and generally form secondary structures including stem loops, hairpins and bulges.[43-46] Alternative splicing of the mRNA in the nucleus can adjust these elements, resulting in unique RNP compositions and ultimately different outcomes for the mRNA.[47,48]

The prevailing model of localization suggests that newly formed RNPs are recognized by other factors, such as proteins linking cargo to molecular motors in the case of transport, and become localized. During this localization, mRNAs are thought to be kept translationally silent. Once at their destination, RNP complexes are then remodeled and repression factors replaced by anchoring and/or translational machinery.

There are three main mechanisms of mRNA localization: active transport, diffusion-entrapment and local protection from mRNA degradation.[49] The ease of culturing and imaging *Drosophila* egg chambers and early embryos and the ability to monitor mRNA localization in both live and fixed tissue has facilitated the dissection of these mechanisms.

Active Transport in the Early Embryo

The embryo has been an especially attractive model for RNA localization due to its amenability to microinjection of fluorescently labeled mRNAs. This led to the first direct evidence of Dynein mediated mRNA transport along microtubules.[50] Shortly after, it was shown that the Egalitarian (Egl) - Bicaudal D (BicD) - Dynein mediates transport of mRNA to the apical side of embryo cells as well as from the nurse cells to the oocyte.[51] This complex has been further dissected to show that Egl, and not BicD, is capable of binding RNA sequences from many different transcripts.[52] A recent biochemical screen identified a novel protein, Lissencephaly-1 (Lis-1), as being required for recruiting the Dynein-Dynactin complex to localizing RNPs and, in *lis-1* mutant embryos, travel distances in the minus-end direction on MTs are reduced.[53] High resolution tracking of mRNA using an in vitro motility assay showed processive and diffusive movements of mRNAs bound to the Dynein-Dynactin complex with mRNA localization signals increasing the processive movements.[54] In addition, MT associated proteins and encounters with the ends of MTs have a clear influence on RNP movement and directionality.[54]

Whether the transport particle is moving as a single mRNA or a collection of many transcripts remains an area of debate. Single molecule fluorescence detection provides strong evidence, in vitro and *in vivo*, that apically localized RNAs are transported individually.[43] Whether this is also the case in for mRNAs in the oocyte is not clear. Future in vitro and RNA injection experiments will likely continue to lead the way for experiments testing the *in vivo* composition of RNP complexes and their stoichiomerty.

Active Transport in Early and Mid-Oogenesis

The predominant mechanism in early and mid-oogenesis is active transport on cytoskeletal tracks by molecular motors. *In vivo* labeling of endogenous *grk*, *osk* and *bcd* mRNA showed that each displays dynamic movements.[23,30,55,56] MTs impact the localization process by dictating where complexes can be transported by motors. In oogenesis, the MT cytoskeleton display three distinct arrangements, with both the population and individual MTs being highly dynamic and displaying random but biased polarity in at least some stages.[57] MTs in the early egg chamber are nucleated from a microtubule-organizing center at the posterior pole of the oocyte and extend anteriorly into the nurse cells.[58,59] *grk* mRNA synthesized in the nurse cells is actively transported by Dynein to the minus end of the MTs at the oocyte posterior pole.[16,21,60]

Following the first re-arrangement of the cytoskeleton in the egg chamber, *grk* mRNA is actively transported in a Egl, Bic-D, and Dynein dependent manner toward the minus ends of MTs which in mid-oogenesis emanate, at least in part, from the anterior and dorsal anterior corner of the oocyte.[61,62] The current model, based on tracking of injected fluorescent RNA directly into the center of the oocyte, suggests two steps are involved to form the D/A cap of *grk* mRNA over the nucleus.[61] In the first

step *grk* mRNA is actively transported to the anterior margin and in the second to the dorsal anterior corner in a step that is disrupted in *squid* or K10 mutants.[17,61] At the D/A corner, injected *grk* RNA enters into large non-membranous electron-dense structures that are lost when anti-Dynein heavy chain is injected.[62] Fluorescence recovery after photobleaching (FRAP) experiments using MS2 tagged *grk* mRNA shows a decrease in the mRNA's dynamics as oogenesis progresses.[56] In K10 and sqd mutants, *grk* mRNA mis-localized at the anterior is more dynamic supporting a model for these proteins in anchoring roles rather than mediating transport.[56] What factor or combination of factors is required for this temporal anchoring of *grk* mRNA still remains unclear as point mutations in the *grk* localization signal (GLS) suggest a least one novel factor.[63] Whether *grk* mRNA is transported with the oocyte nucleus or if it is degraded between the two localization events is unknown. A model where new *grk* mRNA from the adjacent nurse cells enters the oocyte at stage 7–10, is yet to be supported experimentally.

Recent genetic screens continue to identify new proteins required for mRNA localization events.[64,65] While some transfactors appear to be specific to a single mRNA, many proteins are required for more than one localization event. For example, the Egl-BicD-Dynein complex is used to target mRNAs in various *Drosophila* cell types.[51,66,67] Moreover, glutathione-RNA chromatography, an efficient and specific technique for protein purification, reveals that *Drosophila* Syncrip, a homolog of mammalian SYNCRIP/hnRNPQ, binds *grk* and *osk* mRNA in vitro and is required for localization and translation *in vivo*.[68] Co-localization of trans-acting factors with different mRNAs that have discrete localizations raises the question of what the factors that dictates localization are.

To this end, work on *osk* mRNA, which sets up the future pole plasm in mid-oogenesis, has sought to address what specific factors are required for localization. Direct tagging of *osk* mRNA shows a bias random walk mediated by the plus-end directed motor kinesin to the posterior pole on a marginally polarized MT cytoskeleton.[23] *Stau*, *mago*, *barentsz*, and *Tropomyosin II* mutants result in mislocalization of *osk* mRNA.[23] At the posterior, the actin cytoskeleton, the actin binding proteins Lasp, Didum, Spire, Cappuccino, the Myosin-V motor, MTs and Dynein are all required for proper posterior accumulation and maintenance.[69-75] The involvement of both kinesin and Dynein in *osk* mRNA localization suggests that RNP particles are exposed to opposing directional forces during localization. How particles *in vivo* transition between plus and minus end movement remains unclear and an aim for future research. Increasingly data also suggests that localization-incompetent RNA molecules can hitch-hike on transport particles.[76-78] The *osk* 3′UTR mediates hitch-hiking, presumably through RNA-RNA interactions, however the full details of how an RNA is recognized, attached and released from a transport particle is yet to be explained.

bcd mRNA shows two recognizable phases of localization, in mid- and late oogenesis respectively. Unlike *grk* mRNA, which is translated and secreted in both the posterior and D/A locations, *bcd* mRNA is localized to the anterior where it remains translationally silent until after deposition.[32,33] Classic genetic analysis

identified three trans-acting factors that are required for *bcd* localization, Exuperantia (Exu), Swallow (Swa) and Staufen (Stau).[79] It is clear from experiments in which *bcd* mRNA is first injected into a nurse cell, removed and re-injected into an ooctye that Exu, an RNA binding protein, is required in the nurse cells but not in the oocyte for anterior *bcd* accumulation.[80] This was the first evidence that established a specific role in mRNA localization for nurse cell cytoplasm, an area in the cell that is still not fully understood. Swa, which was thought to be the linking factor between *bcd* mRNA and Dynein,[81] has now been shown to act in organizing the cytoskeletal architecture that provides the microtubule tracks for *bcd* mRNA to move to the anterior on.[82] Moreover, *hu li tai shao* (*hts*), an actin regulating factor, has also been shown to play a similar role[83] suggesting that proteins thought to be direct may instead be indirectly functioning in mRNA localization. The other trans-acting factor originally identified in *bcd* mRNA accumulation at the anterior, Stau, is not required until the second phase of localization. Interestingly, a recent genome-wide sequence analysis has shown that Stau bound transcripts have significantly longer 3′ UTRs with specific secondary structures likely to be essential for recognizing specific mRNA.[84]

The observation that *bcd* and *grk* mRNA both require Dynein and a polarized cytoskeleton and accumulate together in stage 7 oocytes raises the question of how they ultimately adopt unique patterns. One possibility is that when *grk* and *bcd* mRNA enter the mid-stage oocyte along the anterior margin, they are simply shuttled toward the minus ends of the MTs. The overall orientation of the dynamic MTs result in *grk* and *bcd* mRNA particles moving along the entire anterior. When *grk* RNPs come into contact with the D/A, they become trapped in a Squid dependent manner while *bcd* remains mobile at the anterior. While direct evidence is lacking, *grk* mRNA is likely degraded after it signals to the follicle cells at the D/A corner and *bcd* mRNA is also likely degraded until the Stau-dependent second phase of localization is complete.

Multiple Mechanisms of Localization in Late Oogenesis

The majority of *bcd* mRNA is localized during late oogenesis. Stage 10b, when streaming and dumping commence, demarcates the second phase of *bcd* mRNA localization where it is estimated that at least 10-fold more of the mRNA accumulates than in mid-oogenesis.[30] Live cell imaging has shown a continual active transport mechanism requiring Dynein and a population of MTs nucleated at the anterior, before being anchored by actin at stage 14, the end of oogenesis.[30,55] Stau, a double stranded RNA binding protein, is essential for *osk* mRNA localization, translational activation and anchoring[12,14,85-87] and is also required for *bcd* mRNA localization, specifically in late oogenesis.[30]

Concurrent with *bcd* late-oogenesis localization, *nos* mRNA becomes distributed throughout the ooplasm by diffusion, facilitated by ooplasmic streaming. *nos* mRNA that encounters the posterior cortex of the oocyte is entrapped in association with germ plasm resident proteins, such as Vas, and anchored to the

actin cytoskeleton at the posterior pole as observed by live cell imaging.[29] *Germ cell-less* and *cyclin B* mRNA also become localized to the posterior pole in this manner.[4] This mechanism is inefficient, leaving the majority (96%) of *nos* mRNA unlocalized. This unlocalized RNA is translationally repressed (see below) and ultimately degraded in the blastoderm embryo.[88] For long-term maintenance of the germ plasm, live imaging shows that Kinesin, Dynein and cortical MTs are required for germ plasm RNP, containing *nos* mRNA, motility which is coordinated with Myosin V mediated tethering.[75] This multiple mechanism model stresses the importance of restricting mRNA to the correct cellular position for extended periods of time.

Superimposition of Translational Regulatory Mechanisms

Regulating translation, both activation and repression, and degradation of mRNA is essential for cell function. At the destination, the RNP encounters or adds factors that maintain the localization and likely undergoes a large scale change in composition that results in translation. One established control mechanism is competition for interaction with the cap-binding eukaryotic initiation factor 4E (eIF4E) by eIF4G and eIF4E-binding proteins.[89] Once eIF4E binds to the 7-methyl guanosine cap at the 5′ end, translation can be initiated by the subsequent binding of eIF4G which, in conjunction with eIF3, leads to recruitment of the 43S ribosomal pre-initiation complex.[90] Unlocalized or repressed mRNAs often have eIF4E-binding proteins bound to eIF4E, thus blocking this initiation. This process is reviewed in detail.[89]

Extensive work on *osk* has shown that repression is sustained through an interaction between the 5′ and 3′ ends. In this mechanism, Cup binds eIF4E via a conserved binding sequence in addition to interacting with Bruno which directly binds, with HRP48, at three sites in the 3′ UTR.[25,91,92] Mutant analysis of *cup* shows that it also negatively regulates *orb*, a CPEB homolog, and blocks its positive autoregulatory loop.[93] Regulation by *cup* is important as Orb protein has been shown to act as a translational activator in mid-oogenesis of both *osk* and *grk* mRNA.[94,95] *Oligomerization* of *osk* mRNA into large silencing particles is thought to be important in protecting transcripts from ribosomes.[96] Proteins are also important in this repression as shown by mutations in Polypyrimidine tract-binding (PTB) protein that decrease the size of *osk* particle and mis-express the mRNA.[97]

Different mRNAs appear to be localized to the same cellular regions but experience different translational outcome. This is the case with *grk* and *bcd* mRNA in stage 8 and 9 oocytes when *grk* is expressed and *bcd* is repressed. In situ hybridization on ultra-thin frozen sections followed by immuno-electron microscopy has shown that both endogenous mRNA species associate at the anterior margin with electron dense bodies that have similar protein distributions to Processing bodies (P bodies), distinct cytoplasmic regions of mRNA control and turnover, well described in yeast.[98-101] *bcd* mRNA

is detected inside the P body where translation is not supported while *grk* mRNA is associated at the edge where the translational activator Orb and ribosomes are present. It remains unclear if degradation follows translation of *grk* mRNA or if *bcd* mRNA is degraded at these stages as well.[101] At the posterior of the oocyte, P bodies are also present and their formation has been shown to be promoted in the germline by *Drosophila* Ge-1.[102] P bodies in the posterior might play a role in regulating the major differences in the translational timing of germ plasm RNAs; *nos* mRNA translated immediately on localization, germ cell-less translated in the early embryo, polar granule component not translated until after pole cell formation, and others not apparently translated until germ cell migration.[103]

Take Home Messages about mRNA Localization

"When" is as critical as "where." mRNA localization can concentrate protein expression in regions where these proteins function and, when coupled with translational control, can prevent toxicity due to production at inappropriate locations. Temporal control can be imposed on the spatial control in two ways: first, transcripts can be transported at different times—second, regardless of when transcripts are transported, they can be translated at a later time in response to a developmental or extracellular cue.

There are conserved aspects across transcripts, tissues and organisms. For example, transport complexes, such as Egl-BicD-Dynein, are used to dynamically target mRNAs in various cell types[51,66,67]; proteins, such as Stau, are required for mRNAs localized in the same tissue to different regions; organisms show conservation of mechanism for organizing mRNAs[1,8]

Consider the tissue since mRNA localization is utilized for different cellular needs. In neurons the requirements are very different from the germline, with speed and readiness being essential for function.

mRNA localization and translational control has implications for human health. Alternate splicing of mRNA and changing RNP complex composition enable cells to alter and tune their transcriptome and proteome in response to internal and external cues. The increasing identification of mutations in mRNA that cause disease has lead to therapeutics targeting mRNAs and RNPs.[104]

Outstanding questions about mRNA localization

To what extent are mRNPs heterogeneous and are these complexes in a state of continued flux? Are mRNA complexes remodelled after nuclear export or are they fully equipped when they set out into the cytoplasm? Does the mRNP shed components after a function is complete? How dynamic are these complexes?

Is there a quality control check point in the formation or localization of mRNPs? Is the complex destroyed or rebuilt if the wrong composition is detected?

In a cell with multiple populations of MTs, how do mRNAs discriminate between these populations? Is there a general mechanism organizing the availability and location of transport components?

What is the complete mechanism of how mRNAs are translationally activated? Are mRNAs translated within the RNPs or do they have to be released from the RNP?

To what extent does mRNA act as a scaffold for protein-protein interactions or miRNA-protein interactions? Are there localized mRNAs that play a structural role rather than serving as templates for protein synthesis?

Is miRNA-based regulation superimposed on mRNA localization? What is the full extent and mechanism of miRNA function with localized transcripts? Are there widespread requirements for miRNA beyond clearance of mRNAs.[105-108]

Future Experimental Approaches

Ongoing proteomic analysis, high throughput biochemical approaches and advanced imaging methods are all working toward addressing these and many more key questions about mRNA localization. The understanding of cellular mechanism will require not only these techniques, but also *in vivo* experimentation.

Use of single molecule analysis is increasing and experiments that follow an individual mRNA through a living tissue while simultaneously visualizing key components in real-time are a realistic goal. Experiments such as this will have to address the formation, remodeling and degradation of RNPs *in vivo*.

System-wide identification of RNA binding proteins[109] and genome-wide predictions of protein binding sites provide an insight into an enormous number of testable *in vivo* hypotheses. Future iterations of the interactome of substantial interest will be in defining the ratios of protein-RNA interactions *in vivo*. Sampling mRNA temporally and spatially and ascertaining complexes stoichiometry *in vivo* will offer key insights for addressing the dynamics of mRNA associations.[110]

CRISPR/Cas genome editing technology promises efficient germline and somatic engineering in *Drosophila*.[111] For example, the manipulation of mRNA protein binding sites *in vivo* will be simplified and made significantly more time efficient. As this technology becomes fully implemented and further advanced, new experimental approaches will be applied.

Fast temporal control over protein function. Current techniques such as photo inactivation by light including chromophore assisted light inactivation (CALI),[112,113] transgenically Encoded Protein Photoinactivation (FIAsH-FALI)[114] and Channelrhodopsin-2 (ChR2)[115] as well as the tobacco etch mosaic virus (TEV) protease[116] and auxin[117] based methods have not been widely adapted to questions of mRNA localization. In part, the specificity and speed of these techniques render them incompatible. Advancements or new methods of switching off a protein in vivo will be important in further understanding questions addressing when and where proteins function.

The *Drosophila* germline tissue is an ideal model for implementing these and other new approaches that will ultimately result in a clear understanding of this common and essential cellular process.

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