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Gratuitous mRNA localization in the *Drosophila* oocyte

Thomas L. Serano[1,2,†] and Robert S. Cohen[1,*]

[1]Department of Biochemistry, University of Kansas, Lawrence, KS 66045, USA
[2]Department 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, 1955). 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 $capu^{G7}$, $spir^{PJ}$ and exu^{QR}, respectively, which have been previously described (Manseau and Schüpbach, 1989; Schüpbach and Wieschaus, 1986). The two *K10* alleles are $K10^{1}$ (Wieschaus et al., 1979) and $K10^{LM00}$, the latter of which was provided by T. Schüpbach and described in Cheung et al. (1992). All markers and balancers are described in Lindsley 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 $K10^{LM00}$ background. $K10^{LM00}$ 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). Photography 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 $K10^{1}$ 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 *Asp*718-*Sal*I 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 *Sal*I site of the 2.3 kb fragment.

The 3′UTR of the *KΔ3′* construct consists of 19 nucleotides of linker DNA followed by the *Dra*I-*Pst*I fragment of *K10* (nucleotides 4349-5345). The *K10 Dra*I-*Pst*I 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 Eco*RV-*Stu*I fragment (nucleotides 4099-4732, Berleth, et al., 1988), 14 nucleotides of linker DNA and the *K10 Dra*I-*Pst*I fragment (described above). The 3′UTR of *K-osk* consists of 19 nucleotides of linker DNA followed by the *osk Dra*I-*Xba*I 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 (olc21 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 w^{1118} 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 $K10^{LM00}$ background were mated to wild-type males. Eggs were collected on yeasted 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

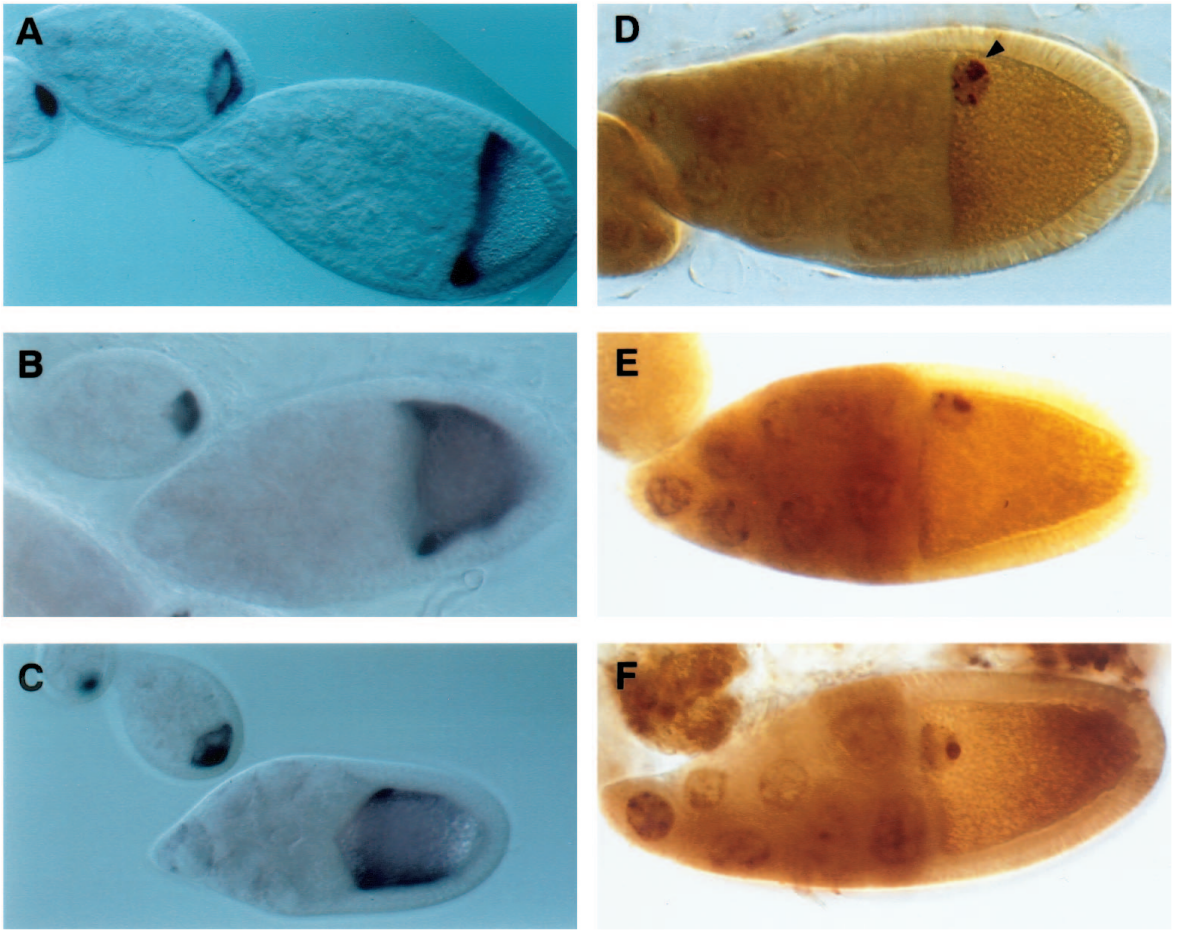

Fig. 1. *K10* mRNA and protein distribution patterns in wild-type and mutant egg chambers. Wild-type (A), *capu* (B) and *spir* (C) stage 6-10 egg chambers are shown following whole-mount in situ hybridization with a digoxigenin-labeled *K10* probe. Note that, in *capu* and *spir* mutants, *K10* transcripts 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.

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

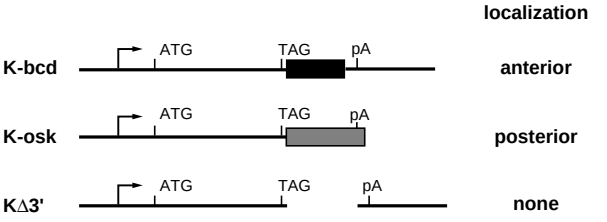

Fig. 2. Schematic diagrams of *K-bcd*, *K-osk* and *KΔ3′* constructs and the oocyte localization patterns of their encoded transcripts. The thin black line represents sequences derived from the *K10* gene. The approximate positions of the *K10* transcription start site (arrow), translation start site (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

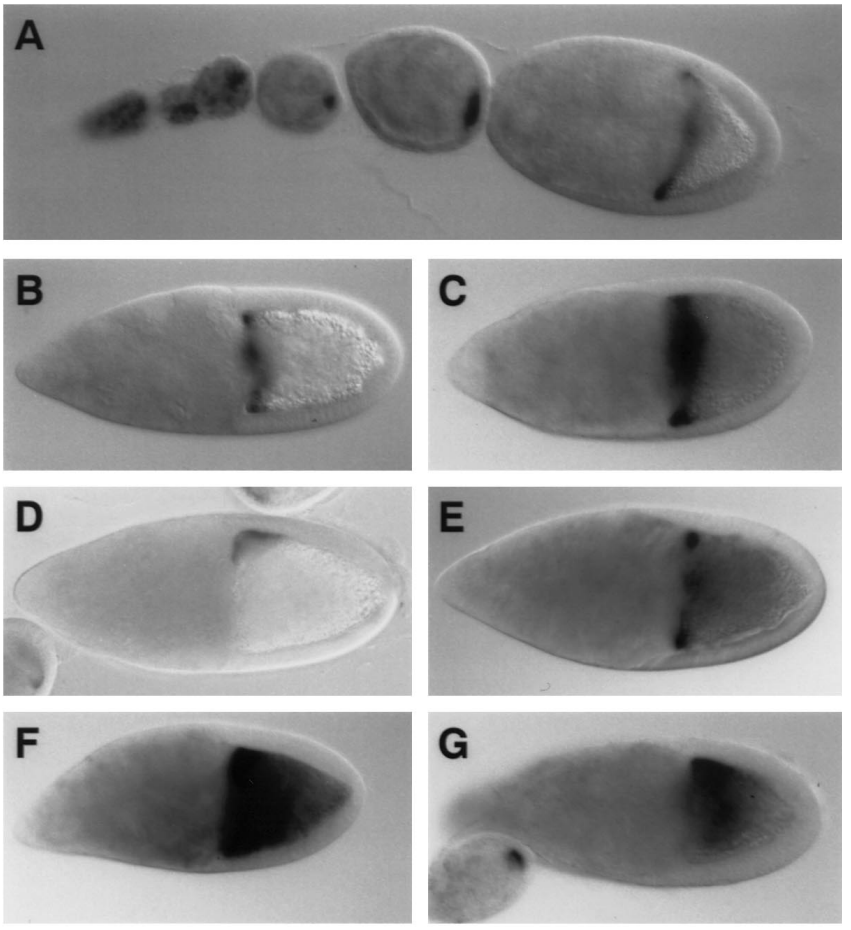

Fig. 3. *K-bcd* and *grk* mRNA distribution patterns in various genetic backgrounds. Egg chambers are shown following whole-mount in situ hybridization with digoxigenin-labeled probes specific for *K-bcd* (A-C,G) or *grk* (D-F) mRNAs. Stage 9 or 10 egg chambers are shown in each panel with the exception of A, in which a series of egg chambers are arranged from left to right in order of increasing stage of development. Egg chamber genotypes are as follows: (A,D) $K10^{LM00}$*; P[K-bcd].* (B,E,F) $K10^{LM00}$*; capu; P[K-bcd].* (C) $K10^{LM00}$*; spir; P[K-bcd].* (G) $K10^{LM00}$*; exu; P[K-bcd].* In these experiments, all flies carried two copies of the *K-bcd* transgene. Note that *K-bcd* mRNA remains localized to the oocyte's anterior cortex in *capu* and *spir* egg chambers (B,C, respectively). The *grk* mRNA distribution pattern is variable in *capu* and *spir* mutants (see text for details). (E,F) The mild and severe extremes of this variability, respectively. Results similar to E and F were seen with *capu*, *spir* and $K10^{LM00}$*; spir; P[K-bcd]* egg chambers (not shown). *capu*, *spir* and *exu* alleles are described in Materials and Methods.

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

		% hatching	
K10 transgene	line #	one copy	two copies
K-bcd	4	14	91
	9	9	87
	12	19	94
	19	5	70
	20	40	86
K-osk	1	79	n.d.
	2	75	n.d.
	3	32	n.d.
	5	94	n.d.
	6	91	n.d.
	7	78	n.d.
	26	60	n.d.
KΔ3′	4	21	72
	5	<1	<1
	9	<1	n.d.
	10	0	n.d.
	27	<1	n.d.

Several independently transformed lines were generated for each *K10* transgene. Transgenes were introduced into a $K10^{LM00}$ background by standard genetic crosses. Eggs were collected from $K10^{LM00}$ 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.

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.

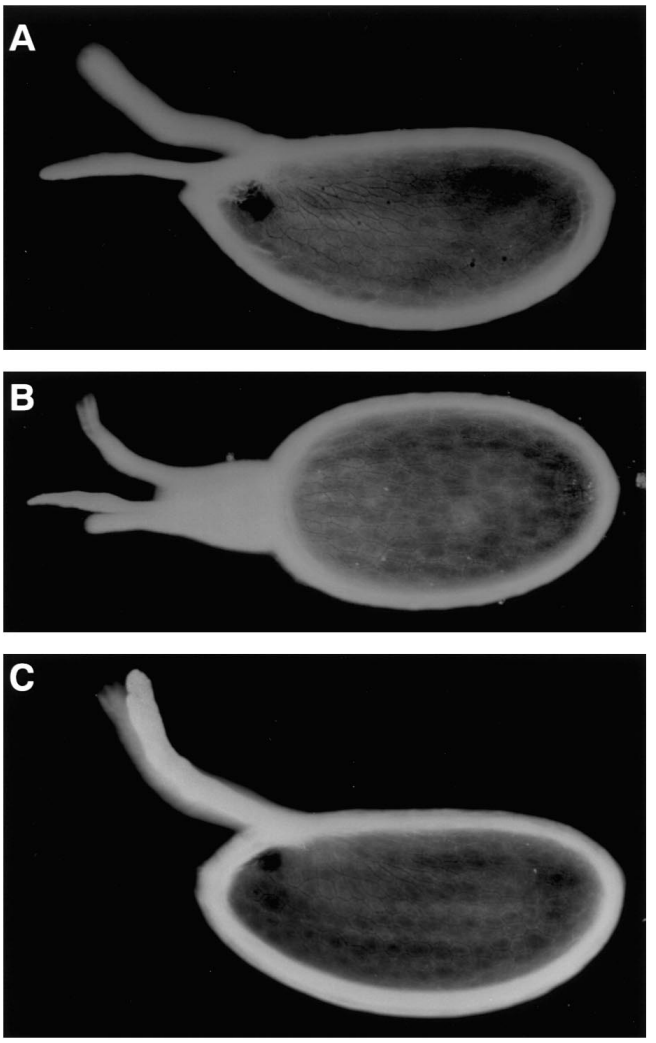

Fig. 4. Non-anteriorly localized *K10* transcripts rescue the dorsoventral pattern defects of *K10* mutant egg shells. Lateral views are shown of egg shells from females of the following genotypes: (A) wild type; (B) $K10^{LM00}$; (C) $K10^{LM00}$ *exu*; *P[K-bcd]*. As described in text, wild-type egg 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 $K10^{LM00}$; *exu*; *P[K-bcd]* females since *exu* is maternal effect lethal due to anteroposterior defects.

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 mislocalized *K10* transcripts to the posterior pole of the oocyte by

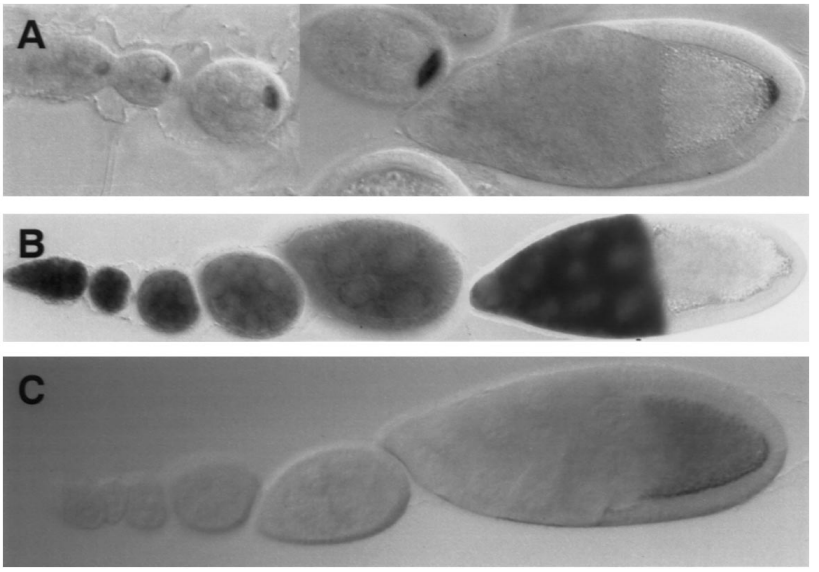

Fig. 5. *K-osk* and *KΔ3′* mRNA distribution patterns. Egg chambers are arranged from left to right in order of increasing stage of development following whole-mount in situ hybridization with a digoxigenin-labeled *K10* probe. Egg chamber genotypes are as follows: (A) $K10^{LM00}$*; K-osk;* (B) $K10^{LM00}$*; KΔ3′;* (C) $K10^{LM00}$. Note that the *K10* probe detects no mRNA in $K10^{LM00}$ egg chambers and thus the transcripts detected in A and B correspond to *K-osk* and *KΔ3′*, respectively.

replacing the *K10* 3′UTR with a portion of the *osk* 3′UTR (Fig. 2; Kim-Ha et al., 1993). The resulting construct, called *K-osk*, produces mRNA that is localized to the oocyte's posterior pole in a manner similar to that of wild-type *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, *K-osk* transgenes produce K10 protein that localizes to the oocyte nucleus. Furthermore, this protein exhibits nearly wild-type activity. *K-osk* transgenes restore up to 94% fertility to *K10* mutant stocks (Table 1). We conclude that the persistent accumulation of *K10* mRNA at the oocyte's anterior cortex during stages 8-10B is not obligatory for *K10* gene function.

K10 transcripts that are not localized produce functional *K10* protein

The one caveat of the above experiment is that *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 *K10* mRNA. Ideally, we would like to examine the function of *K10* transcripts that are transported into the oocyte, but never

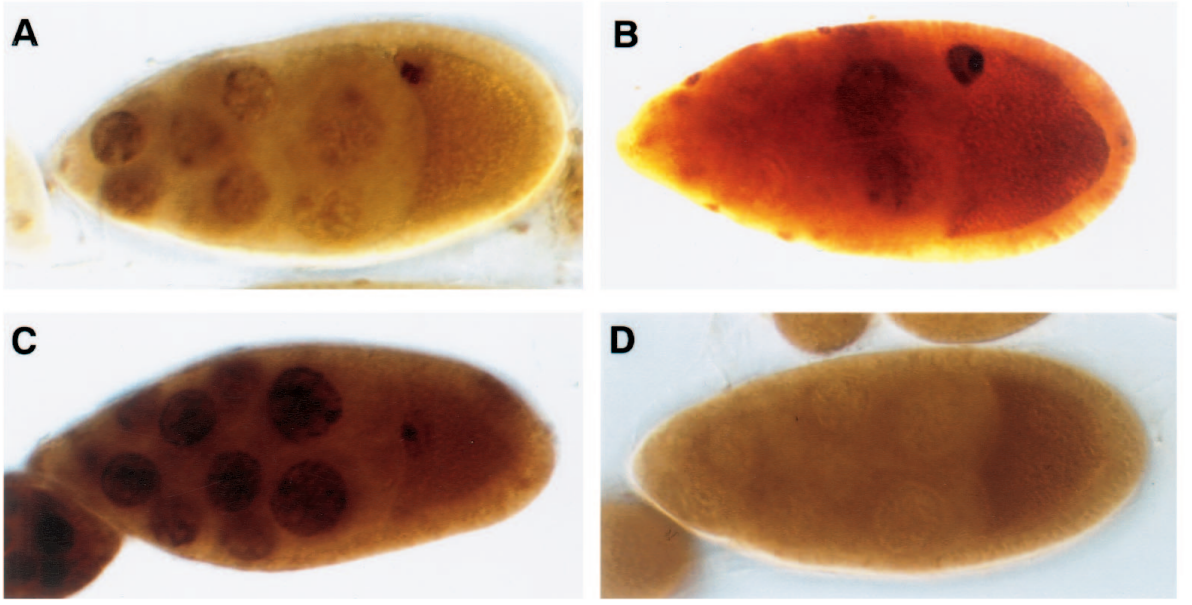

Fig. 6. Protein distribution patterns of *K10* transgenes. Stage 9 or 10 egg chambers are shown following whole-mount immunocytochemistry with a polyclonal anti-K10 antibody. Egg chamber genotypes are as follows: (A) $K10^{1}$; *K-bcd,* (B) $K10^{1}$; *K-osk,* (C) $K10^{1}$; *KΔ3′,* (D) $K10^{1}$. Note that the anti-K10 antibody detects very little, if any, protein in $K10^{1}$ egg chambers and thus is specific for protein produced from *K10* transgenes. K10 protein produced from *KΔ3′* mRNA accumulates primarily in nurse cell nuclei; however, some protein can be detected in the oocyte nucleus (C).

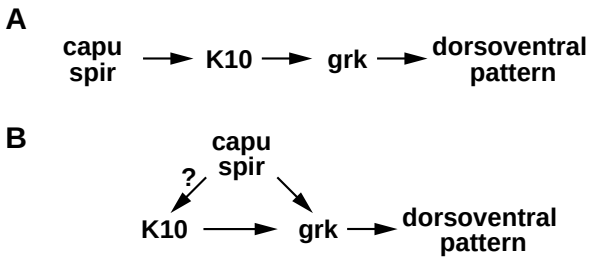

Fig. 7. Possible pathways for dorsoventral patterning. Previous genetic and molecular studies (Manseau and Schüpbach, 1989; Cheung et al., 1992; Neuman-Silberberg and Schüpbach, 1993; Serano et al., 1995) are consistent with two models of *capu* and *spir* gene function. The simplest of these, shown in A, proposes that *capu* and *spir* regulate *grk* mRNA localization exclusively through the activation of *K10*. Our results (described in text), however, favor a second model, shown in B, where *capu* and *spir* act in parallel with *K10* to regulate *grk* mRNA localization. As denoted by the question mark, this model does not rule out the possibility that *capu* and/or *spir* also act upstream of *K10*.

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 *KΔ3′*.

KΔ3′ 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 *KΔ3′* transgenes accumulate in nurse cells (Figs 5B and 6C, respectively). When the *P[KΔ3′]* 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[KΔ3′]* 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 compared to the other four *P[KΔ3′]* lines (data not shown). There are no other detectable differences in *KΔ3′* mRNA distribution between the lines, i.e., in all five lines, transcripts are only detectable in nurse cells (Fig. 5B and data not shown). So, while the transport of *K10* mRNA into the oocyte is normally required for *K10* function, this requirement can be bypassed by over-expressing *KΔ3′* mRNA in nurse cells. Presumably, *KΔ3′* mRNA diffuses into the oocyte, where it is translated into functional protein. Alternatively, K10 protein synthesized from *KΔ3′* mRNA in nurse cells diffuses into the oocyte and functions normally. In either case, functional K10 protein is produced from non-anteriorly localized mRNA. We conclude that the anterior localization of *K10* mRNA within the oocyte is not obligatory for *K10* function.

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 (Fig. 7A). Data presented here, however, indicate that *capu* and *spir* act in parallel with *K10* to regulate *grk* mRNA localization (Fig. 7B). 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.

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