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REVIEW

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Localization and translation control of *slam* in Drosophila cellularization

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ABSTRACT

In this extra view, we comment on our recent work concerning the mRNA localization of the gene *slow as molasses (slam)*. *slam* is a gene essential for the polarized invagination of the plasma membrane and separation of basal and lateral cortical domains during cellularization as well as for germ cell migration in later embryogenesis. We have demonstrated an intimate relationship between *slam* RNA and its encoded protein. *Slam* RNA co-localizes and forms a complex with its encoded protein. *Slam* mRNA localization not only is required for reaching full levels of functional Slam protein but also depends on Slam protein. The translation of *slam* mRNA is subject to tight spatio-temporal regulation leading to a rapid accumulation of Slam protein and zygotic *slam* RNA at the furrow canal. In this extra view, we first discuss the mechanism controlling localization and translation of *slam* RNA. In addition, we document in detail the maternal and zygotic expression of *slam* RNA and protein and provide data for a function in membrane stabilization. Furthermore, we mapped the region of Slam protein mediating cortical localization in cultured cells.

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Introduction

Beside the generic function of being constitutively translated, many mRNAs are subject to translational regulation or specific subcellular localization. 70% of examined transcripts displayed a specific subcellular localization in blastoderm Drosophila embryos [1]. This high prevalence of RNA localization has been confirmed for coding and noncoding RNAs throughout embryogenesis as well as in larval tissues [2]. It is generally hypothesized that localization of an RNA indicates posttranscriptional regulation of gene function. Spatial restriction of an mRNA and its translation may be a mechanism for protein localization, which is potentially more efficient than transport of a protein uniformly synthesized within the cytoplasm. Despite its prevalence and apparent importance, the biological significance and molecular mechanisms for linking RNA localization and translational control are little understood.

The essential gene slam is very suited for investigating the significance and mechanisms of post-transcriptional regulation. *slam* is special in that *slam* mRNA co-localizes, binds to and functionally interacts with its encoded protein [3]. Slam protein lacks obvious motifs and appears to be largely intrinsically disordered. Yet, *slam* serves specific, clearly defined and essential functions in embryonic development. *slam* is required for formation and ingression of the plasma membrane during cellularization [4,5], separation of cortical domains, organization of Rho signaling [6], and germ cell migration [7].

Transcriptional and post-transcriptional control of *slam*

Slam was initially identified as a member of the class of early zygotic genes [4,8]. In addition, maternally derived RNA and protein significantly contribute to *slam* function [5]. The *slam* null phenotype characterized by a complete lack of furrow ingression during cellularization is only observed in embryos maternally and zygotically deficient for *slam* [5]. Using sensitive fluorescent in situ hybridization (FISH) and immunostaining, we examined maternal and zygotic expression of *slam* in detail (Figure 1).

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We scored the zygotic genotype of blastoderm embryos by the number of nuclear *slam* RNA foci corresponding to transcription sites on the chromatin. Wild type embryos (2x $slam^+$) have two, *slam* hemizygous embryos (1x $slam^+$) one and *slam* homozygous embryos (0x $slam^+$ or m^+z^-) no foci. Thus, *slam* signal in embryos without nuclear foci represents maternally derived RNA and protein. We detected nuclear foci and thus nascent zygotic transcripts as early as mitotic cycle 11 in the embryos carrying 2x $slam^+$ gene (Figure 1(a)). Starting from these low levels, the signals for *slam* RNA and protein first gradually and then strongly increase until onset of cellularization in interphase 14. During syncytial cycles, RNA and protein co-localize at the metaphase furrow in mitosis and intercap region in interphase [9]. The peak of *slam* RNA and protein was detected at the onset of cellularization. The increase in RNA levels corresponds to the onset of zygotic transcription as indicated by the prominent nuclear RNA foci during this stage (Figure 1(a) and Yan 2017 [3]). We assayed the maternal contribution in embryos zygotically deficient for *slam* (m^+z^-). In these embryos, we detected cortical and metaphase furrow associated *slam* RNA and protein in interphase and mitosis, respectively, similar to wild type, however with a constant and strongly reduced signal (Figure 1(b)). During the course of cellularization, the signal disappeared. Despite this rapid decay of maternal RNA, furrows forms and slowly ingress

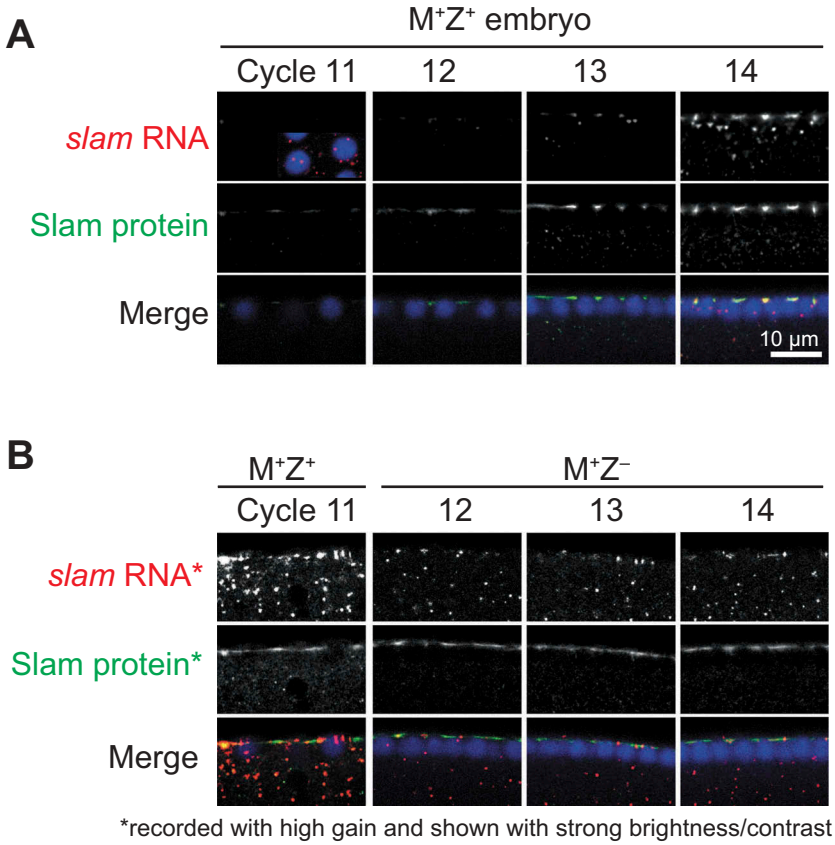

Figure 1. Maternal expression and localization of *slam* RNA and protein in *slam* zygotic deficient embryos.

Embryos were fixed and stained for *slam* RNA by fluorescent RNA in situ hybridization (grey/red), Slam protein by immunostaining (grey/green) and for DNA by DAPI (blue). All embryos were stained in one tube. Images were recorded with the unchanged settings of the confocal microscope and processed under same condition. Embryos were staged by nuclear density and morphology. (a). Expression and localization of slam RNA and protein in blasterderm M^+Z^{++} embryos. Insert shows nascent transcript foci on top view. (b). Expression and localization of *slam* RNA and protein in M^+Z^- embryos. M^+Z^+ embryo in cycle 11 as comparation. To better view the RNA and protein level, a higher gain was used for image recording and a high brightness and contrast for image processing.

during cellularization, indicating that maternally derived slam is able to initiate cellularization but does not suffice for full functionality.

The strong induction of *slam* transcription during the onset of cellularization may in principle suffice for a corresponding increase of Slam protein. However, we found that *slam* RNA is subject to post-transcriptional control involving RNA localization and regulated translation as well as involving a peculiar interaction of *slam* mRNA and Slam protein. Firstly, we found that *slam* RNA is not restricted to the basal domain and region of prospective furrow canal in embryos lacking Slam protein indicating that mRNA localization depends on its encoded protein. In contrast to the RNA, Slam protein is enriched at the target site even in the absence of *slam* RNA. This functional dependence of mRNA on its encoded protein is likely to be mediated by a biochemical interaction, since *slam* RNA and protein are components of a molecular complex as revealed by co-immunoprecipitation. Interaction of *slam* RNA and protein is likely to be indirect as Slam protein does not contain a dedicated RNA binding domain.

Secondly, we tested whether RNA localization and RNA-protein interaction were involved in *slam* function. To generate an RNA encoding Slam protein but impaired in RNA localization and protein interaction, we exchanged most codons with synonymous codons. We termed this modified *slam* gene *slam*[ACU]. *slam*[ACU] is expressed similarly to endogenous *slam* but does not localize, is little translated and does not rescue *slam* mutants, although *slam*[ACU] is normally translated in cultured S2 cells and in reticulate lysate. These findings suggest that translation of *slam* RNA is linked to RNA localization or interaction with Slam protein.

Thirdly we visualized local translation of *slam* RNA at the furrow canal in early embryos. To achieve this, we employed the TRICK assay allowing labelling of not-yet translated RNAs by binding of a fluorescent protein to a PP7 site inserted within the coding sequence. The first passage of ribosomes over the mRNA displaces the fluorescent protein from the PP7 site and RNA. We assayed the completion of translation by inserting the PP7 sites close to the stop codon. As we detected specific and punctate signal at the furrow canal, a significant fraction of *slam* RNA molecules reaches the target site before translation is completed. *Slam*[ACU] was similarly translated as wild type *slam* in generic translation systems such as reticulocyte lysate and transient transfection of cultured Drosophila S2 cells. However, *slam*[ACU] was much less translated than wild type *slam* in embryos. These two observations, local translation of slam-PP7 RNA and inefficient translation of un-localized *Slam* [ACU] RNA, support a mechanism of localization related translation regulation.

Slam translation may be controlled by repression of initiation or elongation. These two options may be distinguished by *slam* constructs in which the PP7 site is inserted close to the N-terminus (Slam-NPP7). Regulation of translational initiation would similarly affect both constructs Slam-NPP7 and Slam-CPP7. In contrast, Slam-NPP7 should not give rise to localized signal, if elongation is regulated. The mechanism of repression may also be addressed by tracking of nascent Slam peptides by the SunTag system, for example [10,11], which is suited to visualize the translation of *slam* RNA with a temporal and spatial resolution.

Rapid accumulation of zygotically expressed Slam protein is a prerequisite to stabilize the FC structure

The region of the plasma membrane forming the furrow canal is highly dynamic during the initial phase of cellularization. Dynamic micrometer-long tubular structures of the plasma membrane are detected during the first 5–10 min of the cellularization extending towards the cytoplasm. These long tubular structures are labelled and depend on by the N-BAR protein Amphiphysin [12–15]. Afterwards the tubular extensions disappear depending on F-actin and the genes *nullo* and *dia* [13,16]. The tubular structures may negatively regulate and inhibit rapid ingression of furrows as the ingression rate positively and negatively correlates with absence and presence the tubular extensions, respectively [15].

Slam is also involved in the formation of the tubular extensions. Firstly, GFP-slam marks these tubular extensions [14]. Secondly, *slam* is required for ingression of the furrow during initial cellularization (slow phase) [4,17]. Based on the model

that loss of tubular extension correlates with furrow ingression, we hypothesized that *slam* would counteract membrane tubulation and would stabilize the membrane in the region of the prospective furrow canal. We stained wild type and *slam* deficient embryos with Amphiphysin and assayed for the presence and the extent of tubular structures (Figure 2). Consistent with previous reports, Amphiphysin-positive tubules were detected only during initial cellularization but not in following stages in wild type embryos. In contrast, the tubular structures prominently persisted throughout the cellularization in *slam* deficient embryos. These data show that *slam* suppresses the presence of endocytic tubules following initial cellularization and suggest that *slam* promotes furrow ingression by stabilization of the membrane in the region of the prospective furrow canal.

The localization element of *slam* RNA and protein

A central feature of *slam* is the specific subcellular localization of the RNA and the protein. To address the mechanism of localization we started to map the parts of RNA and protein required and sufficient for localization. Knowing these elements in the RNA and domains within the protein will allow us to address the mechanism and factors underlying the specific localization. Firstly, we mapped three regions within the 5'UTR untranslated region and the coding sequence of the mRNA, which are sufficient for RNA localization in the presence of Slam protein [3]. The mapping experiments were conducted in a wild type background in the presence of endogenous Slam protein, which is required for RNA localization. Secondly, we have started to map the region within the protein that is sufficient for RNA independent localization. To separate RNA independent and RNA dependent localization we employ *slam*[ACU], a modified *slam* gene, in which the majority of the codons was replaced by synonymous codons. *slam*[ACU] RNA is equally expressed as wild type *slam* but does not localize. Slam protein encoded by *slam*[ACU] localizes correctly however. For initial mapping we have employed an assay in cultured Drosophila S2 cells, which do not express *slam* in detectable levels [3]. We have previously found that Slam protein is localized to the cell cortex [6]. Similar to wild type *slam*, *slam*[ACU] was efficiently translated and gave rise to cortical protein localization, whereas the controls GFP alone or GFP with 5'UTR of *slam* gave rise to cytoplasmic proteins (Figure 3(a)). With a series of N- and C-terminal truncations of *slam*[ACU] we identified the region in the N-terminal half (aa 164–aa 532) as being necessary and sufficient for cortical localization (Figure 3(b)). In future

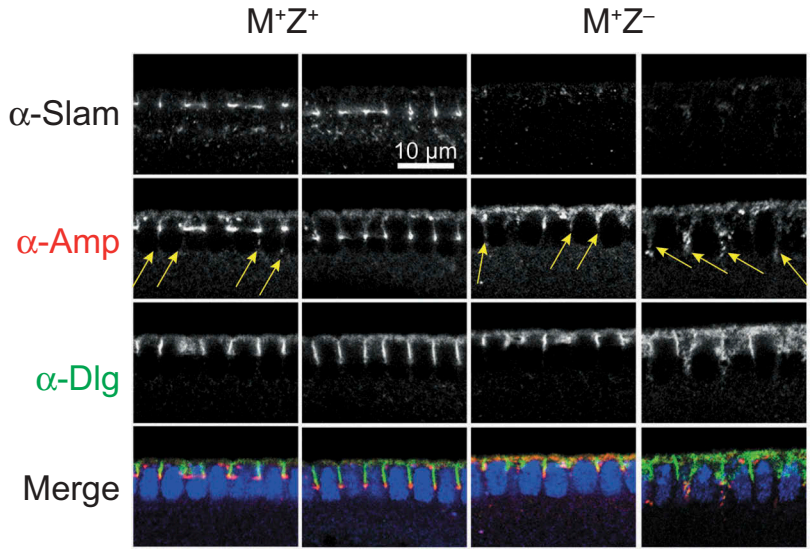

Figure 2. Tubular extensions persist throughout the cellularization in *slam* deficient embryos.
Embryos were fixed and stained for Slam (grey), Amphiphysin (grey/red), Dlg (grey/green) and DNA (blue). Arrows in yellow point to tubular extension in embryos. Slam marks the basal domain of furrow canal; Amphiphysin marks the tubular extension; Dlg, Discs large, marks the lateral plasma membrane of the ingressing furrow.

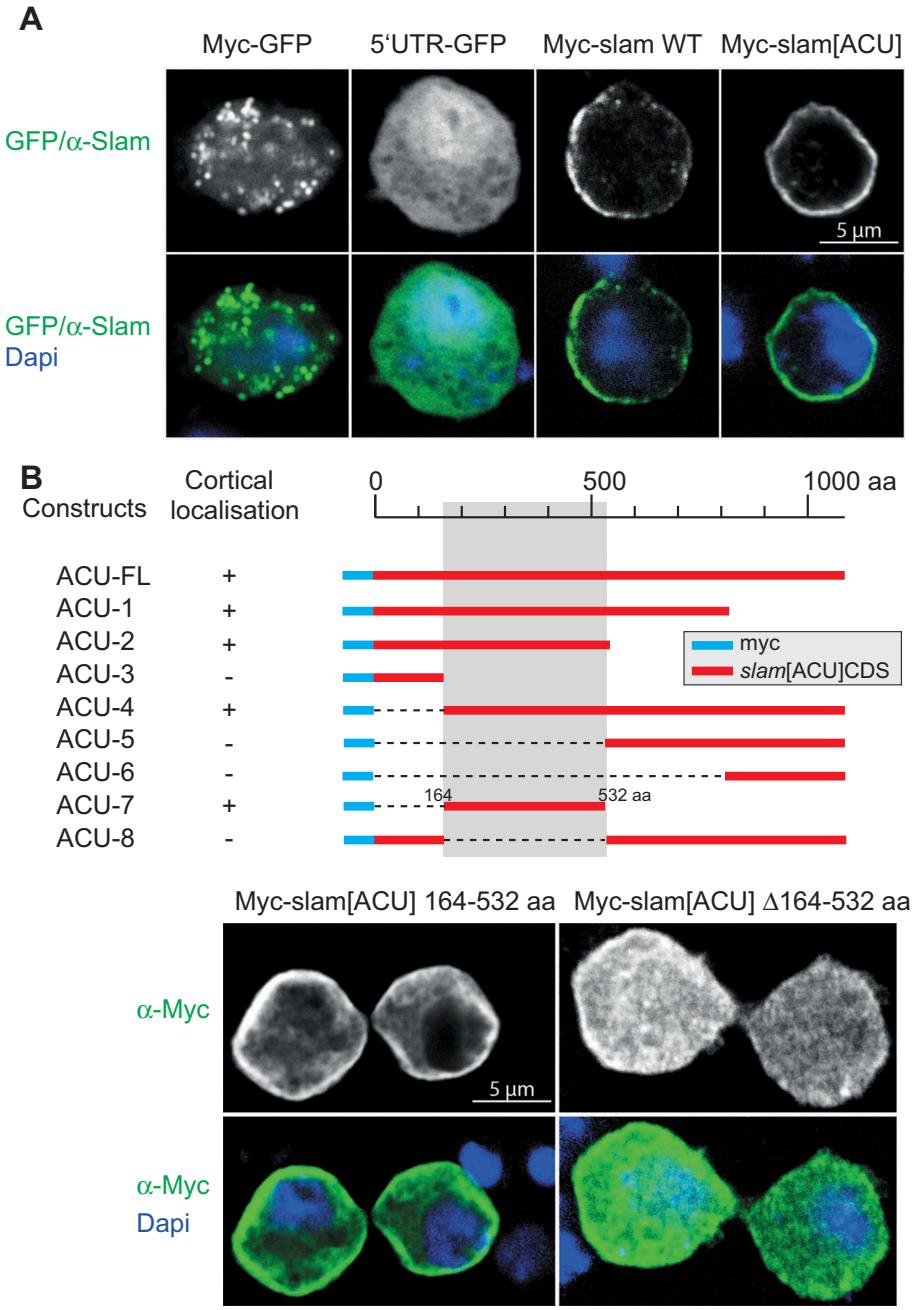

Figure 3. Mapping of cortical localization element of Slam protein in S2 cells.

S2 cells were transiently transfected with slam ACU constructs. S2 cells were fixed and stained with slam/Myc antibody and DNA. (a) Cortical localization of slam protein in slamACU transfected cells. (b) Schematic representation of constructs and localization element of Slam protein.

experiments we will test the activity of this region in embryos, i. e. whether the same region confers localization to the furrow canal during cellularization. Knowing the part of the protein that mediates protein localization will allow to address the way how Slam protein attracts and anchors *slam* RNA to the furrow canal.

Potential regulators for translational control of *slam* in early embryos

Slam RNA is subject to translational regulation in the blastoderm embryo. Whereas wild type *slam* RNA and *slam*[ACU] are equally translated in vitro and in cultured S2 cells, *slam*[ACU] is poorly translated in early embryos [3]. Furthermore, we

found that translation of at least a fraction of *slam* RNA is completed at the furrow canal, suggesting that translation is not initiated or stalled until the mRNA reaches its target site [3].

Translation regulation relies upon general factors, proteins binding to the 5'UTR cap and 3' poly(A) tail of the mRNA as well as on associated proteins: eIF4E, eIF4G, PABP, for example. In addition, specific regulators, such as P body components, are engaged in translation of subsets of or specific transcripts. Beside the localization at the furrow canal, *slam* RNA and Slam protein are detected in particles of variable size in the basal cytoplasm. These 'basal' particles become more prominent during the course of cellularization and disappear in parallel to Slam at the furrow canal. We do not know the identity and function of these particles. It is conceivable that these particles are related to RNA containing particles (RNPs), such as P body. Me31b, a standard marker of P body, has been reported to be involved in regulation of *bicoid, gurken, nanos* and *oskar* RNA translation in embryos and oocytes [18–21]. In embryos, P bodies are present throughout the blastoderm stage and cellularization. The number of P body structures strongly increases during cellularization [22]. In early Drosophila embryos, Me31b, cup and TRAL form a stable complex with eIF4E, which blocks the binding of eIF4G with eIF4E, resulting in translation repression. While Me31b does not bind with eIF4E in S2 cells, eIF4E, PABP and eIF4G form a complex and facilitate translation [23]. This is coincident with our findings, that translation of localization-incapable *Slam*[ACU] is repressed in early embryos but not in S2 cells.

Besides P body components, FMR1 is another candidate regulator for *slam* translation. FMR1 contains dedicated RNA binding domains, two KH domains and one RGG motif (reviewed by [24]). RGG motifs have been proposed to bind to target RNAs by G-quadruplex structures. A well characterized target of FMR1 is Map1B (Drosophila homologue, Futsch), whose translation is inhibited in synapses [25]. Several models for translational regulation by FMR1 are currently discussed, including inhibition of translation initiation, a role of micro RNAs, and stalling ribosomes during translation elongation [24].

In Drosophila, the *Fmr1* gene is important for viability, although some homo- or hemizygous mutant flies can be obtained. Beside its function in the nervous system, *Fmr1* is involved in early embryonic development [26–28]. Embryos from *Fmr1* homozygous females show a delayed and incomplete cellularization, a phenotype reminiscent to weak *slam* alleles. FMR1 has been reported to colocalize with RNA particles (RNP) [27], which are marked by the RNA helicase ME31B and its associated proteins Cup and TRAL (Wang2017) and thus may be related to P bodies [29,30].

FMR1 is associated with the RNA binding protein and translational regulator Caprin in Drosophila embryos ([31] and references therein). *Caprin* is not essential for development and viability in Drosophila. However, embryos from *Caprin* homozygous (or hemizygous) females cellularize more slowly than wild type, which is reminiscent to the phenotype of embryos from *Fmr1* females [31]. *Fmr1* genetically interacts with *Caprin*, as embryos from *caprin* homozygous and *Fmr1* heterozygous double mutant females show a stronger phenotype, including an additional nuclear division prior to cellularization in some of the embryos [31]. The cell cycle phenotype may be due to binding and modulated translation of *frs* and *CycB* mRNAs [31].

Concluding remarks

In our recent paper we demonstrated that *slam* mRNA and its protein build an intimate relationship in functional and biochemical terms. Initially, low levels of cytoplasmic Slam protein, potentially maternally derived, starts to move towards and bind independently of its mRNA to the membrane region that will form the furrow canal. Nascent zygotic transcripts, under either full or partial translation repression, are recruited to the furrow canal region by Slam protein and other so far unknown factors. At the membrane, translation repression is released allowing efficient translation. The increasing amount of Slam protein initiates a positive feedback loop, which ensures that full levels of Slam protein are reached within a short period of time. Slam protein functions together with downstream factors, such as RhoGEF2 and Patj to suppress the long tubular extensions, stabilize

the furrow canal structure and initiate furrow ingression. Future work will define the localization elements, the regions mediating RNA and protein interaction, visualize nascent peptides and identify the regulators of localization and translation. These studies will provide more and detailed insight in the biogenesis of a specific RNA-protein particle.

Materials and methods

Materials and methods were as described in Yan et al [3,14].and Wenzl et al [6].

Abbreviations

slam:	Slow as molasses
FC:	Furrow canal
PP7	bacteriophage PP7
S2 cells	*Drosophila melanogaster* Schneider 2 cells
UTR	untranslated region

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

1. Lecuyer E, Yoshida H, Parthasarathy N, et al. Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. Cell. 2007;131:174–187.
2. Wilk R, Hu J, Blotsky D, et al. Diverse and pervasive subcellular distributions for both coding and long noncoding RNAs. Genes Dev. 2016;30(5):594–609.
3. Yan S, Acharya S, Gröning S, et al. Slam protein dictates subcellular localization and translation of its own mRNA. PLoS Biol. 2017;15(12):e2003315.
4. Lecuit T, Samanta R, Wieschaus E. slam encodes a developmental regulator of polarized membrane growth during cleavage of the Drosophila embryo. Dev Cell. 2002;2:425–436.
5. Acharya S, Laupsien P, Wenzl C, et al. Function and dynamics of slam in furrow formation in early Drosophila embryo. Dev Biol. 2014;386:371–384.
6. Wenzl C, Yan S, Laupsien P, et al. Localization of RhoGEF2 during Drosophila cellularization is developmentally controlled by slam. Mech Dev. 2010;127:371–384.
7. Stein JA, Broihier HT, Moore LA, et al. Slow as molasses is required for polarized membrane growth and germ cell migration in Drosophila. Development. 2002;129:3925–3934.
8. Merrill PT, Sweeton D, Wieschaus E. Requirements for autosomal gene activity during precellular stages of Drosophila melanogaster. Development. 1988;104(3):495–509.
9. Schmidt A, Lv Z, Großhans J. ELMO and Sponge specify subapical restriction of Canoe and formation of the subapical domain in early *Drosophila* embryos. Development. 2018;26(2):145.
10. Tanenbaum ME, Gilbert LA, Qi LS, et al. A protein-tagging system for signal amplification in gene expression and fluorescence imaging. Cell. 2014;159:635–646.
11. Lyon K, Stasevich TJ. Imaging translational and post-translational gene regulatory dynamics in living cells with antibody-based probes. Trends Genet. 2017;33:322–335.
12. Zelhof AC, Bao H, Hardy RW, et al. Drosophila Amphiphysin is implicated in protein localization and membrane morphogenesis but not in synaptic vesicle endocytosis. Development. 2001;128:5005–5015.
13. Sokac AM, Wieschaus E. Local actin-dependent endocytosis is zygotically controlled to initiate Drosophila cellularization. Dev Cell. 2008;14:775–786.
14. Yan S, Lv Z, Winterhoff M, et al. The F-BAR protein Cip4/Toca-1 antagonizes the formin Diaphanous in membrane stabilization and compartmentalization. J Cell Sci. 2013;126:1796–1805.
15. Su J, Chow B, Boulianne GL, et al. The BAR domain of amphiphysin is required for cleavage furrow tip-tubule formation during cellularization in Drosophila embryos. Mol Biol Cell. 2013;24:1444–1453.
16. Grosshans J, Wenzl C, Herz HM, et al. RhoGEF2 and the formin Dia control the formation of the furrow canal by directed actin assembly during Drosophila cellularisation. Development. 2005;132:1009–1020.
17. Figard L, Xu H, Garcia HG, et al. The plasma membrane flattens out to fuel cell-surface growth during Drosophila cellularization. Dev Cell. 2013;27:648–655.
18. Weil TT, Parton RM, Herpers B, et al. Drosophila patterning is established by differential association of mRNAs with P bodies. Nat Cell Biol. 2012;14:1305–1313.
19. Götze M, Dufourt J, Ihling C, et al. Translational repression of the Drosophila nanos mRNA involves the RNA helicase Belle and RNA coating by Me31B and Trailer hitch. RNA. 2017;23:1552–1568.
20. Nakamura A, Amikura R, Hanyu K, et al. Me31B silences translation of oocyte-localizing RNAs through the formation of cytoplasmic RNP complex during Drosophila oogenesis. Development. 2001;128:3233–3242.

21. Wilhelm JE, Hilton M, Amos Q, et al. Cup is an eIF4E binding protein required for both the translational repression of oskar and the recruitment of Barentsz. J Cell Biol. 2003;163:1197–1204.
22. Patel PH, Barbee SA, Blankenship JT, et al. GW-Bodies and P-Bodies constitute two separate pools of sequestered non-translating RNAs. PLoS One. 2016;11(3): e0150291.
23. Wang M, Ly M, Lugowski A, et al. ME31B globally represses maternal mRNAs by two distinct mechanisms during the Drosophila maternal-to-zygotic transition. Elife. 2017;6:6. pii: e27891.
24. Chen E, Fragile JS. X mental retardation protein: A paradigm for translational control by RNA-binding proteins. Biochimie. 2015;114:147–154.
25. Zhang YQ, Bailey AM, Matthies HJ, et al. Drosophila fragile X-related gene regulates the MAP1B homolog Futsch to control synaptic structure and function. Cell. 2001;107:591–603.
26. Georgieva D, Dimitrov R, Kitanova M, et al. New X-chromosomal interactors of dFMRP regulate axonal and synaptic morphology of brain neurons in Drosophila melanogaster. Biotechnol Biotechnol Equip. 2014;28:697–709.
27. Monzo K, Papoulas O, Cantin GT, et al. Fragile X mental retardation protein controls trailer hitch expression and cleavage furrow formation in Drosophila embryos. Proc Natl Acad Sci USA. 2006;103:18160–18165.
28. Deshpande G, Calhoun G, Schedl P. The drosophila fragile X protein dFMR1 is required during early embryogenesis for pole cell formation and rapid nuclear division cycles. Genetics. 2006;174:1287–1298.
29. Barbee SA, Estes PS, Cziko AM, et al. Staufen- and FMRP-containing neuronal RNPs are structurally and functionally related to somatic P bodies. Neuron. 2006;52:997–1009.
30. DeHaan H, McCambridge A, Armstrong B, et al. An in vivo proteomic analysis of the Me31B interactome in Drosophila germ granules. FEBS Lett. 2017;591:3536–3547.
31. Papoulas O, Monzo KF, Cantin GT, et al. dFMRP and Caprin, translational regulators of synaptic plasticity, control the cell cycle at the Drosophila mid-blastula transition. Development. 2010;137:4201–4209.