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# Oocyte-specific transcription of *fs(1)K10*: a *Drosophila* gene affecting dorsal-ventral developmental polarity

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**The expression of the *fs(1)*K10 gene is required in early oogenesis for the establishment of the dorsal-ventral polarity of the oocyte, and later in the embryo. P-element-mediated transformation shows that the *K10* function is located within a fragment of DNA of 5 kb, which encodes four RNA species. A major transcript of 3.1 kb is likely to be responsible for the *K10* function. It is abundant in ovaries and in early developing embryos. Thus its expression profile corresponds closely to that which could be anticipated from the biological characteristics of the mutation. *In situ* hybridization on ovary sections shows that the gene is not only specifically transcribed in the germ line (which is consistent with the germ-line dependence of the mutation), but that its expression is also cell-specific since it is apparently restricted to the oocyte.**

*Key words: Drosophila*/dorsal-ventral polarity/transcription/*in situ* hybridization

## Introduction

The polarity of the embryo underlies all further pattern formation in the development of an organism. However, the problem of the molecular origin of polarity remains a challenge to developmental biology. In *Drosophila*, the establishment of dorsal-ventral polarity of the developing embryo has been shown to be genetically defined, and to depend on the expression of at least 10 maternal effect genes dispersed throughout the genome (Anderson and Nüsslein-Volhard, 1984 a,b; Gans *et al.*, 1975). For each locus, lack of gene function in the mother results in an embryonic phenotype identical to that described for the mutation *dorsal* (*dl*) where all the cells behave as those normally located in the dorsal region of the blastoderm. The resulting embryo thus consists of a long hollow tube of dorsal cuticle.

However, although essential for the dorsal-ventral pattern forming process, all these mutants exhibit a normal morphological polarity of the egg as evidenced by the asymmetrical pattern of the chorion. It follows that the corresponding genes are not responsible for the egg shape asymmetry.

To trace the origin of this inherent asymmetry of the egg and to investigate how it might affect the polarity of the subsequent developing embryo, it is of interest to look at mutations affecting both the pattern of the egg chorion, and that of the resultant developing embryo. To date the recessive female sterile mutation *K10* [fs(1)K10] is unique in so far as it shows abnormalities in both of these (Wieschaus *et al.*, 1978; Wieschaus, 1979). Females homozygous for *K10* lay eggs which look almost cylindrical. The two chorion-derived respiratory appendages constitute the most obvious landmark for the dorsal side of the normal embryo; in *K10* eggs the appendages are fused at their base by material circling the egg as though most of its circumference had become dorsalized. Moreover, when they are fertilized, these eggs develop into highly abnormal larvae with a dorsalized pattern similar to that observed for the 'dorsal-like' group of mutants. However, the dorsalization of *K10* embryos is never as complete as that of the dorsal group mutants. Whereas dorsalization in the latter takes place all along the length of the embryos, in the *K10* egg, dorsalization is more extreme in the anterior region of the embryo, the posterior part becoming progressively less dorsal, thus showing a possible interaction of the *K10* function with information along the anterior-posterior axis.

Although the more striking feature of the mutation thus concerns the chorion which is secreted in the last stages of oogenesis by follicle cells of somatic origin, it has been shown by pole cell transplantation and by mitotic recombination (Wieschaus *et al.*, 1978; Wieschaus, 1980), that the *K10* mutation is strictly germ-line dependent, and that, consequently the corresponding gene has to be expressed in one of the two germ line cell types: either the nurse cells and/or the oocyte. A quantitative analysis of mitotic recombination experiments suggested to Wieschaus and Szabad (1979) that the expression of the *K10* locus is required at, and probably temporally restricted to, oogenesis.

In an earlier report, we have described the cloning and the localization of the *K10* locus with respect to three adjacent loci (Haenlin *et al.*, 1985). Here, we focus on the *K10* locus and show that the corresponding fragment of DNA of 5 kb encodes four overlapping transcripts, with different levels of expression. The major one, of 3.1 kb, is likely to be responsible for the *K10* function. *In situ* hybridization of *K10* probes to ovary sections shows that the *K10* transcript appears early during oogenesis, and that its expression is restricted to the oocyte.

## Results

### *Molecular mapping of the K10 locus*

Genetically mapped in the region at the tip of chromosome X, between 2B17-C1 and 3A2-3 (Wieschaus *et al.*, 1978; Perrimon *et al.*, 1984), the *K10* gene has been localized within a fragment of DNA of 11 kb (Haenlin *et al.*, 1985), by the combined use of chromosome microdissection and microcloning (Scalenghe *et al.*, 1981), chromosomal walking (Bender *et al.*, 1983) and P-element mediated transformation (Rubin and Spradling, 1982; Spradling and Rubin, 1982). This 11-kb region has been shown to encode six transcripts: two of them have been assigned to the two complementation groups which map on either side of the *K10* locus: *kurz (kz)* and *crooked neck (crn)*, respectively, thereby suggesting that the region encoding the *K10* function could be restricted to a smaller fragment of DNA.

To localize more precisely the *K10* function, we resorted to P-element-mediated transformation, making use of the same breeding scheme as previously described. Transformation experiments are facilitated in the case of the *K10* gene because of its expression in the germ line cells, which allows us to detect

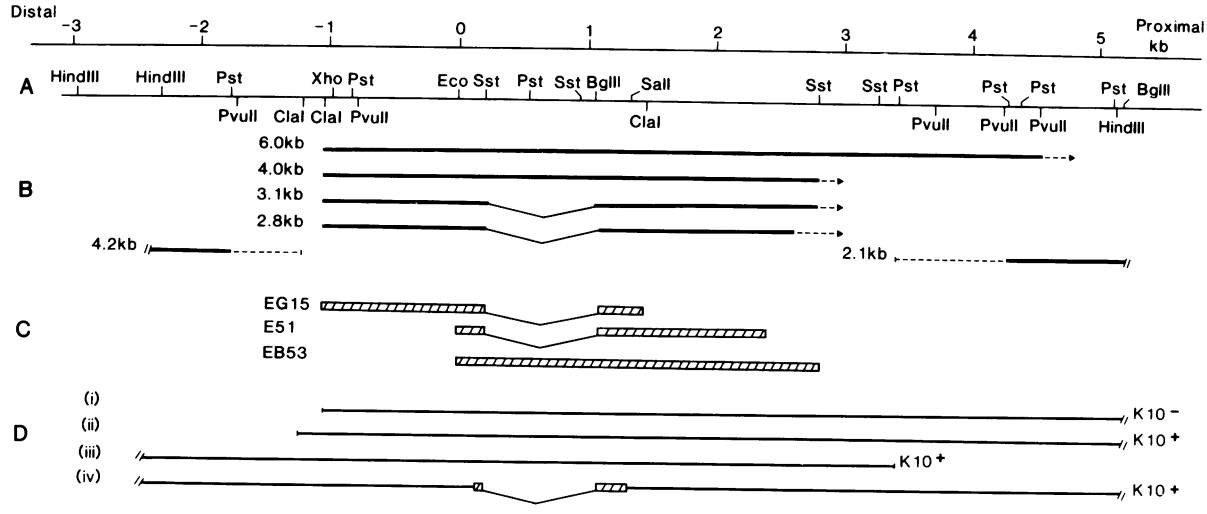


**Fig. 1.** Organization and restriction map of the *K10* region. **(A)** Restriction map of the *K10* region site, formerly position +61 in Haenlin *et al.* (1985). **(B)** Localization of the various transcripts as deduced from Northern analysis. Dashed lines represent the uncertainty of localization. Transcription is from left to right. The 4.2- and 2.1-kb RNAs which map on both sides of the *K10* region correspond to the *kz* and *crn* loci, respectively. **(C)** cDNAs of the *K10* region. Three of the most representative cDNAs isolated from 0–6-h embryo libraries are shown. Most of the cDNAs artificially stop at *Eco*RI site, due to incomplete methylation during library construction. Clone EG15, and others (not shown) which extend beyond the *Eco*RI site, confirm this explanation. cDNA structure was established by comparison of fine restriction mapping with genomic DNA. Hatched boxes represent exonic regions. **(D)** Positioning of the $K10^+$ function by P-element-mediated transformation. Genomic fragments have been tested for their capacity to rescue the *K10* mutation. After cloning in either Carnegie 3 or 4 vectors (Rubin and Spradling, 1983), each of the transposons was coinjected with P$\pi$-25 to all embryos derived from heterozygous *K10,w*/FM3 females crossed with *K10,w* males. Resulting *K10,w* homozygous GO females were tested for their ability to lay wild-type eggs. When no homozygous GO female was found positive, the heterozygous *K10,w*/FM3 injected females were further crossed to GO males, and the same analysis was pursued in G1. **(i)** Smaller *ClaI–XbaI* fragment, **(ii)** larger *ClaI–XbaI* fragment, **(iii)** *XhoI–PstI* fragment, **(iv)** hybrid construction between genomic and cDNA constituents of *K10*. The major part of the construction is of genomic origin and consists of the Xba-15 fragment (Haenlin *et al.*, 1985) in which the *PstI–SalI* at position 0.2–1.35 on the genomic map has been substituted with the corresponding fragment of cDNA E51.

transformants among the GO homozygous females by the restoration of fertility. Subfragments of the region initially defined as the *K10* region were cloned into Carnegie vectors (Rubin and Spradling, 1983) and assayed for their capacity to rescue the *K10* mutation. The relevant fragments are presented in Figure 1D. The distal limit of the $K10^+$ function relative to the centromere has been positioned by both fragments (i) and (ii) and is comprised within the 150 bp which separate the two *ClaI* sites. The fragment (iii) establishes the proximal limit of the $K10^+$ function at the *PstI* site at position +3.4 on the physical map. This extremity was not further characterized. The combined results thus define a region of 4.6 kb still retaining the $K10^+$ biological function, extending from coordinate −1.2 to coordinate +3.4 on the physical map.

*Mapping of the K10 transcripts*

The transcription pattern of the *K10* region was investigated in detail in the light of the transformation results. The combined analysis of transformation experiments, Northern blots and cDNA structures, leads to the results summarized in Figure 1B. A major transcript of 3.1 kb has already been reported. A less abundant transcript of 2.8 kb which was overlooked in our first investigations, because it is essentially detected in ovaries (see below), is also detected at some stages of development. The RNA which was initially described as having an approximate size of 4.5 kb and extending over both the *kz* and *K10* loci, actually consists of two juxtaposed transcripts of about the same size. One of these transcripts, of 4.2 kb, maps on the distal side, with respect to the chromocenter. Its interruption, at the genomic level (the *Kpn*13 fragment in our previous experiments), abolishes the capacity of the corresponding transposon to restore the *kz* function, which suggests that this transcript is a product of the *kz* gene. The other transcript, of 4.0 kb, maps proximally and belongs to the *K10* locus. These two RNAs have a different developmental profile of expression (see below and Figure 3). A fourth transcript of ~6 kb is hardly detectable but since it extends beyond the limits of the *K10* region as defined by transformation, it was not analysed further.

The direction of transcription is the same for all four RNAs and was determined to be distal to proximal by using strand-specific probes of cDNA E51.

We initiated the analysis of the promoter region by hybridization of Northern blots of poly(A)$^+$ RNA with small probes of ~200 bp originating from the 5′ end of the transcribed region, namely the *PstI–ClaI*, *ClaI–ClaI* and *ClaI–XhoI* fragments from coordinates −1.8 to −0.8. The 100-bp *ClaI–XhoI* fragment, extending around the coordinate −1, hybridizes to all four transcripts on Northern blots of poly(A)$^+$ RNA isolated from various stages of development (Figure 2C). In contrast, almost no signal can be detected when the 150-bp-long *ClaI–ClaI* fragment is used as a probe (Figure 2B). These results suggest that the origin of transcription is very close if not identical for all four transcripts. The next distal fragment *PstI–ClaI* hybridizes to a unique 4.2-kb transcript, the developmental profile of which demonstrates that it is the *kz* mRNA (cf. Figure 2A with Figure 3B). Preliminary results of S1 mapping and primer extension analyses lead to the same conclusion (data not shown).

*Analysis of cDNA clones*

To determine the structural organization of the *K10* locus more precisely, cDNA libraries constructed from poly(A)$^+$ RNA isolated from 0–6-h embryos [kindly provided by T.Kornberg (Poole *et al.*, 1985) and by S.Artavanis-Tsakonas] were screened with genomic probes extending on both sides of the *Eco*RI site at coordinate 0. More than 30 clones ranging in size from 1.1 to 2.9 kb were isolated belonging to three classes, one of

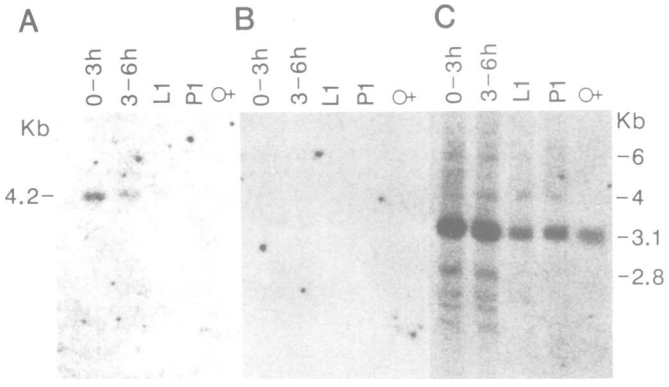


**Fig. 2.** Northern analysis of the *PstI−XhoI* region around coordinate −1. Poly(A)$^+$ RNA (5 μg) from the stages indicated were fractionated on gels, blotted onto nitrocellulose filters and hybridized with the following polyacrylamide-purified fragments: the distal 560-bp *PstI−ClaI* fragment **(A)**, the 150-bp *ClaI−ClaI* fragment **(B)**, and proximal 100-bp *ClaI−XhoI* fragment **(C)**. **(A)** is a re-utilization of blot **(C)** after removal of the probe. One-week exposure.

each of which is represented in Figure 1C. After elimination of the artefactual heterogeneity of the cDNA clones, due to the incomplete methylation of *Eco*RI sites during the construction of the library (T.Kornberg, personal communication), there are in fact only two groups represented on one hand by cDNA EB53 and on the other hand by the others. cDNA EB53 contains an intronic sequence of about 900 bp which is absent from the other cDNA clones. This intron is present as an exonic sequence in both the 4.0- and 6-kb transcripts whereas it has been spliced out of the transcripts of 3.1 and 2.8 kb. This is shown by the hybridization of the fragment *PstI−BglII*, which is entirely contained within the intron, to Northern blots of poly(A)$^+$ RNAs from the larval stage. Lane L1i in Figure 3A shows hybridization to the 4.0-kb transcript and a very faint signal corresponding to the 6-kb transcript.

*Developmental profile of the K10 transcripts*

The observation of multiple transcripts arising from the *K10* locus led us to pose the question: which RNA(s) is (are) responsible for the *K10* function? A first attempt to answer this question consists of identifying and characterizing each of the transcripts by its profile of expression during development, so as to investigate whether or not there is a specificity of expression corresponding to that which can be expected from the biological characteristics of the *K10* mutation.

cDNA E51 was used as a probe and hybridized to Northern blots of poly(A)$^+$ RNA originating from different stages of *Drosophila* development. The results are shown on Figure 3A. A major transcript of 3.1 kb is abundant mostly in ovaries and during early stages of embryogenesis (0−12 h). Even though it decreases ~20- to 50-fold at later stages, as estimated from shorter exposure times, it is present throughout development. It is also present in adult males, although to a lesser extent. The 2.8-kb RNA mimics the developmental profile of the 3.1-kb RNA, but at a lower level of expression, and is likely to differ from the 3.1-kb transcript by its 3′ end. Both the 4.0- and 6-kb RNAs show a different developmental profile since they appear at or after the blastoderm stage (3 h after oviposition) and remain present throughout development. In contrast to the 3.1- and 2.8-kb transcripts, they are barely detectable in ovaries and at early stages of development. Note that the 6-kb transcript has a very low level of expression at all stages. These differences

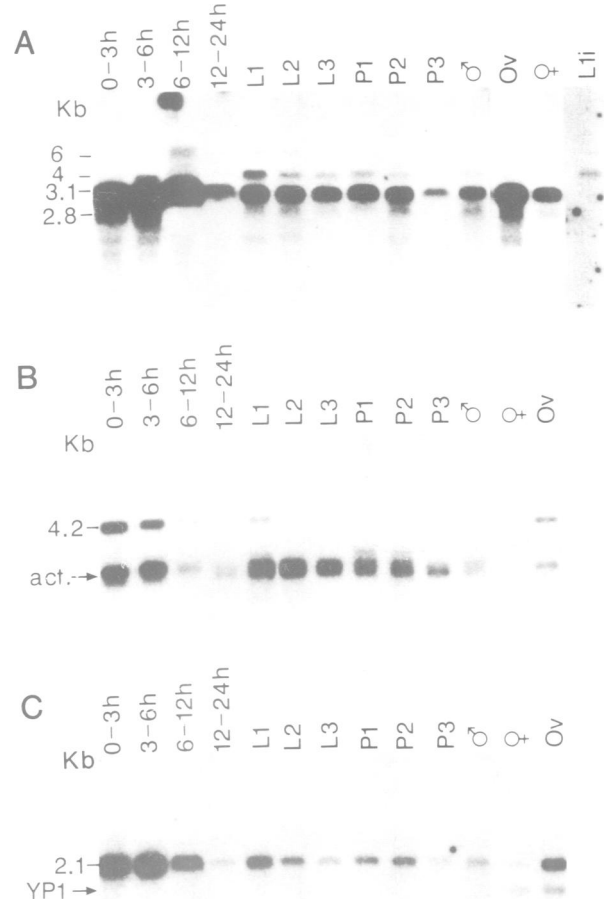


**Fig. 3.** Northern analysis of the transcripts. Poly(A)$^+$ RNA from various stages of the *Drosophila* life cycle was fractionated on formaldehyde-containing agarose gels (0.7%). After transferring the RNA to nitrocellulose filters, the transcripts were detected by hybridization with nick-translated cDNA E51 **(A)**, with the genomic *Hin*dIII−*Hin*dIII fragment from coordinate −3 to −2.3 for the detection of the *kz* transcript **(B)** and with the genomic *PstI−PstI* fragment around position +4 for the detection of the *crn* transcript **(C)**. Filters **(B)** and **(C)** were rehybridized with an actin probe (Fyrberg *et al.*, 1983), and with an YP1 probe, respectively. The quantities of poly(A)$^+$ RNA analysed in blot **(A)** have been re-adjusted on the basis of quantification by the actin probe. In **(A)**, each lane contains 10 μg of poly(A)$^+$ RNA except stages 6−12 h and 12−24 h which contain 20 μg and the lane Ov (ovaries) which contain 5 μg. In **(B)** and **(C)** each lane contains 1 μg of poly(A)$^+$ RNA except ovaries which contain 0.5 μg. Exposure was 1 week for blot **(A)** and 2 days for blots **(B)** and **(C)**. L1, L2, L3 are 1st, 2nd and 3rd instar larvae. P1, P2, P3 are pupae early, mid and late. Ov is ovaries from 2−3-day-old females. L1i in blot **(A)** corresponds to the same blot which was rehybridized with the intron *PstI−BglII* genomic fragment used as a probe. 1st larval instar lane was selected as being the most representative of hybridization pattern.

in the developmental profile of expression of the *K10* transcripts were confirmed by a more detailed study of early stages of development (data not shown).

Out of the six transcripts analysed, including those of *kz* (4.2 kb) and *crn* (2.1 kb) (Figure 3), four are synthesized in the ovaries, and furthermore are present at early stages of development. This behaviour is consistent with their biological properties (Perrimon *et al.*, 1984). The two other transcripts, the 6- and 4.0-kb RNAs, belonging to the *K10* locus, are not detected in the ovaries. Their low level of expression and the presence

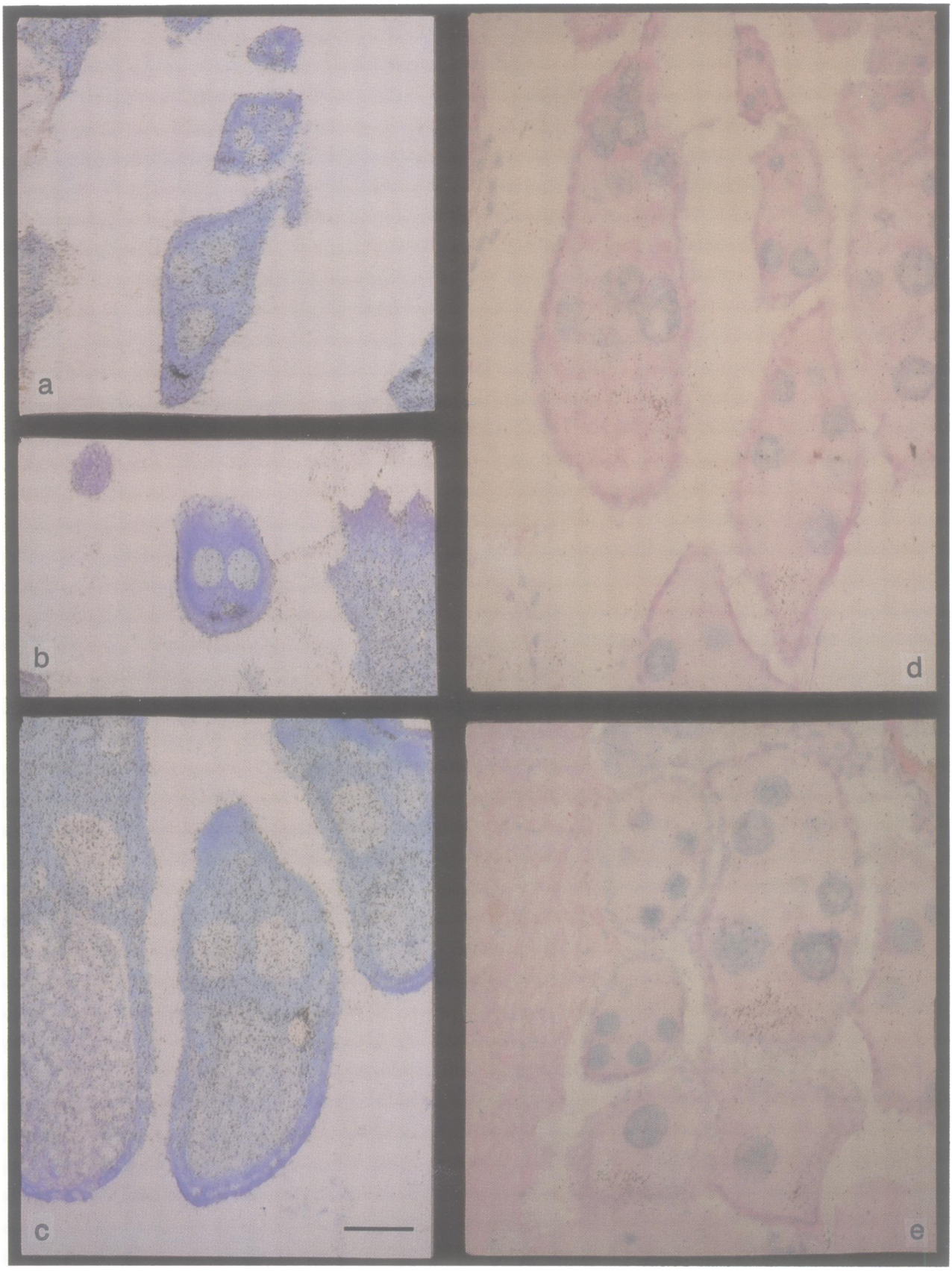


**Fig. 4.** *In situ* hybridization to sections of *Drosophila* ovaries. Wild-type egg chambers from stages 4, 5 and 7 are visible in **(a)**, stage 7 in **(b)** and stage 9 in **(c)**. In **(d)** a group of three egg chambers (stages **4–7**) within the same ovariole are visible to the right and a stage 8 to the left. In **(e)** a group of egg chambers at stages 6–8 is visible. The posterior pole of the egg chamber is oriented downwards on the figures. The left-hand series (**a, b** and **c**) presents hybridizations with a nick-translated E51 probes and Giemsa coloration, the sections being made on dissected ovaries. The right-hand series (**d** and **e**) presents hybridizations with a single-stranded anti-sense E51 probe and a methyl-green pyronin coloration, the sections were made on whole abdomens. The enlargement is the same on all panels, the bar in **(c)** being equal to 25 $\mu$m.

of an intron lead us to conclude that they represent precursor forms of the major 3.1-kb RNA.

*Identification of the K10 transcript responsible for the $K10^+$ function*

To determine which transcript is responsible for the *K10* function, we have modified the *K10* gene structure by constructing a transposon based upon a genomic DNA fragment in which the intron-containing portion has been substituted by the corresponding portion of E51 cDNA (Figure 1C). This construction was injected in the embryos derived from heterozygous *K10* females crossed to *K10* males following the procedure described earlier. This construction is capable of restoring the $K10^+$ function in homozygous *K10* females. Since it is unable to produce either the 6- or 4.0-kb RNA, its capacity to restore the $K10^+$ function implies that the $K10^+$ function can be assigned either to the 3.1-kb and/or the 2.8-kb transcript and rules out both the 4.0- and 6-kb RNAs. However, the fact that the minor species are not essential for the $K10^+$ function does not imply that they are deprived of biological significance. A similar experiment to discriminate between the 3.1- and the 2.8-kb RNAs requires more data concerning the structure of these RNAs.

*Expression of the K10 gene during oogenesis*

Since genetic studies, as well as our Northern analysis, suggest that *K10* is expressed in the ovaries of adult females, it was of interest to examine the localization of *K10* RNA during oogenesis. cDNA E51 was chosen as a probe because it is deprived of any internal repetitive sequence (to be published), and is therefore specifically representative of the *K10* gene. Each ovary in *Drosophila* is made up of 16 ovarioles [for ovarian development see King (1970)]. At the apical end of each ovariole is the germarium which contains the stem cell population of the germ line and the somatic components of the egg chambers. Cellular division of a germ line stem cell results in the formation of a cluster of 16 sister cells interconnected by cytoplasmic bridges. One of these cells will become the oocyte, the others, the nurse cells. This cluster is surrounded by the follicle cells of somatic origin and together these form the egg chamber. The egg chamber leaves the germarium and enters the main body of the ovariole, where vitellogenesis takes place as the egg chamber moves down the length of the ovariole (Mahowald and Kambysellis, 1980). Each ovariole of a mature female contains six or seven egg chambers arranged in a chronological order: the younger and smaller egg chamber being in the more apical position. Oogenesis has been divided into 14 stages (King, 1970), the first being when the egg chamber leaves the germarium and the last corresponding to the mature egg just before oviposition. On this scale vitellogenesis begins at stage 7 and choriogenesis at stage 10.

Figure 4 shows hybridization of cDNA E51-probe to ovary and to abdomen sections. Several egg chambers at various stages of development are visible. In early stages (stage 4 in panel a) there is no specific labelling, whereas at about stage 5 (panel a) a specific label appears at one extremity of the egg chamber, mainly in one cell. This signal intensifies at later stages (7 on panels a and b). The basal position of that cell relative to the general orientation of the maturing egg chamber implies that it will become the oocyte. The diffuse signal visible in the nurse cells with nick-translated probes (panels a–c) almost disappears when hybridization is performed with single-stranded anti-sense RNA (panels d and e) and corresponds to the background signal visible with the sense RNA (data not shown), thus it is not a *K10* specific signal. Later on, when the oocyte has enlarged due to vitellogenesis (stage 9 in panel c), the transcripts are condensed around the oocyte nucleus, adjacent to the follicle cells in the prospective anterior-dorsal region of the egg. In the last stages of oogenesis, the signal is still present but becomes diluted and evenly distributed and this remains the case for early embryonic stages (data not shown).

Hybridization of ovary sections with the *PstI–BglII* fragment, which constitutes part of the intronic sequence, shows the same diffuse background labelling as that visible in the nurse cells when cDNA E51 sense RNA is used as a probe (data not shown). Thus the two larger transcripts are not present in detectable amounts, which is consistent with the Northern data. We conclude that the specific labelling can be assigned to the 3.1-kb and/or 2.8-kb transcript, and therefore corresponds to the expression of the $K10^+$ function.

## Discussion

The female sterile *K10* mutation stands out among the dorsal-like mutants, because in addition to the dorsalized development which is the common feature of these mutations, the *K10* egg appears dorsalized, thus suggesting an almost complete lack of polarity. This mutation reveals that the dorsal–ventral polarity of the egg shape is under genetic control, and suggests the existence of a stage in development when egg shape polarity is coupled with embryonic polarity. It follows that this mutation provides an opportunity to elucidate the mechanism by which the oocyte acquires dorsal–ventral polarity and to determine the extent to which egg shape polarity might influence the subsequent developmental embryonic polarity.

In this paper we have focused on the expression of the *K10* gene. Northern blot analysis shows a major transcript of 3.1 kb with a developmental profile which roughly corresponds to that expected from the characteristics of the *K10* mutation, namely it is most abundant in ovaries and in early developing embryos. However, its presence, even though strongly reduced, at later stages of development, is unexpected for a maternal effect gene, and might argue for a second function of the *K10* gene, a function which might have escaped notice during genetic analysis. On the other hand, the presence of this RNA does not imply a function, as a post-transcriptional regulation could prevent the synthesis of a biologically active protein.

The *in situ* hybridization presented here shows that the *K10* expression corresponds to the predictions which could be made from the biological properties of the mutation. In particular, despite the fact that the most obvious phenotypic feature is expressed in the shape of the chorion made by the somatic follicle cells, the germ line dependence of *K10* implies that its expression should be restricted to cells of the germ line, i.e. the oocyte and/or the nurse cells.

The *in situ* hybridization shows the transcripts close to the oocyte nucleus up to stage 10 of oogenesis, suggesting that *K10* is not only specifically transcribed in the germ line, but that its expression is apparently restricted to the oocyte nucleus. The transcripts begin to accumulate at the basal position of the egg chamber at stage 6 or earlier, at a time when no visible polarity can be detected either in the oocyte or in the egg chamber, but nevertheless after differentiation of the oocyte. Indeed, it is thought that by the time the egg chamber leaves the germarium (stage 1), one of the 16 germ line cells is already committed to differentiate into an oocyte (King, 1970). In this respect the *K10* gene would be one of the first genes expressed as a result of oocyte differentiation.

The fact that *K10* transcription occurs in only one of the two

possible cell types in the germ line is not suprising *per se*. However, it is generally considered that the oocyte remains most silent during oogenesis and receives products synthesized in the nurse cells, products which are passed into the oocyte at stage 10 (Mahowald and Kambysellis, 1980). This is what happens, for instance, in the case of the *dorsal* gene. *In situ* hybridization shows that *dl* is expressed in the nurse cells of the egg chamber, and that the mRNA is transferred from these cells to the growing oocyte where it is stored until the end of oogenesis (Steward *et al.*, 1985). Although we cannot exclude the possibility that the *K10* gene is expressed in the nurse cells and its products rapidly poured into the oocyte, this is unlikely both on the basis of the *in situ* hybridization data and because it would require a specific transport of the *K10* messenger. Therefore, we assume that in the case of *K10* the signal is oocyte specific, which implies that the gene is specifically transcribed within the cell to which it will confer polarity.

However, this cell-specific transcription does not imply that the encoded protein has to be active in the oocyte itself. It might act on other cells, such as the follicle cells, one function of which is the secretion of the protective coverings of the egg, i.e. the chorion and the appendages. In the case of *K10* mutant ovaries, the follicle cells, misinformed, would in turn generate aberrant chorion and appendages. The analysis of the protein encoded by the *K10* gene and its distribution within the ovaries determined by immunofluorescent staining with specific antibodies, will bring complementary information and might help in understanding the mechanism which underlies the establishment of dorsal–ventral polarity of the egg.

Another aim of our work was to find a molecular support for the dorsalized development which is observed in those *K10* eggs that are fertilized. Three hypothesis can be proposed to account for this *dl* resembling development. (i) It is simply a consequence of the absence of polarity in the oocyte and might result from an improper distribution of positional cues normally deposited in the egg by the mother during oogenesis. (ii) Development along the dorsal–ventral axis is under the control of a hierarchy of genes. As *K10* is one of the earliest acting genes, its function might be required for the expression of one or several other genes. (iii) It is the developmental manifestation of the lack of a second, early embryonic, function of the *K10* gene itself. This hypothesis was suggested by the example of *dorsal*. As in the case of the transcript of the *dl* gene, the *K10* 3.1-kb mRNA is likely to be stored in the oocyte for use in early embryos. We might then expect that, if this second activity resembles that of the *dl* gene product, then its loss by mutation, will lead to a dorsalized development of the resulting embryo. In the case of *dl*, the existence of stored information could be shown by rescue experiments based on the injection of wild-type cytoplasm (Santamaria and Nüsslein-Volhard, 1983) into mutant embryos.

A similar experiment was undertaken with *K10* eggs. Because of the low percentage of fertilization (about 1%), it was necessary to inject a large number of eggs. Sixteen developing embryos were thus obtained out of the 1700 eggs injected with wild-type cytoplasm. All of them showed the characteristic cuticle pattern of developing *K10* embryos: the anterior ends of such animals consist of a hollow tube of skin with the hair pattern characteristic of the dorsal side. Posteriorly, the pattern of the larvae becomes more ventralized, and the last few segments (typically four) show the bands of denticles normally found only in the ventral hypoderm (Wieschaus, 1980). None of these larvae showed any sign of rescue which could have been seen most easily by an increase in the number of the posterior segments presenting the ventralized hair pattern. In a second approach, we reasoned that it might be possible to inhibit the translation of the stored transcript by the injection of *K10* anti-sense RNA into wild-type embryos, thus leading to the production of phenocopies showing a dorsalized development (Melton, 1985; Rosenberg *et al.*, 1985). In our hands, the injection of wild-type embryos with the *in vitro* transcribed anti-sense strand of the *K10* cDNAs, cloned into SP6 vectors, did not result in such phenocopies. The interpretation of these experiments is difficult as total blocking of translation might be difficult to achieve.

Although neither of these negative results exclude the hypothesis of an embryonic function of *K10*, they do not favour it. This might argue for either the egg shape polarity influencing the subsequent developmental embryonic polarity, or the *K10* gene having a regulatory function on some of the other genes involved in the development along the dorsal–ventral axis.

## Materials and methods

The P-element transformation, microinjection, RNA extraction and Northern blots were as described in Haenlin *et al.* (1985).

*In situ hybridization to tissue sections*

The tissue consisted of either dissected ovaries or whole abdomens from Oregon R females. The tissue was prepared as described by Hafen *et al.* (1983) except that a treatment in 0.25% acetic anhydride in 0.1 M triethanolamine pH 8.0 for 10 min at room temperature was added just prior to the last dehydration.

Hybridization with nick-translated probes was carried out according to Hafen *et al.* (1983) with all four nucleotides $^{3}$H-labelled (30–100 Ci/mmol, Amersham). The final probe size was ~75 bp and probe concentration was 2.5 ng DNA ($450 \times 10^3$ d.p.m.)/$\mu$l of hybridization solution.

For hybridization with single-stranded probes the cDNA E51 template was subcloned into pSP65 and probes were synthesized according to Melton *et al.* (1984). $^{35}$S-labelled UTP (Amersham) had an activity of 1000 Ci/mmol and was used at 10 $\mu$M final concentration. The synthesized RNA was fragmented in sodium carbonate pH 10.2 as described by Cox *et al.* (1984) to give a final mean fragment size of 75 bp. The probe had a final concentration of 0.5 ng RNA ($500 \times 10^3$ d.p.m.)/$\mu$l of hybridization solution. Hybridization and washing were as described by Ingham *et al.* (1985) except that Denhardt's was omitted from the wash solution and that washing in NTE was done 4 × 5 min immediately prior to RNase treatment (NTE is 500 mM NaCl, 10 mM Tris–HCl pH 7.5, 1 mM EDTA).

The radioactive label was visualized with Kodak NTB2 emulsion diluted in water to give 40% final concentration. Exposition time varied from 2 to 3 weeks for $^{3}$H-labelled nick-translated probes and from 5 to 10 days for $^{35}$S-labelled RNA probes. After developing, slides were coloured for 10 min in a solution of 2.5% pyronin, 1% methylgreen (Merck) and 100 mM sodium acetate pH 4.8 or 2 min in Giemsa diluted 1:20 in 100 mM $KH_2PO_4$ and 10 mM EDTA (pH 6.8 with KOH).

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