Development 114, 653-661 (1992)
Printed in Great Britain © The Company of Biologists Limited 1992

Evidence for a highly selective RNA transport system and its role in establishing the dorsoventral axis of the *Drosophila* egg

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Summary

The specification of cell fates along the dorsoventral axis of the *Drosophila* embryo is dependent on the asymmetric distribution of proteins within the egg and within the egg's outer membranes. Such asymmetries arise during oogenesis and are dependent on multiple cell-cell interactions between the developing oocyte and its neighboring somatic follicle cells. The earliest known such interaction involves the generation of a signal in the oocyte and its reception in the follicle cells lying on the dorsal surface of the oocyte at ~stage 10 of oogenesis. Several independent lines of investigation indicate that the *fs(1)K10 (K10)* gene negatively regulates the synthesis of the signal in the oocyte nucleus. Here we present data that indicate that the accumulation of *K10* protein in the oocyte nucleus is a multistep process involving: (1) the synthesis of *K10* RNA in nurse cells, (2) the rapid transport of *K10* RNA from nurse cells into the oocyte, (3) the localization of *K10* RNA to the anterior margin of the oocyte, and (4) *K10* protein synthesis and localization. *K10* RNA is transported into the oocyte continuously beginning at ~stage 2. This indicates a high degree of selectivity in transport, since most RNAs synthesized in stage 2 and older nurse cells are stored there until stage 11, when nurse cells donate their entire cytoplasm to the oocyte. The sequences responsible for the early (pre-stage 11) and selective transport of *K10* RNA into the oocyte map to the 3′ transcribed non-translated region of the gene. None of the other identified genes involved in dorsoventral axis formation are required for *K10* RNA transport. Two such other genes, *cappuccino (capu)* and *spire (spir)* are, however, required for the subsequent localization of *K10* RNA to the oocyte's anterior end.

Key words: *Drosophila* oogenesis, *K10* gene, RNA transport, *Drosophila* pattern formation, P element transformation.

Introduction

The dorsoventral pattern of the *Drosophila* embryo is generated by the sequential action of eighteen maternal effect genes (reviewed in Rushlow and Arora, 1990; Govind and Steward, 1991). The first six genes of this hierarchy, *K10, capu, spir, cornichon (cni), gurken (gk)* and *torpedo (top),* act during oogenesis to specify somatic cell fates along the dorsoventral axis of the egg chamber. The egg chamber consists of the oocyte, 15 nurse cells, which are connected to the oocyte's anterior end via cytoplasmic bridges, and a surrounding epithelium of somatic follicle cells (King, 1970). Through the action of the remaining dorsoventral maternal genes, dorsal and ventral follicle cells differentially signal the oocyte, thus establishing the ventral and dorsal identities of the future embryo.

Genetic mosaic analyses show that *K10, capu, spir, cni* and *gk* are required exclusively in germ cells (Wieschaus, 1979; Schüpbach, 1987; Manseau and Schüpbach, 1989; Schüpbach and Wieschaus et al., 1991), indicating that follicle cell differentiation is dependent on factors synthesized in germ cells. The *top* gene appears to encode the receptor for one such factor (ligand); *top* is required exclusively in somatic cells (Schüpbach, 1987), and *top* protein, which is found on the oocyte-facing surface of all follicle cells (R. Schweitzer, N. Zak and B. Shilo, personal communication), has extensive sequence homology to the mammalian epidermal growth factor receptor (Price et al., 1989; Schejter and Shilo, 1989). Females lacking wild-type *top* activity produce ventralized egg chambers, i.e., egg chambers whose dorsal and ventral follicle cells behave and differentiate like the ventral follicle cells of wild-type egg chambers (Schüpbach, 1987). This result shows that *top* protein is required for dorsal-like follicle cell differentiation, but not for ventral-like follicle cell differentiation. Females lacking wild-type *K10, capu* or *spir* activity produce dorsalized egg chambers, indicating that these genes are required

to repress the delivery of *top* ligand to ventral follicle cells (Wieschaus, 1979; Manseau and Schüpbach, 1989). The alternative view that *K10*, *capu* and *spir* encode factors that actively promote ventral-like follicle cell differentiation is disfavored, since ventral follicle cells differentiate normally in females doubly mutant for *top* and *K10, capu* or *spir* (Schüpbach, 1987; Manseau and Schüpbach, 1989).

Although it is not clear how *top* ligand is specifically targeted to dorsal follicle cells, it is tempting to speculate, as previously suggested, that the necessary asymmetry in the system comes from the dorsal migration of the oocyte nucleus at stage 9 of oogenesis (Parks and Spradling, 1987; Manseau and Schüpbach, 1989). The first visible sign of dorsal versus ventral follicle cell differentiation appears during stage 10. In this model, *K10* protein is thought to repress (partially) the transcription of the *top* ligand in the oocyte nucleus, such that the ligand is synthesized in amounts only sufficient to induce those follicle cells that lie closest (i.e., dorsal follicle cells) to the ligand source. Consistent with the idea that *K10* encodes a transcription factor, *K10* protein is localized to the oocyte nucleus and contains structural motifs similar to several procaryotic and eucaryotic transcription factors (Prost et al., 1988).

In this paper we investigate the mechanism of *K10* RNA accumulation in the oocyte. In contrast to a previous study, which reported that *K10* RNA is synthesized directly in the oocyte (Haenlin et al., 1987), our data provide strong evidence that *K10* RNA is synthesized principally in nurse cells and, beginning at stage 2, rapidly transported into the oocyte. *K10* RNA transport must be a selective process, since most of the RNAs synthesized in nurse cells are stored there until stage 11 (Mahowald and Tiefert, 1970; Mahowald and Kambysellis, 1980). Sequences responsible for *K10* RNA transport have been mapped to the 3′ end of the gene, which includes a long ~1400 bp transcribed nontranslated region with potential to form multiple stem-loop structures. *K10* genes lacking this sequence produce wild-type or near wild-type amounts of RNA, but the RNA is not transported into the oocyte until stage 11.

An unexpected finding of the studies presented here is that *K10* RNA becomes localized to the anterior margin of the oocyte at stage 8, just before or coincident with the onset of *K10* protein synthesis. In *capu* and *spir* mutant ovaries, *K10* RNA accumulates in the oocyte on schedule, but never localizes to the anterior margin. This result indicates that *capu* and *spir* act upstream of *K10* in dorsoventral patterning and that the localization of *K10* RNA to the anterior margin of the oocyte is important for *K10* function, perhaps facilitating the subsequent localization of *K10* protein to the oocyte nucleus.

Materials and methods

Fly stocks

The wild-type stock was Oregon R. The $K10^{LMOO}$ RNA null mutation was provided by T. Schüpbach. The $capu^{RK12}$, $capu^{G7}$, $spir^{PJ56}$ mutations are described in Manseau and Schüpbach (1989). ry^{506} flies served as recipients for P element transformation experiments. All mutant chromosomes carried visible markers for identification. Flies were reared under standard conditions. Staging of egg chambers was according to King (1970).

Germ-line transformations

Embryos were prepared for microinjections as previously described (Cohen and Meselson, 1985). Test DNAs (Fig. 1) were cloned into the Carnegie 20 transformation vector (Rubin and Spradling, 1983) and injected at a concentration of ~300 μg/ml. Transposase was provided by the helper plasmid, p13πwc (Rubin and Spradling, 1982; Spradling and Rubin, 1982; Cohen and Meselson, 1985), which was coinjected with the test constructs at a concentration of ~75 μg/ml. Between 3 and 8 transformed lines were generated and analyzed for each construct. Transformants were maintained as homozygous or balanced heterozygous stocks.

β-gal histochemical assays

Ovaries were dissected in PBS* (Phosphate-buffered saline containing 2 mM $MgCl_2$, 2 mM spermidine, 0.02% sodium deoxycholate and 0.02% Nonidet P-40) and fixed for 10 minutes in PBS* containing 2% freshly made paraformaldehyde. Fixed tissues were rinsed with PBS* and then placed in PBS containing 0.2% X-gal, 1.1 mM $K_3Fe(CN)_6$ and 1.1 mM $K_4Fe(CN)_6$ and 2 mM $MgCl_2$ (Glaser et al., 1986). Staining reactions were carried out at 37°C until the desired intensity was obtained, typically 15-30 minutes. Stained tissues were mounted in Polyaquamount (Polysciences, Inc.) and photographed with Nomarski optics.

Gene constructs

The *KZK* construct (Fig. 1) was made in several steps to yield the following: *K10* sequences from −483 bp to +333, where +1 corresponds to the first nucleotide of *K10* mRNA, and +318 corresponds to the first nucleotide of the translation start site; *E. coli lacZ* sequences from just downstream of the translation start site to just downstream of the translation stop site; *K10* sequences from +2628 to +4861, where the K10 protein coding region ends at nucleotide body, and where the poly(A) addition site corresponds to +3997 (ref. 8). All *K10* sequences derive from plasmid Pst22 (kindly provided by E. Mohier). *K10* coordinates come from Prost et al. (1988). The *lacZ* sequence derives from plasmid pMC1871 (Pharmacia). The *hsp26* nurse cell enhancer-*Sgs3* promoter DNA fragment of the *26gZS* and *26gZK* constructs consists of two copies of the *hsp26* sequence −543 to −373 linked to the *Sgs3* sequence −127 to +38 (L. Frank et al., 1991). The *Sgs3* sequence contains a translation start site (Garfinkel et al., 1983). The *Sgs8* sequence, which constitutes the 3′ end of the *KZS*, *KKZS*, and 26gZS constructs (Fig. 1), derives from plasmid ploxba (kindly provided by V. Corbin) and includes a functional poly(A) addition site (Garfinkel et al., 1983). Further details of these constructs will be made available upon request.

In situ hybridization

Probe labeling with digoxigenin-dUTP and whole-mount *in situ* hybridization was carried out according to Tautz and Pfeifle (1988), except that dimethyl sulfoxide was added to the initial fixation buffer to a final concentration of 10%. In addition, the length of proteinase K treatment was increased to 30 minutes. *K10* RNA was detected using two different random-primed probes, each of which gave identical results.

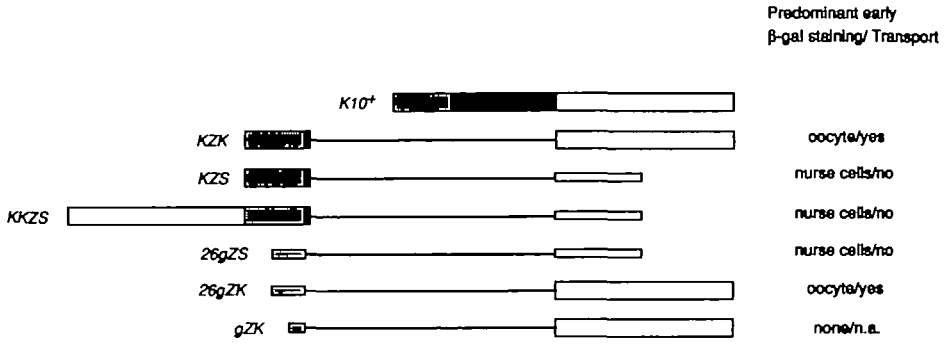


Fig. 1. Structure and expression summary of *K10-lacZ* fusion constructs. The $K10^{+}$ construct at the top of the figure depicts the minimal amount of *K10* DNA needed to completely rescue $K10^{-}$ flies when introduced by P element transformation (Prost et al., 1988). The highlighted domains of this 5.3 kb DNA fragment correspond to the following: lightly shaded rectangle, *K10* 5′ flanking DNA plus 5′ non-translated mRNA sequences (nurse cell transcriptional control sequences reside in this region, see text); deeply shaded rectangle, the entire *K10* protein coding sequence (derived from genomic and cDNA sequences); non-shaded rectangle, *K10* 3′ transcribed non-translated sequences plus 3′ flanking DNA. In *KZK*, the bulk of the *K10* protein coding sequence is replaced with *E. coli lacZ* sequences (thin line). In *KZS*, the *K10* sequence at the 3′ end of *KZK* is replaced with a sequence from the 3′ end of the *Drosophila Sgs8* gene (thin rectangle), which encodes a poly(A) addition site. In *KKZS*, the 3′ *K10* sequence of *KZK* is added to the 5′ end of the *KZS* construct. In *26gZS*, a DNA fragment (lightly shaded thin rectangle) consisting of the *hsp26* nurse cell enhancer linked to the *Sgs3* (glue) promoter-translation start site is substituted for the *K10* portion of the *KZS* gene. In *26gZK*, the 3′ *Sgs8* sequence of *26gZS* is replaced with the 3′ *K10* sequence of *KZK*. In *gZK*, the *hsp26* nurse cell enhancer is deleted from the *26gZK* construct. Three to eight lines were established with each construct and analyzed by staining for β-gal enzyme activity as described in Materials and methods. Predominant early β-gal staining/ transport column indicates which germ cells stain most intensely in pre-stage 11 egg chambers, i.e., prior to the time when nurse cells indiscriminately donate their cytoplasm to the oocyte. Early oocyte staining is thought to reflect transcription of the gene in nurse cells, and the immediate transport of the resulting RNAs into the oocyte (see Fig. 3 and text). Apart from the *gZK* lines, which do not stain for β-gal activity, lines generally showed staining within 15 minutes of the addition of the X-gal color substrate. The intensity of staining amongst different lines carrying the same construct varied only slightly (≤5-fold).

For the experiment presented in Fig. 4, the probe corresponded to the 3′ end of the *K10* mRNA (nucleotide residues +2628 to +3997). The other *K10* probe derived from the 5′ end of the *K10* transcription unit. The *lacZ* hybridization probe was generously provided by P. Feinstein. Hybridization was visualized using an alkaline phosphatase-conjugated anti-digoxigenin antibody (Boehringer Mannheim) and color development with X-phosphate according to manufacturer's instructions. Stained tissues were mounted and photographed as described above for X-gal-stained tissues.

Results

Sequences at the 3′ end of the K10 gene promote the early accumulation of K10 RNA in the oocyte

To identify sequences responsible for the early accumulation of *K10* RNA in the oocyte, we employed a transgenic fly assay system. We started with a 5.3 kb DNA fragment, consisting of *K10* genomic and cDNA sequences previously shown to rescue completely $K10^{-}$ flies upon P element transformation (Haenlin et al., 1987). We reintroduced this fragment into the *Drosophila* germ-line after substituting the structural portion of the bacterial *lacZ* gene for the bulk of the *K10* protein coding region to create *KZK* (Fig. 1). The ovarian expression pattern of the *KZK* gene was analyzed in several independently transformed lines using a histochemical assay for β-galactosidase (β-gal) activity. As seen in Fig. 2, β-gal-staining activity is greatly enriched in oocytes as compared to nurse cells, and is not detected in somatic follicle cells. β-gal staining activity is first detectable in oocytes at ~stage 6 and steadily increases in intensity thereafter. This expression pattern is very similar to that reported for authentic *K10* protein (Prost et al., 1988) and we therefore conclude that the *KZK* construct contains most or all of the *cis*-acting sequences required for wild-type *K10* expression during oogenesis.

To delimit further the sequences required for wild-type *K10* expression, we replaced the 3′ *K10* portion of the *KZK* gene, which includes ~1400 bp of transcribed non-translated sequence and ~800 bp of 3′ flanking DNA, with the poly(A)/transcription termination region of the *Drosophila Sgs8* gene to create *KZS* (Fig. 1). In contrast to *KZK* transformants, the oocytes of *KZS* transformants exhibit no β-gal-staining activity until stage 11, when nurse cells indiscriminately donate their entire cytoplasmic contents to the oocyte (Fig. 2 and data not shown). Prior to stage 11, the β-gal-staining activity of *KZS* transformants is restricted to nurse cells (Fig. 2). Thus, the 3′ end of the *K10* gene is required for the early (pre-stage 11) accumulation of *K10* gene products in the oocyte.

Because the *KZK* and *KZS* genes encode identical proteins (Fig. 1), the difference in the ovarian β-gal staining patterns of *KZK* and *KZS* transformants should reflect differences in the distribution patterns of *KZS* and *KZK* RNA. Indeed, *in situ* hybridization analyses show that prior to stage 11 *KZK* RNA accumulates predominantly in oocytes, while *KZS* RNA accumulates predominantly in nurse cells (Fig. 3). It is also clear from such analyses that the absolute amount of *KZK* RNA in pre-stage 11 oocytes is much greater than the absolute amount of *KZS* RNA in pre-

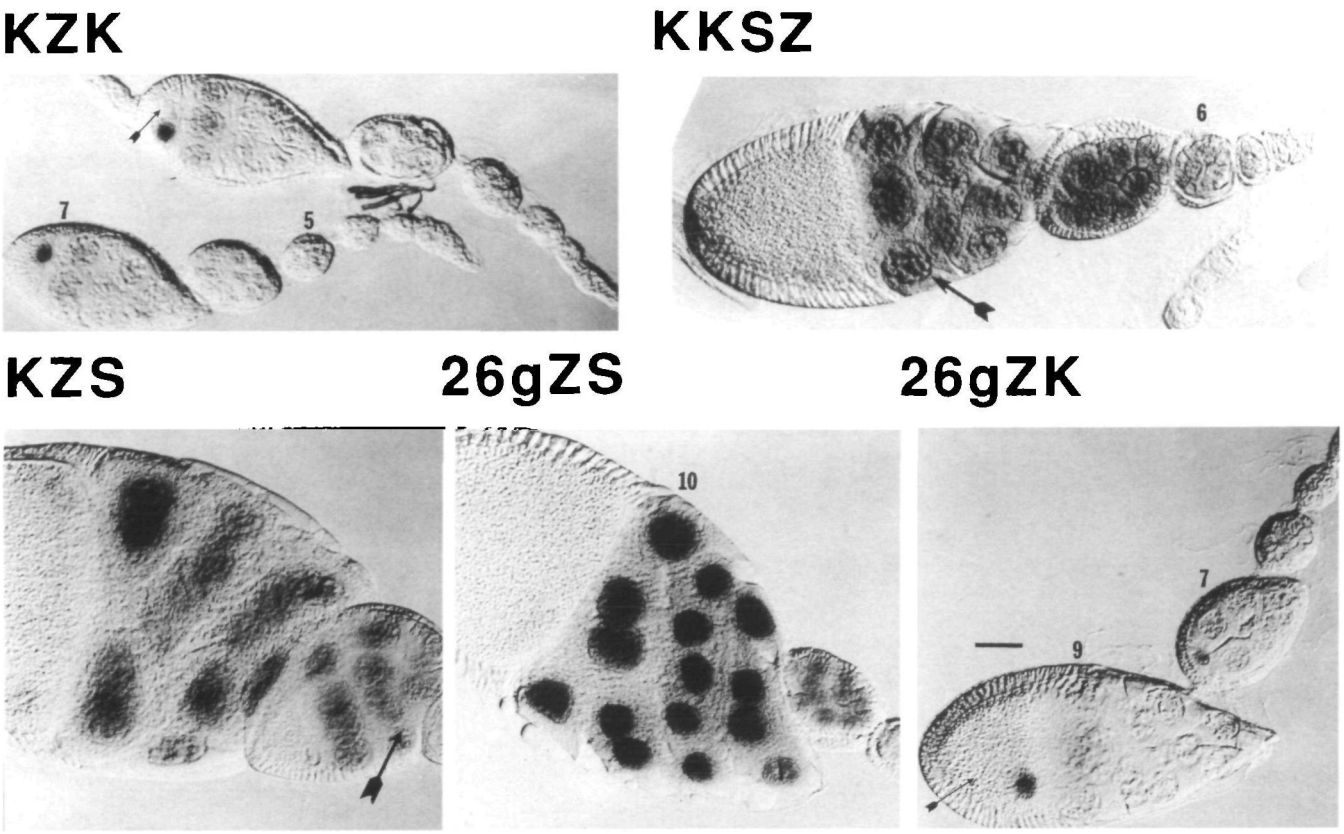


Fig. 2. Ovaries from flies carrying the indicated *lacZ* fusion genes following histochemical staining for β-gal activity. Stages of selected egg chambers are denoted with numbers according to King (1970). The orientation of the egg chambers are such that the oocyte (thin arrows) lies to the left of the nurse cells (thick arrows). In all lines, staining (dark areas) is restricted to ~stage 6 and older egg chambers. In the *KZK* and *26gZK* lines, staining is seen predominantly in the oocyte, although a low-level of staining is also seen in nurse cells, especially in those nurse cells that lie closest to the oocyte. In contrast, in the *KKZS, KZS* and *26gZS* lines, nurse cells stain much more intensely than does the oocyte, until stage 11 (not shown), when nurse cells indiscriminately donate their contents into the oocyte (Mahowald and Kambysellis, 1980). Note that β-gal staining localizes to nuclei. Scale bar equals 30 μm.

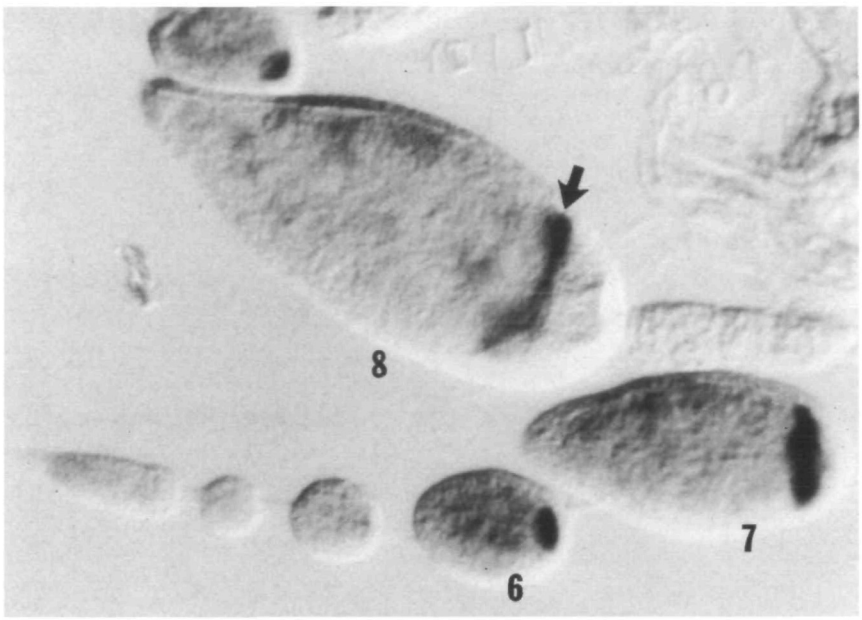
stage 11 oocytes. This is true even though Northern blots indicate that the total amount of *KZS* RNA in the ovaries of *KZS* transformants is several fold higher than the total amount of *KZK* RNA in the ovaries of *KZK* transformants (data not shown). Whether the difference in the steady state concentrations of *KZK* and *KZS* RNAs reflect differences in the efficiency at which these genes are transcribed or differences in the stability of the respective RNAs is not clear.

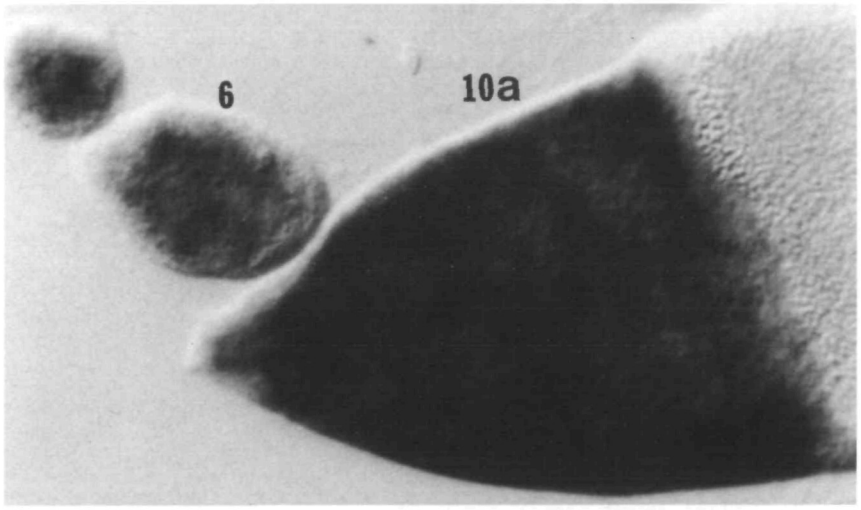
The 3′ K10 sequence is sufficient to cause a heterologous RNA to accumulate in the oocyte early during oogenesis

To test whether the 3′ *K10* sequence can cause a heterologous RNA to accumulate in the oocyte prior to stage 11, we substituted this sequence for the 3′ end of an *hsp26-lacZ-Sgs8* fusion gene *(26ZS)* to create *26ZK*. While the β-gal staining activity of *26ZS* transformants is concentrated in nurse cells prior to stage 11, we find that β-gal-staining activity of *26ZK* transformants is concentrated in oocytes during such stages (Fig. 2). We interpret this result to mean that the 3′ *K10* sequence is sufficient to cause a heterologous RNA to accumulate in the oocyte prior to stage 11.

The 3′ K10 sequence functions only when incorporated into the transcribed portion of a gene

The 3′ end of the *K10* gene could control the early accumulation of *K10* RNA in the oocyte at the level of transcription initiation and/or post-transcriptionally. In the case of control at the level of transcription initiation, the 3′ sequence would have to contain sequences that both promote transcription in the oocyte and repress transcription in nurse cells, since, as noted above, a deletion of the 3′ sequence leads both to an increase in the amount of *K10* RNA found in nurse cells and a decrease in the amount of *K10* RNA found in oocytes. To distinguish between control at the level of transcription initiation and post-transcriptionally, we investigated the positional requirements of the 3′ sequence. If the sequence functions post-transcriptionally, it should work only when incorporated into the transcribed portion of a gene. Conversely, if the sequence functions at the level of transcription initiation, it should work when placed outside or inside of





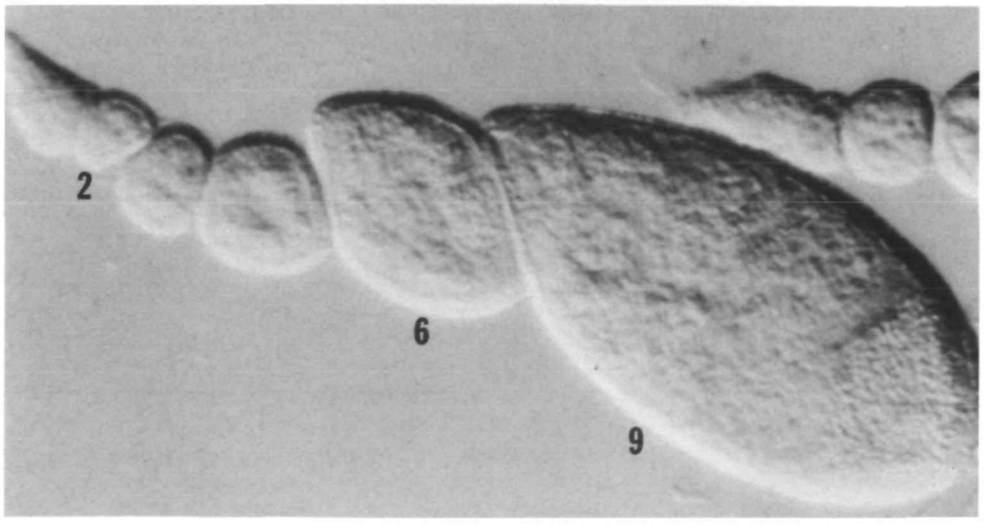


Fig. 3. *lacZ* RNA distribution in P transformed and wild-type ovaries. Each panel shows a series of egg chambers following hybridization with a digoxigenin-labeled *lacZ* probe and immuno-color detection. Numbers are as indicated in Fig. 2. The orientation of the egg chambers is reversed compared to that of Fig. 2, however, such that the oocyte now lies to the right of the nurse cell cluster. Note that *KZK* (top panel) and *KZS* (middle panel) RNAs show the same temporal distribution, but that *KZK* RNA is localized predominantly to the oocyte, while *KZS* RNA is localized predominantly to nurse cells. No labeling is seen in non-transformed control ovaries (bottom panel). Also note that in stage 8 and older egg chambers, *KZK* RNA is localized to the anterior margin of the oocyte (arrow). Endogenous *K10* transcripts are similarly localized in such oocytes (see Fig. 4 and text).

a transcription unit. As seen in Fig. 2, the 3′ *K10* sequence has no effect on the expression pattern of the *KZS* gene when placed 5′ to the promoter (compare the β-gal-staining patterns of the *KKZS* and *KZS* genes). We tentatively conclude from this experiment that the 3′ *K10* sequence functions post-transcriptionally.

To rule out the possibility that the 3′ *K10* sequence affects oocyte expression at the level of transcription initiation, but only when placed 3′ to a gene, we moved the sequence back to its original location, 3′ to the *lacZ* gene, but stripped the promoter down to a minimal sequence (sequences from −127 to +38, which includes a TATA box and transcription start site, were retained). Transformants carrying this construct *(gZK)* exhibit no β-gal staining whatsoever (Fig. 1 and data not shown). Together with our previous findings, we conclude that the 3′ *K10* sequence functions post-transcriptionally.

K10 RNA distribution in wild-type ovaries

The simplest interpretation of the data presented above is that *K10* is transcribed principally in nurse cells, and that the resulting transcripts are rapidly transported into the oocyte. Although previous *in situ* hybridization experiments using ^{35}S probes only detected *K10* RNA in oocytes (Haenlin et al., 1987), we thought that we might be able to detect *K10* RNA in nurse cells prior to their transport into the oocyte with digoxigenin-labeled probes, which in our hands are more sensitive than

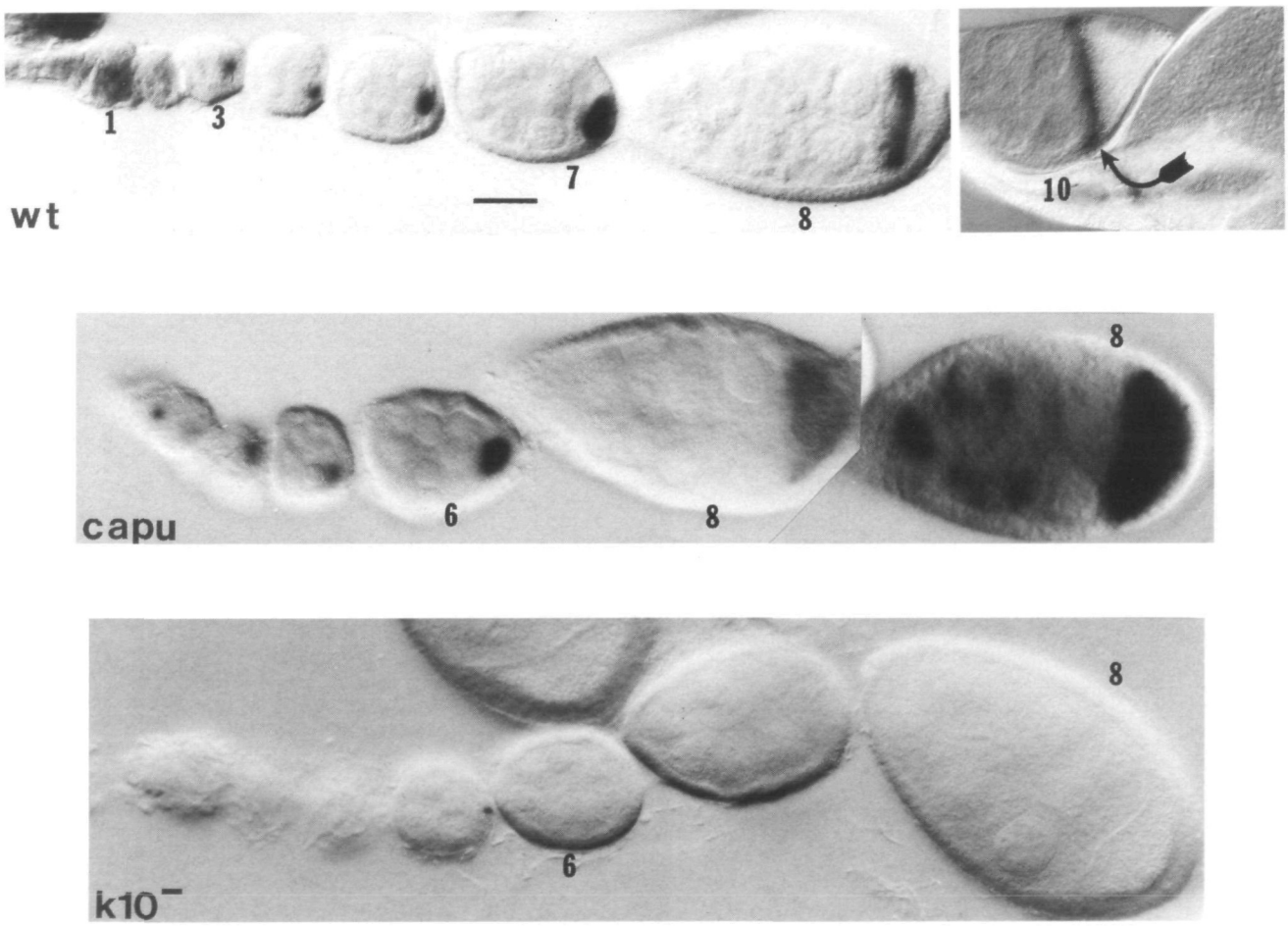


Fig. 4. *K10* RNA distribution in wild-type and mutant ovaries. Each panel shows a series of egg chambers following hybridization with a digoxigenin-labeled *K10* probe and immuno-color detection. Numbers and scale bar are as indicated in Fig. 2. Relevant genotypes are as follows; wt, Oregon R; capu, $capu^{RK12}/capu^{RK12}$; K10⁻, $K10^{LM00}/K10^{LM00}$. Note that in young (~stage 1) wild-type egg chambers, *K10* transcripts are equally abundant in nurse cells and oocytes (i.e., all stage 1 germ cells label with equal intensity), while in older egg chambers, the oocyte labels much more intensely than do the nurse cells. By ~stage 8, when the oocyte has enlarged due to its uptake of yolk protein, *K10* RNA is clearly localized to the anterior margin of the oocyte. This is most obvious in older oocytes (see arrow in wild-type stage 10 egg chamber). Note that *K10* RNA does not localize to the anterior margin of *capu* (capu panel) and *spir* (not shown) oocytes. The color reaction was carried out for an extended time in the right-most *capu* egg chamber to emphasize the delocalization of *K10* transcripts within the oocyte. Under such conditions, *K10* RNA is detected in nurse cells, even in wild-type ovaries (not shown). The $K10^{LM00}$ mutation is fully penetrant and gives severely dorsalized egg chambers, eggs and embryos. The $capu^{RK12}$ is a strong *capu* allele that disrupts dorsoventral and anteroposterior patterning, but is unlikely to be a null mutation (Manseau and Schüpbach, 1989).

radiolabeled probes. To control for background labeling, we also examined ovaries from flies carrying various *K10* mutant alleles. One such allele, $K10^{LM00}$, proved to be an RNA null (Fig. 4). During early oogenesis (e.g. stage 1) in wild-type ovaries, we find that *K10* transcripts are equally abundant in nurse cells and oocytes (Fig. 4). Thereafter, *K10* RNA decreases in abundance in nurse cells, approaching background levels by stage 3, and increases in abundance in the oocyte through stage 8, when it becomes localized to the periphery of the oocyte's anterior margin. *K10* RNA remains localized to the oocyte's anterior margin at least through the end of stage 10. (*K10* RNA cannot be detected reliably in stage 11 and older oocytes using the whole-mount hybridization procedure employed here, due to the secretion of the chorion by the somatic follicle cells). *K10* RNA reappears in nurse cells at ~stage 9 and accumulates there until stage 11, when it is apparently transported into the oocyte with the rest of the nurse cell cytoplasm.

K10 RNA distribution in mutant ovaries

We also examined *K10* RNA distribution in *capu* and *spir* mutant ovaries (Fig. 4 and data not shown). Two strong mutant alleles of *capu* ($capu^{RK12}$, $capu^{G7}$) and one of *spir* ($spir^{PJ56}$) were examined (Manseau and Schüpbach, 1989). In each case, *K10* RNA accumulates normally through stage 7, i.e., the RNA preferentially accumulates in the oocyte. In contrast to what is seen in wild-type oocytes, however, *K10* RNA never localizes to the anterior margin of *capu* and *spir* mutant oocytes. Instead the RNA remains uniformly distributed throughout the ooplasm or, less frequently, organizes itself into several, randomly distributed, diffuse islands. Weak alleles of *capu* and *spir*, which give anteroposterior pattern defects, but no dorsoventral pattern defects were not examined. Mutations in other dorsoventral group genes also were not examined, since epistasis tests indicate that all other such genes act downstream of *K10*.

Discussion

The first visible sign of dorsoventral patterning during *Drosophila* development is the migration of the oocyte nucleus towards the dorsal surface of the cell at stage 9 of oogenesis (King, 1970). During stage 10, the follicle cells lying on the dorsal surface of the oocyte differentiate from those lying on its ventral surface (Margaritis et al., 1980; Parks and Spradling, 1987). Such differentiation is dependent on wild-type *K10* gene activity (Wieschaus, 1979). *K10* protein specifically accumulates in the oocyte nucleus beginning at stage 8 or 9 and increases in abundance through stage 12 (Prost et al., 1988), where it has been suggested to partially repress the synthesis of factors that stimulate dorsal-like follicle cell differentiation (Manseau and Schüpbach, 1989). As a result of such repression, only those follicle cells that lie closest to the oocyte nucleus are stimulated.

Here we show that the accumulation of *K10* protein in the oocyte nucleus is a multistep process involving: (1) the synthesis of *K10* RNA in nurse cells, (2) the rapid transport of *K10* RNA from nurse cells into the oocyte, (3) the localization of *K10* RNA to the anterior margin of the oocyte and (4) the translation of *K10* RNA and the localization of *K10* protein to the oocyte nucleus.

K10 RNA synthesis and transport

Tritiated uridine incorporation studies indicate that the vast majority of the RNAs present in the mature egg and early embryo originate in nurse cells (Mahowald and Tiefert, 1970). This is not a surprising finding, since nurse cells replicate their DNA throughout oogenesis, reaching an average chromosome number of over 1,000 by stage 10, while the oocyte remains diploid (Mahowald and Kambysellis, 1980). In addition, there is some evidence that oocyte transcription is actively repressed during stages 5-8 (Mahowald and Tiefert, 1970). Based on these findings alone, one might expect that the preferential accumulation of *K10* transcripts in the oocyte during oogenesis results from *K10* transcription in nurse cells and the transport of its transcripts into the oocyte. The data presented here meet two key predictions of this model, and argue against the alternative view (Haenlin et al., 1987) that most *K10* transcripts are synthesized directly in the oocyte. Specifically, we show that the 5′ end of the *K10* gene contains transcriptional control sequences active in nurse cells, and that the 3′ end of the gene contains sequences necessary for the preferential accumulation of *K10* transcripts in the oocyte. Moreover, the 3′ sequences function only when incorporated into the transcribed portion of the gene.

Presumably the early (pre-stage 11) transport of *K10* RNA into the oocyte is important for the early (pre-stage 11) accumulation of *K10* protein in the oocyte nucleus, and hence, for *K10* function. Consistent with this idea, we have recently shown (unpublished) that *K10* genes specifically deleted for sequences required for the early accumulation of its RNA in the oocyte, do not, or only weakly, rescue *K10* mutants.

The transport of *K10* RNA into the oocyte must be a fairly selective process, since most RNAs are retained by nurse cells until stage 11 (Mahowald and Tiefert, 1970). The RNAs of the *bicoid (bcd)* and *oskar (osk)* genes also preferentially accumulate in the oocyte prior to stage 11 (St. Johnston et al., 1989; Kim-Ha et al., 1991; Ephrussi et al., 1991) and may, therefore, be transported into the oocyte via the same system that transports *K10* RNA into the oocyte. If so, mutations that disable the system should disrupt anteroposterior patterning as well as dorsoventral patterning; *bcd* and *osk* are required for anterior and posterior patterning, respectively (Nüsslein-Volhard et al., 1987). We have examined several mutations that disrupt dorsoventral, or both dorsoventral and anteroposterior patterning, but have not found any defects in *K10* RNA transport (data not shown). Perhaps the genes that regulate *K10* RNA transport and, possibly, *bcd* and *osk* RNA transport, also regulate the transport of molecules

required for oocyte differentiation. Consistent with this idea, we have found that loss-of function *Bic-D* and *egal* mutations lead to the uniform accumulation of *K10* transcripts in all 16 germ cells (data not shown, also see Suter and Steward, 1991; Mohler and Wieschaus, 1986). *Bic-D* loss-of-function mutations also lead to the uniform accumulation of *osk* RNA in all 16 germ cells, although prior to egg chamber formation, *osk* RNA temporarily localizes to a single germ cell, i.e., to the presumptive oocyte (Suter and Steward, 1991). Also, gain-of-function *Bic-D* mutations interfere with the translocation of *osk* RNA from the oocyte's anterior to posterior end during stage 9 (Kim-Ha et al., 1991; Ephrussi et al., 1991). Nevertheless, it remains unclear whether the absence of efficient RNA transport in *Bic-D* loss-of-function mutants reflects a direct involvement of *Bic-D* in such transport, or simply the fact that no oocyte is made in such mutants.

K10 RNA localization within the oocyte

Beginning at ~stage 8, *K10* RNA becomes localized to the anterior margin of the oocyte, where it remains through at least the end of stage 10. Interestingly, *bcd* and *osk* RNAs also localize to the oocyte's anterior end at ~stage 8 (St Johnston, 1989; Kim-Ha et al., 1991). In the case of *osk* RNA, such localization is only transient; *osk* RNA stably relocalizes to the oocyte's posterior pole during stage 9 (Kim-Ha et al., 1991; Ephrussi et al., 1991). Sequences required for the anterior localization of *bcd* mRNA have been mapped to a putative stem loop structure at the 3′ end of the molecule (Macdonald and Struhl, 1988; Macdonald, 1990). Computer-assisted analyses of *K10* RNA secondary structure reveal multiple possible stem-loop structures, but none of these are similar in primary sequence to the *bcd* localization sequence (data not shown). Similar analyses of *osk* RNA also reveal no striking sequence similarities to the *bcd* localization sequence (data not shown). It is likely, therefore, that the genes that regulate *bcd* RNA localization are different than those that regulate *K10* and *osk* RNA localization. Consistent with this idea, mutations in *exuperantia* and *swallow,* which disrupt *bcd* RNA localization (St. Johnston et al., 1989), specifically disrupt anterior pattern formation (Frohnhöfer and Nüsslein-Volhard, 1987). (As alluded to above, mutations that interfere with *K10* and *osk* function would be expected to interfere with dorsoventral and posterior pattern formation, respectively). Similarly, the *capu* and *spir* mutations that disrupt *osk* and *K10* RNA localization (Kim-Ha et al., 1991; Ephrussi et al., 1991; this paper), specifically interfere with dorsoventral and posterior pattern formation (Manseau and Schüpbach, 1989). The fact that *capu* and *spir* mutations disrupt *osk* and *K10* RNA localization raises the possibility that these RNAs are localized to the oocyte's anterior end in a similar fashion.

The finding that *K10* RNA does not localize to the oocyte's anterior end in *capu* and *spir* mutants suggests that such localization is important for *K10* function. The localization of *K10* RNA to the oocyte's anterior end could, for example, facilitate *K10* protein synthesis and/or the subsequent localization of this protein to the oocyte nucleus, which lies in the anterodorsal quadrant of the oocyte. At present, however, we can rule out neither the possibility that *K10* function is independent of *K10* RNA localization within the oocyte, nor the possibility that *capu* and *spir* mutations disrupt dorsoventral pattern formation in ways other than the mislocalization of *K10* RNA.

We thank Trudi Schüpbach and Christiane Nüsslein-Volhard for sending mutant strains, Paul Macdonald for sharing unpublished data, and Vicki Corbin, Jane Dodd, Richard Mann, Gary Struhl, Robin Wharton, and members of the Cohen lab for comments on the manuscript. This work was funded by grants from the NIH and the NSF to R.S.C.

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(Accepted 25 November 1991)