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Report

# Temporal and Spatial Control of Germ-Plasm RNAs

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## Summary

**In many species, germ cells form in a specialized germ plasm, which contains localized maternal RNAs [1–5]. In the absence of active transcription in early germ cells, these maternal RNAs encode germ-cell components with critical functions in germ-cell specification, migration, and development [6, 7]. For several RNAs, localization has been correlated with release from translational repression, suggesting an important regulatory function linked to localization [3, 4, 8, 9]. To address the role of RNA localization and translational control more systematically, we assembled a comprehensive set of RNAs that are localized to polar granules, the characteristic germ-plasm organelles. We find that the 3′-untranslated regions (UTRs) of all RNAs tested control RNA localization and instruct distinct temporal patterns of translation of the localized RNAs. We demonstrate necessity for translational timing by swapping the 3′UTR of *polar granule component* (*pgc*), which controls translation in germ cells, with that of *nanos*, which is translated earlier. Translational activation of *pgc* is concurrent with extension of its poly(A) tail length but appears largely independent of the *Drosophila* CPEB homolog ORB. Our results demonstrate a role for 3′UTR mediated translational regulation in fine-tuning the temporal expression of localized RNA, and this may provide a paradigm for other RNAs that are found enriched at distinct cellular locations such as the leading edge of fibroblasts or the neuronal synapse.**

## Results and Discussion

### Translation of Germ-Plasm RNAs Is Temporally Regulated by Their 3′UTRs

To investigate the translational state of germline-localized RNAs, we assembled a list of RNAs localized to germ plasm by using publicly available databases and published reports. We used data from the Berkeley *Drosophila* Genome Project (BDGP) in situ database, the embryo database by Lecuyer et al., and literature searches to assemble a list of RNAs present in germ cells and then tested these RNAs for their mode of germ-cell localization [10, 11]. We based our analysis on the expression patterns of RNAs previously known to be localized to the germ plasm such as *nanos, germ cell less* (*gcl*), and *polar granule component* (*pgc*). These RNAs were shown by electron microscopy to be localized to the polar granules, which are integral RNA-protein components of germ plasm [12, 13]. *nanos, gcl*, and *pgc* are initially localized in the form of a crescent at the posterior pole of the embryo (stage 1–2) and are then incorporated into developing germ cells (stage 3–4). Our analysis suggests that about ~33% (58/171) of germ-cell RNAs are localized in a manner similar to *nanos*, *gcl*, and *pgc*, whereas the remaining RNAs are protected in germ cells by selective stabilization without prior localization (see Tables S1 and S2 available online). The majority of maternally synthesized RNA is not localized or protected and this RNA is degraded at the transition from maternal to zygotic gene expression (stage 4–5) [8, 14–18]. Of the 58 genes with expression patterns comparable to *nanos, gcl*, and *pgc,* we selected 11 for further analysis (Table 1).

In the case of *nanos*, RNA translation in the embryo is linked to its localization to the germ plasm, and both aspects of RNA regulation are mediated by the *nanos* 3′UTR [8]. In order to determine whether this link between RNA localization and translation applies more generally to RNAs localized to the germ plasm and is mediated by 3′UTRs, we generated reporter constructs containing the 3′UTRs of selected localized RNAs and used previously described reporters for *nanos* and *orb* [19, 20]. We fused the maternally active *nanos* promoter and its 5′UTR to the green fluorescent protein (GFP) coding region, flanked by HA-tags at both the N and C termini, and we then added to this reporter cassette the 3′UTRs of selected localized RNAs (Figure 1A). We assayed the resulting transgenic lines for localization to the germ plasm by using in situ hybridization analysis for *GFP* RNA. For each of the seven transgenes generated, the 3′UTR was sufficient for germ-plasm localization as well as degradation of the uniformly distributed RNA that is found throughout the embryo (Table 1, Figures 1B–1D, Figures S1B–S1K). To determine whether the localization pattern of these hybrid transgenic constructs was germ plasm dependent, we tested a reporter construct containing the *pgc* 3′UTR (*pnos::HA-GFP-HA pgc* 3′UTR) in an *oskar* (*osk*) mutant background in which germ plasm is not formed (Figure S2). *GFP* RNA was not localized to the posterior pole in embryos from *osk* mutant mothers. We confirmed this result by crossing the transgene into females that carried an *osk-bcd* 3′UTR transgene; in this genetic background, germ plasm is formed ectopically at the anterior pole because of OSK-mediated assembly of germ plasm at the anterior pole, and the expression of the reporter was found at the anterior (Figure S2) [21]. Thus, in both assays, localization of the hybrid *pgc* reporter construct was dependent on a functional germ plasm.

The reporter constructs demonstrated that 3′UTRs were sufficient to localize RNAs to the germ plasm, so we wanted to analyze the translational state of these RNAs beginning at the germ-plasm stage (stage 1) through stage 8 of

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Table 1. RNAs Localized to Germ Plasm

| RNA | Function | Germ Plasm[a] | RNA Islands[a] | 3′UTR Sufficiency | References |
|---|---|---|---|---|---|
| *nos* | translational control | + | + | + | [45] |
| *bruno* | translational control | + | + | + | this study |
| *pgc* | transcriptional silencing | + | + | + | this study |
| *gcl* | Germ-cell formation | + | + | + | this study |
| *CG5292* | RNA binding[b] | + | + | + | this study |
| *sra* | $Ca^{2+}$ signaling | + | + | + | this study |
| *CG18446* | zinc ion binding[b] | + | + | + | this study |
| *CG2774* | endocytosis[b] | + | + | + | this study |
| *cyclin B* | cell cycle | + | + | + | [46] |
| *orb* | translational control | + | + | + | this study, [20] |
| *rapgap1* | GTPase | + | + | ND | [47] |

Column 1, genes studied; column 2, known or predicted function; column 3, localization pattern of RNAs as described [10, 11] and confirmed by RNA in situ hybridization; column 4, RNA island formation is a characteristic feature of germ plasm-localized RNAs, first described for *nanos* RNA [48]. Islands of germ plasm form when nuclei migrate into the germ plasm at nuclear cycle 9; all posteriorly localized RNAs studied form RNA islands; column 5, sufficiency of 3′UTR to localize reporter construct to the germ plasm (see also Figure S1); column 6, references for data in column 5. ND, not determined.

[a] Localization data from BDGP [11] or Lecuyer et al. [10] confirmed in this study.

[b] Predicted function.

embryogenesis, when zygotic transcription is initiated in germ cells [22]. In addition to following the expression of GFP protein translated from each respective reporter construct, we analyzed the expression of endogenous proteins when antibodies were available. The results are summarized in Figure 1E and Figures S3–S6. In general, we found that all localized RNAs we tested were translationally regulated. With the exception of *cyclin B,* the reporter RNAs were not translated outside the germ plasm in the early embryo [24]. Although all of the RNAs analyzed showed an apparently identical localization to germ plasm, the onset of translation varied, and we observed distinct patterns, which we assigned to five different classes. Class I RNAs such as *nanos* and *orb* are already translated in the germ plasm (stage 1) (Figure S3). Class II RNAs such as *gcl* are repressed in germ plasm and translated at nuclear cycle 9 just before germ-cell formation (stage 2–3) (Figure S4). Class III RNAs, including *pgc*, *sra*, *CG5292*, *CG18446*, and *rapgap1*, are translationally repressed in the germ plasm and become translationally active concurrent with germ-cell formation (stage 4) (Figure S5). Class IV RNAs such as *bruno* and *CG2774* are not translated in germ cells during embryogenesis (Figure S6). Class V includes *cyclin B,* which is translationally repressed in germ plasm and germ cells and activated at stage 16 when germ cells have reached the somatic gonad [23, 24]. By utilizing available antibodies or published protein expression patterns, we found that the onset of translation was identical between the respective reporter constructs and the endogenous proteins for PGC, GCL, NANOS, and BRUNO [25–28]. However, GFP protein often persisted in germ cells beyond detection of the endogenous protein (Figure S7), likely resulting from differences in protein stability. To address whether the amount of RNA localized may affect the timing of translation, we compared the onset of translation in embryos that received two copies of the *pnos::HA-GFP-HA pgc* 3′UTR transgene from their mother to embryos that had received one copy of the transgene and embryos with reduced germ plasm (derived from mothers heterozygous for *oskar*). Although the amount of RNA localized to the germ plasm clearly differed, the onset of translation was not affected (Figure S8). Taken together, our analysis of multiple localized RNAs suggests that RNA localization per se does not trigger translation and demonstrates that discrete information encoded by specific 3′UTRs dictates the exact timing of expression of a localized RNA.

### Translation of *pgc* Is Associated with Polyadenylation

To further explore the link between 3′UTR-mediated localization and subsequent activity, we chose to focus on Class III RNAs, namely *pgc* and four other RNAs, for which translation was repressed during the early cleavage stages of embryogenesis and was activated upon germ-cell formation (Figure 1E, Figure S5). This particular pattern suggests that these protein products may perform functions required specifically in newly formed germ cells. Indeed, *pgc,* the best-studied representative of this class of RNAs, controls transcriptional silencing in germ cells at this stage [25, 29]. To determine how translation is induced upon germ-cell formation, we determined whether *pgc* activation is mediated by poly(A) tail elongation, a common mechanism of 3′UTR regulation [30]. We collected cDNA from adult ovaries and timed embryo collections by using progeny from wild-type and *oskar* (*osk*) mutant flies and performed poly(A) tail length (PAT) assays [31]. By using RT-PCR we could detect an amplicon for *pgc* RNA during oogenesis in both wild-type and *osk* mutant flies (Figure 2, Figures S9A and S9B). During embryogenesis, we detected a strong *pgc* RNA signal between stages 1 and 4 (0–150 min after egg deposition [AED]) and a weaker signal until stage 8 (330 min AED) in wild-type embryos; this result is consistent with the degradation of the majority of unlocalized *pgc* RNAs during the maternal to zygotic transition of gene expression at stage 4–5 and protection of the localized RNA in germ cells [14]. Indeed, in *osk* mutants in which germ plasm and germ cells fail to form, we detected *pgc* until stage 3–4 but not at later stages (Figure 2A, Figure S9B). By using the PAT assay, we detected a prominent RNA species with a short poly(A) tail of about 100 nucleotides (nt) during oogenesis and embryogenesis in wild-type and *osk* mutant embryos; an additional, longer poly(A) tail of about 200–250 nt was present in the 30–90 min (stage 2–3) collection from wild-type embryos (Figure 2A, Figure S9B). Only 12% of the total RNA was shifted to the longer tail length (Figure S9C). These data are consistent with previous studies showing that only a small fraction of the total *nanos* and *oskar* RNA, 4% and 18%, respectively, are localized [32]. The long poly(A) tail species of *pgc* RNA were detected only in wild-type embryos, but not in *osk* mutant embryos, so we conclude that the long poly(A) tail is present only when *pgc* RNA is localized to the germ plasm and translated in germ cells. We also analyzed the length of the poly(A) tail of the Class IV RNA *bruno*, which is localized but not translated during germ-cell formation. During oogenesis when *bruno* is translated, it has a long poly(A) tail of 350–400 nt (Figure S9D) [28]. However, during embryogenesis, *bruno* is not translated and has a short poly(A) tail of about 75 nt. A short poly(A) tail is also observed for *bruno* in *osk* mutants (Figure S9D). Taken together, our data show that translation of localized RNA correlates with

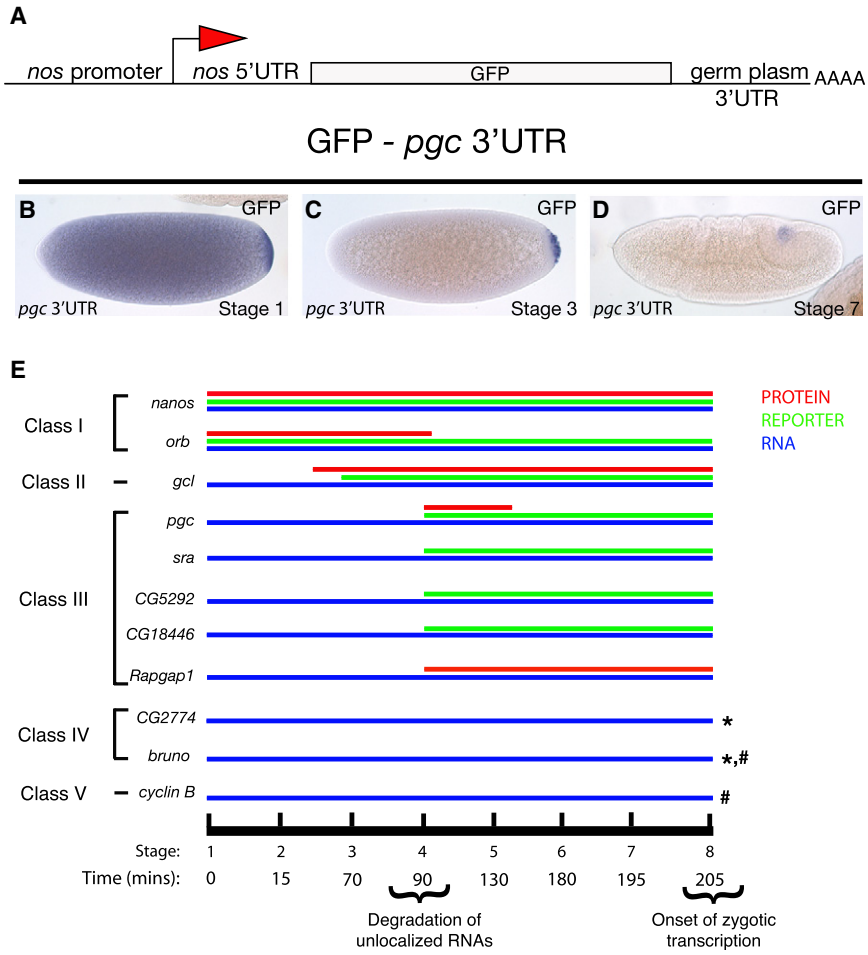


Figure 1. Translational Regulation of Germ-Plasm RNAs

(A) Diagram shows the GFP-HA-3′UTR reporter cassette used in this study. For *nanos* 3′UTR, GFP was fused to Moesin instead of HA [19]. *Orb* 3′UTR was fused to LacZ [20].

(B–D) *pgc* 3′UTR recapitulates endogenous RNA localization. In situ hybridization for *GFP* RNA at different stages of embryogenesis shows degradation of unlocalized *pgc* RNA and protection of localized RNAs in germ cells. Stages as indicated; posterior of the embryo is to the right.

(E) Classification of germ plasm-localized RNAs according to onset of translation. Blue line represents endogenous RNA. Green line represents translation of the reporter construct under control of respective 3′UTRs. Red line represents endogenous protein expression when antibodies were available. Stages of embryonic development and corresponding developmental time after egg deposition are indicated by the black line. Lines marked with asterisk were tested for reporter expression and showed no expression of GFP/HA. Lines marked with hatch mark were tested for expression of endogenous protein and showed no protein expression.

an increase in poly(A) tail length and suggest that polyadenylation is one of the mechanisms that triggers 3′UTR-mediated translational timing.

One mechanism by which the poly(A) tail length of *pgc* RNA may be controlled is by regulated access of the cytoplasmic polyadenylation element binding protein (CPEB) to the RNA. In neuronal granules, as well as during oocyte maturation of *Xenopus laevis* eggs, repressed RNAs are activated by poly(A) elongation via the activity of CPEBs [33]. *Drosophila* has two CPEBs; of these the one encoded by the *orb* gene is predominantly expressed in the germ line. *orb* RNA and protein are both present in germ plasm and in germ cells (Table 1; Figure S10). However, genetic analysis of ORB's role in germ-plasm translation is difficult. ORB plays essential roles during oogenesis including positively regulating the translation of *osk* at the posterior pole of the oocyte [34, 35]. Indeed, the weak $orb^{mel}$ allele has a phenotype similar to that of *osk*, and embryos laid by $orb^{mel}$ mothers fail to assemble germ plasm or form germ cells, precluding the direct analysis of a later role of ORB in germ plasm or germ cells [36].

To assess whether ORB is required for *pgc* RNA poly(A) tail elongation and translational activation, we circumvented the necessity for *orb* in the translation of *osk* and thus the formation of germ plasm. We localized *osk* RNA to the anterior pole of the embryo by utilizing the *bcd* 3′UTR [15]. Embryos from $orb^{mel}/orb^{343}$ mutant mothers carrying both the *osk-bcd* 3′UTR and *pnos::GFP-HA-pgc* 3′UTR transgenes were collected and stained for VASA, a germ-cell marker, and the HA tag to detect expression from the *GFP-HA-pgc* 3′UTR transgene (Figures 2B and 2C). The mutant embryos had no germ cells at the posterior end, confirming the role of ORB protein in the synthesis of endogenous OSK protein. VASA-positive cells, however, were detected at the anterior pole, which also stained positively for HA (Figures 2B and 2C). Thus, *orb* does not affect the translation of *pgc* and *germ cell less* (*gcl*), which are required for germ-cell specification and formation downstream of *osk* [26]. Low levels of ORB activity present in the $orb^{mel}$ mutant could be sufficient for *pgc* and *gcl* translation but not for *osk* translation. Alternatively, the *Drosophila* poly(A) polymerase, *hiiragi* (*hrg*), which has been shown to act cooperatively with ORB for *osk* translation, could act independently of ORB for *pgc* and *gcl* regulation [37]. Another possibility is that deadenylation may be the regulated component that controls the onset of *pgc* and *gcl* translation rather than polyadenylation. Indeed, the CCR4-Not-Pop2 deadenylation complex has been shown to control *Cyclin B* RNA translational repression in early germ cells [24].

## Polar Granules Coordinate Translation of Germ-Plasm RNAs

The role of polar granules in the regulation of germ-cell RNAs remains elusive. In somatic cells, processing (P) bodies are known centers of RNA repression [38]. Because polar granules share common components with P bodies, it has been proposed that polar granules are centers of RNA repression [6, 39]. However, EM studies have also shown that polar granules contain ribosomes, thereby predicting a more active role in translation [13]. Among the localized germ-plasm RNAs that we investigated, *nanos*, *gcl*, and *pgc* are found in polar granules by electron microscopy at the germ-plasm stage [12, 13]. These three RNAs are translated at different time points, namely at the germ-plasm (stage 1), germ-bud (stage 2–3),

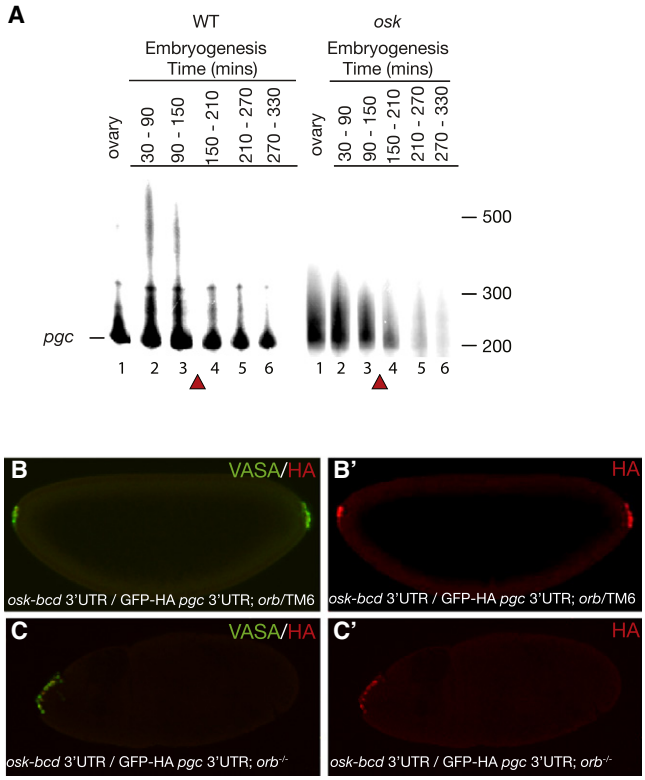


Figure 2. Translation of *pgc* Is Concurrent with Poly(A) Tail Extension and Is CPEB Independent

(A) PAT assay was performed for *pgc* RNA as indicated in Supplemental Experimental Procedures and products were run on a urea denaturing acrylamide gel. Poly(A) tail length of ovary and embryo RNA from wild-type and *osk* mutant females. Lane 1, ovary; lane 2, 30–90 min AED (stage 1–3); lane 3, 90–150 min AED (stage 3–4); lane 4, 150–210 min AED (stage 4–5); lane 5, 210–270 min AED (stage 5–7); lane 6, 270–330 min AED (stage 8–10). The baseline band indicated by a line corresponds to the shortest amplified fragment at 200 nt. Poly(A) tail length is measured from this line. Red triangles mark the maternal to zygotic transition during which unlocalized maternal RNAs are degraded. A loading control for RNA from wild-type and *osk* mutant embryos is shown in Figure S9.

(B and B′) Germ cells are formed at both anterior as well as posterior poles of embryos from *osk-bcd* 3′UTR/ *pnos::HA-GFP-HA-pgc* 3′UTR; $orb^{mel}$/*TM6* mothers. (B) Merge of both VASA and HA antibody; (B′) stained for GFP-HA reporter.

(C and C′) Germ-cell formation only at anterior pole and not posterior pole in embryos from *osk-bcd* 3′UTR/ *pnos::HA-GFP-HA-pgc* 3′UTR; $orb^{mel}$ /$orb^{343}$ mothers. (C) Merge of both VASA and HA antibody; (C′) stained for GFP-HA reporter. Posterior of the embryo is to the right.

and germ-cell (stage 4) stages, respectively. If polar granules had a solely repressive or activating role, one might expect that the association of these RNAs with polar granules would change during development as each RNA becomes translated. We used a transgenic line that expressed an Aubergine-GFP fusion protein (AUB-GFP) to mark polar granules [40, 41] while also assessing *nos, pgc*, and *gcl* RNA localization by fluorescent in situ hybridization. We found that all three RNAs colocalize with polar granules during all stages of germ-cell formation (Figures S11 and S12). Although it is possible that small amounts of RNA leave the granules and are then translated, we favor the hypothesis that polar granules are dynamic centers of RNA regulation that control both RNA repression and translation.

## Regulation of Translation by 3′UTR Is Important for Proper Development

Our results show that germline RNAs are translationally regulated during embryogenesis in a temporally restricted manner. We next wanted to determine whether altering the temporal expression of these RNAs by switching 3′UTRs had consequences for proper germline or somatic development. We chose *pgc* because of its role as a global transcriptional repressor in germ cells, a function that is required when germ cells form [29]. Furthermore, ectopic expression of *pgc* causes transcriptional silencing in somatic tissues [25]. We swapped the *pgc* 3′UTR, which restricts translation to the germ-cell stage (stage 4), with the *nanos* 3′UTR, which confers translational activation earlier as oocytes mature during late oogenesis and in germ plasm (Figures 3B1–3B4′) [42]. In transgenic lines that carry *pgc* under the control of the *nanos* 3′UTR, somatic cells located adjacent to the germ cells failed to cellularize properly and nuclei fell into the yolk, leaving a "pole hole" in ~50% (≥3 cells) of embryos (n = 75) compared to ~10% in wild-type (n = 70) (Figures 3C and 3D). PGC protein represses transcription in a global manner, so we asked whether ectopic PGC protein reduces the expression of zygotically expressed genes that are required for somatic cell formation. We therefore analyzed the status of RNA Polymerase II activity by staining embryos with an antibody that recognizes phosphorylation of Ser2 (pSer2) in the carboxy-terminal domain (CTD) of RNA Polymerase II, a marker for active transcription. In embryos with precocious PGC translation, the pSer2 epitope was reduced in the nuclei of the posterior blastoderm and consequently these nuclei expressed lower levels of proteins like SLAM that are required for somatic cellularization (Figures 3E–3H). We conclude that temporal regulation of germ plasm-restricted RNAs like *pgc* is important to segregate the germline program from the somatic program.

## Conclusion

By systematically analyzing RNAs localized to germ plasm in the embryonic germline, we show that 3′UTRs play an instructive role in the spatial and temporal control of germline expression, a role made especially critical because of the lack of active transcription in early germ cells. In general, sequences within the 3′UTR restrict and protect RNAs that function in germline biology to the germ cells. Moreover, the 3′UTRs also harbor specific programs to repress and activate translation at distinct times of development. Thus, contrary to previous findings with *oskar* and *nanos* RNA, which suggested a mechanism by which translational repression was relieved concomitant with localization, our results suggest that additional mechanisms regulate translation during different stages of germline development. Our results suggest that association with polar granules may not be limited to translationally active or repressed RNAs. Transcription is repressed in germ cells, so intrinsic timing mechanisms need to control the activity of *trans*-acting factors or the accessibility of RNA structures to relieve repression within polar granules. Large-scale RNA localization is not unique to the *Drosophila* germ plasm but has also been observed in migrating fibroblasts and in neuronal dendrites [43, 44]. So far, only a small number of RNAs have been analyzed in detail for their regulation. A more global analysis of regulated RNAs should provide new insight into the logic contained in 3′UTRs that instructs specific translational outcomes.

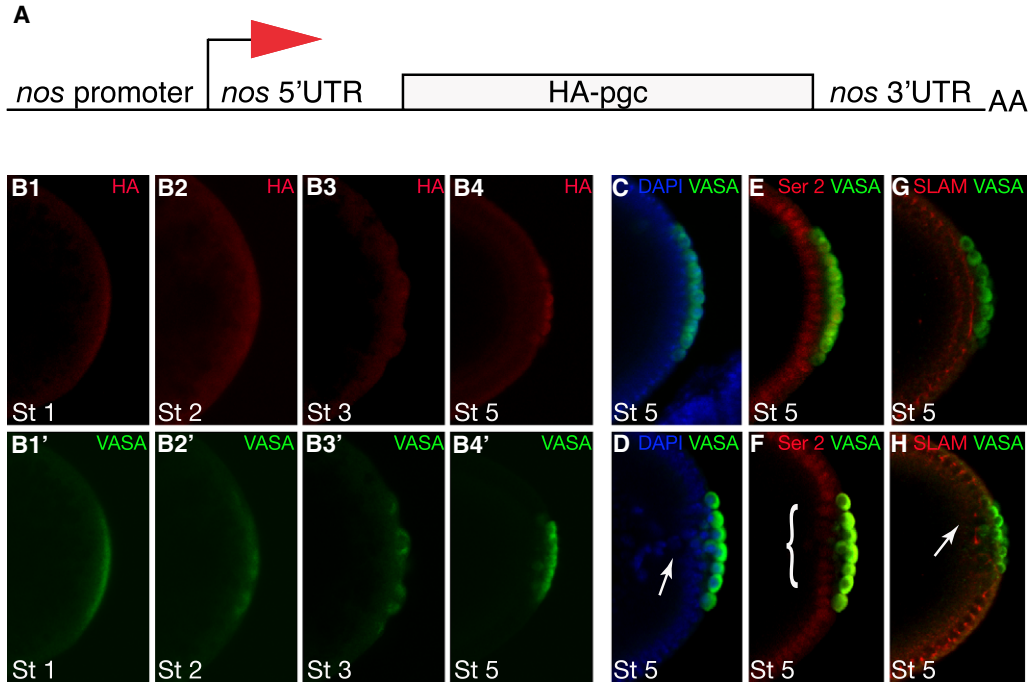


Figure 3. Precocious Expression of PGC Affects Embryonic Development

(A) Diagram showing the *pnos::PGC-HA-nos* 3′UTR construct used in this experiment to translate *pgc* in the germ plasm under the control of the *nos* 3′UTR.
(B) NOS-like translation pattern of embryos from mothers carrying *pnos::PGC-HA-nos* 3′UTR reporter construct. (B1–B4) Immunostaining for HA shows translation in germ plasm and germ cells. (B1′–B4′) Immunostaining for VASA shows staging of germline development.
(C and D) Wild-type embryo (C) and embryo from *pnos::PGC-HA-nos* 3′UTR female (D) stained for DNA in blue (DAPI) and germ cells in green (VASA). The somatic cells adjacent to the germ cells form a continuous epithelial layer in the wild-type (C) but fail to cellularize properly and fall back into the yolk ("Pole hole phenotype") in the embryo that precociously expresses PGC (arrow).
(E and F) Wild-type embryo (E) expresses high levels of active RNA Pol II (detected by antibodies against the P-Ser2 epitope in the CTD of RNA Pol II [in red]) in somatic cells adjacent to the germ cells (VASA, green); note that germ cells are transcriptionally silent because of PGC function. Embryo from *pnos::PGC-HA-nos* 3′UTR female (F) expresses reduced levels of the P-Ser2 epitope (red) in somatic cells (marked by bracket) adjacent to germ cells (VASA, green) because of expanded expression of PGC.
(G and H) Wild-type embryo (H) immunostained for Slow as molasses (SLAM) (red), a zygotically expressed gene required for somatic cellularization; VASA (in green) marks germ cells. Expression of SLAM is disrupted in embryo from female carrying the *pnos::PGC-HA-nos* 3′UTR transgene ([H], arrow).
Stages as indicated, posterior is to the right.

## Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, 12 figures, and 2 tables and can be found with this article online at http://www.current-biology.com/supplemental/S0960-9822(08)01630-8.

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## References

1. King, M.L., Messitt, T.J., and Mowry, K.L. (2005). Putting RNAs in the right place at the right time: RNA localization in the frog oocyte. Biol. Cell *97*, 19–33.
2. Knaut, H., Steinbeisser, H., Schwarz, H., and Nusslein-Volhard, C. (2002). An evolutionary conserved region in the vasa 3′UTR targets RNA translation to the germ cells in the zebrafish. Curr. Biol. *12*, 454–466.
3. Ephrussi, A., Dickinson, L.K., and Lehmann, R. (1991). Oskar organizes the germ plasm and directs localization of the posterior determinant nanos. Cell *66*, 37–50.
4. Kim-Ha, J., Smith, J.L., and Macdonald, P.M. (1991). oskar mRNA is localized to the posterior pole of the *Drosophila* oocyte. Cell *66*, 23–35.
5. Illmensee, K., and Mahowald, A.P. (1974). Transplantation of posterior polar plasm in *Drosophila*. Induction of germ cells at the anterior pole of the egg. Proc. Natl. Acad. Sci. USA *71*, 1016–1020.
6. Seydoux, G., and Braun, R.E. (2006). Pathway to totipotency: lessons from germ cells. Cell *127*, 891–904.
7. Cinalli, R.M., Rangan, P., and Lehmann, R. (2008). Germ cells are forever. Cell *132*, 559–562.
8. Gavis, E.R., and Lehmann, R. (1994). Translational regulation of nanos by RNA localization. Nature *369*, 315–318.
9. Gunkel, N., Yano, T., Markussen, F.H., Olsen, L.C., and Ephrussi, A. (1998). Localization-dependent translation requires a functional interaction between the 5′ and 3′ ends of oskar mRNA. Genes Dev. *12*, 1652–1664.
10. Lecuyer, E., Yoshida, H., Parthasarathy, N., Alm, C., Babak, T., Cerovina, T., Hughes, T.R., Tomancak, P., and Krause, H.M. (2007). Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. Cell *131*, 174–187.
11. Tomancak, P., Berman, B.P., Beaton, A., Weiszmann, R., Kwan, E., Hartenstein, V., Celniker, S.E., and Rubin, G.M. (2007). Global analysis

of patterns of gene expression during *Drosophila* embryogenesis. Genome Biol. *8*, R145.

12. Nakamura, A., Amikura, R., Mukai, M., Kobayashi, S., and Lasko, P.F. (1996). Requirement for a noncoding RNA in *Drosophila* polar granules for germ cell establishment. Science *274*, 2075–2079.
13. Amikura, R., Kashikawa, M., Nakamura, A., and Kobayashi, S. (2001). Presence of mitochondria-type ribosomes outside mitochondria in germ plasm of *Drosophila* embryos. Proc. Natl. Acad. Sci. USA *98*, 9133–9138.
14. Tadros, W., Houston, S.A., Bashirullah, A., Cooperstock, R.L., Semotok, J.L., Reed, B.H., and Lipshitz, H.D. (2003). Regulation of maternal transcript destabilization during egg activation in *Drosophila*. Genetics *164*, 989–1001.
15. Bashirullah, A., Halsell, S.R., Cooperstock, R.L., Kloc, M., Karaiskakis, A., Fisher, W.W., Fu, W., Hamilton, J.K., Etkin, L.D., and Lipshitz, H.D. (1999). Joint action of two RNA degradation pathways controls the timing of maternal transcript elimination at the midblastula transition in *Drosophila melanogaster*. EMBO J. *18*, 2610–2620.
16. Tadros, W., Goldman, A.L., Babak, T., Menzies, F., Vardy, L., Orr-Weaver, T., Hughes, T.R., Westwood, J.T., Smibert, C.A., and Lipshitz, H.D. (2007). SMAUG is a major regulator of maternal mRNA destabilization in *Drosophila* and its translation is activated by the PAN GU kinase. Dev. Cell *12*, 143–155.
17. Bushati, N., Stark, A., Brennecke, J., and Cohen, S.M. (2008). Temporal reciprocity of miRNAs and their targets during the maternal-to-zygotic transition in *Drosophila*. Curr. Biol. *18*, 501–506.
18. Ding, D., Parkhurst, S.M., Halsell, S.R., and Lipshitz, H.D. (1993). Dynamic Hsp83 RNA localization during *Drosophila* oogenesis and embryogenesis. Mol. Cell. Biol. *13*, 3773–3781.
19. Sano, H., Renault, A.D., and Lehmann, R. (2005). Control of lateral migration and germ cell elimination by the *Drosophila melanogaster* lipid phosphate phosphatases Wunen and Wunen 2. J. Cell Biol. *171*, 675–683.
20. Lantz, V., and Schedl, P. (1994). Multiple cis-acting targeting sequences are required for orb mRNA localization during *Drosophila* oogenesis. Mol. Cell. Biol. *14*, 2235–2242.
21. Ephrussi, A., and Lehmann, R. (1992). Induction of germ cell formation by oskar. Nature *358*, 387–392.
22. Van Doren, M., Williamson, A.L., and Lehmann, R. (1998). Regulation of zygotic gene expression in *Drosophila* primordial germ cells. Curr. Biol. *8*, 243–246.
23. Asaoka-Taguchi, M., Yamada, M., Nakamura, A., Hanyu, K., and Kobayashi, S. (1999). Maternal Pumilio acts together with Nanos in germline development in *Drosophila* embryos. Nat. Cell Biol. *1*, 431–437.
24. Kadyrova, L.Y., Habara, Y., Lee, T.H., and Wharton, R.P. (2007). Translational control of maternal Cyclin B mRNA by Nanos in the *Drosophila* germline. Development *134*, 1519–1527.
25. Hanyu-Nakamura, K., Sonobe-Nojima, H., Tanigawa, A., Lasko, P., and Nakamura, A. (2008). *Drosophila* Pgc protein inhibits P-TEFb recruitment to chromatin in primordial germ cells. Nature *451*, 730–733.
26. Jongens, T.A., Hay, B., Jan, L.Y., and Jan, Y.N. (1992). The germ cell-less gene product: a posteriorly localized component necessary for germ cell development in *Drosophila*. Cell *70*, 569–584.
27. Wang, C., and Lehmann, R. (1991). Nanos is the localized posterior determinant in *Drosophila*. Cell *66*, 637–647.
28. Webster, P.J., Liang, L., Berg, C.A., Lasko, P., and Macdonald, P.M. (1997). Translational repressor bruno plays multiple roles in development and is widely conserved. Genes Dev. *11*, 2510–2521.
29. Martinho, R.G., Kunwar, P.S., Casanova, J., and Lehmann, R. (2004). A noncoding RNA is required for the repression of RNApolII-dependent transcription in primordial germ cells. Curr. Biol. *14*, 159–165.
30. Richter, J.D. (1999). Cytoplasmic polyadenylation in development and beyond. Microbiol. Mol. Biol. Rev. *63*, 446–456.
31. Salles, F.J., Richards, W.G., and Strickland, S. (1999). Assaying the polyadenylation state of mRNAs. Methods *17*, 38–45.
32. Bergsten, S.E., and Gavis, E.R. (1999). Role for mRNA localization in translational activation but not spatial restriction of nanos RNA. Development *126*, 659–669.
33. Richter, J.D. (2007). CPEB: a life in translation. Trends Biochem. Sci. *32*, 279–285.
34. Christerson, L.B., and McKearin, D.M. (1994). orb is required for anteroposterior and dorsoventral patterning during *Drosophila* oogenesis. Genes Dev. *8*, 614–628.
35. Lantz, V., Chang, J.S., Horabin, J.I., Bopp, D., and Schedl, P. (1994). The *Drosophila* orb RNA-binding protein is required for the formation of the egg chamber and establishment of polarity. Genes Dev. *8*, 598–613.
36. Castagnetti, S., and Ephrussi, A. (2003). Orb and a long poly(A) tail are required for efficient oskar translation at the posterior pole of the *Drosophila* oocyte. Development *130*, 835–843.
37. Juge, F., Zaessinger, S., Temme, C., Wahle, E., and Simonelig, M. (2002). Control of poly(A) polymerase level is essential to cytoplasmic polyadenylation and early development in *Drosophila*. EMBO J. *21*, 6603–6613.
38. Parker, R., and Sheth, U. (2007). P bodies and the control of mRNA translation and degradation. Mol. Cell *25*, 635–646.
39. Thomson, T., Liu, N., Arkov, A., Lehmann, R., and Lasko, P. (2008). Isolation of new polar granule components in *Drosophila* reveals P body and ER associated proteins. Mech. Dev. *125*, 865–873.
40. Jones, J.R., and Macdonald, P.M. (2007). Oskar controls morphology of polar granules and nuclear bodies in *Drosophila*. Development *134*, 233–236.
41. Harris, A.N., and Macdonald, P.M. (2001). Aubergine encodes a *Drosophila* polar granule component required for pole cell formation and related to eIF2C. Development *128*, 2823–2832.
42. Forrest, K.M., Clark, I.E., Jain, R.A., and Gavis, E.R. (2004). Temporal complexity within a translational control element in the nanos mRNA. Development *131*, 5849–5857.
43. Bramham, C.R., and Wells, D.G. (2007). Dendritic mRNA: transport, translation and function. Nat. Rev. Neurosci. *8*, 776–789.
44. Mili, S., Moissoglu, K., and Macara, I.G. (2008). Genome-wide screen reveals APC-associated RNAs enriched in cell protrusions. Nature *453*, 115–119.
45. Gavis, E.R., and Lehmann, R. (1992). Localization of nanos RNA controls embryonic polarity. Cell *71*, 301–313.
46. Dalby, B., and Glover, D.M. (1992). 3′ non-translated sequences in *Drosophila* cyclin B transcripts direct posterior pole accumulation late in oogenesis and peri-nuclear association in syncytial embryos. Development *115*, 989–997.
47. Chen, F., Barkett, M., Ram, K.T., Quintanilla, A., and Hariharan, I.K. (1997). Biological characterization of *Drosophila* Rapgap1, a GTPase activating protein for Rap1. Proc. Natl. Acad. Sci. USA *94*, 12485–12490.
48. Gavis, E.R., Curtis, D., and Lehmann, R. (1996). Identification of cis-acting sequences that control nanos RNA localization. Dev. Biol. *176*, 36–50.