Gratuitous mRNA localization in the *Drosophila* oocyte

Thomas L. Serano¹,²,† and Robert S. Cohen¹,*

¹Department of Biochemistry, University of Kansas, Lawrence, KS 66045, USA
²Department of Biochemistry and Molecular Biophysics, Columbia University College of Physicians and Surgeons, 630 West 168th Street, New York, NY 10032, USA

*Author for correspondence
†Present address: Department of Molecular and Cell Biology, Howard Hughes Medical Institute, University of California, Berkeley, CA 94720, USA

SUMMARY

Many of the genes that control pattern formation in *Drosophila* encode mRNAs that are localized to discrete regions of the oocyte during oogenesis. While such localization is generally assumed to be important for the pattern-forming activities of these genes, this has been rigorously demonstrated in only a few cases. Here we address the role of mRNA localization for the dorsoventral patterning gene *K10*. *K10* mRNA is localized to the oocyte's anterior cortex following its transport into the cell during early stages of oogenesis. We show that mutations in *cappuccino* and *spire*, which permit *K10* mRNA transport, but prevent subsequent anterior localization, do not disrupt the synthesis or localization of *K10* protein. We also show that modified *K10* transgenes that produce transcripts which are uniformly distributed throughout the oocyte, or which are mislocalized to the oocyte’s posterior pole, produce localized and functional *K10* protein. We conclude that the anterior localization of *K10* mRNA is not important for *K10* protein targeting or gene function. We propose that the anterior localization of *K10*, and probably other mRNAs, is a by-product of mRNA transport and does not necessarily reflect a requirement for localization per se.

Key words: mRNA localization, *Drosophila* oogenesis, *K10*, *cappuccino*, *spire*, dorsoventral patterning

INTRODUCTION

A number of vertebrate and invertebrate mRNAs have been identified that are asymmetrically distributed within the cells that express them (reviewed in Macdonald, 1992; Ding and Lipshitz, 1993; Wilhelm and Vale, 1993). However, a functional requirement for mRNA localization has only been rigorously demonstrated in a few cases, e.g., for the *Drosophila* bicoid (*bcd*), oskar (*osk*) and gurken (*grk*) mRNAs. *bcd* and *osk* mRNAs are localized to the anterior and posterior poles of the oocyte, respectively, and define the anteroposterior axis of the future embryo (Berleth et al., 1988; Kim-Ha et al., 1991; Ephrussi et al., 1991). *grk* mRNA is localized to the oocyte’s anterodorsal corner and defines the dorsoventral axis of the egg and future embryo (Neuman-Silberberg and Schüpbach, 1993). The role of mRNA localization in the case of *bcd*, *osk* and *grk* is that of protein targeting. Mutations that alter the mRNA distribution patterns of these genes similarly alter protein distribution patterns and result in pattern formation defects (Berleth et al., 1988; Driever and Nüsslein-Volhard, 1988; St. Johnston et al., 1989; Kim-Ha et al., 1991; Ephrussi et al., 1991; Neuman-Silberberg and Schüpbach, 1993; Serano et al., 1995; P. Macdonald, personal communication).

While there is a clear rationale for *bcd*, *osk* and *grk* mRNA localization, the role of localization for mRNAs that do not colocalize with their respective proteins is enigmatic. One such mRNA is encoded by the dorsoventral patterning gene *fs(1)K10* (*K10*). *K10* mRNA is localized to the oocyte’s anterior cortex, whereas *K10* protein is localized to the oocyte nucleus (Cheung et al., 1992; Prost et al., 1988; Cohen and Serano, 1995). Like other mRNAs localized within the *Drosophila* oocyte, *K10* mRNA is synthesized in nurse cells and selectively transported through cytoplasmic bridges to the posterior pole of the oocyte during early stages of oogenesis (Cheung et al., 1992). We have previously shown that such transport is essential for *K10* function, but were unable to address the significance of the mRNA’s subsequent movement to, and stable localization at, the oocyte’s anterior cortex (Cheung et al., 1992; Cohen and Serano, 1995). While it seems unlikely that the anterior localization of *K10* mRNA plays a role in protein targeting – nuclear targeting sequences within the *K10* protein itself would be expected to serve this function – such localization may be required for other functions, e.g. the translation and/or post-translational modification of *K10* protein. Two observations are consistent with the idea that the anterior localization of *K10* mRNA is important for *K10* gene function. First, mutations in *cappuccino* (*capu*) and *spire* (*spir*) block the anterior localization of *K10* mRNA and produce dorsoventral patterning defects similar to those of *K10* mutants (Wieschaus, 1979; Manseau and Schüpbach, 1989; Cheung et al., 1992). Second, an analysis of inducible *K10* transgenes indicates that *K10* protein activity is not required prior to the...
anterior localization of its mRNA at stage 8 (Serano et al., 1995). Nevertheless, it is possible that only the transport of K10 mRNA into the oocyte is required for K10 function, i.e., the subsequent localization of the mRNA to the oocyte’s anterior cortex may not be important for K10 function.

Here we investigate the role of K10 mRNA anterior localization. We show that K10 transgenes that produce transcripts that are dispersed throughout the oocyte, or that are mislocalized to the posterior pole of the oocyte, produce localized and functional K10 protein. Thus, while the transport of K10 mRNA into the oocyte is important for K10 function, its subsequent localization within the oocyte is not. In addition, we demonstrate that the dorsoventral pattern defects of capu and spir mutants are not due to the inability of these mutants to localize K10 mRNA.

MATERIALS AND METHODS

Fly stocks

The wild-type stock is Oregon R. The capu, spir and exu alleles are capuG7, spirP1 and exuO8, respectively, which have been previously described (Manseau and Schüpbach, 1989; Schüpbach and Wieschaus, 1986). The two K10 alleles are K10I (Wieschaus et al., 1979) and K10LM00, the latter of which was provided by T. Schüpbach and described in Cheung et al. (1992). All markers and balancers are described in Lindsay and Zimm (1992).

In situ hybridization

In situ hybridization to whole-mount ovaries was carried out according to Tautz and Pfeifle (1989) with modifications described in Cheung et al. (1992). Digoxigenin-labeled DNA probes were produced by random-priming according to Feinberg and Vogelstein (1983). The K10 probe corresponds to nucleotides 757-1763 (Prost et al., 1988). In situ hybridization to K-bcd, K-osk and KΔ3 transcripts was carried out in a K10LM00 background. K10LM00 produces no detectable K10 mRNA (Cheung et al., 1992; and see Fig. 5C). The grk probe corresponds to grk cDNA nucleotides 505-1029 (Neuman-Silberberg and Schüpbach, 1993). Photographs was carried out as previously described (Serano et al., 1995).

Immunocytochemistry

Immunocytochemistry to whole-mount ovaries was carried out according to Macdonald et al. (1991), with modifications described in Serano et al. (1995). The anti-K10 polyclonal antibody was previously described (Cohen and Serano, 1995). Immunocytochemistry to K10 protein produced from K-bcd, K-osk and KΔ3 transcripts was carried out in a K10I background in which very little, if any, endogenous K10 protein can be detected (see Fig. 6D).

Transformation constructs

All transformation constructs contain the same -2.3 kb Asp718-Sall fragment derived from K10 [corresponding to K10 nucleotides 1-3112 (Prost et al., 1988), except that the intron is omitted]. This fragment includes the K10 nurse cell enhancer/promoter, the entire K10 protein coding region and the first 65 nucleotides of the K10 3’UTR (Prost et al., 1988; Cheung et al., 1992). Constructs differ from each other in their respective 3’UTRs, which directly abut the Sall site of the 2.3 kb fragment.

The 3’UTR of the KΔ3’ construct consists of 19 nucleotides of linker DNA followed by the DraI-PstI fragment of K10 (nucleotides 4349-5345). The K10 DraI-PstI fragment contains the last 133 nucleotides of the K10 3’UTR, including the poly(A)-addition site. KΔ3’ thus lacks 1236 nucleotides of the K10 3’UTR. The 3’UTR of the K-bcd construct consists, from 5’ to 3’, of 15 nucleotides of linker DNA, the bcd EcoRV-Std fragment (nucleotides 4099-4732, Berleth, et al., 1988), 14 nucleotides of linker DNA and the K10 DraI-PstI fragment (described above). The 3’UTR of K-osk consists of 19 nucleotides of linker DNA followed by the osk DraI-Xbal fragment (nucleotides 2796-3658, Kim-Ha et al., 1991), which includes the osk poly(A)-addition site. While K-osk transcripts are localized to the posterior pole of the oocyte, recombinant osk transcripts that contain a similar portion of the osk 3’UTR (olec21 in Kim-Ha et al., 1993) have been reported to be deficient for such localization. Transformation constructs similar to K-bcd and K-osk, but including larger portions of the bcd and osk 3’UTRs, were also made (details available upon request). Such transgenes produced localized mRNAs that were not translated and thus were not analyzed further.

Transformations and rescue experiments

The three K10 constructs described above were cloned into the pCaSpeR4 transformation vector (Pirrotta, 1988) and introduced into w1118 flies by P element-mediated transformation (Rubin and Spradling, 1982; Spradling and Rubin, 1982). Transposase activity was provided by the p13pwc helper plasmid (Cohen and Meselson, 1985). At least five independently transformed lines were generated and analyzed for each construct. The K10 transgenes were introduced into desired mutant backgrounds by standard genetic crosses. For rescue assays, 5-10 females carrying the K10 transgene in a K10LM00 background were mated to wild-type males. Eggs were collected on yeast apple plates and hatching frequency was recorded. In cases where rescue to hatching could not be examined (e.g., in capu, spir and exu, which are maternal effect lethal due to anteroposterior patterning defects), rescue was assayed by examining the grk mRNA distribution pattern, or by a visible inspection of egg shells (see text for details). Egg shells were prepared and photographed as previously described (Serano et al., 1995).

RESULTS

K10 protein distribution in capu and spir mutants

The localization of K10 mRNA to the oocyte’s anterior cortex is a dynamic process. During stages 1-7 of oogenesis, K10 mRNA is transcribed in nurse cells and transported through cytoplasmic bridges to the posterior pole of the oocyte (Cheung et al., 1992; Serano and Cohen, 1995; and see King, 1970, for a description of the 14 stages of oogenesis). Then, during stage 8, the mRNA moves to the oocyte’s anterior cortex, where it remains localized through stage 10B (Fig. 1A). After stage 10B, K10 mRNA becomes delocalized and is degraded prior to the onset of embryogenesis (Serano and Cohen, unpublished data). In capu and spir mutants, K10 mRNA is efficiently transported to the posterior pole of the oocyte, but fails to accumulate, or only partially accumulates, at the oocyte’s anterior cortex (Fig. 1B,C; Cheung et al., 1992). Instead, the mRNA becomes more uniformly distributed throughout the oocyte cytoplasm. To determine whether the failure to localize K10 mRNA to the anterior cortex in capu and spir mutant oocytes affects K10 protein synthesis and/or localization, we carried out whole-mount immunocytochemistry using an anti-K10 antibody. In contrast to their mRNA distribution patterns, the K10 protein distribution patterns of capu and spir mutants are indistinguishable from that of wild-type flies (Fig. 1D-F). In each case, K10 protein accumulates specifically in the oocyte nucleus and to a similar level. We thus conclude that capu and spir are not required for the synthesis or nuclear localization of K10 protein. By extension, we conclude that the anterior
localization of K10 mRNA within the oocyte is not required for the synthesis or nuclear localization of K10 protein.

**capu and spir mediate dorsoventral patterning independently of K10 mRNA localization**

The above results suggest that the dorsoventral patterning defects seen in *capu* and *spir* mutants do not arise from the inability of these mutants to localize K10 mRNA to the oocyte’s anterior cortex. To test this idea more directly, we examined the dorsoventral pattern of *capu* and *spir* mutants that carry a K10 transgene engineered to produce anteriorly localized transcripts in a *capu* and *spir*-independent fashion. This transgene, called K-bcd, was constructed by replacing the K10 3' untranslated region (3'UTR), which contains all of K10's transport and localization control sequences (Cheung et al., 1992; Cohen and Serano, 1995), with those of the bcd gene, which previous studies have shown directs mRNA transport and anterior localization in a *capu*- and *spir*-independent fashion (Fig. 2; Macdonald and Struhl, 1988; Macdonald et al., 1993; Serano and Cohen, unpublished). As seen in Fig. 3A, transcripts

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**Fig. 1.** K10 mRNA and protein distribution patterns in wild-type and mutant egg chambers. Wild-type (A), *capu* (B) and *spir* (C) stage 6-10 egg chambers are shown following whole-mount in situ hybridization with a digoxigenin-labeled K10 probe. Note that, in *capu* and *spir* mutants, K10 transcripts are not localized to the oocyte’s anterior cortex during stages 8-10 and instead are distributed throughout the oocyte cytoplasm. Wild-type (D), *capu* (E) and *spir* (F) stage 9-10 egg chambers are shown following whole-mount immunocytochemistry with a polyclonal anti-K10 antibody. K10 protein is detected as one major, and several minor, foci of staining within the oocyte nucleus (see arrow in D). Lesser amounts of protein are seen in the oocyte nuclei of younger egg chambers (not shown). Alleles are described in Materials and Methods. For this and all other figures, anterior is to the left and dorsal is up.

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**Fig. 2.** Schematic diagrams of K-bcd, K-osk and KΔ3' constructs and the oocyte localization patterns of their encoded transcripts. The thin black line represents sequences derived from the K10 gene. The approximate positions of the K10 transcription start site (arrow), translation start site (ATG), stop codon (TAG) and poly(A) addition site (pA) are indicated. In K-bcd and K-osk, most of the K10 3'UTR is replaced by a portion of the bcd 3'UTR (represented by the black box) or a portion of the osk 3'UTR (represented by the gray box), respectively. Note that osk sequences provide the poly(A) addition site for K-osk. The same region of the K10 3'UTR that is replaced in K-bcd, is deleted in KΔ3' without the inclusion of additional sequences. See Materials and Methods for precise coordinates. The localization pattern of K-bcd, K-osk and KΔ3' transcripts in stage 8-10 oocytes is indicated to the right of the gene diagrams.
produced by K-bcd transgenes are transported into the oocyte and subsequently localized to the oocyte’s anterior cortex in a manner similar to those produced by wild-type bcd and K10 genes. Moreover, K-bcd transcripts produce protein that localizes to the oocyte nucleus (Fig. 6A) and restores fertility to K10 mutants (Table 1). The mRNA and protein distribution patterns of K-bcd transgenes are not altered following their introduction into a capu or spir mutant background (Fig. 3B, C and data not shown).

We next compared dorsoventral patterning in capu and spir stocks that carried or lacked the K-bcd transgene. If capu and/or spir mediate dorsoventral patterning solely via K10 mRNA localization, then capu and/or spir mutants carrying the K-bcd transgene should exhibit no dorsoventral pattern defects. Conversely, if capu and/or spir are required for dorsoventral patterning events other than K10 mRNA localization, then capu and/or spir mutants that carry the K-bcd transgene should exhibit dorsoventral patterning defects. Dorsoventral patterning was assessed by examining grk mRNA localization. The dorsoventral pattern of the egg chamber, egg and future embryo is dependent on the localization of grk mRNA to the oocyte’s anterodorsal corner during stages 8-10B (Schüpbach, 1987; Neuman-Silberberg and Schüpbach, 1993, 1994). Mutations in K10, capu and spir cause grk mRNA to accumulate along the entire anterior cortex of the oocyte and lead to the production of dorsalized egg chambers, eggs and embryos (Neuman-Silberberg and Schüpbach, 1993; Wieschaus, 1979; Manseau and Schüpbach, 1989).

As seen in Fig. 3D, grk mRNA is localized to the anterodorsal corner of K10; P[K-bcd] oocytes. In contrast, grk mRNA does not localize, or only inefficiently localizes, to the anterodorsal corner of K10; capu; P[K-bcd] and K10; spir; P[K-bcd] oocytes. Instead, the mRNA generally accumulates along the entire anterior cortex of these oocytes (Fig. 3E and data not shown). Not surprisingly, these stocks produce egg shells with dorsoventral pattern defects indistinguishable from those produced by capu and spir mutants (data not shown). Thus, the K-bcd transgene does not circumvent the need for wild-type capu and spir gene activities even though it produces a mRNA that is localized to the oocyte’s anterior cortex in a capu- and spir-independent fashion. We conclude that capu and spir do not mediate dorsoventral patterning via K10 mRNA localization.

Although the above experiments show that capu and spir do not mediate dorsoventral patterning via K10 mRNA localization, they do not show that capu and spir mediate dorsoventral patterning independent of K10 function. It is possible that capu and spir are necessary for some post-translational modification of K10 protein. A second observation made during the course of the above experiments, however, strongly support the idea that capu and spir do in fact mediate dorsoventral patterning independent of K10 function. Specifically, we noticed that grk mRNA is more severely delocalized in capu and spir mutants than it is in K10 null mutants; while some anterodorsal localization of grk mRNA is consistently seen in K10 null mutants (Roth and Schüpbach, 1994; Serano et al., 1995), such localization is less often seen in capu and spir mutants. Indeed, in some capu and spir mutant oocytes, grk mRNA is uniformly distributed throughout the oocyte cytoplasm (Fig. 3F). This finding is inconsistent with the idea that capu and spir mediate dorsoventral patterning through K10, in which case one would expect the grk mRNA distribution pattern to mirror that of K10 mutants, or, if the

![Fig. 3. K-bcd and grk mRNA distribution patterns in various genetic backgrounds.](image-url)
capu and spir mutations analyzed are hypomorphic rather than null, to more closely resemble wild type.

**Tight anterior localization of K10 transcripts is not required for K10 function**

The experiments described above show that the translation and nuclear localization of K10 protein is not dependent on the localization of K10 mRNA to the oocyte’s anterior cortex. We next wanted to determine whether anterior localization is important for the production of functional K10 protein. To this end, we examined K-bcd gene activity in an exuperantia (exu) mutant background. Wild-type exu gene activity is required for the stable localization of bcd mRNA to the oocyte’s anterior cortex, but is not normally required for dorsoventral patterning (Berleth et al., 1988; St. Johnston et al., 1989). Since exu mediates bcd mRNA localization through the bcd 3’UTR (Macdonald et al., 1993), we reasoned that K-bcd mRNA localization would be disrupted in exu mutants. If anterior localization is not required for K10 function, then K-bcd transgenes should rescue the dorsoventral patterning defects of K10 mutants even if such mutants lack exu activity. Conversely, if anterior localization is required for K10 function, then the K-bcd transgene should rescue the dorsoventral patterning defects of K10 mutants, but not those of K10; exu double mutants.

Dorsoventral patterning was assessed by examining egg shell phenotypes. Wild-type egg shells exhibit a distinct dorsoventral pattern that is most recognizable by the position of the two dorsal appendages; one dorsal appendage projects out from each side of the dorsal midline (Fig. 4A). K10 mutant egg shells are dorsalized. Their dorsal appendage material projects circumambiently from the anterior of the egg (Fig. 4B). As seen in Fig. 4C, K-bcd transgenes restore normal dorsoventral pattern to K10 mutant egg shells even in the absence of wild-type exu activity. This rescue occurs despite the fact that K-bcd transcripts are not tightly localized to the anterior cortex of exu mutant oocytes, particularly during stages 9 and 10 (see Fig. 3G). We conclude that the tight anterior localization of K-bcd transcripts is not required for the production of fully functional K10 protein.

**K10 transgenes that produce posteriorly-localized transcripts rescue K10 mutants**

The above results demonstrate that K-bcd transcripts need not be tightly localized to the oocyte’s anterior cortex to produce functional protein. To determine whether anterior localization is completely dispensable for wild-type K10 function, we mis-localized K10 transcripts to the posterior pole of the oocyte by

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**Table 1. The ability of various K10 transgenes to restore fertility to K10 mutants**

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<td>K-bcd</td>
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Several independently transformed lines were generated for each K10 transgene. Transgenes were introduced into a K10LM00 background by standard genetic crosses. Eggs were collected from K10LM00 females that carried either one or two copies of each K10 transgene as noted. For each genotype, at least 100 eggs were examined. The percentage of eggs that were rescued to hatching are indicated. n.d., not determined.

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**Fig. 4.** Non-anteriorly localized K10 transcripts rescue the dorsoventral pattern defects of K10 mutant egg shells. Lateral views are shown of egg shells from females of the following genotypes: (A) wild type; (B) K10LM00; (C) K10LM00 exu; P[K-bcd]. As described in text, wild-type egg shells (A) contain a distinct dorsoventral pattern, which is absent in K10 mutants (B) and restored in K10; exu double mutants carrying the K-bcd transgene (C). exu single mutant egg shells do not display any pattern defects (not shown). Rescue to hatching could not be tested for eggs produced by K10LM00; exu; P[K-bcd] females since exu is maternal effect lethal due to anteroposterior defects.
replacing the \textit{K10} 3'UTR with a portion of the \textit{osk} 3'UTR (Fig. 2; Kim-Ha et al., 1993). The resulting construct, called \textit{K-osk}, produces mRNA that is localized to the oocyte’s posterior pole in a manner similar to that of wild-type \textit{osk} mRNA; transcripts are transported to the oocyte’s posterior pole and, after transiently accumulating along the oocyte’s anterior cortex early in stage 8, move back to the posterior pole of the oocyte by late stage 8, where they remain through the end of oogenesis (Fig. 5A). As seen in Fig. 6B, \textit{K-osk} transgenes produce \textit{K10} protein that localizes to the oocyte nucleus. Furthermore, this protein exhibits nearly wild-type activity. \textit{K-osk} transgenes restore up to 94% fertility to \textit{K10} mutant stocks (Table 1). We conclude that the persistent accumulation of \textit{K10} mRNA at the oocyte’s anterior cortex during stages 8-10B is not obligatory for \textit{K10} gene function.

\textbf{\textit{K10} transcripts that are not localized produce functional \textit{K10} protein}

The one caveat of the above experiment is that \textit{K-osk} mRNA transiently accumulates along the anterior cortex of the oocyte early in stage 8. It is possible that this brief anterior accumulation satisfies a requirement for anteriorly localized \textit{K10} mRNA. Ideally, we would like to examine the function of \textit{K10} transcripts that are transported into the oocyte, but never
localized within it; such a localization pattern has not been reported for any mRNA. However, modified K10 transgenes, whose mRNAs are not transported or localized and which normally do not rescue K10 mutants, can partially restore K10 gene function when over-expressed (Serano and Cohen, unpublished). This is presumably due to the diffusion of K10 mRNA and/or protein from nurse cells into the oocyte. This phenomenon is most evident for $K\Delta 3'$. $K\Delta 3'$ was constructed by deleting virtually all of the K10 3'UTR, including all cis-regulatory elements required for transport and anterior localization (Fig. 2; Cheung et al., 1992; Cohen and Serano, 1995). Messenger RNA and protein produced from $K\Delta 3'$ transgenes accumulate in nurse cells (Figs 5B and 6C, respectively). When the $P[K\Delta 3']$ transgenes of four independently transformed lines are crossed into K10 mutants, less than 1% fertility is restored (Table 1). In contrast, the transgene of a fifth $P[K\Delta 3']$ line restores significant fertility to K10 mutants (see line #4 in Table 1). Most of the eggs produced by K10 mutants carrying two copies of this transgene hatch. This appears to be due to an increased level of transcription, as mRNA levels are several fold higher in this line

DISCUSSION

Both molecular and genetic approaches have been used to identify localized mRNAs in a number of systems (reviewed in Ding and Lipshitz, 1993; Wilhelm and Vale, 1993). However, the biological significance of localization has not been determined for most of these mRNAs. We have previously shown that the localization of K10 mRNA to the Drosophila oocyte is important for K10 function (Cheung et al., 1992; Serano and Cohen, 1995). Here, we have examined the role of the subsequent localization of K10 mRNA within the oocyte. Our data show that the anterior localization of K10 mRNA within the oocyte is not required for K10 function.

The role of capu and spir in dorsoventral patterning and mRNA localization

Mutations in K10, capu and spir disrupt grk mRNA localization and lead to the production of dorsalized eggs and embryos (Neuman-Silberberg and Schüpbach, 1993; Wieschaus, 1979; Manseau and Schüpbach, 1989). Together with the finding that capu and spir are required for K10 mRNA localization (Cheung et al., 1992), this suggested that K10, capu and spir represent a single genetic pathway, where capu and spir are required to localize K10 mRNA, and K10, in turn, is required to localize grk mRNA. Since the localization of other mRNAs, such as osk, also require capu and spir gene activities (Kim-Ha et al., 1991; Ephrussi et al., 1991), it seems likely that mutations in capu and spir non-specifically disrupt mRNA localization. This is supported by the recent observation that cytoplasmic streaming, which begins at stage 10B in wild-type oocytes, is prematurely initiated at stage 8 in capu and spir mutants (Theurkauf, 1994). Such premature cytoplasmic streaming may dislocate mRNAs from the localization machinery, i.e. from microtubules (Theurkauf, 1994).

The localization of K10, grk and osk mRNAs requires capu and spir gene activities, but bcd mRNA localization does not. bcd mRNA may be more tightly coupled to microtubules and thus more resistant to cytoplasmic streaming than other mRNAs. Alternatively, bcd mRNA may become anchored to non-microtubule components of the cytoskeleton immediately upon its entry into the oocyte. Other mRNAs, such as osk, may become anchored to non-microtubule components of the cytoskeleton only after stage 8. This follows from the observation that osk mRNA is sensitive to cytoplasmic streaming when initiated prematurely (i.e., stage 8), but not when initiated at the normal stage (i.e., stage 10B). Still other mRNAs, such as K10 and grk, may never become anchored as they are always sensitive to cytoplasmic streaming. These mRNAs are delocalized at stage 8 in capu and spir mutant oocytes and at stage 10B/11 in wild-type oocytes (Cheung et al., 1992; Neuman-Silberberg and Schüpbach, 1993; Serano and Cohen, unpublished).

mRNA transport and anterior localization: one phenomenon

Early in stage 8, mRNAs that have been transported into the oocyte accumulate along the oocyte’s anterior cortex. Several of these mRNAs later move to the posterior pole, or in the case of grk, to the anterodorsal corner, but the majority persist at the oocyte’s anterior cortex through at least stage 10B. We propose that the oocyte’s anterior cortex serves as a default localization site for mRNAs that, like K10, need to be trans-
ported into, but not localized within, the oocyte. Implicit in this idea is that mRNA transport and anterior localization are one process. An examination of the K10 and bcd cis-acting localization elements supports this idea. In an extensive deletion analysis of the K10 3′UTR, we have identified a 44 nucleotide sequence that directs both mRNA transport and anterior localization (Serano and Cohen, unpublished). Another small sequence, BLE1, directs both bcd mRNA transport and anterior localization through stage 10 (Macdonald et al., 1993). The fact that, in both of these cases, a single small sequence directs both transport and anterior localization strongly suggests that these two events are mechanistically one process.

This idea is further supported by the distribution pattern of microtubules (Theurkauf et al., 1992), which appear to mediate mRNA transport and localization during oogenesis (Pokrywka and Stephenson, 1991, 1995; Clark et al., 1994). Before stage 7, the microtubule organizing center (or minus end) is located at the posterior pole of the oocyte and microtubules extend outward through cytoplasmic bridges into the nurse cells. During stages 7 and 8, microtubules reorganize within the oocyte such that the minus end becomes located along the oocyte’s anterior cortex, where it persists at least through stage 10. Therefore, both mRNA transport to the posterior pole of the oocyte during early stages of oogenesis and localization to the oocyte’s anterior cortex during stages 8-10 could be mediated by the continuous association of the mRNA with a minus end-microtubule motor.

In addition to K10 and bcd, at least five other mRNAs have been reported to be localized at the anterior of the oocyte during stages 8-10B: Adducin-like, Bicaudal-D (Bic-D) and yemanuclein-α (yem-α) (Ding et al., 1993; Suter et al., 1989; Yue and Spradling, 1992; Lantz et al., 1992; Aït-Ahmed et al., 1992). While the significance of K10 and bcd mRNA anterior localization has been addressed, it remains to be seen whether anterior localization is required for the functions of these other mRNAs. We predict that anterior localization will not be important for yem-α, which encodes a nuclear protein (Aït-Ahmed et al., 1992), and for Bic-D and orb, which encode proteins that are uniformly distributed throughout the oocyte’s cytoplasm or along its cortex, respectively, during stages 8-10B (Wharton and Struhl, 1989; Christerson and McKearin, 1994; Lantz et al., 1994). It remains to be seen whether there are gratuitously localized mRNAs in other systems.

We thank Trudi Schüpbach and Gary Struhl for fly stocks, F. Shira Neuman-Silberberg and Trudi Schüpbach for the gsr cDNA clone, and Paul Macdonald for the bcd and osk clones. Special thanks to Kam Cheung, who generated the anti-K10 antibody. Finally, we thank Vicki Corbin, Xiangyi Lu, Doug Ruden, Kathy Suprenant and members of the Cohen laboratory for helpful discussions and comments on the manuscript. R. S. C. was supported by a grant from the NSF (IBN-08821) and by the General Research Fund of the University of Kansas.

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(Accepted 5 June 1995)