MOLECULAR AND CELLULAR BIOLOGY, Apr. 1994, p. 2235–2242
0270-7306/94/$04.00+0

Vol. 14, No. 4

Multiple *cis*-Acting Targeting Sequences Are Required for *orb* mRNA Localization during *Drosophila* Oogenesis

VALERIE LANTZ[1] AND PAUL SCHEDL[2]*

Department of Biology, Washington University, St. Louis, Missouri 63130,[1] and Moffett Laboratory, Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544[2]

Received 28 July 1993/Returned for modification 28 September 1993/Accepted 15 December 1993

The targeting of positional information to specific regions of the oocyte or early embryo is one of the key processes in establishing anterior-posterior and dorsal-ventral polarity. In many developmental systems, this is accomplished by localization of mRNAs. The germ line-specific *Drosophila orb* gene plays a critical role in defining both axes of the developing oocyte, and its mRNA is localized in a complex pattern during oogenesis. We have identified a 280-bp sequence from the *orb* 3′ untranslated region capable of reproducing this complex localization pattern. Furthermore, we have found that multiple *cis*-acting elements appear to be required for proper targeting of *orb* mRNA.

The localization of mRNAs to specific subcellular regions or compartments provides an important mechanism for targeting proteins to the sites where their activity is required. In neurons, MAP2 mRNA, which encodes a dendrite-specific microtubule-associated protein, accumulates in the dendrites but not in the cell bodies (7, 9). Similarly, in fibroblasts, actin mRNA is localized to the periphery of the cell close to the actin filament network (19). The use of mRNA targeting to localize proteins is not restricted to differentiated somatic cells. In fact, it appears to play an especially important role in the early development of many animals in which it is one of the key mechanisms for distributing positional information in a defined spatial pattern. In *Xenopus* oocytes, Vg1 mRNA is restricted to the vegetal cortex of the egg (23, 25, 34). Vg1 encodes a transforming growth factor beta homolog, and the asymmetric distribution of the Vg1 message has led to the suggestion that it may function in mesodermal induction in the early *Xenopus* embryo. In *Drosophila melanogaster*, the establishment of anterior-posterior and dorsal-ventral polarity also requires the localization of specific mRNAs to different regions of the developing oocyte and early embryo (28). Formation of the abdominal region of the early embryo and the assembly of polar granules (required for germ cell determination) depend on the targeting of mRNAs for several posterior group genes (e.g., *oskar*, *nanos*, and *tudor*) to the posterior pole during oogenesis (8, 10, 14, 33). Similarly, the definition of the dorsal-ventral axis appears to involve the asymmetric distribution of mRNAs in the developing oocyte during oogenesis (3).

One plausible model for mRNA localization is that the targeted RNAs contain *cis*-acting signal sequences that specify a particular subcellular address (20). Such sequences would be recognized by RNA-binding proteins that function either in the transport of mRNAs from their site of synthesis to the appropriate subcellular destination or in the anchoring of the mRNAs at these sites. We have recently identified a germ line-specific *Drosophila* gene, *orb*, which may function in the transport and/or anchoring of mRNAs during *Drosophila* oogenesis (4, 17, 18). The predicted protein product of the *orb* gene contains two domains showing sequence similarity to the RNA recognition motif-type family of RNA-binding proteins (2, 13). Genetic studies indicate that *orb* is required throughout much of oogenesis. Early in oogenesis, it is essential for the formation and differentiation of the egg chamber, which consists of the germ line-derived oocyte and 15 nurse cells and the somatic follicle cells (4, 18). In severe loss-of-function *orb* alleles, the oocyte-nurse cell complex is not formed, while in moderate alleles, the oocyte-nurse cell complex is formed, but the oocyte fails to properly differentiate. In addition to disrupting the development of the egg chamber, these *orb* mutations perturb the localization of mRNAs that are normally targeted to the oocyte at very early stages of oogenesis (18). Later in oogenesis, *orb* seems to function in the definition of polarity axes, and embryos produced from mothers carrying a weak maternal effect *orb* allele show defects in both anterior-posterior and dorsal-ventral polarity (4). Defects in mRNA localization during mid and late stages of oogenesis are also evident in this maternal effect allele (4). Like the products of some of the other genes involved in the development of the egg chamber and the establishment of polarity, *orb* mRNA and protein are localized to specific regions of the developing oocyte (4, 17, 18). Moreover, the distribution of Orb protein in the egg chamber during the course of oogenesis (18) is consistent with the idea that it might function in the transport and/or anchoring of localized mRNAs in the oocyte (or that it controls, for example, the translation or stability of localized mRNAs).

The proper localization of *orb* mRNA at different points during the development of the *Drosophila* egg is likely to be important in the functioning of the Orb protein. Consequently, it was of interest to identify the *cis*-acting elements responsible for generating the complex spatial and temporal patterns of accumulation. In the studies reported here, we have shown that the *orb* 3′ untranslated region (UTR) contains a relatively short sequence capable of localizing a heterologous mRNA in the same pattern as the endogenous *orb* message. Furthermore, it appears that this complex distribution pattern is not dependent on a single sequence element but depends on multiple elements that may have different functions.

MATERIALS AND METHODS

Construction of transformation vectors. The transformation vectors were constructed as follows. To construct plasmid D5lacZ, a 624-base *Hpa*I fragment from pC4AUGBgal (31)

* Corresponding author. Phone: (609) 258-5003. Fax: (609) 258-5323.

containing *lacZ* coding sequences was ligated in frame into the unique *Hpa*I site of the *orb* D5 cDNA. In order to use *orb* polyadenylation signals, this plasmid (D5HpalacZ/BS+) was cut with *Hin*dIII to remove the last 1,000 bases of the 3′ UTR of the cDNA, and a 2.6-kb *Hin*dIII *orb* genomic fragment (8E2) containing the last 1,000 bases of the *orb* 3′ UTR and 1.6 kb of genomic sequences downstream was ligated into the *Hin*dIII site. The *Sal*I site located in the BS+ polylinker was converted to *Not*I to create D5HpalacZ8E2Hd/BS+Not. The *Not*I fragment from this plasmid was ligated into pHS83Ca SpeR (12a, 35).

For all constructs containing simian virus 40 (SV40) poly(A), various *orb* fragments were converted to *Xba*I fragments and were ligated into the *Xba*I site of SV40 polyA/BS-Not 2X. A *Not*I fragment from the resulting plasmid was ligated into the *Not*I site of *hsp83Adh-lacZ*/CaSpeRNot vector. SV40 polyA/BS-Not 2X was constructed by ligating a 0.85-kb *Xba*I-*Eco*RI fragment from pC4AUGBgal containing SV40 polyadenylation sequences into BS-Not2X. (BS-Not2X was generated by converting the *Kpn*I site in BS− into a *Not*I site so that there are *Not*I sites on either side of the *Xba*I and *Eco*RI sites of the BS− polylinker.) To construct the *hsp83Adh-lacZ*/CaSpeRNot transformation vector, the *Eco*RI-*Xba*I fragment from pC4 AUGBgal containing *Adh-lacZ* was converted to a *Bam*HI fragment. This *Bam*HI fragment was ligated into the *Bam*HI site of pUC18. An *Eco*RI-*Pst*I fragment containing *Adh-lacZ* from this pUC18 plasmid was ligated into the *Eco*RI-*Pst*I sites of pHS83CaSpeR. The *Pst*I site in the polylinker at the 3′ end of *lacZ* in the resulting plasmid was converted to a *Not*I site.

For the 8E2Hd construct, the 2.6-kb *Hin*dIII 8E2 genomic fragment was converted to a *Not*I fragment and ligated into the *Not*I site of *hsp83Adh-lacZ*/CaSpeRNot. For the AE and CE constructs, deletions of the E4/BS+ plasmid, which contains a truncated ovarian cDNA (17), were made by using exonuclease III and S1 nuclease to generate nested deletions (12). Selected deleted plasmids were sequenced. A DNA fragment from the *Asn*I or *Cfo*I restriction site to the site of the deletion at nucleotide 4333 of the D5 ovarian cDNA was converted to an *Xba*I fragment and ligated into the *Xba*I site of SV40 polyA/BS-Not 2X.

D5lacZΔHN was constructed as follows. A *Hin*dIII fragment containing the last 200 bases of the *orb* 3′ UTR and 1.6 kb of downstream genomic sequences was isolated by cutting 8E2Hd/BS+ with *Nde*I and adding *Hin*dIII linkers. The resulting 1.8-kb fragment was ligated into D5HpalacZ/BS-Not, which was made by cutting D5HpalacZ8E2Hd/BS+Not with *Hin*dIII to remove the *orb* 8E2 *Hin*dIII genomic fragment. The *Not*I fragment containing D5HpalacZ8E2NdeI-HindIII was ligated into the *Not*I site of HS83CaSpeR.

P-element transformation. A $w(ry^{+}\Delta 2\text{-}3)$ stock was used as a recipient strain for injection of most of the plasmids (26, 27). Several of the plasmids were injected along with the pUChsπΔ2-3wc (P-turbo plasmid) (32). The resulting injected adults were crossed to w^{1} or w^{1118} stock, and the progeny were examined for rescue of the *white* mutant phenotype.

Characterization of the localization constructs. Ovaries were dissected from females homozygous for each independent insert and examined by whole-mount in situ hybridization (30) with a *lacZ* antisense PCR probe or an antisense RNA *lacZ* probe. Only one line was examined for D5lacZ and hsp83lacZ (line 25 [12a]). At least five independent lines were examined for each of the other constructs. For each whole-mount in situ hybridization experiment, flies carrying the *Adh-lacZ* construct and flies carrying a construct expressing a chimeric *orb-lacZ* RNA which is localized in the wild-type *orb* pattern were included as controls. In those cases in which no localized RNA was observed, it was possible to distinguish a hybridization signal from background by comparing the staining in germ line and follicle cells. The *hsp83* promoter is more active in the germ line nurse cells than it is in the somatic follicle cells, and in the experiments presented here, a difference in signal between these two cell types was evident even for RNAs that showed no apparent localization. That functional mRNA was produced by the different constructs was also confirmed by Western blotting (immunoblotting) of ovarian proteins using antibodies directed against β-galactosidase.

RESULTS

Distribution of *orb* mRNA during oogenesis. The distribution of *orb* mRNA during oogenesis is shown in Fig. 1A to C. Localized *orb* mRNA is first evident early in oogenesis in region 2a of the germarium. This part of the germarium contains the newly formed 16-cell cysts which are generated by a series of four mitotic divisions of a cystoblast (the product of a stem cell) with incomplete cytokinesis (reviewed in reference 22). At this stage, *orb* mRNA accumulates preferentially in only one cell in the cyst, the presumptive oocyte. The 16-cell cyst (which contains 15 nurse cells and the oocyte) is then surrounded by somatically derived follicle cells to form a stage 1 egg chamber. From this stage until the onset of vitellogenesis, at the end of stage 7, most of the *orb* message is in the oocyte, and only low levels are present in the nurse cells. Moreover, *orb* mRNA is preferentially localized at the very posterior end of the oocyte. Between stages 8 and 10a, *orb* mRNA is no longer observed at the posterior end. Instead, it accumulates at the anterior end of the oocyte along the nurse cell/oocyte border, where it is distributed asymmetrically along the dorsal-ventral axis (17). Finally, during the last stages of oogenesis, it is uniformly distributed within the oocyte.

An *hsp83* promoter-*orb* construct is capable of reproducing the endogenous *orb* mRNA distribution. If the localization pattern is dependent on signals in the *orb* mRNA and not, for example, on some special features of the *orb* promoter, it should be possible to reproduce the wild-type distribution by expressing an *orb* cDNA from a heterologous promoter. For this purpose, we used the *hsp83* promoter, which is constitutively active in the female germ line and is expressed at high levels in nurse cells (1). To distinguish the mRNA produced by the construct from the endogenous *orb* mRNA, we inserted a 624-bp *lacZ* fragment in frame into the protein-coding region of the *orb* cDNA. Finally, to ensure that the polyadenylation signals were intact, we replaced the 3′ UTR of the *orb* cDNA with a genomic *Hin*dIII fragment containing the same UTR sequences plus 1.6 kb of downstream genomic sequences. The structure of the resulting construct, D5lacZ, is shown in Fig. 2A. The construct was introduced into the germ line of the fly by P-element-mediated transformation (26, 27), and the distribution of *orb-lacZ* mRNA was determined by in situ hybridization of a *lacZ* probe to ovaries isolated from females carrying two copies of the construct. Figure 1D shows that the *lacZ*-tagged mRNA is distributed in the same pattern as the endogenous *orb* mRNA (i.e., preferential accumulation in the oocyte, posterior accumulation up to stage 7, and anterior accumulation at stages 8 to 10). Thus, all of the sequences required for wild-type *orb* mRNA localization appear to be contained within the D5lacZ construct.

The *orb* 3′ UTR is sufficient for localization of a heterologous RNA. If localization is mediated by distinct *cis*-acting elements in the *orb* mRNA, these elements should be capable of localizing a heterologous message. To identify these putative localization elements, we used a reporter construct, an

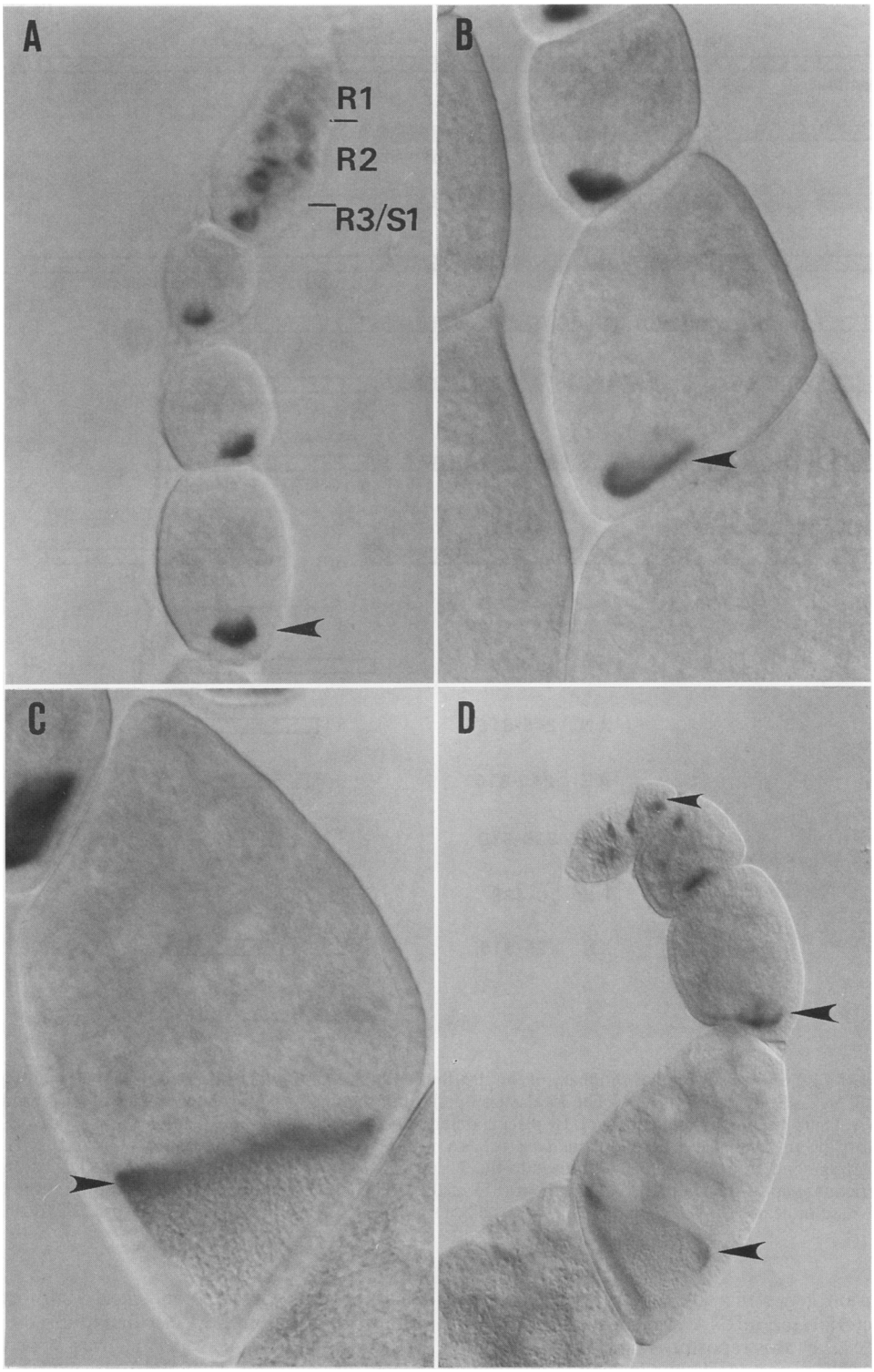

FIG. 1. The distribution of *orb* mRNA in wild-type ovaries compared with the distribution of *orb-lacZ* RNA expressed from the D5lacZ construct. (A to C) Distribution of *orb* mRNA in wild-type ovaries. (A) Early stages. Regions 1, 2, and 3 of the germarium are indicated. *orb* mRNA preferentially accumulates in the oocyte and toward the posterior end during stages ~2 to 6. (B) Stages ~6 and 7. *orb* mRNA is localized at the posterior of the oocyte. (C) Stage 9. *orb* mRNA is present at the anterior of the oocyte. (D) D5lacZ construct. The distribution of the D5lacZ RNA is identical to that of the wild type. Arrowheads indicate RNA localization to or within the oocyte.

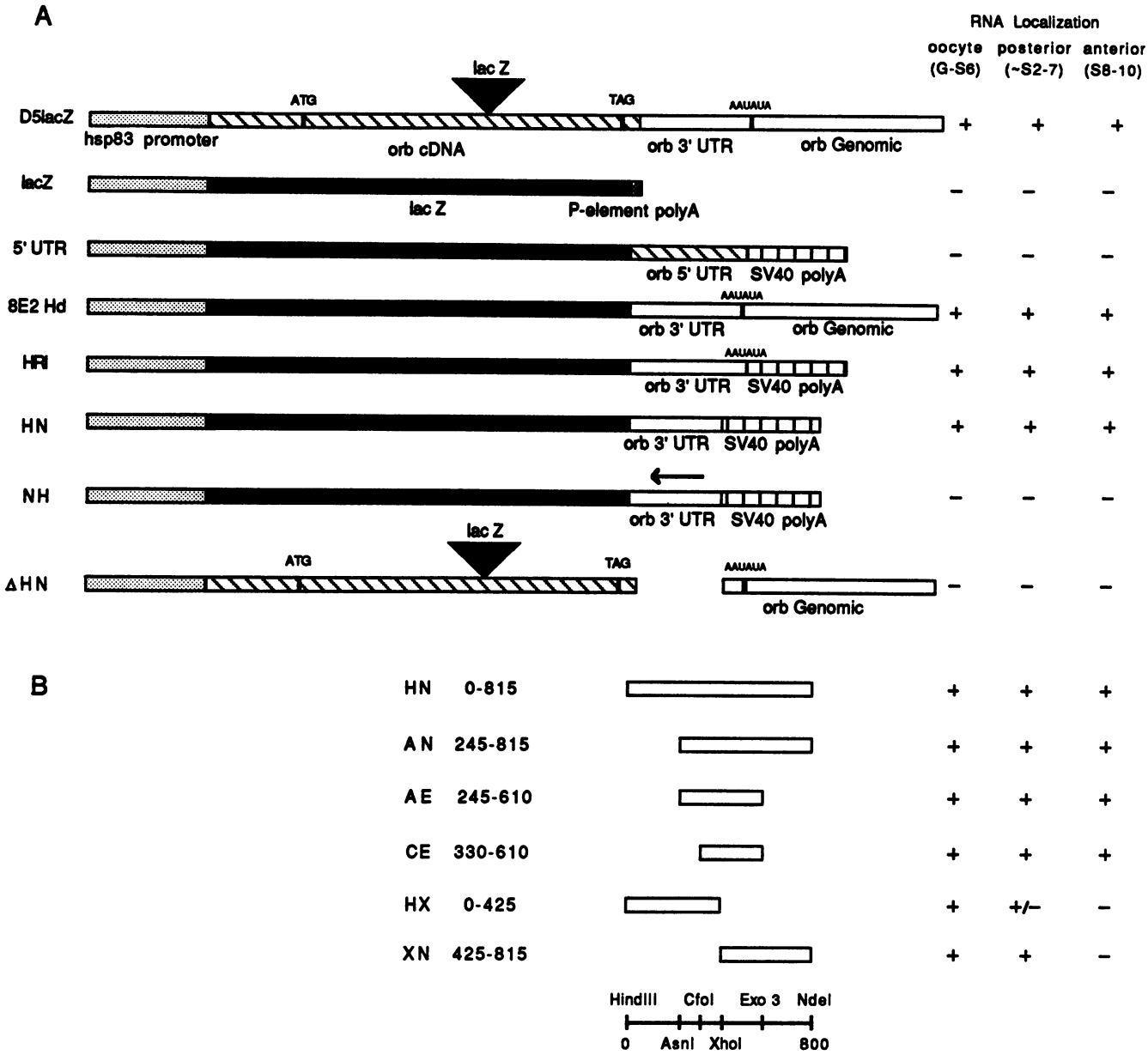

FIG. 2. Structures of *lacZ-orb* constructs and summary of the distribution of the *lacZ-orb* construct RNA. The heterologous *hsp83* promoter was used to express all *lacZ-orb* hybrid RNAs. (A) The localization pattern of the endogenous *orb* mRNA was first reproduced by expressing a *lacZ*-tagged *orb* cDNA, D5 (plus 3′ genomic sequence). By placing different *orb* sequences at the 3′ end of *Adh* leader-*lacZ* reporter, we mapped *cis*-acting elements capable of localizing a heterologous *lacZ* mRNA in the same pattern as the endogenous *orb* RNA to the 3′ UTR. (B) Constructs containing fragments from the *orb* 3′ UTR fused to the 3′ end of *Adh-lacZ* are depicted. SV40 polyadenylation signals were used in all constructs. A restriction map of the *Hin*dIII-*Nde*I fragment (815 of the 1,200 bases) of the *orb* 3′ UTR is shown at the bottom. Distributions of the construct RNAs are indicated. +, the construct RNA is localized similarly to the wild type; −, the construct RNA is uniformly distributed.

hsp83-Adh-lacZ fusion gene (Fig. 2A, lacZ), which constitutively expresses bacterial *lacZ* mRNA. As shown in Fig. 3A, the *lacZ* mRNA expressed by this reporter is not localized in any discernible pattern during oogenesis but instead is uniformly distributed throughout the nurse cell-oocyte complex. We then tested different *orb* sequences from D5lacZ for the ability to localize the *lacZ* mRNA. We first inserted a D5lacZ fragment containing the *orb* 5′ UTR downstream of *lacZ*. (To provide a polyadenylation signal, a DNA fragment from SV40 was placed 3′ to the *orb* sequence; Fig. 2A.) As shown in Fig. 3B, the 5′ UTR does not contain signals capable of localizing a heterologous message, and *lacZ* RNA expressed from this construct is uniformly distributed in the nurse cell-oocyte complex at all stages just as was observed for the initial reporter construct. That RNA was expressed by this construct (and other constructs showing no localized transcripts; see below) was confirmed by comparing hybridization signals in germ line and follicle cells and by Western blotting using β-galactosidase antibodies (see Materials and Methods).

We next tested an *orb* fragment from D5lacZ containing the 3′ UTR and genomic DNA downstream of the poly(A) site (construct 8E2Hd). As can be seen in Fig. 3C, this fragment is

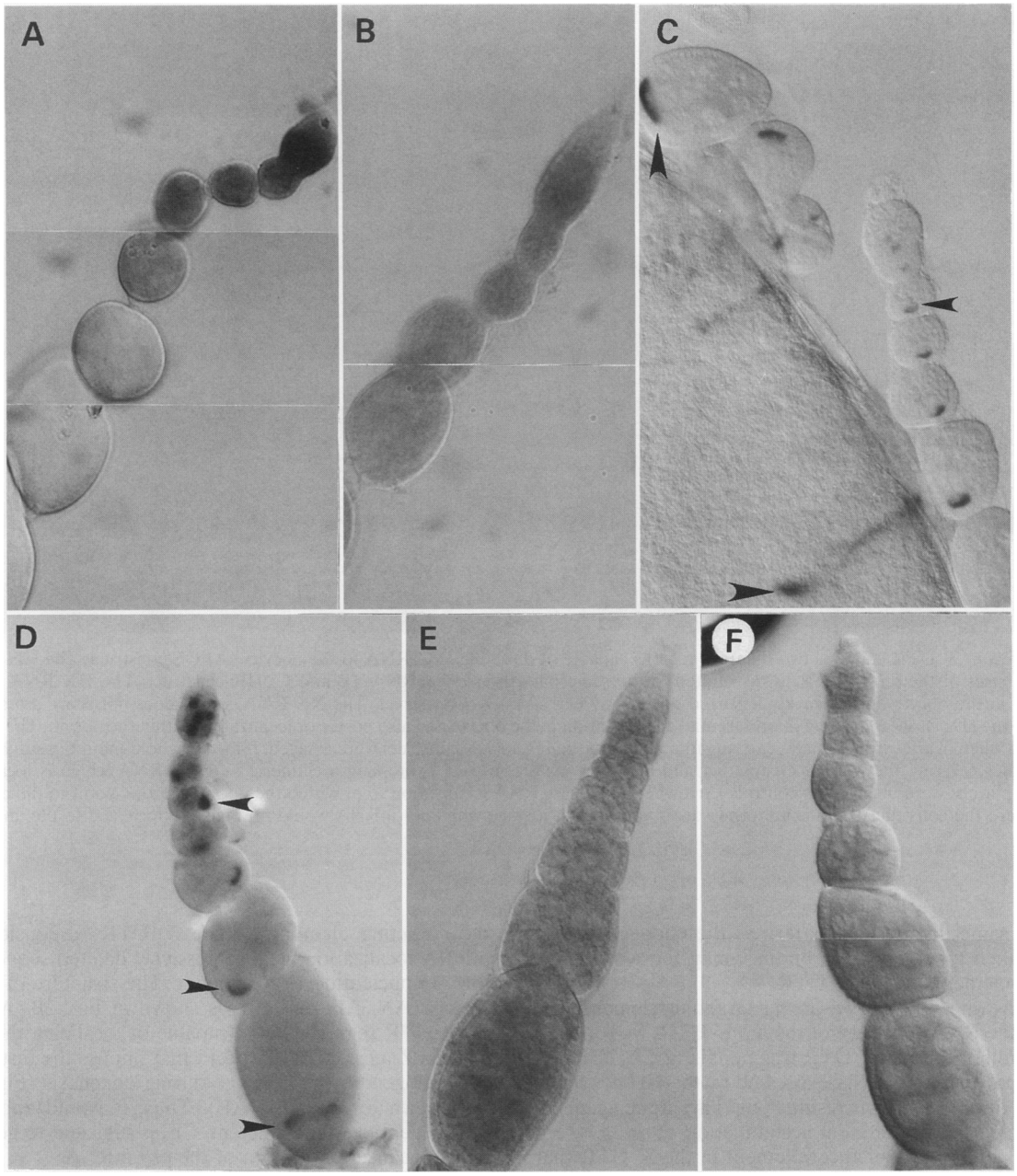

FIG. 3. The distribution of *lacZ-orb* hybrid RNA in ovaries from transformed females. (A) *lacZ* construct. *lacZ* RNA in ovaries from females transformed with *hsp83-lacZ* is uniformly distributed. (B) 5′ UTR construct. The distribution of *lacZ* RNA with the *orb* 5′ UTR at the 3′ end is uniformly distributed. (C) 8E2Hd construct. The 8E2Hd construct RNA shows the same distribution as wild-type *orb* mRNA. (D) HRI construct. The HRI construct RNA, which contains *orb* 3′ UTR sequences from the *orb* D5 cDNA, also shows the same distribution as the wild type. (E) NH construct. When the orientation of the *Hin*dIII-*Nde*I fragment of the *orb* 3′ UTR is reversed and fused to the 3′ end of *lacZ*, the NH construct RNA is uniformly distributed. (F) ΔHN construct. The RNA expressed from the ΔHN construct, which contains the *lacZ* tagged-*orb* cDNA minus 815 bp (HN) of the 3′ UTR, is uniformly distributed. Arrowheads indicate RNA localization to or within the oocyte.

able to target the heterologous *lacZ* mRNA in a pattern indistinguishable from that of the endogenous *orb* mRNA. In addition to mapping the localization sequences to the 3′ end of the *orb* gene, this finding would rule out a cotranslational targeting mechanism (16) (like that used in directing proteins to the endoplasmic reticulum) in which a peptide near the amino terminus of the *orb* protein specifies the targeting of polysomes containing the *orb* mRNA to the oocyte.

The localization of the *lacZ* mRNA by the 8E2Hd fragment does not require the *orb* poly(A) addition signal or sequences in the region downstream of the poly(A) site. This was demonstrated using two fragments from the *orb* D5 cDNA containing only 3′ UTR sequences. The first, a 1-kb *Hin*dIII-*Eco*RI fragment (Fig. 2A, HRI), spans nearly the entire 3′ UTR sequence present in the 8E2Hd construct, while the second, an 815-bp *Hin*dIII-*Nde*I fragment (Fig. 2A, HN), lacks 200 bp from the 3′ end of the UTR. *lacZ* RNA expressed from both the HRI (Fig. 3D) and HN (not shown) constructs

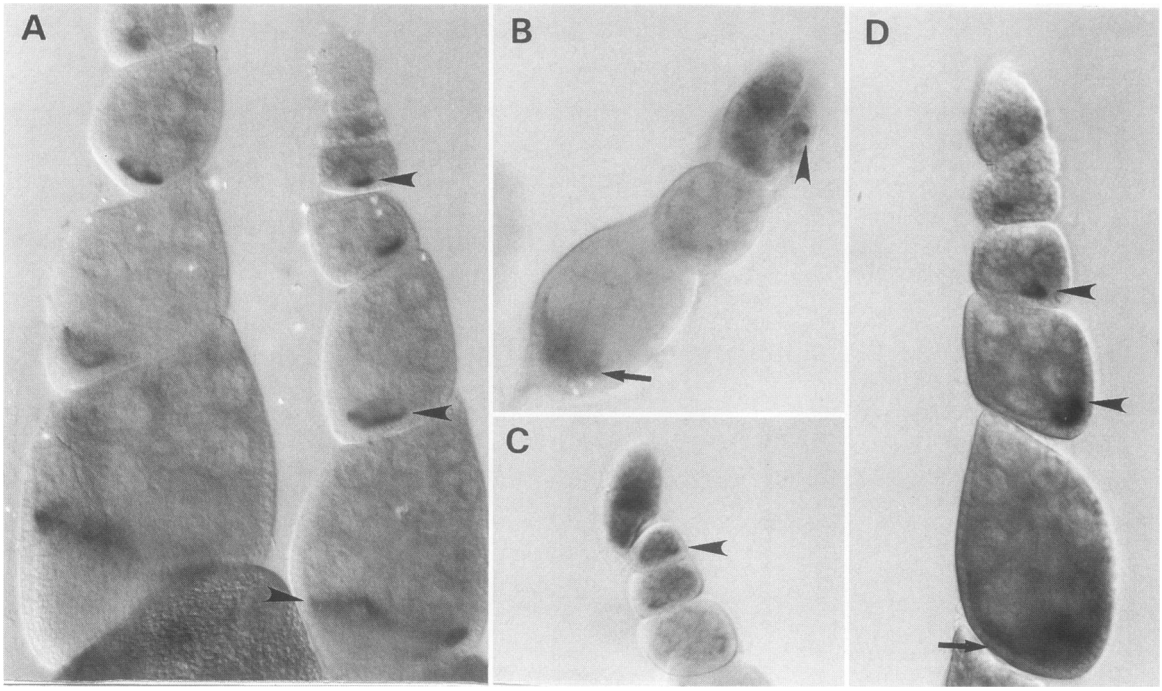

FIG. 4. The *orb* 3′ UTR contains two different regions capable of directing *lacZ* RNA to the oocyte. (A) CE construct. The CE RNA, which contains 280 bases of the *orb* 3′ UTR, is distributed in the same pattern as *orb* mRNA. (B and C) HX construct. The HX RNA accumulates preferentially in the oocyte and is weakly localized posteriorly early. (D) XN construct. The XN RNA, which does not share *orb* sequences in common with the HX RNA, is capable of preferential accumulation in the oocyte and also posterior localization within the oocyte. The same results were obtained when ovaries from females carrying four copies of the HX or XN construct were assayed. For each whole-mount in situ hybridization experiment, flies carrying the *Adh-lacZ* construct and flies carrying a construct expressing a chimeric *orb-lacZ* RNA which is localized in the wild-type *orb* pattern were included as controls. Arrowheads indicate RNA localization to or within the oocyte. Arrows point to the oocyte of egg chambers where the construct RNA is uniformly distributed but at a stage when *orb* mRNA would normally be localized to the anterior of the oocyte.

showed the same distribution pattern as the endogenous *orb* message. Taken together, these findings map the *orb* mRNA localization elements to the 3′ UTR.

To demonstrate that the *cis*-acting targeting elements are encoded in the RNA sequence of the *orb* 3′ UTR, we reversed the orientation of the HN 3′ UTR fragment (Fig. 2A, NH). As shown in Fig. 3E, *lacZ* mRNA expressed by the NH construct is uniformly distributed. This result would also argue against a model in which the preferential accumulation of *orb* RNA in the oocyte is due to an enhancer element in the 3′ UTR that specifically directs expression in the oocyte.

The 3′ UTR is required for *orb* mRNA localization. The findings described above indicate that an 815-nucleotide sequence from the *orb* 3′ UTR is able to target a heterologous mRNA in the same pattern as that observed for the endogenous *orb* message. It was important to ascertain whether this UTR sequence is actually required for the localization of the *orb* message itself. To answer this question, we deleted this same 815-bp 3′ UTR sequence from the *lacZ*-tagged *orb* construct, D5lacZ, described above to create ΔHN (Fig. 2A). We found that the tagged *orb* RNA expressed by ΔHN displays no discernible pattern of localization and instead is uniformly distributed during oogenesis (Fig. 3F). This result indicates that the 3′ UTR is critical for correctly targeting the *orb* mRNA, while sequences elsewhere in the *orb* transcript (e.g., the 5′ UTR or the protein-coding sequences) are by themselves unable to localize the message.

The *orb* 3′ UTR contains at least two regions capable of directing a heterologous RNA to the oocyte. To further delimit the *cis*-acting elements in the 3′ UTR responsible for *orb* mRNA localization, we progressively deleted sequences from the 815-nucleotide HN fragment. The structure of these constructs (AN, AE, and CE) is shown in Fig. 2B. All three of these UTR segments were capable of localizing the heterologous *lacZ* message in a pattern that mimics the distribution of the endogenous *orb* message during oogenesis (Fig. 4A; data not shown for AN and AE). Thus, it would appear that a 280-nucleotide UTR segment, CE, is sufficient to generate the entire localization pattern of the *orb* mRNA.

We were able to further subdivide the targeting sequence by cutting the 815-nucleotide HN fragment at an *Xho*I site that lies near the middle of the 280-bp CE sequence (Fig. 2B). We then inserted the resulting subfragments, HX and XN, into our *hsp83-Adh-lacZ* reporter construct. Figures 4B to D show that while both HX and XN are able to target the heterologous *lacZ* mRNA (at least to some extent), neither is capable of reproducing the complex localization pattern observed for the endogenous *orb* message.

The targeting activity of the HX fragment is most severely impaired. As can be seen in Fig. 4B and C, HX *lacZ* mRNA preferentially accumulates in the oocyte during the early stages of oogenesis (region 2A of the germarium through stage 7-8). Since *lacZ* RNA expressed from the control reporter construct *hsp83-Adh-lacZ* is not concentrated in the oocyte during these stages, this result would suggest that HX has a *cis*-acting element which is capable of directing the preferential accumulation of transcripts in the oocyte. On the other hand, the localization of the HX *lacZ* transcripts within the oocyte is

abnormal. First, HX *lacZ* mRNA does not accumulate strongly at the posterior pole of the oocyte in egg chambers between stages ~2 and 7; instead it is usually distributed uniformly throughout the oocyte (especially in the older chambers). In some of the earlier chambers, weak accumulation at the posterior pole can occasionally be observed. Second, no anterior localization is evident in stage 8-10 chambers.

The distribution of *lacZ* mRNA from the XN construct is much more similar to that of the endogenous *orb* mRNA than HX (Fig. 4D). Not only does the XN RNA preferentially accumulate in the oocyte at early stages, but we also observed localization at the posterior pole between stages ~2 and 7. This result would suggest that the XN RNA has fully functional targeting elements specifying oocyte accumulation and posterior localization. However, at stages 8 to 10, XN *lacZ* mRNA appears to be uniformly distributed in the oocyte. Hence, the XN fragment would appear to lack the targeting element responsible for the anterior localization of the *orb* message.

DISCUSSION

We have shown that *cis*-acting elements within the *orb* mRNA are responsible for targeting the *orb* message during *Drosophila* oogenesis. These *cis*-acting elements are encoded in the *orb* 3′ UTR and are capable of targeting a heterologous *lacZ* RNA expressed from a heterologous promoter in the same distribution pattern as the endogenous *orb* message. Moreover, we have demonstrated that these same targeting elements are essential for the localization of the *orb* mRNA itself. The presence of targeting sequences in the 3′ UTR may be a common theme in mRNA localization; targeting elements have been identified in a number of other mRNAs (e.g., *bicoid*, *nanos*, *K10*, *cyclin B*, *Vg1*, and the *Drosophila* pair-rule genes *even-skipped*, *fushi tarazu*, and *hairy*) (3, 5, 6, 11, 20, 21, 24), and as for *orb*, the critical sequences are also located in the 3′ UTRs of these messages.

The *cis*-acting sequences sufficient to generate the same localization pattern as that observed for endogenous *orb* mRNA are in a relatively small 280-nucleotide sequence, CE, from the 1.2-kb *orb* 3′ UTR. Our results suggest that this localization sequence may actually be composed of multiple signalling elements. First, nonoverlapping fragments containing either the 5′ (HX) or 3′ (XN) half of the CE sequence are capable of directing the preferential accumulation of *lacZ* mRNA in the oocyte. This finding suggests that there are at least two elements which can specify oocyte accumulation. On the other hand, these two elements may normally function together, as the level of residual *lacZ* mRNA in nurse cells appears to be consistently higher with the HX and XN construct than it is for constructs in which the CE sequence is intact. Second, the XN fragment contains an element that directs posterior localization between stages ~2 and 7. Whether this element is the same as the one specifying oocyte accumulation is not clear. It is possible that XN contains two distinct elements, one that directs transport from the site of synthesis in the nurse cells to oocyte (and perhaps even targeting to the posterior) and a second that is responsible for tethering the mRNA once it arrives at the posterior pole. Interestingly, posterior localization is also observed for the HX RNA. However, in contrast to the XN construct, posterior localization with the HX construct is evident only in early egg chambers and is usually quite weak and variable. This result could indicate, for example, that HX contains an element specifying translocation to the posterior pole but lacks an element required to maintain the RNA at the pole. Finally, neither HX nor XN is able to direct the accumulation of *lacZ* mRNA at the anterior of the oocyte between stages 8 and 10. This finding raises the possibility that there is another element or a combination of elements specifying anterior localization. In the former case, the sequences constituting this anterior localization element could be disrupted in the two constructs because they span or are close to the *Xho*I restriction site (Fig. 2B). In the later case, two (or more) elements may be required for anterior localization, one in the HX fragment and one in the XN fragment.

While our results argue that multiple elements are required to generate the *orb* localization pattern, it is not entirely clear whether these elements are distinct. It could be argued, for example, that the complex localization pattern is generated by a single reiterated element. In this view, different aspects of the localization pattern would depend on the number of reiterated elements; localization to the oocyte, for example, would require fewer of these reiterated elements than localization to the anterior. On the other hand, there are no obvious reiterated sequences in the *orb* 3′ UTR. For this reason, we favor the idea that the different elements in each fragment are distinct sequences and that they are able to function, at least to some extent independently, to specify particular aspects of the localization pattern. In this regard, it is of interest that Kim-Ha et al. (15) have recently found that there are multiple localization elements in the 3′ UTR of *oskar* RNA and that these elements are responsible for different aspects of the localization pattern.

The presence of multiple *cis*-acting elements raises the possibility that the targeting of *orb* mRNA (as well as other mRNAs) during oogenesis involves interactions with several different RNA-binding proteins. If this is the case, these proteins would likely function at different steps in the localization process. In the nurse cells in which *orb* RNA is transcribed, there would be specific RNA-binding proteins which facilitate the transport of the RNA into the oocyte most probably via the microtubule network. Once in the oocyte, other proteins might be involved in transporting the *orb* RNA to specific locations (e.g., the posterior pole). In addition to RNA-binding proteins which function in translocation, it seems likely that other proteins might be required to anchor the RNA to the cortex of the oocyte once it has reached its appropriate destination. Presumably, this would involve interactions with the cytoskeleton.

While only a handful of mRNAs that are localized during oogenesis have been identified, several distinct but apparently overlapping distribution patterns are observed. One group which includes *K10* and *Bic-D* has a localization pattern much like *orb* (3, 29). Prior to the onset of vitellogenesis, the RNAs in this group are preferentially localized to the posterior pole of the developing oocyte, while after the onset of vitellogenesis, the RNAs at the posterior pole disappear and instead accumulation is observed at the anterior of the oocyte along the nurse cell-oocyte border. *tudor* RNA also accumulates at the posterior pole during the early stages of oogenesis; however, unlike the RNAs in the *orb* group, it does not localize to the anterior region of the oocyte after the beginning of vitellogenesis (11). Similarly, although the *oskar* RNA localization pattern resembles that of the *orb* group, it differs in that *oskar* RNA persists at the posterior pole after stage 8 (8, 14, 15). Finally, a rather different distribution is observed for *hu-li tai shao* (*hts*) RNA (36). During the early stages of egg chamber development, *hts* RNA appears to accumulate around the entire cortex of the oocyte, while at later stages it is localized only along the anterior margin of the oocyte. Conceivably, these diverse localization patterns might be gen-

erated by different combinations of *cis*-acting elements. In such a model, each RNA would contain several different sequence elements that specify particular aspects of the localization pattern, e.g., oocyte, posterior, or anterior. RNAs in the *orb*-like group, which display a similar (although not precisely identical) distribution pattern, might share many of the same *cis*-acting elements. Other RNAs such as *tudor* could contain some of the *cis*-acting elements found in the *orb*-like group (e.g., oocyte and posterior) but would lack other elements (e.g., anterior). Further studies will be required to test this combinatorial model.

ACKNOWLEDGMENTS

We thank J. Horabin for the *hsp83-lacZ* transformant line and the pHS83CaSpeR plasmid and J. Chang, R. Steward, T. Schüpbach, J. Horabin, and M. Samuels for helpful comments on the manuscript.

This work was supported by a grant from NIH.

REFERENCES

1. **Ambrosio, L., and P. Schedl.** 1984. Gene expression during *Drosophila melanogaster* oogenesis: analysis by in situ hybridization to tissue sections. Dev. Biol. **105:**80–92.
2. **Bandziulis, R., M. Swanson, and G. Dreyfuss.** 1989. RNA-binding proteins as developmental regulators. Genes Dev. **3:**431–437.
3. **Cheung, H.-K., T. Serano, and R. S. Cohen.** 1992. Evidence for a highly selective RNA transport system and its role in establishing the dorsoventral axis of the *Drosophila*. Development **114:**653–661.
4. **Christerson, L., and D. McKearin.** *orb* is required for anteroposterior and dorsoventral patterning during *Drosophila* oogenesis. Genes Dev., in press.
5. **Dalby, B., and D. M. Glover.** 1992. 3′ Non-translated sequences in *Drosophila* cyclin B transcripts direct posterior pole accumulation late in oogenesis and perinuclear association in syncytial embryos. Development **115:**989–997.
6. **Davis, I., and D. Ish-Horowicz.** 1991. Apical localization of pair-rule transcripts requires 3′ sequences and limits protein diffusion in the *Drosophila* blastoderm embryo. Cell **67:**927–940.
7. **Davis, L., G. A. Banker, and O. Steward.** 1987. Selective dendritic transport of RNA in hippocampal neurons in culture. Nature (London) **330:**477–479.
8. **Ephrussi, A., L. K. Dickenson, and R. Lehmann.** 1991. *oskar* organizes the germ plasm and directs localization of the posterior determinant nanos. Cell **66:**37–50.
9. **Garner, C. C., R. P. Tucker, and A. Matus.** 1988. Selective localization of messenger RNA for cytoskeletal protein MAP2 in dendrites. Nature (London) **336:**674–677.
10. **Gavis, E., and R. Lehmann.** 1992. Localization of *nanos* RNA controls embryonic polarity. Cell **71:**301–313.
11. **Golumbeski, G. S., A. Bardsley, F. Tax, and R. E. Boswell.** 1991. *tudor*, a posterior-group gene of *Drosophila melanogaster*, encodes a novel protein and an mRNA localized during mid-oogenesis. Genes Dev. **5:**2060–2070.
12. **Henikoff, S.** 1987. Unidirectional digestion with exonuclease III in DNA sequence analysis. Methods Enzymol. **155:**156–165.

12a. **Horabin, J.** Personal communication.

13. **Kenan, D. J., C. C. Query, and J. D. Keene.** 1991. RNA recognition: towards identifying determinants of specificity. Trends Biochem. Sci. **16:**214–220.
14. **Kim-Ha, J., J. L. Smith, and P. M. Macdonald.** 1991. *oskar* mRNA is localized to the posterior pole of the *Drosophila* embryo. Cell **66:**23–35.
15. **Kim-Ha, J., P. J. Webster, J. L. Smith, and P. M. Macdonald.** 1993. Multiple RNA regulatory elements mediated distinct steps in localization of *oskar* mRNA. Development **119:**169–178.
16. **Landry, S. J., and L. M. Gierasch.** 1991. Recognition of nascent polypeptides for targeting and folding. Trends Biochem. Sci. **16:**159–163.
17. **Lantz, V., L. Ambrosio, and P. Schedl.** 1992. The *Drosophila orb* gene is predicted to encode sex-specific germline RNA binding proteins and has localized transcripts in ovaries and early embryos. Development **115:**75–88.
18. **Lantz, V., J. S. Chang, J. I. Horabin, D. Bopp, and P. Schedl.** The *Drosophila orb* RNA binding protein is required for the formation of the egg chamber and the establishment of polarity. Genes Dev., in press.
19. **Lawrence, J., and R. H. Singer.** 1986. Intracellular localization of messenger RNAs for cytoskeletal proteins. Cell **45:**407–415.
20. **Macdonald, P. M.** 1992. The means to the ends: localization of maternal messenger RNAs. Semin. Dev. Biol. **3:**413–424.
21. **Macdonald, P. M., and G. Struhl.** 1988. Cis-acting sequences responsible for anterior localization of *bicoid* mRNA in *Drosophila* embryos. Nature (London) **336:**595–598.
22. **Mahowald, A. P., and M. P. Kambysellis.** 1980. Oogenesis, p. 141–224. *In* M. Ashburner and T. R. F. Wright (ed.), The genetics and biology of *Drosophila*, vol. 2d. Academic Press, New York.
23. **Melton, D. A.** 1987. Translocation of a localized maternal mRNA to the vegetal pole of *Xenopus* oocytes. Nature (London) **328:**80–82.
24. **Mowry, K. L., and D. A. Melton.** 1992. Vegetal messenger RNA localization directed by a 340-nt RNA sequence element in *Xenopus* oocytes. Science **255:**991–994.
25. **Rebagliati, M. R., D. L. Weeks, R. P. Harvey, and D. A. Melton.** 1985. Localized maternal mRNAs in *Xenopus laevis* eggs. Cell **42:**769–777.
26. **Robertson, H. M., C. R. Preston, R. W. Phillis, D. Johnson-Schlitz, W. K. Benz, and W. R. Engels.** 1988. A stable genomic source of P element transposase in *Drosophila melanogaster*. Genetics **118:**461–470.
27. **Spradling, A. L., and X. Rubin.** 1992. Transformation of cloned P-elements into the *Drosophila* germline chromosomes. Science **218:**341–347.
28. **St. Johnston, D., and C. Nusslein-Volhard.** 1992. The origin of pattern and polarity in the *Drosophila* embryo. Cell **68:**201–219.
29. **Suter, B., L. Romberg, and R. Steward.** 1989. *Bicaudal-D*, a *Drosophila* gene involved in developmental asymmetry: localized transcript accumulation in ovaries and sequence similarity to myosin heavy chain tail domains. Genes Dev. **3:**1957–1968.
30. **Tautz, D., and C. Pfeifle.** 1989. A non radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals a translational control of the segmentation gene *hunchback*. Chromosoma **98:**81–85.
31. **Thummel, C. S., A. M. Boulet, and H. D. Lipshitz.** 1988. Vectors for *Drosophila* P-element-mediated transformation and tissue culture transfection. Gene **74:**445–456.
32. **Tomlinson, A., B. E. Kimmel, and G. M. Rubin.** 1988. *rough*, a *Drosophila* homeobox gene required in photoreceptors R2 and R5 for inductive interactions in the developing eye. Cell **55:**771–784.
33. **Wang, C., and R. Lehmann.** 1991. *nanos* acts as the posterior determinant in *Drosophila*. Cell **66:**637–647.
34. **Weeks, D. L., and D. A. Melton.** 1987. A maternal mRNA localized to the vegetal hemisphere in *Xenopus* eggs codes for a growth factor related to TGF-B. Cell **51:**861–867.
35. **Xiao, H., and J. T. Lis.** 1989. Heat shock and developmental regulation of *Drosophila melanogaster hsp83* gene. Mol. Cell. Biol. **9:**1746–1753.
36. **Yue, L., and A. Spradling.** 1993. *hu-li tai shao*, a gene required for ring canal formation during *Drosophila* oogenesis, encodes a homolog of adducin. Genes Dev. **6:**2443–2454.