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.

**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 posttranscriptional 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](image.png)

**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 blastoderm 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 comparison. 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 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 ingress 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 \textit{slam} would counteract membrane tubulation and would stabilize the membrane in the region of the prospective furrow canal. We stained wild type and \textit{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 \textit{slam} deficient embryos. These data show that \textit{slam} suppresses the presence of endocytic tubules following initial cellularization and suggest that \textit{slam} promotes furrow ingression by stabilization of the membrane in the region of the prospective furrow canal.

**The localization element of \textit{slam} RNA and protein**

A central feature of \textit{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 \cite{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 \textit{slam}[ACU], a modified \textit{slam} gene, in which the majority of the codons was replaced by synonymous codons. \textit{slam}[ACU] RNA is equally expressed as wild type \textit{slam} but does not localize. Slam protein encoded by \textit{slam}[ACU] localizes correctly however. For initial mapping we have employed an assay in cultured Drosophila S2 cells, which do not express \textit{slam} in detectable levels \cite{3}. We have previously found that Slam protein is localized to the cell cortex \cite{6}. Similar to wild type \textit{slam}, \textit{slam}[ACU] was efficiently translated and gave rise to cortical protein localization, whereas the controls GFP alone or GFP with 5'UTR of \textit{slam} gave rise to cytoplasmic proteins (Figure 3(a)). With a series of N- and C-terminal truncations of \textit{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 \textit{slam} deficient embryos.](image)

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.
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 ingress. 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**

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