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The PIWI protein Aubergine recruits eIF3 to activate translation in the germ plasm

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Piwi-interacting RNAs (piRNAs) and PIWI proteins are essential in germ cells to repress transposons and regulate mRNAs. In Drosophila, piRNAs bound to the PIWI protein Aubergine (Aub) are transferred maternally to the embryo and regulate maternal mRNA stability through two opposite roles. They target mRNAs by incomplete base pairing, leading to their destabilization in the soma and stabilization in the germ plasm. Here, we report a function of Aub in translation. Aub is required for translational activation of nanos mRNA, a key determinant of the germ plasm. Aub physically interacts with the poly(A)-binding protein (PABP) and the translation initiation factor eIF3. Polysome gradient profiling reveals the role of Aub at the initiation step of translation. In the germ plasm, PABP and eIF3d assemble in foci that surround Aub-containing germ granules, and Aub acts with eIF3d to promote nanos translation. These results identify translational activation as a new mode of mRNA regulation by Aub, highlighting the versatility of PIWI proteins in mRNA regulation.

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INTRODUCTION

Translational control is a widespread mechanism to regulate gene expression in many biological contexts. This regulation has an essential role during early embryogenesis, before transcription of the zygotic genome has actually started. In Drosophila, embryonic patterning depends on the translational control of a small number of maternal mRNAs. Among them, nanos (nos) mRNA encodes a key posterior determinant required for abdominal segmentation and development of the germline. nos mRNA is present in the whole embryo, but a small proportion accumulates at the posterior pole in the germ plasm, a specialized cytoplasm in which the germline develops. Localization and translational control of nos mRNA are linked, such that the pool of nos mRNA present in the bulk of the embryo is translationally repressed, whereas the pool of nos mRNA localized in the germ plasm is translationally activated to produce a Nos protein gradient from the posterior pole. Both repression of nos mRNA translation in the bulk of the embryo and activation in the germ plasm are required for embryonic development.

The coupling between mRNA localization and translational control depends in part on the implication of the same factors in both processes. The Smaug (Smg) RNA binding protein specifically recognizes nos mRNA through binding to two Smaug recognition elements (SRE) in its 3′UTR. Smg is both a translational repressor of nos, and a localization factor through its role in mRNA deadenylation and decay in the bulk of the embryo, by recruitment of the CCR4-NOT deadenylation complex. Smg directly interacts with the Oskar (Osk) protein that is specifically synthesized at the posterior pole of oocytes and embryos and drives germ plasm assembly. Smg interaction with Osk prevents Smg binding to nos mRNA, thus contributing to relieving both Smg-dependent translational repression and mRNA decay in the germ plasm. Osk is therefore a key player in the switch of nos and other germ cell mRNA regulation between soma and germ plasm of the embryo.

More recently, we have demonstrated the role of Aubergine (Aub) in the localization of germ cell mRNAs to the germ plasm. Aub is one of the three PIWI proteins in Drosophila. PIWI proteins belong to a specific clade of Argonaute proteins that bind 23–30 nucleotides (nt)-long small RNAs referred to as Piwi-interacting RNAs (piRNAs). piRNAs and PIWI proteins have an established role in the repression of transposable elements in the germline of animals. piRNAs target transposable element mRNAs through complementarity and guide interaction with PIWI proteins that, in turn, cleave targeted mRNAs through their endonucleolytic activity. In addition to this role, piRNAs have a conserved function in the regulation of cellular mRNAs in various biological contexts. In the Drosophila embryo, Aub loaded with piRNAs produced in the female germline is present both at low levels in the bulk of the embryo and at higher levels in the germ plasm. Aub binds maternal germ cell mRNAs through incomplete base pairing with piRNAs. Aub binding to these mRNAs induces their decay in the bulk of the embryo, either by direct cleavage or recruitment together with Smg of the CCR4-NOT deadenylation complex. In contrast, in the germ plasm...
Aub recruits Wispy, the germline-specific cytoplasmic poly(A) polymerase, leading to poly(A) tail elongation and stabilization of Aub-bound mRNAs. Thus, Aub and piRNAs play a central role in the localization of germ cell mRNAs through two opposite functions in mRNA stability: mRNA destabilization in the bulk of the embryo and stabilization in the germ plasm. The role of piRNAs and PIWI proteins in cellular mRNA regulation in other contexts, including mouse spermiogenesis and sex determination in Bombyx, also depends on their function in the regulation of mRNA stability.
Here, we describe translational activation as a new mechanism of mRNA regulation by piRNAs and PIWI proteins. Using ectopic expression of Osk in the whole embryo to mimic the germ plasm, we show that Aub and piRNAs are required for nos mRNA translation. Mass spectrometry analysis of Aub interactors in early embryos identifies several components of the translation machinery, including translation initiation factors. We find that Aub physically interacts with the poly(A)-binding protein (PABP) and several subunits of the translation initiation complex eIF3. Furthermore, PABP and eIF3d accumulate in foci that assemble around and partially overlap with Aub-containing germ granules in the germ plasm. Polysome gradient analysis indicates that Aub activates translation at the initiation step. Finally, functional experiments involving the concomitant decrease of Aub and eIF3d show that both proteins act together in nos mRNA translation in the germ plasm. These results identify translational activation as a new level of mRNA regulation by PIWI proteins. Moreover, they expand the role of the general eIF3 translation initiation complex in translation regulatory mechanisms required for developmental processes.

RESULTS

Aub is required for nos mRNA translation

Only a low amount (4%) of nos mRNA is localized to the germ plasm and actually translated. nos mRNA stabilization and translation in the germ plasm depend on the presence of Osk. Therefore, we ectopically expressed Osk in the whole embryo using UASp-osk and the germline-specific driver nos-Gal4, to increase translated nos mRNA levels and address the mechanisms of translational activation. Osk overexpression (osk-OE) in embryos from UASp-osk/+; nos-Gal4/+ females led to increased and ectopic Nos protein synthesis in whole embryo (Fig. 1a). Quantification of Nos protein levels in osk-OE embryos, either following Nos visualization using immunostaining or western blot, revealed a 2-fold increase compared to wild-type (WT) embryos (Fig. 1a, b). In contrast, nos mRNA levels quantified using RT-qPCR were similar in osk-OE and WT embryos (Fig. 1c). This is consistent with the presence of high amounts of nos mRNA in the bulk of embryos, and nos spatial regulation depending mostly on translational control at these stages (0–2 h embryos). Therefore, Osk overexpression in 0–2 h embryos led to ectopic translational activation of nos mRNA without changes in nos mRNA levels.

Aub protein is present at low levels in the bulk of WT embryos and highly accumulates in the germ plasm. Ectopic expression of Osk led to an homogeneous redistribution of Aub in the embryo (Fig. 1a). Strikingly, the lack of Aub in osk-OE embryos resulted in the lack of Nos protein synthesis (Fig. 1a, b), although nos mRNA levels were not decreased (Fig. 1c). This result suggested that Aub was required for nos mRNA translational activation in the presence of Osk. Importantly, the level of Osk protein was not significantly affected by aub mutation, indicating that the lack of Nos protein did not result from the lack of Osk (Fig. 1b). Of note, the UASp-osk transgene almost exclusively overexpressed the long Osk isoform of the two isoforms, Short-Osk and Long-Osk (Supplementary information, Fig. S1a). Long-Osk can induce germ plasm assembly when overexpressed although less actively than Short-Osk. Long-Osk levels were poorly affected by aub mutations, making this UASp-osk transgene a useful tool to address direct nos mRNA regulation by Aub and piRNAs (Fig. 1b; Supplementary information, Fig. S1a).

We analyzed the role of Armitage (Armi), another component of the piRNA pathway with a prominent role in piRNA biogenesis, in nos mRNA translational activation. Nos protein levels were strongly reduced in osk-OE; armi−/−/embryos, compared to osk-OE embryos, although again, the levels of Osk protein were not significantly decreased (Fig. 1b). nos mRNA levels quantified using RT-qPCR remained unaffected by armi mutation (Fig. 1c), revealing a role of Armi in nos mRNA translational control. Armi does not localize to the germ plasm. Instead the defect in nos mRNA translational activation in armi mutant might depend on highly reduced piRNA levels in this mutant, suggesting that piRNAs were required in Aub binding to nos mRNA for its role in translational activation. This is consistent with Aub iCLIP assays showing that an Aub double point mutant in the PAZ domain, AubAA that is unable to load piRNAs, was also unable to bind mRNAs. To confirm the role of piRNAs in Aub-dependent translational activation of nos, we took advantage of the nos (ΔpirooΔapi412) transgene, in which two piRNA target sites (from roo and 412 transposable elements) in close proximity to a prominent Aub-binding site in nos 3′UTR have been deleted (Fig. 1d). We have shown before that deletion of these piRNA target sites affected nos mRNA localization to the germ plasm, without affecting its level. Using single molecule fluorescence in situ hybridization (smFISH), we confirmed the posterior localization defect of nos mRNA from this transgene: 36% of embryos showed a reduced domain of posterior localization (Fig. 1e, g, i). As previously reported, this defect was moderate because several Aub-binding sites remained unaffected in the nos (ΔpirooΔapi412) transgene (Fig. 1d).13 Recording nos mRNA translation in nos(ΔpirooΔapi412)−/+; nosBW/BW embryos using immunostaining showed a similar percentage of embryos (38%) with reduced protein accumulation (Fig. 1f, h, j). However, protein synthesis appeared to be more affected than mRNA localization in these embryos since 3.8% of them did not produce any Nos protein, a defect (no localization) that did not occur with nos mRNA (Fig. 1e, g). In addition, when taking into account all embryos, immunofluorescence intensity was reduced in nos mRNA using UASp-GFP-Aub nos-Gal4 and UASp-osk/+; UASp-GFP-Aub nos-Gal4/+ embryos with anti-GFP antibody, showing the distribution of Aub protein (bottom panels). Posterior is to the right. Western blots of WT, osk-OE, osk-OE; aub−/− and osk-OE; armi−/− embryos revealed with anti-Nos, anti-Osk and anti-e-Tubulin antibodies. Tubulin served as a loading control. Quantification of Osk translation was performed using the ImageJ software with 5 biological replicates. Error bars represent SEM. ****P < 0.0001, **P < 0.01, *P < 0.05, ns not significant, using the unpaired Student’s t-test. Quantification of nos mRNA using RT-qPCR in WT, osk-OE, osk-OE; aub−/− and osk-OE; armi−/− embryos. mRNA levels were normalized with Rpl32 mRNA. Quantification of 4–8 biological replicates. Error bars represent SEM. ns not significant, using the unpaired Student’s t-test. Schematic representation of nos mRNA and 3′UTR targeting with piRNAs. Thin boxes are 5′UTR and 3′UTR, lines are introns and thick boxes are exons. Clusters of Aub crosslink sites are indicated in red. The sequence of the region with the strongest crosslink sites and base pairing with representative roo and 412 piRNAs are shown. The deletions overlapping with the piRNA target sites in the nosΔp∆api412 target are boxed. Arrowhead, the prominent Aub-binding site in nos(ΔpirooΔapi412)−/−; nosBW/BW embryos. Posterior of embryos with the three types of staining: wild type, reduced size or reduced intensity, are shown. Scale bars, 20 μm. g-j Quantification of posterior staining shown in e and f using the ImageJ software. For each genotype, the percentage of embryos with each staining category was recorded for nos mRNA (g) and Nos protein (h). ****P < 0.0001 using the χ2 test. Scatter plots of size and fluorescence intensity of posterior staining for each embryo, for nos mRNA (i) and Nos protein (j). Two-way ANOVA showed significant difference (P < 0.001) in fluorescence intensity of posterior staining for nos mRNA and Nos protein between genotypes.
Fig. 2 Ectopic expression of Osk nucleates RNA granules related to germ granules in the soma. a–d″ Immunostaining of UASp-osk/++; UASp-GFP-Aub nos-Gal4/+ embryos with anti-Osk (red) and anti-GFP (green) to visualize Aub (a–a″); anti-Nos (red) and anti-GFP (green) (c–c″); and anti-Smg (red) and anti-GFP (green) (d–d″); and smFISH of embryos with the same genotype revealing nos mRNA and GFP-Aub through GFP fluorescence (b–b″). DNA was visualized using DAPI. Scale bars, 10 μm. e Quantification of colocalization of immunostaining and smFISH shown in a–d″, using the Imaris software. Granules (> 0.5 μm) and foci (> 0.2 μm) were quantified around nuclei and in the cytoplasm between nuclei, respectively.
Δ(piρ0Δpi412)/1-; nos^{BN/BN} embryos compared to WT, again a defect that did not occur with smFISH (Fig. 1i, j). Therefore, deletion of piRNA target sites in nos mRNA affected Nos protein synthesis, in addition to reducing mRNA localization.

These data are consistent with a direct role of Aub and piRNAs in nos mRNA translation through piRNA-guided binding of Aub to nos. In this hypothesis, a piRNA pathway component specifically involved in transposable element regulation should not interfere with nos mRNA translational control. We used Panoramix (Panx), a key factor in Piwi-dependent transcriptional silencing of transposable elements, which acts downstream of Piwi and has no function in piRNA biogenesis.28,29 panx mutants had no effect on
Fig. 3 Identification of Aub-interacting partners. a–d Volcano plots showing the mass spectrometry analysis of GFP-Aub immunoprecipitation from 0–2 h embryos. Embryos expressing cytoplasmic GFP were used as control. UASp-GFP-Aub nos-Gal4 embryos (a, b); osk<sup>−/−</sup>; UASp-GFP-Aub/nos-Gal4 embryos (c, d). The analysis was based on four biological replicates. The red line indicates the significance threshold (P = 0.05). Known Aub interactors and RNA-binding proteins are indicated in red and purple, respectively (a, c); translation initiation factors are indicated in blue (b, d). e GO analysis of proteins identified as Aub interactors by mass spectrometry. If Validation of Aub interactors using the LUMIER assay. Left: schematic representation of the assay (FFL: Firefly luciferase; RL: Renilla luciferase). Right: graph plotting the IP efficiency of the indicated proteins. The values are IP efficiencies of the coprecipitation of the RL fusion proteins (IP/Input) normalized by the IP/Input values for FLAG-FFL-Aub. Error bars represent SD. Stars indicate values significantly greater than six times the mean value obtained in the control IPs without anti-FLAG antibody (Control). Scalloped (Sd) and Cherry proteins were used as negative controls. ***P < 0.001, **P < 0.01, ns not significant, using the Z-test.

Nos protein levels in osk-OE embryos, consistent with a role of Aub and piRNAs in nos mRNA translation, independent of their role in transposable element regulation (Supplementary information, Fig. S1b).

Finally, most aub mutant embryos fail to develop, although they are fertilized. To address whether the lack of Nos protein in osk OE; aub<sup>−/−</sup> embryos could result from their arrest of embryonic development, we quantified Nos protein levels in osk OE unfertilized eggs that are activated by egg laying but do not develop. Nos levels were similar in osk OE unfertilized eggs and embryos, demonstrating that the defect in Nos protein synthesis in osk OE; aub<sup>−/−</sup> embryos did not result from their lack of embryonic development (Supplementary information, Fig. S1c).

Together, these results show that piRNA-guided Aub binding to nos mRNA plays a direct role in translational activation in the presence of Osk.

Ectopic expression of Osk leads to the formation of granules related to germ granules in the soma

In the germ plasma, Osk leads to the assembly of germ granules that are large ribonucleoprotein particles containing mRNAs required for germ cell specification and development. In addition to Osk, Aub is a core component of germ granules. We asked whether Osk ectopic expression in the somatic part of the embryo could lead to the formation of RNA granules related to germ granules, containing Aub and nos mRNA. Immunostaining of osk OE embryos also expressing GFP-Aub revealed that Osk was present in the bulk of the embryo where it accumulated in cytoplasmic foci that became larger around nuclei (Fig. 2a). GFP-Aub was also present in cytoplasmic foci in the bulk of osk OE embryos and in larger foci around nuclei. Small foci of either Osk or GFP-Aub were dispersed in the cytoplasm and did not colocalize. However, Osk and GFP-Aub colocalized in larger foci that surrounded nuclei, indicating a different composition of these large foci (Fig. 2a–a′, e). smFISH of nos mRNA in embryos of the same genotype showed that nos mRNA accumulated in larger foci around nuclei where it colocalized with GFP-Aub (Fig. 2b–b′, e). Strikingly, Nos protein also accumulated around nuclei and partially colocalized with GFP-Aub in large foci, suggesting that nos mRNA translation occurred in the vicinity of these granules (Fig. 2c–c′, e). In contrast, in osk OE embryos, Smg protein was present in foci that did not concentrate around nuclei and did not colocalize with large GFP-Aub foci (Fig. 2d–d′, e). This result was consistent with the reorganization of Smg into small foci in the germ plasma as compared to the somatic region in WT embryos, which suggested that Smg interaction with Osk did not take place within germ granules.

We conclude that the presence of Osk in the somatic part of osk OE embryos induces the formation of RNA granules that share functional similarities with germ granules, in which Aub and nos mRNA accumulate and at the proximity of which nos mRNA is translated.

Aub interacts with translation initiation factors

To further decipher the function of Aub, we identified Aub interactors in embryos. GFP-Aub was immunoprecipitated from UASp-GFP-Aub nos-Gal4 0–2 h embryos and the coprecipitated proteins were analyzed using mass spectrometry. Embryos expressing GFP alone were used as negative controls (Supplementary information, Fig. S2a). 107 proteins were significantly enriched in GFP-Aub immunoprecipitation (IP) (P < 0.05) (Supplementary information, Table S1). Known Aub interactors were identified, including Tudor (Tud) that is restricted to the germ plasm and required for Aub accumulation in the germ plasm, three components of the nos translation repressor complex, Tudor hitch (TraI), Belle (Bel) and Cup, and Capsuleen/PRMT5 (Csl), the methyltransferase responsible for Aub arginine dimethylation. Several RNA-binding proteins were also found in GFP-Aub IP (Fig. 3a). Importantly, six translation initiation factors were identified as Aub interactors, among which are PABP, three subunits of elf3 (elf3d, elf3k and elf3b), and elf4E, another component of nos translation repressor complex (Fig. 3b). In addition, 48 ribosomal proteins coprecipitated with Aub (Supplementary information, Fig. S2b). Gene Ontology (GO) term enrichment analysis using FlyMine (http://www.flymine.org) identified Translation as the most enriched term among Aub interactors (Fig. 3e). We also analyzed Aub interactors in osk<sup>−/−</sup> mutant embryos that do not form germ plasm, with the aim of identifying specific Aub interactors in the germ plasm, which might be lost in osk mutant embryos. However, mass spectrometry of GFP-Aub IP from osk<sup>−/−</sup> mutant embryos identified a very similar set of proteins to that identified in osk<sup>−/−</sup> embryos (Fig. 3c, d; Supplementary information, Fig. S2c, Table S1). These data suggested that Osk might not affect Aub interaction with most of its protein interactors, but rather their activity. Indeed, PABP and elf4E are found in the nos translational repression complex, although they do not activate translation in this complex. elf3 subunits were found to be in complex with Aub in the absence of Osk, suggesting that they might also be present in the nos repressed mRNP. The presence of Osk, by remodeling the mRNP, would allow to switch on their activity in translational activation.

We used quantitative luminescence-based coIP (LUMIER) assays to validate Aub interactions with translation initiation factors. Aub was fused to FLAG-tagged Firefly luciferase (FFL), whereas potential interactors were fused to Renilla luciferase (RL). Following transient expression in Drosophila S2R<sup>+</sup> cells, Aub was immunoprecipitated with anti-FLAG antibodies, or without antibodies as negative control, and interactors coIP was quantified by recording Renilla and Firefly luciferase activities (Fig. 3f). PABP, four subunits of elf3 (elf3b, elf3d, elf3g and elf3h) among six tested subunits, and elf4E were found to significantly coprecipitate with Aub in these assays. Thus, although elf3k that was identified as an Aub interactor by mass spectrometry could not be confirmed with the LUMIER assay, elf3d and elf3b interaction with Aub was confirmed, and two other elf3 subunits, elf3g and elf3h were found to be in complex with Aub. Differences in the interaction between Aub and elf3 individual subunits between embryos and S2R<sup>+</sup> cells likely resulted from differences in these two experimental systems.

These results reveal that Aub physically interacts with the translation machinery and are consistent with a direct function of Aub in translation regulation.
Aub interaction with PABP and eIF3d

Because PABP and eIF3d showed the strongest association with Aub in the mass spectrometry analysis, and have key roles in translation initiation, we further investigated their interaction with Aub. We used coIP to address Aub physical interaction with PABP in embryos. PABP coprecipitated with GFP-Aub in 0–2 h embryos; however, this coprecipitation was strongly reduced in the presence of RNase (Fig. 4a). In the reverse experiment, PABP was also able to coprecipitate Aub, but this coprecipitation was abolished in the presence of RNase (Fig. 4b). These results could indicate either that Aub and PABP did not interact directly and coprecipitated through their binding to the same mRNAs, or that Aub direct interaction with PABP was stabilized by mRNA in a tripartite association. To address this question, we analyzed direct interaction between Aub and PABP using GST pull-down assays. Aub has three domains characteristic of Argonaute proteins (PAZ, MID and PIWI) and was separated into two parts, Aub (1–482) that contains the N-terminal and PAZ domains, and Aub (476–866) that contains the MID and PIWI domains (Fig. 4c). PABP is composed of four RNA recognition motifs (RRM1-4), a proline-rich linker region and a PABP C-terminal (PABC) domain. Each RRM and the PABC domain were fused
independently translated, or directly through its interaction with partial overlap of both proteins (Fig. 4e Supplementary information, Fig. S3a) either colocalized with, or plasma large proportion of Aub-containing germ granules (79.4%, Supplementary information, Fig. S3a). 

through its binding to 5′UTR of specific mRNAs, elf3d appears to act as a master regulator of Nos translation, either through binding to 5′UTR of specific mRNA, leading to cap-independent translation, or directly through its interaction with the cap structure. 

elf3d is composed of twelve subunits and one associated factor, and coordinates several steps of translation initiation. 

Interestingly, in addition to this role in basal translation, elf3d plays regulatory roles in the translation of specific mRNAs. elf3d associates with Nos-Gal4 activator proteins containing 5′UTR regulatory elements, either through binding to 5′UTR of specific mRNA, leading to cap-independent translation, or directly through its interaction with the cap structure. 

Mechanism of Aub-dependent translational activation 

Aub association with translation initiation factors suggested that Aub might activate nos mRNA translation at the level of initiation. We directly addressed this question using polysome profiling in which mRNA-protein complexes are separated by fractionation through linear sucrose gradients. mRNA localization within the sucrose gradient reflects its translation status: migration in the light RNP or monosomal fractions of the gradient indicates a lack of translation, whereas migration in the heavy polysomal fractions indicates active translation. Polysome profiling was performed with 0–2 h WT, osk-OE, and osk-OE; aub embryos. The abundance of polysomes was reduced in osk-OE embryos compared to WT, indicating that ectopic expression of Osk in the whole embryo affected basal translation (Fig. 5a). In contrast, polysome abundance was partially restored in osk-OE; aub embryos, revealing that translation was active in these embryos (Fig. 5a). Thus, the level of basal translation was affected oppositely to the level of Nos protein. This is consistent with Aub being involved in a regulatory mode of translation occurring on specific mRNAs. To confirm this point, we used smg mRNA as a control, since it is highly translated in the whole embryo upon egg activation. Smg protein levels were not decreased in osk-OE; aub embryos (Fig. 5b, c). Western blot analysis further supports the specificity of Aub-dependent translational activation (Supplementary information, Fig. S4a). Western blot analysis of the gradient fractions revealed co-sedimentation of Aub with actively translating mRNAs in the heavy polysomal fractions, and the presence of PABP in these fractions (Fig. 5b, c). To confirm Aub association with actively translating mRNAs, we treated embryo lysates with puromycin which causes premature termination of elongating ribosomes. Puromycin treatment efficiency was validated by the complete disassembly of polysomes visualized by absorbance measurement of OD at 254 nm, and the shift of ribosomal proteins to monosomal and lighter fractions containing 60S and 40S ribosomal subunits (Fig. 5b, c). Aub shifted to the light mRNP fractions in the presence of puromycin, indicating its bona fide association with translating mRNAs. In contrast, although PABP was shifted towards lighter fractions of the gradient in the presence of puromycin, a certain amount remained present in most fractions, suggesting the presence of heavy RNA complexes containing PABP in Drosophila embryos (Fig. 5c). This is consistent with the presence of mRNAs in heavy fractions of sucrose gradients independently of translation, in polysome gradients from early embryos. We then quantified mRNA through polysome gradients using RT-qPCR. nos mRNA was mostly present in initiation and light polysomal fractions in WT embryos, in agreement with a low amount of nos mRNA being actively
translated (Fig. 5d). In osk-OE embryos, the level of nos mRNA decreased in the initiation fractions whereas it increased in the heavy polysomal fractions, consistent with the 2-fold increase of Nos protein levels in these embryos (Figs. 1a, b, 5d). Quantification of nos mRNA through the gradient in the presence of puromycin confirmed that the pool of nos present in the heavy fractions was indeed associated with actively translating polysomes (Supplementary information, Fig. S5b−d). Interestingly, in osk-OE; aub−/− embryos, the distribution of nos mRNA was similar to that in WT embryos, with higher amounts of mRNA in initiation fractions and lower amounts in heavy polysomal fractions (Fig. 5d). These results suggested the role of Aub at the initiation step of translation. To
Aub and eIF3d functionally interact for nos mRNA translation. a Percentage of embryonic lethality of single or double aub and eIF3d heterozygous mutants. The genotypes are indicated. ***P < 0.001, using the χ² test. b Immunostaining of single and double aub and eIF3d heterozygous mutant embryos with anti-Nos antibody. Posterior of embryos with the three types of staining: wild type, reduced size or reduced intensity, are shown. Scale bars, 20 μm. c Quantification of posterior staining shown in b using the ImageJ software. For each genotype, the percentage of embryos with each staining category was recorded. ****P < 0.0001, ns not significant, using the χ² test. d Model of Aub-dependent translational activation. In the somatic part of the embryo, nos mRNA translation is repressed by two mechanisms: a cap-dependent mechanism that involves Cup binding to eIF4E, and a cap-independent mechanism that involves the coating of the mRNA by Me31B and TraI. Both mechanisms might depend on the CCR4-NOT complex recruited by Smg and Aub. In the germ plasm, Smg binding to Osk precludes its interaction with nos mRNA, leading to depletion of CCR4-NOT and remodeling of the mRNP. This would lead to the dissociation of Me31B/Trai from the mRNA. Aub interaction with PABP and eIF3 subunits would allow unconventional translation, bypassing eIF4E requirement. The recruitment of Wispy poly(A) polymerase by Aub leading to polyadenylation is likely to also contribute to translation activation. Note that eIF3 might be present in the repressor complex in the soma, since eIF3 was found as Aub interactor in osk mutant embryos; however, its activity in translation activation would be repressed.

Further confirm the role of Aub in translational activation of specific mRNAs, we quantified smg and mRpl43 mRNA through the polysome gradients. Consistent with smg active translation in early embryos, most smg mRNA was present in heavy polysomal fractions, and this profile was not affected in osk-OE and osk-OE; aub−/− embryos, indicating that smg translation was independent of both Osk and Aub (Fig. 5e). mRpl43 was used as a control mRNA that is not bound by Aub[15] and similarly, its distribution through the gradient was not strongly affected in osk-OE and osk-OE; aub−/− embryos (Fig. Sf).

These results show that Aub plays a role in the translation of specific mRNAs and are consistent with Aub acting at the level of translation initiation.

eIF3d plays a role in Aub-dependent translational activation

To address the biological relevance of Aub/eIF3d physical interaction, we analyzed the effect of the concomitant reduction of aub and eIF3d gene dosage by half. Although single aub or eIF3d heterozygous mutant embryos showed a low level of lethality (2%–3%), embryonic lethality significantly increased up to 21% in double heterozygous mutants, suggesting that Aub and eIF3d act together in embryonic development (Fig. 6a). nos mRNA translation was then recorded in these embryos using immunostaining. The Nos protein level visualized by immunofluorescence at the posterior cortex was quantified. In WT, 85% of embryos showed a full accumulation of Nos protein at the posterior pole, whereas 15% had a reduced accumulation (Fig. 6b, c). Nos accumulation in heterozygous aub or eIF3d mutant embryos was similar to that in WT. In contrast, in aub−/−; eIF3d−/− double heterozygous mutants, the percentage of embryos with reduced Nos accumulation significantly increased to 34% (Fig. 6b, c; Supplementary information, Fig. S5a). This reduction of Nos accumulation in double heterozygous mutants did not correlate with reduced Osk accumulation or reduced nos mRNA localization at the posterior pole (Supplementary information, Fig. S5b–e), indicating a direct defect in nos mRNA translation. We conclude that Aub/eIF3d physical interaction is required for nos mRNA translational activation.
DISCUSSION

Several studies have reported the role of PIWI proteins in cellular mRNA regulation at the level of stability, piRNA-dependent binding of mRNAs by PIWI proteins leads to their decay in different biological systems.16 In addition, in Drosophila embryos, mRNA binding by the PIWI protein Aub also leads to their stabilization in a spatially regulated manner.13 Here, we report a novel function of Aub in direct translational control of mRNAs. Using nos mRNA as a paradigm, we show that Aub is required for nos mRNA translation. Nos protein levels are also strongly reduced in armi mutant, in which piRNA biogenesis is massively affected,27 suggesting that Aub loading with piRNAs is necessary for its function in translational activation. Consistent with this, we find that deletion of two piRNA target sites in nos mRNA decreases its translation. Importantly, Nos levels are not affected in a panx mutant background. Panx is a piRNA factor required for transcriptional repression of transposable elements, but has no function in piRNA biogenesis.28,29 In addition, as is the case for aub and armi mutants, panx mutant embryos do not develop.7,28,29 Finally, Nos levels are similar in unfertilized eggs and embryos overexpressing Osk, demonstrating that Nos protein synthesis is independent of embryonic development. Together, these results strongly argue for a direct role of Aub and piRNAs in nos mRNA translational control, independently of their role in transposable element regulation or developmental defects in piRNA pathway mutants.

Mass spectrometry analysis of Aub interactors points to a strong link with the translation machinery. In addition, polysome gradient analyses reveal Aub association with actively translated mRNAs in polysomal fractions. A link has been reported previously between the PIWI proteins Miwi and Mili and the translation machinery in mouse testes, where Miwi and Mili were found to associate with the cap-binding complex.46,47 However, the role of Miwi and Mili in translational control has not been characterized. We now decipher the molecular mechanisms of Aub function in translational activation of germ cell mRNAs in the Drosophila embryo. We demonstrate a physical interaction between Aub and the translation initiation factors PABP, eIF4E and subunits of the eIF3 complex. These interactions are in agreement with polysome gradient analyses in WT and aub mutant backgrounds that indicate a role of Aub in translation initiation.

Recent data have identified specific roles of elf3 in the regulation of translation. elf3 is the most elaborate of translation initiation factors containing twelve subunits and an associated factor, elf3j. This complex promotes all steps of translational initiation and does so in part through direct association with other translation initiation factors, contributing to their functional conformations on the small ribosomal subunit surface.79 In addition to this role in basal translation, the elf3a, b, d and g subunits were shown to directly bind 5′UTR of specific mRNAs, leading to cap-dependent translation activation or repression.40 The elf3d subunit that attaches to the edge of the complex appears to play an especially important role in various modes of elf3-dependent translational control: (1) elf3d is involved in the translational repression of Drosophila sex-lethal mRNA through binding to its 5′UTR.78 (2) elf3d was reported to directly bind the cap structure of specific mRNAs in mammalian cells, thus bypassing the requirement of elf4E binding to the cap for translation initiation.71 (3) In the same line, elf3d was involved in cap-dependent translational activation of specific mRNAs for neuronal remodeling in Drosophila larvae, in a context where elf4E is blocked by 4E-binding protein (4E-BP).79 Other studies have reported the role of elf3 in promoting cap-independent translation, thus highlighting elf3 functional versatility in the control of translation. elf3 was shown to directly bind methylated adenosine m6A, in mRNA 5′UTRs to induce cap-independent translation under stress conditions.80 Furthermore, PABP bound to the poly(A) tail was also shown to cooperate with elf3 for its binding to mRNA 5′UTR triggering cap-independent translation.51

Here, we described a new mode of elf3-dependent translational activation through its recruitment by the PIWI protein Aub. Based on previous information on the nos translation repressor complex and data presented here on translational activation, we propose the following model (Fig. 6d). nos mRNA translation is repressed in the somatic part of the embryo by two mechanisms.11,13 First, the 4E-BP protein Cup in complex with Smg binds to elf4E and prevents elf4E recruitment and cap-dependent translation.11,52 The detailed mechanism of Cup recruitment to the repressor complex has not been clarified, but Cup was shown to directly associate with the Not1 subunit of the CCR4-NOT complex and this interaction might stabilize Cup association with elf4E.95 CCR4-NOT itself is recruited to nos mRNA by Smg and Aub.96,97 Second, two translational repressors, the RNA helicase Me31B (Drosophila DDX6) and its partner Tral coat the length of nos mRNA and prevent translation through a cap-independent mechanism.85 Again the mode of Me31B/Tral specific recruitment to nos mRNA has not been determined, but the CCR4-NOT complex might also be involved since DDx6 directly binds the Not1 subunit of CCR4-NOT.54,55 Aub coprecipitation with components of the nos translational repressor complex is consistent with its association with the CCR4-NOT complex in the soma18 and suggests that Aub might be involved in translational repression, in addition to mRNA decay. In the germ plasm, Osk interaction with Smg prevents Smg binding to nos mRNA and this contributes to CCR4-NOT displacement from the mRNP complex. Consistent with this, CCR4 is depleted in the germ plasm.18 The lack of CCR4-NOT on nos mRNA might preclude the recruitment of Me31B/Tral and relieve the cap-independent mechanism of translational repression (Fig. 6d). We find that Aub physically interacts with PABP and several subunits of elf3. We propose that these associations would lead to translational activation independently of elf4E through binding of elf3 to nos 5′UTR, followed by direct recruitment of the 40S ribosome by elf3 and PABP, as previously reported for translation of XIAP mRNA.51 Alternatively, elf3 might act through direct binding of elf3d to the cap structure; however, we do not favor this hypothesis. Indeed, if elf3d interaction with the cap was involved, overexpression of the point mutant elf3dhelix11 that is unable to bind the cap,51 would be expected to induce negative dominant defects, due to the lack of translation mediated by this interaction.96,97 However, overexpression of elf3dhelix11 with the nos-Gal4 driver did not induce any defects in embryonic development or Nos protein synthesis (Supplementary information, Fig. S6).

Germ granules coordinate germ cell mRNA regulation with piRNA inheritance through the role of PIWI proteins.13,19,20 How do germ granules accommodate translational control has remained more elusive. In Drosophila embryos, germ granules contain mRNAs that are translated sequentially.58 We demonstrate a direct role of Aub in translational activation. Strikingly, PABP and elf3d tend to colocalize with Aub at the periphery of germ granules. This is reminiscent of a study analyzing translational control in relation to RNA granules in Drosophila oocytes, in which translational repressors such as Me31B were found to concentrate in the granule core with repressed mRNAs, whereas the translational activator Orb was localized at the edge of the granules where mRNAs docked for translation.59 Similarly, germ granules in embryos might be partitioned into functional subdomains involved in various steps of mRNA regulation, including storage (in an internal region of granules) and translational activation (at the granule periphery).
Our work reveals the central role of Aub in activation of translation. Future studies will undoubtedly address the complexity of mRNA regulation by PIWI proteins in relation with germ granules.

While this manuscript was under review, a role of Miwi and piRNAs in translational activation during mouse spermiogenesis has been demonstrated. Miwi was shown to be in complex with PABP and several subunits of eIF3 for its function in translational activation, which is required for spermatid development. This reveals a striking evolutionary conservation of PIWI protein function in translational control for key developmental processes.

### MATERIALS AND METHODS

**Drosophila lines**

\(w^{1118}\) was used as a control. Mutant alleles and transgenic lines were \(aub^{OC2-}\), \(cn^{bw}/CyO\) and \(aub^{MO2-cn^{bw}}/CyO\), nos-Gal4-VP16, UASp-osk-K10, panx\(^{547}\) and panx\(^{64}\), armi\(^{63}\), armi\(^{2.64}\), nos\(^{54}\), nos\(^{41}\), nos\(^{TM2}\), w; osk\(^{54}\) nos-Gal4-VP16/TM3 Sb, w; osk\(^{54}\) nos-Gal4-VP16/TM3 Sb and yw; osk\(^{54}\) e UASp-GFP-Aub/TM3 Sb, UASp-GFP-Aub, UASp-GFP cytoplasmic (gift from J.M. Dura), eIF3d\(^{7075735}\) (#20072) and eIF3d\(^{654}\) (#43437) (Bloomington Drosophila Stock Center). The UASp-HA-eIF3d and UASp-HA-eIF3d\(^{58}\) lines were generated in this study by insertion of PhiC31 recombination into attP40 site (BestGene). The genotypes of embryos (aged 0–2 h) indicated throughout were the genotypes of mothers. Females of the indicated genotypes were crossed with WT males.

**smFISH**

Dechorionated embryos were fixed at the interface of a 1:1 solution of 10% formaldehyde:100% heptane for 5 min, followed by 100% methanol devitellinization. Embryos were rehydrated, blocked in 1% BSA for 1 h and incubated overnight with primary antibodies. Secondary antibody incubation, after washes in PBS, 0.1% Tween, was performed for 1 h at room temperature. Embryos were mounted in Vectashield (Vector Laboratories) for analysis.

Embryos were washed in Wash Buffer at 37 °C and then in 2× SCC, 0.1% Tween 20, the membrane was incubated for 1 h at room temperature. After washes, HRP-conjugated secondary antibody was used. The quality of the samples was assessed by silver staining (SilverQuest, Invitrogen). For colP experiments, 0.15–0.18 mg of 0–2 h embryos were crushed in IP buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 0.2% NP-40, 1.5 mM DTT, 10 mM EGTA, protease inhibitor) with either 40 U/µL RNase A or 100 U/µL RNase inhibitor. Extracts were centrifuged at 10,000×g for 10 min at 4 °C and incubated on pre-equilibrated magnetic beads with anti-GFP (Chromotek) or anti-HA (Pierce) antibody for 2.5 h at 4 °C. After incubation, the beads were washed five times with IP buffer and immunoprecipitated proteins were eluted from beads by incubation with 2x Laemmli buffer supplemented with 10% β-mercaptoethanol for 5 min at 95 °C. Samples were then analyzed by western blot. For western blot analysis, protein extracts obtained from 30 embryos crushed in 30 µL of 2x Laemmli buffer supplemented with 10% β-mercaptoethanol were boiled for 5 min at 95 °C. Samples were then loaded onto 10% SDS-PAGE gels before transfer to a nitrocellulose membrane. The membrane was blocked for 1 h in 5% milk diluted in 1x PBS, 0.1% Tween 20 before proceeding to primary antibody incubation (overnight, 4 °C on a rotating plate). Antibodies and dilutions for western blot were: rabbit anti-Nos (1:1:000, gift from A. Nakamura), rabbit anti-Osk (1:2000, gift from P. Lasko), mouse anti-HA (1:2000, ascites produced from clone 12CA5), rabbit anti-GFP (1:1:000, Invitrogen), mouse anti-GFP (1:1:000, Roche), goat anti-mouse IgG Cy3 (1:1:000, Jackson Immunoresearch), goat anti-rabbit IgG Alexa-488 (1:800, Invitrogen) and donkey anti-rabbit Cy3 (1:1:000, Jackson Immunoresearch). Microscopy was performed using a Leica SP8 confocal scanning microscope. Data were processed and analyzed using the ImageJ software.

**RNA extraction and RT-qPCR**

Total RNA was prepared from 30 embryos using Trizol (Invitrogen) following recommendations from the manufacturer. For RT-qPCR, 1 µg of total RNA was reverse transcribed using Superscript III (Invitrogen) and random hexamers. Quantitative PCR (qPCR) was performed on a LightCycler LC480 (Roche) with Lightcycler 480 SYBR green master (Roche) and primers are listed in Supplementary information, Table S3. Quantifications were performed in triplicate.

**Communoprecipitation and western blot**

GFP immunoprecipitations for mass spectrometry were performed as follows: 0.5 g of 0–2 h-dechorionated embryos were crushed in DXB buffer (25 mM HEPES, 250 mM sucrose, 1 mM MgCl\(_2\), 1 mM DTT, 150 mM NaCl, protease inhibitor) with 0.1% Triton X-100 and RNasin and incubated on ice for 30 min. Lysates were centrifuged for 10 min and the supernatant was transferred to a new tube. Lysates were incubated on equilibrated GFP-trap beads (Chromotek) overnight at 4 °C on a wheel. Beads were washed seven times in DXB buffer complemented with 1% Triton X-100 and RNasin. Beads were suspended in 2x NuPAGE Blue supplemented with 50 mM DTT and incubated for 10 min at 95 °C. The quality of the samples was assessed by silver staining (SilverQuest, Invitrogen). For colP experiments, 0.15–0.18 mg of 0–2 h embryos were crushed in IP buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 0.2% NP-40, 1.5 mM DTT, 10 mM EGTA, protease inhibitor) with either 40 U/µL RNase A or 100 U/µL RNase inhibitor. Extracts were centrifuged at 10,000×g for 10 min at 4 °C and incubated on pre-equilibrated magnetic beads with anti-GFP (Chromotek) or anti-HA (Pierce) antibody for 2.5 h at 4 °C. After incubation, the beads were washed five times with IP buffer and immunoprecipitated proteins were eluted from beads by incubation with 2x Laemmli buffer supplemented with 10% β-mercaptoethanol for 5 min at 95 °C. Samples were then analyzed by western blot. For western blot analysis, protein extracts obtained from 30 embryos crushed in 30 µL of 2x Laemmli buffer supplemented with 10% β-mercaptoethanol were boiled for 5 min at 95 °C. Samples were then loaded onto 10% SDS-PAGE gels before transfer to a nitrocellulose membrane. The membrane was blocked for 1 h in 5% milk diluted in 1x PBS, 0.1% Tween 20 before proceeding to primary antibody incubation (overnight, 4 °C on a rotating plate). Antibodies and dilutions for western blot were: rabbit anti-Nos (1:1:000, gift from A. Nakamura), rabbit anti-Osk (1:2000, gift from P. Lasko), mouse anti-Aub (4D10, 1:5000), mouse anti-Smg (1:2000, gift from C. Smibert), rabbit anti-PABP (1:500, gift from A. Vincent), rabbit anti-Smg (1:2000), guinea pig anti-Smg (1:2000, gift from C. Smibert), rabbit anti-GFP (1:1:000, Invitrogen), rabbit anti-HA (1:1:000, Covance) and mouse anti-α-Tubulin (1:5000, Sigma). After washes in 1× PBS, 0.1% Tween 20, the membrane was incubated for 1 h at room temperature with secondary antibody coupled to HRP (Jackson Immunoresearch). After washes, HRP-conjugated secondary antibodies were revealed by chemiluminescent detection (Pierce). Quantifications were performed with the ImageJ software using the Gels tool.

**Mass spectrometry**

Total protein elute was loaded on 10% SDS-PAGE gels (Mini-Protean TGX Precast gels, Bio-Rad). For each sample, one band was cut after stacking migration. Gel pieces were destained with three washes in 50% acetonitrile and 50 mM TEABC (trimethyl ammonium bicarbonate buffer). After protein reduction (10 mM DTT in 50 mM TEABC at 60 °C for 30 min) and alkylation (55 mM iodoacetamide in TEABC at room temperature in the dark for 30 min), proteins were in-gel digested using 1 µg Trypsin (Trypsin Gold, Promega). Digested products were dehydrated in a vacuum.
centrifuge. Obtained peptides were analyzed online using Q-Exactive Plus mass spectrometer (Thermo Fisher Scientific) interfaced with a nano-flow HPLC (RSLC U3000, Thermo Fisher Scientific). Samples were loaded onto a 15 cm reverse phase column (Acclaim Pepmap 100, NanoViper, Thermo Fisher Scientific) and separated using a 103-min gradient of 2%–40% of buffer B (80% acetonitrile, 0.1% formic acid) at a flow rate of 300 nL/min. MS/MS analyses were performed in a data-dependent mode (Xcalibur software 4.1, Thermo Fisher Scientific). Full scans (375–1500 m/z) were acquired in the Orbitrap mass analyzer with a 70,000 resolution at 200 m/z. The twelve most intense ions (charge states ≥ 2) were sequentially isolated and fragmented by HCD (high-energy collisional dissociation) in the collision cell and detected at 17,500 resolution. The spectral data were analyzed using the Maxquant software (version 1.5.5.1) with default settings. All MS/MS spectra were searched by the Andromeda search engine against a decay database consisting of a combination of Drosophila melanogaster entries rather than automatic ones, highest evidence for protein sequences into pPHW (UASP-HA-attR1-ccdB-attR2-SV40 3′UTR) in which an attB (pPHW-attB) site has been inserted. The resulting clones were used in gateway cloning to insert the coding sequences into pAct-HA-Renilla-RfA72 (pAHW (DGRC) in which the FFL-coding sequence has been added). To produce HA-RL tagged versions of eIF3b (DGRC, FI08008), eIF3d, eIF3f (DGRC, LD47792), eIF3k (DGRC, LD03569) and eIF4E (from E. Wahle), the coding sequences were amplified using PCR from plasmids. The resulting plasmids were used in gateway cloning to insert the coding sequences into pAct-HA-Renilla-RfA72 (pAHW (DGRC) in which the FFL-coding sequence has been added). To produce GST-PABP clones, the coding sequences of five pAbp domains, RMR1, RMR2, RMR3, RMP4 and PABC were amplified by PCR from a plasmid provided by E. Wahle. A stop codon (TAA) was added at the end of each domain. The different fragments were cloned into pGEX-4T-1 (Sigma) digested with EcoRI and Xhol. The plasmids containing HA-Aub-1(482-866) and HA-Aub(476-866) fragments were generated previously. The primers used to generate the constructs and the constructs are listed in Supplementary information, Tables S3 and 4, respectively.
GST pull-down assays

The plasmids containing GST-RRM1, GST-RRM2, GST-RRM3, GST-RRM4 and GST-PABCD were introduced in E. coli BL21. Protein production was induced by IPTG treatment overnight at 18°C, or at 37°C for GST-RRM2. GST-fused proteins were affinity-purified on glutathione-Sepharose 4B beads (GE Healthcare); the beads were incubated overnight at 4°C in PBT, cComplete™ EDTA-free Protease Inhibitor Cocktail (Roche) and 5% BSA. HA-Aub proteins were synthesized in vitro using the TnT Coupled reticulocyte lysate system (Promega), and were incubated with immobilized GST fusion proteins in 400 µL binding buffer (50 mM HEPES, pH 7.5, 500 mM NaCl, 0.2 mM EDTA, 1 mM DTT, 0.5% NP-40, cComplete™ EDTA-free Protease Inhibitor Cocktail (Roche)) containing 0.2 µg/µL RNase A. Incubations were performed for 1 h at 4°C, followed by 30 min at room temperature. Glutathione-Sepharose beads were then washed four times with binding buffer at room temperature. Recombinant proteins were dissociated from the beads by boiling for 5 min in Laemmli buffer and separated on a SDS-PAGE gel. Western blots were revealed with mouse anti-HA antibody (Covance, MMS-101R) at dilution of 1:1000.

Quantification and statistical analysis

Statistical analysis of mass spectrometric data. Individual LFQ values per detected peptides were first quantile normalized given the experimental condition by using the ProStar (prostar-proteomics.org) software with the default parameter set. After normalization an imputation step was applied in cases where only one value was missing in each condition group by replacing the missing data by the mean of the observed value for this peptide in their respective experimental condition. Then, each individual experiment was combined into one data matrix. To account for batch effects, ComBat from the R package sva was used. After quality controls, differential expression analysis was done using Reproducibility-Optimized Test Statistic (ROTS)14 for each different comparison. P-values and FDR were extracted and plotted using self-written R scripts. Significant proteins were annotated using the FlyMine database.75

Immunofluorescence quantification. Fluorescent images were acquired using a Leica SP8 confocal scanning microscope. Quantification of fluorescent signal was performed using ImageJ tool Measure.

Colocalization quantification. Quantification of colocalization in Fig. 2 was performed in 3D using the Imaris software. For colocalization in granules (around nuclei), spots were defined with a minimal size of 0.5 µm and a PSF correction was applied to account for confocal acquisition deformation. Spot colocalization was determined within a radius of 0.25 µm around the center of the spot. For colocalization in foci (between nuclei), spots were defined with a minimal size of 0.2 µm and a PSF correction was applied to account for confocal acquisition deformation. Spot colocalization was determined within a radius of 0.25 µm around the center of the spot. Quantification of colocalization and overlapping signals in Supplementary information, Fig. S3 was performed using ImageJ, with four embryos per staining. Lines were drawn across each GFP-Aub germ granules to obtain the intensity profiles of GFP-Aub and PABD, or GFP-Aub and HA-elf3d; background signal was subtracted. Each GFP-Aub peak was manually categorized as colocalized, overlapping (single or double) or separated with peaks from the other channel, as depicted in Supplementary information, Fig. S3a, d.

DATA AVAILABILITY

The mass spectrometry proteomics data have been deposited to the ProteomeX-change Consortium via the PRIDE partner repository with the dataset identifier PXD016399.

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AUTHOR CONTRIBUTIONS

M.S. designed the study. A.R., M.-R.G.-S., C.J., R.N.-S., J.D., C.G. and A.C. conducted the experiments and analyzed the data; V.P. and J.C. performed bioinformatic analyses; M.D. performed mass spectrometry experiments; A.B. helped with polysome gradients; F.J. produced clones and provided advices for LUMIER assays. M.S. and A.R. wrote the manuscript; all authors commented on the manuscript.

ADDITIONAL INFORMATION

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