

A Novel Function for the Sm Proteins in Germ Granule Localization during *C. elegans* Embryogenesis

Scott A. Barbee,¹ Alex L. Lublin,¹
and Thomas C. Evans^{1,2,3}

¹Program in Cell and Developmental Biology

²Department of Cellular and Structural Biology, B111
University of Colorado Health Sciences Center
4200 East Ninth Avenue
Denver, Colorado 80262

Summary

General mRNA processing factors are traditionally thought to function only in the control of global gene expression. Here we show that the Sm proteins, core components of the spliceosome, also regulate germ granules during early *C. elegans* development. Germ granules are large cytoplasmic particles that localize to germ cells and their precursors during embryogenesis of diverse organisms [1]. In *C. elegans*, germ granules, called P granules, are segregated to the germline precursor cells during embryogenesis by asymmetric cell division, and they remain in germ cells at all stages of development [2]. We found that at least some Sm proteins are components of P granules. Moreover, disruption of Sm activity caused defects in P granule localization to the germ cell precursors during early embryogenesis. In contrast, loss of other splicing factor activities had no effect on germ granule control in the embryo. These observations suggest that the Sm proteins control germ granule integrity and localization in the early *C. elegans* embryo and that this role is independent of pre-mRNA splicing. Thus, a highly conserved splicing factor may have been adapted to control both snRNP biogenesis and the localization of components important for germ cell function.

Results and Discussion

To identify genes that control embryonic asymmetry, a *C. elegans* embryonic cDNA library was functionally screened by RNA interference (RNAi). From this screen, we identified the *C. elegans* ortholog of human SmE as a regulator of P granule localization (see below). SmE is one of seven conserved Sm proteins that form a heptameric complex required for the biogenesis and function of the snRNPs that catalyze mRNA splicing (Figure 1; [3, 4]). Orthologs to each of the Sm proteins are found in *C. elegans*, and each shows high amino acid identity throughout its length with its vertebrate counterparts (Figure 1). In addition, the Sm proteins in *C. elegans* associate with known snRNAs, suggesting that they function in both *cis*- and *trans*-splicing in worms [5].

To determine the localization of Sm proteins in embryos and germ cells, we stained embryos and adult gonads with several different Sm antibodies. With most of these antibodies, staining was detected in nuclei and

in the cytoplasm of many cell types, consistent with the localization of snRNPs in other organisms (Figures 2C and 2E; our unpublished data). Strikingly, Sm antibodies also stained cytoplasmic particles that colocalized with the P granule component PGL-1 within germ cells and their embryonic precursors (Figures 2A–2F). Sm staining was observed in nucleus-associated (perinuclear) P granules of adult germ cells and older embryos (Figures 2A and 2E), as well as in cytoplasmic P granules of oocytes and early embryos (Figure 2C; our unpublished data). P granules were stained by several monoclonal and polyclonal antibodies that recognize different epitopes on Sm proteins [6–8] (see Supplementary Material available with this article online). Furthermore, the staining of P granules by anti-Sm antibodies was greatly reduced after RNAi of Sm proteins but not after RNAi of RNA polymerase II (compare Figure 2E with 2G; Supplementary Material; our unpublished data). In addition, antibodies to U2AF65, another splicing factor, stained nuclei but did not stain P granules (our unpublished data). Therefore, these results show that at least some Sm proteins are specific and constitutive components of P granules.

To test the functional significance of Sm protein association with P granules, we inhibited Sm activity by RNAi and stained the gonads and embryo progeny of injected worms for PGL-1. In normal embryos, P granules localize to the germ cell precursors (P1–P4) at each of several asymmetric cell divisions (Figure 3A; [2]). After RNAi of one or more Sm proteins, P granules were detected in multiple cells in many embryos between the 8- and 36-cell stages, in contrast to embryos from control animals (Table 1; Figures 3B–3E; see also Figure 2H). Sm depletion also caused embryonic arrest at the 50- to 100-cell stage, with defects in morphogenesis and differentiation (Table 1; Supplementary Material). The effects of Sm depletion on P granule distribution was largely limited to embryos older than the eight-cell stage; most two- and four-cell embryos localized their P granules normally after Sm depletion (Supplementary Material). These results suggest that loss of Sm activity causes a defect in the localization of P granules after the four- to eight-cell stages, during the division of P2 or P3. Because only PGL-1 was examined, it is not known if Sm activity affects the entire P granule structure or if it is restricted to only some components. In addition, because the maternal pool of Sm proteins is reduced but not eliminated in these RNAi experiments (Supplementary Material), the observed phenotypes probably result from a partial loss of function. Longer incubations of *SmE(RNAi)* animals caused increased penetrance of these P granule defects, but they also caused sterility and cell division defects in the embryo (Table 2; Supplementary Material).

The subcellular distribution and size of P granules within germ cell precursors was also altered by Sm depletion. Beginning at the four-cell stage of normal germ cell precursors, P granules become associated with the nuclear envelope during interphase of the cell

³Correspondence: tom.evans@uchsc.edu

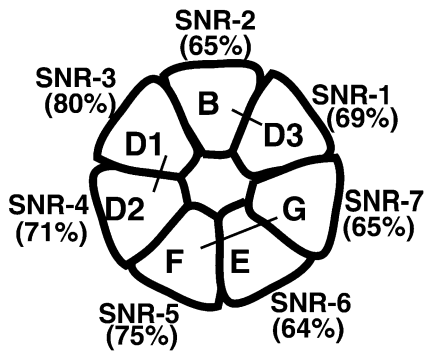


Figure 1. Model of the Sm Complex that Binds the snRNAs
This model is based on structural analysis of mammalian and yeast Sm proteins [4]. The *C. elegans* Sm protein designations and the percent amino acid identity between *C. elegans* and human Sm proteins are indicated. Sm proteins can also form subcomplexes in cells (BD3, D1D2, and FEG), as indicated by lines connecting the interacting subunits [4]. For simplicity, the *C. elegans* SNR proteins are referred to by their common Sm names.

cycle ([2, 9]; Figure 3A). This perinuclear localization is most pronounced after the birth of P4 at the 16-cell stage (see Figure 3D). After Sm depletion, P granule association with nuclei was disrupted in 16- to 40-cell-stage embryos (Table 1; compare Figure 3B with 3C and 3D with 3E). In some *SmE(RNAi)* embryos, P granules remained large but were primarily cytoplasmic (Figure 3C). In other embryos, PGL-1-containing particles were small or very diffuse within the cytoplasm (Figure 3E). This range of effects on P granules suggests that the Sm proteins may be required both for the perinuclear attachment of PGL-1-containing particles and for the integrity of the P granule structure or its PGL-1 component.

To separately perturb the three known Sm subcomplexes (see Figure 1), we used RNAi to disrupt different combinations of Sm proteins. Each combination of double-stranded RNAs (dsRNAs) produced similar phenotypes (Table 1). These results suggest that several Sm's, if not the entire heptamer, are required for P granule control. However, because not all Sm proteins have been depleted individually, some may not be involved. RNAi of several of the *C. elegans* "Sm-like" proteins (the Lsm's) had no effect on P granule regulation (our unpublished data). The Lsm proteins also function in splicing and other processes in eukaryotes [10] and are the most similar to the Sm proteins in *C. elegans*. Therefore, the observed *Sm(RNAi)* phenotypes are not likely to be caused by RNAi of homologous mRNAs or inactivation of linked genes cotranscribed with the *Sm* genes.

The effects of Sm depletion on P granule distribution could be due to a defect in mRNA splicing or to a splicing-independent role of Sm proteins in P granule regulation. To examine this issue, we disrupted the expression of the core splicing factors, U2AF, U170K, and Sap49, and of the large subunit of RNA polymerase II by RNAi. Each of these splicing factors plays central roles in mRNA splicing in many species, including *C. elegans* [11–16]. RNAi of these factors caused developmental arrest of embryos at the 50- to 100-cell stage, with cell differentiation and morphogenesis defects similar to those caused by Sm depletion (Table 1), consistent with

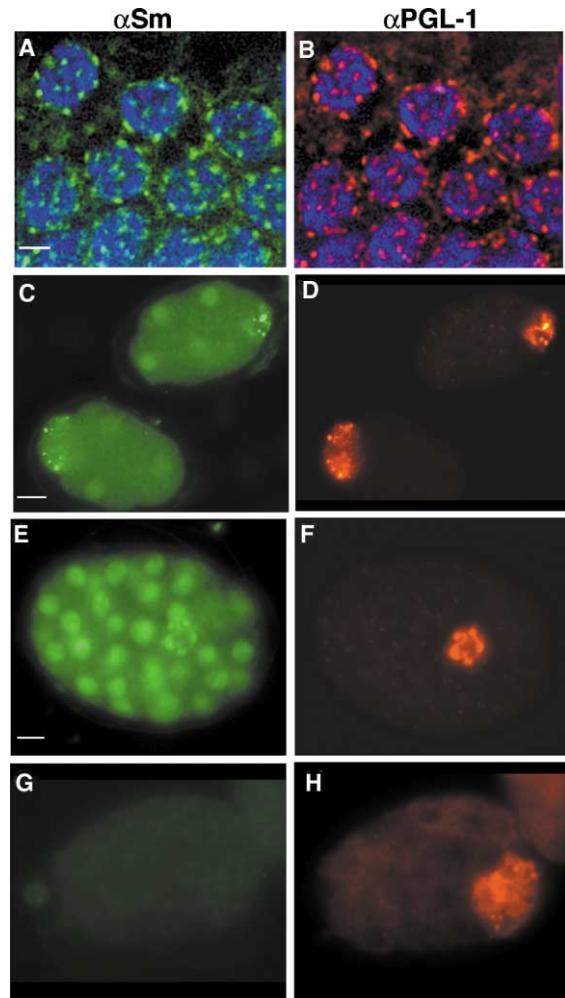


Figure 2. The *C. elegans* Sm Proteins Localize to P Granules
(A and B) Meiotic pachytene germ cells of the adult germline stained with the anti-Sm Y12 monoclonal antibody (A) and PGL-1 (B). DAPI staining of nuclei is also shown.
(C and D) Four-cell and eight-cell embryos stained with Sm human sera (C) and PGL-1 (D). Merged images show that PGL-1 and Sm staining are coincident within P granules (data not shown). The Sm monoclonals KSm4 and 7.13, as well as an SmG polyclonal, also stained P granules (Supplementary Material).
(E–H) Embryos of approximately 50 cells were stained with Sm human sera (E, G) or PGL-1 (F, H). The embryo in (G) and (H) was from an *SmE,D1,D3(RNAi)* animal, while those in (E) and (F) are from a noninjected control animal. The exposures in (E) and (G) are equivalent. Quantitation of the reduction of Sm staining by Sm depletion with several Sm antibodies is presented in the Supplementary Material (Table S2). PGL-1 particles are detected in four cells in (H). The scale bars are 2.5 μ m (A, B), 10 μ m (C, D), and 5 μ m (E–H).

strong disruption of gene expression in the embryo [17]. However, RNAi of these other splicing factors, either alone or in combination, had no effect on the localization of P granules to the germ precursor cells in embryos or on their perinuclear association (Table 1; our unpublished data). When animals were incubated for longer times after splicing-factor RNAi, many injected animals died or became sterile, and some of the few embryos produced arrested prior to the 50-cell stage, consistent with further depletion of both maternal and zygotic

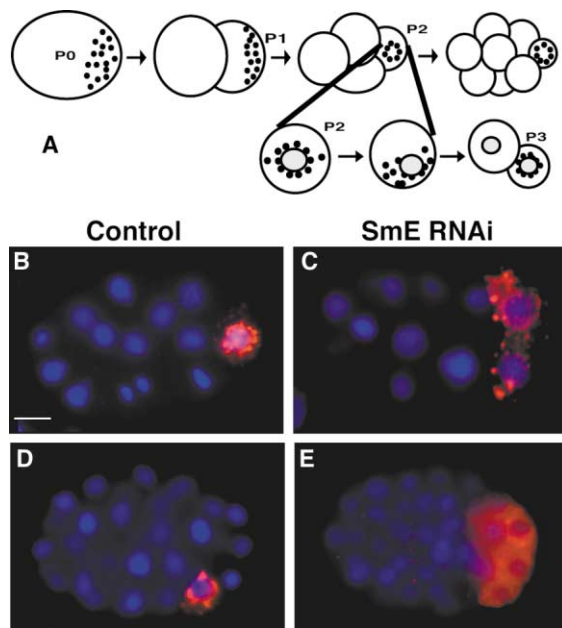


Figure 3. Loss of SmE Activity Disrupts P Granule Localization and Subcellular Distribution in the Embryo

(A) The localization of P granules during early embryogenesis. P granules (black dots) are delivered to the embryo from the oocyte. In the first two cell divisions, P granules are localized to the germ cell precursors (P1 and P2) by cytoplasmic flow [9]. After this stage, most P granules reattach to the nuclear envelope during interphase and are then localized to P3 and P4.

(B–E) RNAi of Sm proteins causes defects in P granule localization during later germ cell divisions. Panel (B) is a 12-cell embryo from a noninjected control; only P3 contains P granules (in red), most of which are associated with its nucleus (in blue). Panel (C) is a 12-cell embryo after RNAi of SmE; large P granules are seen in two cells (P3 and sister cell C); not all nuclei are seen in this focal plane. Panels (D) and (E) are 36- to 50-cell embryos from a noninjected control (D) or an *SmE(RNAi)* animal (E). PGL-1 particles are tightly associated with the nucleus of P4 but are small and diffuse in four cells of the *SmE(RNAi)* embryo. The scale bar is 10 μ m.

mRNAs (our unpublished data). However, no defects in P granule localization or subcellular distribution were detected in the recovered embryos (Table 1). A small percentage of Pol II (RNAi) embryos did show P granule defects, although all of these embryos had abnormal nuclear morphologies and were likely to have arrested development (Table 1; our unpublished data). In contrast, even partial loss of Sm activity caused significant defects in P granule control in the embryo (Tables 1 and 2). Sm depletion caused highly penetrant P granule effects in embryos under conditions in which GLP-1 and PGL-1, both made from maternal mRNAs [18, 19], were not obviously reduced (Figures 2 and 3; our unpublished data) and in which injected animals were still making functional oocytes and embryos that developed to at least the 50-cell stage (Table 2). These observations suggest that Sm depletion disrupted P granule regulation under conditions in which severe reduction in general maternal mRNAs had not occurred, whereas depletion of other splicing factors did not affect P granule control even when both maternal and zygotic activities were reduced (see also below).

Surprisingly, P granule regulation in the embryo is distinct from its control in adult germ cells. Inhibition of transcription or mRNA export in the adult gonad rapidly disrupts the integrity of perinuclear P granules (J. Pitt and J. Priess, personal communication). We have observed similar effects in adult germ cells after RNAi of U2AF, Sap49, or the *Sm* genes (our unpublished data). Together these results suggest that P granule (or PGL-1) accumulation on nuclei of adult germ cells requires active mRNA transcription and processing. However, this requirement is not related to the function of the Sm proteins in early embryos. First, partial depletion of SmE caused significant defects in P granule control in the early embryo when P granule integrity in adult germ cells was unaffected (Table 2). Second, no P granule localization defects were seen in embryos after U2AF and U170K RNAi under conditions in which adult germ cell P granules were severely disrupted. Forty-eight

Table 1. The Effects of Sm and Other Splicing Factor Activities on P Granule Regulation in Embryos

RNAi ^a	Time after Inject	Percentage of Embryos Arrested ^d	PGL-1 Localization Defect ^b (Percentage of Embryos)	PGL-1 Nuclear Association Defect ^c (Percentage of embryos)
none	–	0%	0%	0%
Sm B + D3	24 hr	77% \pm 23%	65% \pm 2%	64% \pm 16%
Sm F + E + G	24 hr	87% \pm 7%	57% \pm 5%	62% \pm 6%
Sm D1 + D2	24 hr	100%	62% \pm 22%	79% \pm 1%
U170K + Sap49	24 hr	98% \pm 2%	0%	0%
U2AF65 + U2AF35	24 hr	100%	0%	0%
U2AF65 + U170K	29 hr	100%	0%	0%
U2AF65 + U170K	48 hr	100%	0%	0%
Pol II	24 hr	100%	0%	0%
Pol II	48 hr	100%	9% \pm 2%	0%

^aThe indicated dsRNAs were injected into adult animals, which were incubated for the times indicated at 20°C.

^bOnly 16- to 40-cell embryos were scored for P granule localization (after birth of P4 and prior to its division). Embryos in which two or more cells had PGL-1 staining of near equal intensity were scored as localization defective.

^cSixteen- to forty-cell embryos in which most or all PGL-1-stained granules were cytoplasmic and not in a ring around any nucleus were scored as nuclear-association defective. Numbers are averages of two experiments \pm the variance, n = 10–36 embryos for each experiment.

^dThe percent of embryos collected 1 hr prior to fixation that failed to hatch is shown (n = 20–150 for each experiment).

Table 2. Effects of SmE RNAi on Embryo and Germline P Granules over Time

Time of SmE dsRNA Feeding	24 hr	36 hr	48 hr	60 hr
Percentage of embryos with PGL-1 localization defect ^a	0%	45%	65%	91%
Percentage of embryos with PGL-1 nuclear association defect ^a	9%	68%	79%	100%
Percentage of gonads with PGL-1 nuclear association defect ^b	0%	0%	0%	53%
Percentage of embryo arrest ^c	31%	100%	100%	n.d.
Percentage of embryos with early nuclear defects ^d	0%	0%	0%	22%

Wild-type L4 larvae were placed on plates with bacteria expressing SmE dsRNA for the indicated times.

^aDetermined as in Table 1. n = 23–49 for each time point.

^bGonad distal arms with cytoplasmic PGL-1 staining and loss of perinuclear PGL-1 from more than 20% of germ nuclei were scored as nuclear-association defective. n = 15–20 gonads for each time point.

^cThrough 48 hr, all embryos arrested with ≥ 50 cells (data not shown). At 60 hr, some embryos may have arrested earlier.

^dEmbryos with < 20 cells and enlarged, fragmented, and disorganized DAPI foci.

hours after RNAi of U170K and U2AF65, PGL-1 staining was displaced from germ cell nuclei in the distal arms of 87% of gonads (n = 8), but all of the 16–40 cell embryos (n = 10) had normally localized P granules. These results strongly suggest that regulation of P granules in adult germ cells is distinct from their control in the early embryo, which depends specifically on Sm activity.

The results of this study suggest that at least some Sm proteins, core components of the spliceosome, are also constitutive components of *C. elegans* P granules that control granule integrity and localization during early embryogenesis. P granule regulation is very sensitive to Sm depletion but is not affected by depletion of other splicing factors. These observations are consistent with a unique function of Sm proteins for early embryonic regulation of P granules; this function is separate from their role in mRNA splicing. This novel function of Sm proteins in P granule control could be direct or indirect. For example, the Sm proteins could control P granules indirectly by regulating the fate of the germ cell precursor. Alternatively, the presence of Sm proteins in P granules may indicate that the Sm complex controls P granule localization or integrity by some direct cytoplasmic mechanism.

Several molecular functions for the Sm proteins in P granule regulation can be envisioned. One simple idea is that the Sm complex promotes the attachment of some or all P granule components to the germ precursor cell nucleus. Nuclear attachment is probably important for P granule localization after the four-cell stage of the embryo [9] (see Figure 3A). The Sm complex could directly promote P granule transport to the nuclear pore by a mechanism similar to its proposed role in snRNP nuclear import [3]. The Sm's could be bound to small RNAs or mRNAs that contain a tri-methyl cap [20, 21], providing the same signals that promote snRNP recruitment to nuclear pores [22–24]. Consistent with this idea, P granules appear to have intimate associations with the nuclear-pore complex [25]. Thus, germ cell precursors may have adapted a conserved general transport function to promote the cytoplasmic partitioning of components that control germ cell function. Alternatively, the Sm complex may control P granule localization more indirectly, by regulating P granule integrity or other functions. The Sm complex could regulate mRNP assembly, mRNA translation/stability, or nuclear trafficking of novel snRNPs in germ cell precursors. Because a variety of

different maternal mRNAs associate with P granules, P granules could play important roles in mRNP assembly or activity in germ cells [26, 27]. In addition to being a core UsnRNP component, the Sm complex is also a component of telomerase in budding yeast [28]. The related Lsm proteins also have other molecular functions in addition to splicing [10]. Thus, the Sm proteins could have diverse functions in eukaryotic cells.

Germ granules also exist in other organisms, and they share some of their components and properties with the P granules of *C. elegans* [1]. In mouse spermatocytes, Sm antibodies stain large perinuclear granules that, like nematode P granules, also contain a homolog of fly VASA [29, 30]. These observations hint at conserved structures with conserved functions in germ cells of diverse species. Novel roles of Sm proteins raise the possibility that other general factors could also have alternative and specific roles in development. Consistent with this view, factors that control general splicing, translation, and mRNA export have also been implicated in specific developmental functions [31–34]. These studies suggest that the adaptation of general functions to specific developmental pathways may be widespread.

Supplementary Material

Analysis and discussion of Sm antibody specificity, *Sm(RNAi)* phenotypes, and the Experimental Procedures are available at <http://images.cellpress.com/supmat/supmatin.htm>.

Acknowledgments

We thank J. Pitt and J. Priess for communicating results prior to publication. We would also like to thank T. Blumenthal, J. James, J. Kimble, R. Luhrmann, S. Strome, B. Wood, and G. Zieve for generously providing antibodies. The Y12 antibody and human anti-Sm sera, provided by T. Blumenthal, were originally gifts of J. Steitz and I. Mattaj, respectively. We also thank G. Seydoux for generously providing plasmids and information on their use prior to publication and Y. Kohara for providing many cDNA clones. We especially want to thank D. Evans, M. Kumar, P. MacMorris, and T. Blumenthal for supplying some of the splicing-factor RNAs used and for communicating unpublished results on splicing factors and Sm proteins in *C. elegans*. We also thank J. Ahringer, T. Blumenthal, D. Bentley, J. Hooper, S. Britt, and P. MacMorris for helpful comments on the manuscript. This work was supported by a March of Dimes Basil O'Connor award, NSF grant 9982944, a Howard Hughes Institutional Research Award, and seed grants from the American Cancer Society.

Received: April 24, 2002
Revised: June 18, 2002

Accepted: July 4, 2002
Published: September 3, 2002

References

1. Houston, D.W., and King, M.L. (2000). Germ plasm and molecular determinants of germ cell fate. *Curr. Top. Dev. Biol.* **50**, 155–181.
2. Strome, S., and Wood, W.B. (1982). Immunofluorescence visualization of germ-line-specific cytoplasmic granules in embryos, larvae, and adults of *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **79**, 1558–1562.
3. Will, C.L., and Luhrmann, R. (2001). Spliceosomal UsnRNP biogenesis, structure and function. *Curr. Opin. Cell Biol.* **13**, 290–301.
4. Kambach, C., Walke, S., and Nagai, K. (1999). Structure and assembly of the spliceosomal small nuclear ribonucleoprotein particles. *Curr. Opin. Struct. Biol.* **9**, 222–230.
5. Thomas, J.D., Conrad, R.C., and Blumenthal, T. (1988). The *C. elegans* trans-spliced leader RNA is bound to Sm and has a trimethylguanosine cap. *Cell* **54**, 533–539.
6. Hermann, H., Fabrizio, P., Raker, V.A., Foulaki, K., Hornig, H., Brahm, H., and Luhrmann, R. (1995). snRNP Sm proteins share two evolutionarily conserved sequence motifs which are involved in Sm protein-protein interactions. *EMBO J.* **14**, 2076–2088.
7. Fury, M., Andersen, J., Ponda, P., Aimes, R., and Zieve, G.W. (1999). Thirteen anti-Sm monoclonal antibodies immunoprecipitate the three cytoplasmic snRNP core protein precursors in six distinct subsets. *J. Autoimmun.* **12**, 91–100.
8. Puijn, G.J., Schoute, F., Thijssen, J.P., Smeenk, R.J., and van Venrooij, W.J. (1997). Mapping of SLE-specific Sm B cell epitopes using murine monoclonal antibodies. *J. Autoimmun.* **10**, 127–136.
9. Hird, S.N., Paulsen, J.E., and Strome, S. (1996). Segregation of germ granules in living *Caenorhabditis elegans* embryos: cell-type-specific mechanisms for cytoplasmic localisation. *Development* **122**, 1303–1312.
10. He, W., and Parker, R. (2000). Functions of Lsm proteins in mRNA degradation and splicing. *Curr. Opin. Cell Biol.* **12**, 346–350.
11. Zorio, D.A., and Blumenthal, T. (1999). Both subunits of U2AF recognize the 3' splice site in *Caenorhabditis elegans*. *Nature* **402**, 835–838.
12. Merendino, L., Guth, S., Bilbao, D., Martinez, C., and Valcarcel, J. (1999). Inhibition of msl-2 splicing by Sex-lethal reveals interaction between U2AF35 and the 3' splice site AG. *Nature* **402**, 838–841.
13. Wu, S., Romfo, C.M., Nilsen, T.W., and Green, M.R. (1999). Functional recognition of the 3' splice site AG by the splicing factor U2AF35. *Nature* **402**, 832–835.
14. Kohtz, J.D., Jamison, S.F., Will, C.L., Zuo, P., Luhrmann, R., Garcia-Blanco, M.A., and Manley, J.L. (1994). Protein-protein interactions and 5'-splice-site recognition in mammalian mRNA precursors. *Nature* **368**, 119–124.
15. Hilleren, P.J., Kao, H.Y., and Siliciano, P.G. (1995). The amino-terminal domain of yeast U1-70K is necessary and sufficient for function. *Mol. Cell. Biol.* **15**, 6341–6350.
16. Igel, H., Wells, S., Perriman, R., and Ares, M., Jr. (1998). Conservation of structure and subunit interactions in yeast homologues of splicing factor 3b (SF3b) subunits. *RNA* **4**, 1–10.
17. Powell-Coffman, J.A., Knight, J., and Wood, W.B. (1996). Onset of *C. elegans* gastrulation is blocked by inhibition of embryonic transcription with an RNA polymerase antisense RNA. *Dev. Biol.* **178**, 472–483.
18. Kawasaki, I., Shim, Y.H., Kirchner, J., Kaminker, J., Wood, W.B., and Strome, S. (1998). PGL-1, a predicted RNA-binding component of germ granules is essential for fertility in *C. elegans*. *Cell* **94**, 635–645.
19. Evans, T.C., Crittenden, S.L., Kodoyianni, V., and Kimble, J. (1994). Translational control of maternal glp-1 mRNA establishes an asymmetry in the *C. elegans* embryo. *Cell* **77**, 183–194.
20. Van Doren, K., and Hirsh, D. (1990). mRNAs that mature through trans-splicing in *Caenorhabditis elegans* have a trimethylguanosine cap at their 5' termini. *Mol. Cell. Biol.* **10**, 1769–1772.
21. Liou, R.F., and Blumenthal, T. (1990). trans-spliced *Caenorhabditis elegans* mRNAs retain trimethylguanosine caps. *Mol. Cell. Biol.* **10**, 1764–1768.
22. Hamm, J., Darzynkiewicz, E., Tahara, S.M., and Mattaj, I.W. (1990). The trimethylguanosine cap structure of U1 snRNA is a component of a bipartite nuclear targeting signal. *Cell* **62**, 569–577.
23. Fischer, U., and Luhrmann, R. (1990). An essential signaling role for the m3G cap in the transport of U1 snRNP to the nucleus. *Science* **249**, 786–790.
24. Fischer, U., Sumpster, V., Sekine, M., Satoh, T., and Luhrmann, R. (1993). Nucleo-cytoplasmic transport of U snRNPs: definition of a nuclear location signal in the Sm core domain that binds a transport receptor independently of the m3G cap. *EMBO J.* **12**, 573–583.
25. Pitt, J.N., Schisa, J.A., and Priess, J.R. (2000). P granules in the germ cells of *Caenorhabditis elegans* adults are associated with clusters of nuclear pores and contain RNA. *Dev. Biol.* **219**, 315–333.
26. Subramaniam, K., and Seydoux, G. (1999). nos-1 and nos-2, two genes related to *Drosophila* nanos, regulate primordial germ cell development and survival in *Caenorhabditis elegans*. *Development* **126**, 4861–4871.
27. Schisa, J.A., Pitt, J.N., and Priess, J.R. (2001). Analysis of RNA associated with P granules in germ cells of *C. elegans* adults. *Development* **128**, 1287–1298.
28. Seto, A.G., Zaug, A.J., Sobel, S.G., Wolin, S.L., and Cech, T.R. (1999). *Saccharomyces cerevisiae* telomerase is an Sm small nuclear ribonucleoprotein particle. *Nature* **401**, 177–180.
29. Moussa, F., Oko, R., and Hermo, L. (1994). The immunolocalization of small nuclear ribonucleoprotein particles in testicular cells during the cycle of the seminiferous epithelium of the adult rat. *Cell Tissue Res.* **278**, 363–378.
30. Toyooka, Y., Tsunekawa, N., Takahashi, Y., Matsui, Y., Satoh, M., and Noce, T. (2000). Expression and intracellular localization of mouse Vasa-homologue protein during germ cell development. *Mech. Dev.* **93**, 139–149.
31. Cline, T.W., Rudner, D.Z., Barbash, D.A., Bell, M., and Vutien, R. (1999). Functioning of the *Drosophila* integral U1/U2 protein Snf independent of U1 and U2 small nuclear ribonucleoprotein particles is revealed by snf(+) gene dose effects. *Proc. Natl. Acad. Sci. USA* **96**, 14451–14458.
32. Amiri, A., Keiper, B.D., Kawasaki, I., Fan, Y., Kohara, Y., Rhoads, R.E., and Strome, S. (2001). An isoform of eIF4E is a component of germ granules and is required for spermatogenesis in *C. elegans*. *Development* **128**, 3899–3912.
33. Hachet, O., and Ephrussi, A. (2001). *Drosophila* Y14 shuttles to the posterior of the oocyte and is required for oskar mRNA transport. *Curr. Biol.* **11**, 1666–1674.
34. Iida, T., and Kobayashi, S. (1998). Essential role of mitochondrially encoded large rRNA for germ-line formation in *Drosophila* embryos. *Proc. Natl. Acad. Sci. USA* **95**, 11274–11278.