Maintaining Sufficient Nanos Is a Critical Function for Polar Granule Component in the Specification of Primordial Germ Cells

Girish Deshpande, Emma Spady,1 Joe Goodhouse, and Paul Schedl2
Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544

ABSTRACT Primordial germ cells (PGC) are the precursors of germline stem cells. In Drosophila, PGC specification is thought to require transcriptional quiescence and three genes, polar granule component (pgc), nanos (nos), and germ cell less (gcl) function to downregulate Pol II transcription. While it is not understood how nos or gcl represses transcription, pgc does so by inhibiting the transcription elongation factor b (P-TEFb), which is responsible for phosphorylating Ser2 residues in the heptad repeat of the C-terminal domain (CTD) of the largest Pol II subunit. In the studies reported here, we demonstrate that nos are a critical regulatory target of pgc. We show that a substantial fraction of the PGCs in pgc embryos have greatly reduced levels of Nos protein and exhibit phenotypes characteristic of nos PGCs. Lastly, restoring germ cell-specific expression of Nos is sufficient to ameliorate the pgc phenotype.

The germline of Drosophila arises from a special group of primordial germ cells (PGC). PGCs are formed during nuclear cycles 9 and 10 when nuclei migrate from the center of the embryo into the posterior pole plasm. These nuclei induce cellularization, incorporating the maternal germline determinants that are assembled in the pole plasm during oogenesis. In addition to precocious cellularization, PGCs differ from the surrounding soma in a number of important respects [reviewed in Santos and Lehmann (2004), Seydoux and Bruan (2006), and Wylie (1999)]. One of these is transcription. Whereas somatic nuclei turn on transcription, it is shut down in PGCs, and they remain transcriptionally quiescent until after they exit the gut much later in development (Zalokar 1976; Seydoux and Dunn 1997). Global down-regulation of transcription in PGCs correlates with the phosphorylation status of the heptad repeats in the C-terminal domain (CTD) of the largest Pol II subunit. There are two Serine (Ser) residues in each heptad, Ser2 and Ser5, which are phosphorylated at different steps.

Ser5 phosphorylation is coordinated with initiation, and Ser2 phosphorylation accompanies elongation (Phatnani and Greenleaf 2006; Hirose and Ohkuma 2007). While both of these modifications are elevated in somatic nuclei of blastoderm embryos, this is not true in PGCs: the elongation phosphorylation, PSer2, is absent, and there are only low levels of the initiation phosphorylation, PSer5 (Seydoux and Dunn 1997; Deshpande et al. 2005). Flies are not the only organism in which primordial germ cells downregulate transcription. Transcriptional quiescence is also a hallmark of germ line progenitors in C. elegans and Xenopus (Seydoux and Dunn 1997; Lai et al. 2012).

The establishment of transcriptional quiescence in fly PGCs is mediated by at least three maternally deposited pole plasm determinants, germ cell-less (gcl), polar granule component (pgc), and nanos (nos) (Asaoka et al. 1998, 1999; Deshpande et al. 1999, 2004; Leatherman et al. 2002; Martinho et al. 2004). These three maternal factors have different (though potentially overlapping) gene targets for downregulation, act at slightly different times, and use different mechanisms. gcl functions when PGCs are formed and targets genes that are activated prior to the mid-blastula transition. pgc and nos are required later, and while they both prevent transcription of somatic mid-blastula transition genes, there are differences in their targets. pgc blocks zen and tailess, whereas nos is required to prevent pair rule genes like even-skipped from being activated. Blocking Pol II activity appears to be important for PGC development. While PGCs from gcl and pgc mothers can go on to form functional germline stem cells (GSC), the number of PGCs in coalesced stage 14–15 mutant gonads is substantially reduced. Even more drastic effects are evident in nos embryos. nos PGCs fail to maintain PGC identity, and unlike either
gen or pgc PGCs, they never develop into functional GSCs (Kobayashi et al. 1996; Sato et al. 2007). Further supporting the importance of nos-dependent transcriptional quiescence, nos PGCs can be partially rescued by mutations in one of the nos target genes Sex-lethal (Deshpande et al. 1999).

As for mechanisms, nothing is known about how gen blocks transcription, whereas nos is thought to act by repressing the translation of an unknown general transcription factor. For pgc, the mechanism is well understood (Hanyu-Nakamura et al. 2008). Pgc protein interacts with transcription elongation factor b (P-TEFb), which is responsible for phosphorylating Ser2 residues in the CTD heptad repeat. The association of Pgc with P-TEFb prevents P-TEFb from being recruited to sites of paused polymerase. Consistent with this biochemical mechanism, PGCs in blastoderm-stage pgc mutants have high levels of CTD-PSer2. Moreover, targeting transcriptional elongation seems to be a conserved mechanism for imposing transcriptional quiescence, as the C. elegans PIE-1 protein is also thought to arrest transcription by inhibiting P-TEFb (Seydoux et al. 1996; Batchelder et al. 1999; Nakamura and Seydoux 2008).

Although it has been suggested that Pgc and its target P-TEFb are the central players in establishing transcriptional quiescence in newly formed PGCs (Cinalli et al. 2008; Nakamura and Seydoux 2008), it is striking that loss of pgc does not fully disrupt the specification of PGC fate or their eventual transition into functional GSCs. This would suggest either that the establishment and/or maintenance of transcriptional quiescence is not a necessary step in PGC development in Drosophila or that pgc has an important, but not absolutely essential, role in this process. For these reasons, we have re-examined the functioning of pgc in germline development and explored its relationship to the PGC/GSC-determinant nos.

**MATERIALS AND METHODS**

**Fly stocks and culture**

Flies were grown at room temperature (22°C) on standard medium. The following stocks obtained from Bloomington Stock Center were used for analysis: nanos-Gal4; VP16, twist-Gal4, nosBN, nosBC. Also used were two different extensively characterized loss-of-function alleles of pgc: pgcD10093B and pgcF17794D (Martinho et al. 2004). The loss of Nos protein in pgc PGCs was confirmed using AS-26, antisense pgc transgene (Nakamura et al. 1996). Nos-tubulin 3' UTR transgenes were a kind gift from Liz Gavis, Princeton University. Typically, virgin females homozygous for the transgene were crossed and stained for fluorescence-based stainings, to begin with, using AS-26, and stained for GSCs using AS-26, to confirm PGC numbers. If it does, one would expect to find that some pgc PGCs exhibit characteristic nos-like phenotypes, while others do not. Although both pgc and nos are known to play important roles in establishing transcriptional quiescence, they interfere with polymerase activity at different steps. Elegant studies by Hanyu-Nakamura et al. (2008) have shown that Pgc imposes transcriptional quiescence by specifically inhibiting the P-TEFb-dependent transcriptional elongation CTD phosphorylation, PSer2. By contrast, nos appears to downregulate transcription in PGCs at an earlier step in the transcription cycle, as the levels of both the initiation CTD phosphorylation, PSer5, and the elongation CTD phosphorylation, PSer2, are elevated in all nos mutant PGCs (Deshpande et al. 2008). If the loss of Nos in pgc PGCs affects their specification, then we would expect to find that PSer2 is upregulated in cells that have reduced amounts of Nos. Before testing this possibility, we first confirmed previous reports that PSer2 is elevated in all pgc PGCs (data not shown). We next examined PSer5 in wild-type, nos, and pgc PGCs. As shown for one of the pgc alleles in Figure 2, although PSer5 is

**RESULTS AND DISCUSSION**

**Subset of pgc pole cells displays loss of Nos protein**

Although Nakamura et al. (1996) have reported that nos mRNA levels in PGCs are reduced when pgc activity is compromised by an antisense pgc transgene, it has been argued that this diminution of nos mRNA is too slight and takes place too late to be relevant for pgc function (Martinho et al. 2004). However, Nos protein accumulation in PGCs compromised for pgc was not examined, and it seemed possible that the loss of pgc activity might have a greater effect on protein expression than it does on nos message levels. For this reason, we examined Nos accumulation in syncytial and cellular blastoderm-stage in both antisense embryos (see supporting information, Figure S1) and embryos produced by two different pgc mutant alleles (Figure 1). In all three cases, we found that PGCs compromised for pgc activity exhibit an unusual, heterogeneous pattern of Nos accumulation. As illustrated for a stage 4 pgc embryo in Figure 1, the pattern of Nos protein accumulation in pgc PGCs is quite different from that in wild-type. In wild-type embryos, Nos protein accumulates to essentially the same level in most all PGCs (Figure 1; see also Figure 2 and Figure S1). By contrast, Nos levels are quite variable in pgc PGCs (Figure 1; see also Figure 2 and Figure S1). Some PGCs have near wild-type levels of Nos, others have intermediate amounts, and still others have little or no Nos. This highly heterogeneous pattern of Nos accumulation is evident soon after PGC formation and continues beyond the cellular blastoderm stage. Overall, over half of the pgc PGCs (58%; 75 out of 126 PGCs) in syncytial and cellular blastoderm-stage embryos have clearly reduced levels of Nos protein, whereas in wild-type, Nos levels are reduced in less than 10% (7%; 6 out of 91 PGCs) of the PGCs. From these findings, we conclude that the previously reported diminution of nos mRNA impacts Nos accumulation, but significantly, it does so only in a subset of the PGCs.

To determine whether the effects on Nos are specific, we examined the accumulation of another germline-specific translation factor, Vasa. As shown in Figure 1, Vasa levels in blastoderm-stage pgc PGCs are unaffected and resemble wild-type PGCs. However, later in development, Vasa levels are often substantially reduced in pgc PGCs that are undergoing apoptosis.

**Initiation CTD phosphorylation PSer5 is upregulated in a subset of pgc PGCs**

An intriguing question is whether the reduction in Nos protein in a subset of the pgc PGCs has any impact on the development of these cells. If it does, one would expect to find that some pgc PGCs exhibit characteristic nos-like phenotypes, while others do not. Although both pgc and nos are known to play important roles in establishing transcriptional quiescence, they interfere with polymerase activity at different steps. Elegant studies by Hanyu-Nakamura et al. (2008) have shown that Pgc imposes transcriptional quiescence by specifically inhibiting the P-TEFb-dependent transcriptional elongation CTD phosphorylation, PSer2. By contrast, nos appears to downregulate transcription in PGCs at an earlier step in the transcription cycle, as the levels of both the initiation CTD phosphorylation, PSer5, and the elongation CTD phosphorylation, PSer2, are elevated in nos mutant PGCs (Deshpande et al. 2005).
clearly present in wild-type PGCs, the level of this CTD phosphorylation is substantially reduced compared with nearby somatic nuclei \((n = 70)\). Similar results were obtained for the other \(pgc\) allele and for the \(pgc\) antisense. By contrast, in \(nos\) embryos, essentially all PGCs have levels of PSer5 approaching that in the surrounding somatic nuclei (see Figure S2). As was observed for Nos protein, there is a quite heterogeneous pattern of PSer5 in \(pgc\) PGCs. As shown in Figure 2, some \(pgc\) PGCs resemble wild-type and have little PSer5. However, a subset of the PGC nuclei have levels of PSer5 approaching that found in somatic nuclei. A careful analysis of multiple \(pgc\) embryos indicates that the extent of upregulation of PSer5 is variable and that elevated levels of this CTD phosphorylation are seen unambiguously in only about 50% of stage 4 or 5 PGCs \((n = 100)\). In the remaining PGCs, there was either no increase or only a marginal increase.

We next asked whether the \(pgc\) PGCs with elevated nuclear PSer5 are the ones with reduced amounts of Nos. To test this possibility, we examined PGCs in stage 5 (cellular blastoderm embryos) coimmunostained with Pser5 and Nos antibodies. Figure 2 shows that the subset of PGCs with reduced Nos corresponds closely to the subset with elevated PSer5. In this experiment, we found that 61% \((32 \text{ out of } 53)\) of the \(pgc\) pole cells had reduced amounts of Nos. Of the pole cells with reduced Nos, PSer5 was elevated in nearly 90% \((27 \text{ out of } 30)\). Significantly, none of the PGCs with normal levels of Nos had elevated PSer5. We next examined stage 4 embryos to test whether Nos protein

---

**Figure 1** Nos protein levels are diminished in \(pgc\)-PGCs. Wild-type (panels A–C and G–I) and \(pgc\) (panels D–F and J–L) blastoderm-stage embryos were probed with Vasa (green) and Nos (red) antibodies. Panels A, D, G, and J show the merged images; panels B, E, H, and K show Vasa alone; and panels C, F, I, and L show Nos alone. Although levels of Nos are reduced, sometimes substantially in a subset of \(pgc\) PGCs, there is no apparent alteration in the levels of Vasa protein at the blastoderm stage. The difference and heterogeneity in Nos level is apparent in panels G–L, which show magnified images of two pole cells each. Similar results were obtained in three independent trials. The numbers in the text are from one of these experiments.

**Figure 2** Loss of Nanos protein is in \(pgc\) PGCs is correlated with increased CTD PSer5. Stage 5 embryos from either wild-type mothers (A–C) or \(pgc\) mothers (D–F) were coimmunostained with Nos (red) and Pser5 (green) antibodies. Many \(pgc\) PGCs have reduced levels and/or uneven distribution of Nos (compare panels E and K). PGCs with reduced Nos have elevated Pser5 (panels F and L). One of the two different \(pgc\) pole cells displays higher levels of Nos protein and corresponding decreases in Pser5 levels. By contrast, wild-type PGCs show only low levels of signal compared with surrounding somatic nuclei (panels D–F). Similar results were obtained in two independent experiments. The numbers in the text are from one of the experiments. Arrows point to \(pgc\) PGC with reduced Nos and elevated Pser5.
levels are diminished in pgc PGCs in stage 4 embryos. As can be seen from embryo shown in Figure S3, Nos protein levels are non-uniform in pgc PGCs even at this earlier stage. Moreover, in the PGCs with reduced Nos, there is concomitant increase in PSer5 levels.

**Does loss of Nos in pgc PGCs have other phenotypic consequences?**

The results described above argue that the CTD initiation phosphorylation is upregulated in a subset of pgc mutant PGCs because they lack sufficient Nos. If this conclusion were correct, then we would expect an approximately similar fraction of the pgc PGCs to exhibit other phenotypes characteristic of nos PGCs, such as migration defects, upregulation of Cyclin B, and premature division, cell death, and a failure to initiate the transition from PGC to GSC identity. Consistent with these expectations, Nakamura et al. (1996) reported that a subset of the PGCs in progeny of antisense pgc mothers exhibited migration defects and died. We confirmed the migration defects in progeny pgc mutant mothers. We also found that the coalesced gonads of stage 14 mutant embryos had fewer PGCs. Whereas wild-type have 9.5 PGCs/gonad (n = 100), pgc embryos have on average 3.5 PGCs/gonad (n = 75). To address this issue further, we determined whether pgc PGCs exhibit other nos-like phenotypes.

Premature cell division in nos PGCs is due to the inappropriate expression of the mitotic cyclin Cyclin B (Asaoka et al. 1999). In wild-type PGCs, Nos together with Pumilio repress the translation of cyclinB mRNA and PGCs arrest the mitotic cycle in G2. To determine whether loss of Nos in pgc PGCs results in the premature expression of Cyclin B protein, we probed wild-type and pgc embryos with Cyclin B antibodies. As shown in Figure 3, a substantial fraction of pgc PGCs in stage 10 embryos (70%; >100 PGCs) have Cyclin B, whereas Cyclin B is infrequently detected in PGCs of wild-type embryos (5%; >100 PGCs) of the same stage. In fact, in wild-type embryos, Cyclin B is not upregulated until much later in development after gonad coalescence (Asaoka et al. 1999).

Cell death in nos PGCs is due to activation of the head involution defective apoptosis pathway Sato et al. (2007) and Maezawa et al. (2009) have shown that over 20% of the PGCs in stages 12–16 nos embryos express the cell death marker cleaved Caspase3. We used antibodies specific for cleaved Caspase3 to test whether this pathway is also activated in pgc PGCs. As Figure 4 shows, all pgc embryos examined had at least one PGC that was positive for cleaved Caspase3. Many of the activated Caspase3-positive PGCs also had greatly reduced Vasa (arrows). As Vasa differed from Nos in that it was not lost in cellular blastoderm pgc PGCs, we suspect that this is a consequence of cell death rather than some more direct function of pgc in sustaining Vasa protein levels.

The transition from PGC to GSC identity begins with assembly of a germ cell-specific organelle called the spectrosome (Lin et al. 1994; Lin and Spradling, 1995). Spectrosome-like structures can be detected at stage 11, just after the PGCs exit the midgut and start migrating through the mesoderm. Between stages 11 and 15 of embryogenesis as PGCs complete their migration and coalesce into the embryonic gonad, the spectrosome enlarges progressively. By stage 15, it is spherical in shape and closely resembles the structure found in adult GSCs.

Wawersik and Van Doren (2005) have shown that nos is required to initiate and maintain the assembly of spectrosomes in migrating PGCs of stages 11 and 12 embryos, and in nos mutants, spectrosomes are not detected in more than 90% of PGCs. To test whether spectrosome assembly is also disrupted in the progeny of pgc mothers, we probed wild-type and pgc embryos with spectrin antibodies. We found that newly formed spectrosomes in PGCs of stages 11 and 12 pgc embryos are typically smaller than the spectrosomes in wild-type embryos of the same age (not shown). As shown in Figure 5, abnormalities in spectrosome assembly were even more apparent after gonad coalescence. In wild-type stage 14 gonads (Figure 5A), all PGCs are typically smaller than the spectrosomes in wild-type embryos. Whereas Cyclin B is infrequently observed in wild-type stage 10 PGCs (5%; 1 out of 19), the majority of the pgc PGCs express detectable levels of Cyclin B at this stage of development (68%; 17 out of 25). Three independent experiments yielded similar results. Also, note that inappropriate expression of Cyclin B can be detected in pgc PGCs from younger blastoderm-stage embryos; however, Cyclin B–positive PGCs are much less frequent at earlier stages. Arrows in panels E and F indicate PGCs with elevated levels of Cyclin B, while the arrowhead indicates a PGC with little or no Cyclin B.

**Is nos a critical pgc target?**

The results described above indicate that in addition to inappropriately upregulating CTD initiation phosphorylation, several nos phenotypes evident in PGCs of older embryos are recapitulated in pgc mutants. One hypothesis to explain this connection is that a critical function of pgc in PGC specification and development is to ensure proper Nos accumulation. In this scenario, PGCs in pgc embryos that have reduced Nos would not be properly specified, have defects in migration, and undergo apoptosis. Moreover, all of these phenotypes would be the consequence of failing to maintain sufficient amounts of Nos. In contrast, the pgc PGCs that are able to coalesce properly with the somatic gonad and develop into functional GSCs would be limited (at the minimum) to those that maintain sufficient levels of Nos. If this hypothesis were correct, it should be possible to rescue pgc PGCs by providing Nos protein.
For this purpose, we used the Gal4/UAS system. Because the nos 3’ UTR contains elements that control localization, translation, and stability, we used an UAS transgene carrying the nos coding sequence fused to the tubulin 3’ UTR (Bergsten and Gavis 1999). To drive expression specifically in the germline, we used a nos:Gal4 transgene.

As the nos promoter is not activated in PGCs until after they exit the midgut and begin migrating toward the somatic gonad, we anticipated that if supplying Nos rescues the pgc PGCs, rescue should be at best incomplete because of this delay. Figure 6 shows that the effects of pgc on the migration and viability of PGCs can be partially rescued by providing Nos. While embryonic gonads of pgc mutants have on average 3.5 PGCs (n = 32), pgc mutants carrying the nos:Gal4/UAS: nos-Tublin 3’ UTR transgene combination had on average 7 (n = 35; P value = 1.918 × 10⁻⁵). The effects of supplying Nos can also be seen in the upward shift in the number of PGCs in embryonic gonads (e.g. nearly 40% of the pgc embryos have 3 or fewer PGCs/embryonic gonad, whereas about 90% of the rescued embryos had 4 PGCs or more).

The finding that PGCs in pgc mutant embryos can be rescued by supplying Nos indicates that one critical pgc function is to ensure that there are sufficient amounts of Nos. This conclusion fits with the nos-like phenotypes evident in a subset of pgc mutant PGCs. At the blastoderm stage, the subset of PGCs that lose Nos also fail to prevent upregulation of the CTD initiation phosphorylation PSer5, which is the target for nos-dependent transcriptional quiescence. Although it wasn’t possible to draw a similar connection in the older pgc embryos, we nevertheless found that a substantial fraction of the PGCs exhibit phenotypes characteristic of nos mutations (migration defects, premature Cyclin B expression, failure to initiate spectrosome assembly, and activation of apoptosis). Because rescue in our experiment was incomplete, it could be argued that pgc has functions that are important for the specification and development of PGCs in addition to maintaining high levels of Nos. On the other hand, not all pgc PGCs lose Nos (at the blastoderm stage). Consequently, it remains possible that all that is needed for pgc PGCs to form fully functional GSCs is that they retain sufficient levels of Nos. If this were true, then the only important (but not absolutely essential) function of pgc would be to ensure that the levels of Nos in PGCs remain high enough so that it can properly specify PGC/GSC fate.

While our findings argue that maintaining full nos activity is a critical function of pgc, it is not clear which of the known nos regulatory

Figure 4 pgc pole cells undergo apoptosis. Stages 13–15 wild-type (not shown) and pgc embryos were probed with activated Caspase3 (green) and Vasa (red) antibodies. Activated caspase3 is only present in cells undergoing apoptosis. Panels A, C, and G show both Vasa and activated Caspase3; panels B, D, and F show only activated Caspase3. Arrows indicate Vasa-positive cells with activated Caspase3. In some cases, little Vasa remains. No activated Caspase3 was detected in PGCs of similarly staged wild-type embryos (n = 20 embryos; >100 PGCs). For this reason, the control is not shown here. The experiment was repeated twice; numbers in the text represent a single trial.

Figure 5 Defective spectrosomes in pgc PGCs. Wild-type and pgc embryos were probed with alpha-Spectrin (green) and Vasa (red) antibodies. Panels A, C, and E show both the signals, whereas panels B, D, and F show only the Spectrin-specific signal. Wild-type germ cells in coalesced embryonic gonads have characteristic large, spherical, GSC-like spectrosomes (panel B), whereas pgc germ cells either lack spectrosomes altogether (panel D) or have incompletely formed spectrosomes (panel F). Arrows indicate GSC-like spectrosomes in wild-type and pgc PGCs.
Figure 6  Germ-cell loss in pgc mutants is partially rescued by ectopic Nos. Blue and red bars correspond to PGCs gonad in pgc and pgc; nos: Gal4/UAS nos: tubulin 3′ UTR embryos, respectively. The embryonic gonads were classified based on total number of surviving PGCs at stage 13 and beyond. This experiment was done twice. Both experiments gave similar distributions and are tabulated together in the text.

targets is primarily responsible for the detrimental effects on PGC development. Previous studies indicate that an important function for nos in PGC development is to maintain transcriptional quiescence (Deshpande et al. 1999). Moreover, as is observed in nos mutant PGCs, the initiation CTD phosphorylation Ser5 is elevated in pgc PGCs that have reduced levels of Nos protein but not in PGCs that have wild-type levels of Nos. Although this correlation would seem to point to nos-dependent transcriptional quiescence, misexpression of two known nos transcriptional targets, Sxl and even-skipped, was not detected in pgc PGCs (Deshpande et al. 2004; Martinho et al. 2004). Thus, it is possible that the detrimental effects of reduced Nos in pgc PGCs arises from a failure to regulate one of the other nos targets, for example Cyclin B mRNA translation. On the other hand, while Nos protein is reduced in amount in a subset of blastoderm-stage pgc PGCs, it is not completely eliminated at this point in development, and further reductions might occur as embryogenesis proceeds. In this case, it is possible that some of the nos transcriptional targets become activated a bit later in development. An additional caveat is that the methods used to assay Sxl and even-skipped expression were not the most sensitive, and a low level of expression of these genes in a subset of the blastoderm pgc PGCs could have been missed.

The idea that pgc might play a subordinate role to nos in germline development and that its primary function is to ensure that Nos can specify PGC/GSC fate would dovetail nicely with recent studies of Lai et al. (2012) on the development of the primordial germline in the vertebrate Xenopus. As has been previously reported for the functioning of nos during germline development in the Drosophila embryo, Lai and coauthors found that Xenopus nos is required for transcriptional quiescence, for germ cell survival and migration to the somatic gonad, and for the process of germ cell specification. In this context, it is also notable that the nos gene is widely conserved across the animal kingdom, as is its function in the process of PGC/GSC specification [for example, see Suzuki et al. (2007) and Tsuda et al. (2003)]. By contrast, nos function in abdominal segmentation in Drosophila appears to be a specialized adaption that is likely restricted to insects. Similarly, the pgc gene is not well conserved. These differences would be consistent with the speculation that a pgc-like activity evolved in flies (and presumably other insects) to ensure that the functioning of nos in germline development is not compromised by the quite different requirements for its activity and its regulation in the development of the posterior soma.

ACKNOWLEDGMENTS

We would like to acknowledge Yu-Chiu Wang and Liz Gavis for discussions and advice on this project. For antibodies and stocks, we thank Liz Gavis, Akira Nakamura, Paul Lasko, Paul Macdonald, and Bloomington Stock Center. Gordon Gray provided the fly food. Research was supported by a grant from the National Institutes of Health (GM-043432).

LITERATURE CITED

Asaoka, M., H. Sano, Y. Obara, and S. Kobayashi, 1998 Maternal Nanos regulates zygotic gene expression in germline progenitors of Drosophila melanogaster. Mech. Dev. 78(1–2): 153–158.
Asaoka, M., M. Yamada, A. Nakamura, K. Hanyu, and S. Kobayashi, 1999 Maternal pompilio acts together with Nanos in germline development in Drosophila embryos. Nat. Cell Biol. 1: 431–437.
Batchelder, C., M. A. Dunn, B. Choy, C. Cassie, E. Y. Shim et al., 1999 Transcriptional repression by the Caenorhabditis elegans germ-line protein PLE-1. Genes Dev. 13: 202–212.
Bergsten, S. E., and E. R. Gavis, 1999 Role for mRNA localization in translational activation but not spatial restriction of nanos RNA. Development 126: 659–669.
Brand, A. H., and N. Perrimon, 1993 Targeted gene expression as a means of altering cell fates and generating dominant expression. Development 118: 401–415.
Cinalli, R. M., P. Rangan, and R. Lehmann, 2008 Germ cells are forever. Cell 132: 559–562.
Deshpande, G., J. Stukey, and P. Schedl, 1995 scute (sis-b) function in Drosophila sex determination. MCB 15: 4430–4440.
Deshpande, G., G. Calhoun, J. Yanowitz, and P. Schedl, 1999 Novel functions of nanos in downregulating mitosis and transcription during the development of Drosophila germine. Cell 99: 271–281.
Deshpande, G., G. Calhoun, and P. D. Schedl, 2004 Overlapping mechanisms function to establish transcriptional quiescence in the embryonic Drosophila germine. Development 131: 1247–1257.
Deshpande, G., G. Calhoun, T. M. Jinks, A. D. Polydorides, and P. Schedl, 2005 Nanos downregulates transcription and modulates CTD phosphorylation in the soma of early Drosophila embryos. Mech. Dev. 122: 645–657.
Hanyu-Nakamura, K., H. Sonobe-Nojima, A. Tanigawa, P. Lasko, and A. Nakamura, 2008 Drosophila Pgc protein inhibits P-TEFb recruitment to chromatin in primordial germ cells. Nature 451: 730–733.
Hirose, Y., and Y. Okhuma, 2007 Phosphorylation of the C-terminal domain of RNA polymerase II plays central roles in the integrated events of euarkyotic gene expression. J. Biochem. 141: 601–608.
Kobayashi, S., M. Yamada, M. Asaoka, and T. Kitamura, 1996 Essential role of the posterior morphogen nanos for germine development in Drosophila. Nature 280: 708–711.
Lai, F., A. Singh, and M. L. King, 2012 Xenopus Nanos1 is required to prevent endoderm gene expression and apoptosis in primordial germ cells. Development 139: 1476–1486.
Leatherman, J. L., L. Levin, J. Boero, and T. A. Jongens, 2002 Germ cell-less acts to repress transcription during the establishment of the Drosophila germ cell lineage. Curr. Biol. 12: 1681–1685.
Lin, H., and A. C. Spradling, 1995 Fusome asymmetry and oocyte determination in Drosophila. Dev. Genet. 16: 6–12.
Lin, H., L. Yue, and A. C. Spradling, 1994 The Drosophila fusome, agermine-specific organelle, contains membrane skeletal proteins and functions in cyst formation. Development 120: 947–956.
Maeszawa, T., K. Arita, S. Shigenobu, and S. Kobayashi, 2009 Expression of the apoptosis inducer gene head involution defective in primordial germ cells of the Drosophila embryo requires eiger, p53, and loki function. Dev. Growth Differ. 51: 453–461.
Martinho, R. G., P. S. Kunwar, J. Casanova, and R. Lehmann, 2004 A non-coding RNA is required for the repression of RNA pol II-dependent transcription in primordial germ cells. Curr. Biol. 14: 159–165.
Nakamura, A., and G. Seydoux, 2008  
Less is more: specification of the germline by transcriptional repression.  
Development 135: 3817–3827.

Nakamura, A., R. Amikura, M. Mukai, S. Kobayashi, and P. F. Lasko,  
1996  
Requirement for a noncoding RNA in Drosophila polar granules  
for germ cell establishment. Science 274: 2075–2079.

Phatnani, H. P., and A. L. Greenleaf, 2006  
Phosphorylation and functions of the RNA polymerase II CTD.  
Genes Dev. 20: 2922–2936.

Santos, A. C., and R. Lehmann, 2004  
Germ cell specification and migration  
in Drosophila and beyond. Curr. Biol. 14: R578–R589.

Sato, K., Y. Hayashi, Y. Ninomiya, S. Shigenobu, K. Arita et al.,  
2007  
Maternal Nanos represses hid/skl-dependent apoptosis to maintain  
the germ line in Drosophila embryos. Proc. Natl. Acad. Sci. USA 104:  
7455–7460.

Seydoux, G., and R. E. Bruan, 2006  
Pathway to totipotency: lessons from germ cells.  
Cell 127: 891–904.

Seydoux, G., and M. A. Dunn, 1997  
Transcriptionally repressed germ cells  
lack a subpopulation of phosphorylated RNA polymerase II in early  
embryos of Caenorhabditis elegans and Drosophila melanogaster.  
Development 124: 2191–2201.

Seydoux, G., C. C. Mello, J. Pettitt, W. B. Wood, J. R. Priess et al., 1996  
Repression of gene expression in the embryonic germ lineage of C. elegans.  
Nature 382: 713–716.

Suzuki, A., M. Tsuda, and Y. Saga, 2007  
Functional redundancy among Nanos proteins and a distinct role of Nanos2  
during male germ cell development. Development 134: 77–83.

Tsuda, M., Y. Sasaoka, M. Kiso, K. Abe, S. Haraguchi et al., 2003  
Conserved role of nanos proteins in germ cell development. Science 301: 1239–1244.

Wawersik, M., and M. Van Doren, 2005  
Nanos is required for formation of the spectrosome, a germ cell specific organelle.  
Dev. Dyn. 234: 22–27.

Wylie, C., 1999  
Germ cells. Cell 96: 165–174.

Zalokar, M., 1976  
Autoradiographic study of protein and RNA formation during early development of  
Drosophila eggs. Dev. Biol. 49: 425–437.

Communicating editor: B. J. Andrews