

Localization of vasa, a component of *Drosophila* polar granules, in maternal-effect mutants that alter embryonic anteroposterior polarity

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Summary

Cytoplasm at the posterior pole of the early *Drosophila* embryo, known as polar plasm, serves as a source of information necessary for germ cell determination and for specification of the abdominal region. Likely candidates for cytoplasmic elements important in one or both of these processes are polar granules, organelles concentrated in the cortical cytoplasm of the posterior pole. Females homozygous for any one of the maternal-effect mutations, *tudor*, *oskar*, *staufen*, *vasa*, or *valois* give rise to embryos that lack localized polar granules, fail to form the germ cell lineage and have abdominal segment deletions. Using antibodies against a polar granule component, the vasa protein, we find that vasa synthesis or localization is affected by these mutations. In *vasa* mutants, synthesis of vasa protein is absent or severely restricted. In *oskar* and *staufen* mutant females, vasa

synthesis appears normal, but the vasa protein is not localized. In *tudor* and *valois* mutant females, vasa is localized to the posterior pole of oocytes, but this localization is lost following egg activation. In addition to the posterior localized vasa, there is a low level of vasa distributed throughout the embryo. A function for this distributed vasa is postulated based on the observation that embryos from *Bicaudal-D* mothers, in which abdominal determinants are incorrectly localized to the anterior pole, do not show any ectopic vasa localization, though abdomen development at the anterior end depends on the amount of vasa protein in the embryo.

Key words: vasa, polar granules, maternal-effect mutants, cytoplasmic determinants.

Introduction

Asymmetric segregation of cytoplasmic components is observed during early stages of embryogenesis of many vertebrate and invertebrate embryos (Davidson, 1986). One well-known example is the polar plasm of *Drosophila*, which contains large numbers of organelles called polar granules (Mahowald, 1962, 1968). Polar granules are electron-dense, RNA-rich cytoplasmic organelles, and are concentrated to the posterior pole of late stage oocytes and early embryos (Mahowald, 1968, 1971b). Several lines of evidence indicate that these structures are required for the localization and/or function of posteriorly localized cytoplasmic determinants required for the formation of the germ cell lineage and for proper abdomen specification. First, mutations of *vasa*, which codes for a component of polar granules, abolish the formation of germ cells and alter abdominal segment specification (Schüpbach and Wieschaus, 1986; Hay *et al.* 1988a,b; Lasko and Ashburner, 1988). Secondly, six other maternal-effect mutations (*tudor*, Boswell and Mahowald, 1985; Schüpbach and Wieschaus, 1986; *staufen*, *valois*, Schüpbach and Wieschaus, 1986; *oskar*, Lehmann and Nüsslein-Volhard, 1986; and *cap-puccino* and *spire*, Manseau and Schüpbach, 1989), which disrupt formation of the germ cell lineage and

abdomen specification, also produce embryos without localized polar granules. Finally, the temporal distribution of RNA-rich polar granules in wild-type oocytes and embryos coincides with the ability of polar plasm to induce germ cell determination (Mahowald, 1971b; Illmensee *et al.* 1976; Niki, 1986).

Synthesis of polar granule components and their localization to the posterior embryonic pole occurs during oogenesis. Assembly of the granule probably occurs in the nurse cells, germline feeder cells, because these cells contain large cytoplasmic masses (nuage) which resemble polar granules, and which contain an identified component of polar granules, the product of the *vasa* locus (Hay *et al.* 1988a). During the second half of oogenesis (stages 10–14 of King, 1970), the nurse cells inject essentially all of their cytoplasmic contents into the growing oocyte through intercellular bridges located at the anterior pole of the oocyte. Cytoplasmic mixing rapidly distributes this material throughout the ooplasm (Gutzeit and Koppa, 1982). This is the period during which localization of polar granules to the posterior pole occurs (Mahowald, 1971a). This posterior localization, in spite of entry at the anterior pole and rapid mixing, probably involves specific transport mechanisms which target polar granules to the posterior pole. Maintenance of this posterior localization may in

turn require specific interactions between receptors at the posterior pole and certain polar granule components.

To understand the requirements for polar granule localization and to identify stages involved in this process, one may follow the fate of known granule components in mutants that lack localized polar granules. Here we report experiments in which we have followed the fate of the *vasa* protein, a component of polar granules, during oogenesis and embryogenesis in a number of mutants. Implications of *vasa* function and of mechanisms of granule localization will be discussed.

Materials and methods

Stocks

Drosophila melanogaster were raised on standard cornmeal-yeast-agar medium at 25°C. Normal flies were of Oregon-R (OR) wildtype strain. Balanced stocks of *vasa* (*vasa*^{PD23} and *vasa*^{DL}), *tudor* (*tudor*^{WC8}), a deficiency that uncovers the *tudor* locus (Df(2L)PL3), *staufer*, *staufer*^{HL54}, a deficiency that uncovers the *staufer* locus (Df(2L)PC4), *valois*, (*valois*^{RB}) and a deficiency that uncovers the *valois* locus (Df(2L)TW2), were provided by Trudi Schüpbach, Princeton University, Princeton, NJ. Other stocks of *tudor* (*tudor*², *tudor*³, *tudor*⁴) were provided by Robert Boswell, University of Colorado, Boulder, CO. Stocks of the two deficiencies Df(2L)A267 and Df(2L)TE116-GW18, which in heterozygous combination delete part of the *vasa* locus, *staufer* (*staufer*^{D3}), *oskar* (*oskar*¹⁶⁶, *oskar*³³⁶, *oskar*³⁰¹), a deficiency that uncovers the *oskar* locus, stocks of *valois* (*valois*^{PE}), and stocks of *Bicaudal-D* (*Bicaudal-D*⁷¹³⁴, *Bicaudal-D*^{111E}) were provided by Ruth Lehmann, Whitehead Institute, Cambridge, MA.

Fly crosses

For most experiments, homozygous females were collected from balanced stocks and mated with OR males. For the creation of a deletion of a part of the *vasa* locus, virgin Df(2L)A267/CyO females, which are deleted for a part of the *vasa* locus, were crossed to Df(2L)TE116-GW18/CyO males, which are also deleted for a part of the *vasa* locus, and non-CyO progeny selected. The only transcription unit affected by both deficiencies in these flies is *vasa* (Lasko and Ashburner, 1988).

Production of polyclonal anti-*vasa* rabbit serum

A *trpE-vasa* fusion protein was used as the immunogen. Site directed mutagenesis was carried out to create an *NdeI* restriction site at the initial methionine of a full-length *vasa* cDNA cloned into bluescript SK- (Stratagene) as described (Kunkel *et al.* 1987). A pair of oligonucleotides was synthesized, phosphorylated and annealed using standard techniques (Maniatis *et al.* 1982) such that *Bam*HI and *NdeI* sites were created as opposite ends of the oligonucleotide. A gel-purified *NdeI-XbaI* fragment containing the entire *vasa* coding region was mixed with these oligonucleotides and ligated into the vector PATH1, cut with *Bam*HI and *XbaI*. This vector contains the *trpE* coding sequence (Dieckmann and Tzagaloff, 1985). Bacteria containing either a *trpE-vasa* construct or *trpE* alone were grown and induced according to Driever *et al.* (1988). Fusion protein was isolated according to Rio *et al.* (1986), dissolved in sample buffer (Laemmli, 1970) and subjected to preparative SDS-PAGE. Protein bands

were visualized by incubating the gel with 0.25 M KCl in H₂O. The gel slice containing the *trpE-vasa* fusion protein was cut out and chopped into small bits. These were then suspended in complete Freund's adjuvant (incomplete for booster injections). Rabbits (Berkeley Antibody Company, Berkeley, CA) were injected at multiple subcutaneous and intramuscular sites, boosted after 4 weeks, and bled at biweekly intervals thereafter. Two rabbits produced high titers against the *vasa* protein. To purify antibodies specific for the *vasa* portion of the fusion protein, the following procedure was carried out. Bacterial extract from cells expressing either the *trpE-vasa* protein or *trpE* alone were dialyzed into coupling buffer (0.1 M Hepes (pH 7.5), 10% glycerin, 5 mM EDTA). Precipitated material was sedimented at 10 000 g, 10 min. The supernatants were used to prepare affinity columns (Affigel 10/15, 1:1 ratio; Biorad) according to the manufacturer's protocol. Following coupling, these columns were washed exhaustively with 0.5 M NaCl, 10 M Tris-HCl, pH 7.5, and then with elution buffer containing 0.2 M glycine, 0.15 M HCl, 0.5% Tween-20, pH 3.5. The column was then equilibrated with antibody-binding buffer (50 mM Tris-HCl, pH 7.5, 5 mM EDTA). Rabbit serum was dialyzed into antibody-binding buffer and then incubated overnight at 4°C batchwise with column material containing bacterial extract induced to express *trpE* alone. The unbound material was incubated batchwise for four hours at room temperature with affigel containing extract from the *trpE-vasa* expressing bacteria. This affigel was then poured into a column and washed with 50 column volumes of antibody-binding buffer. Bound protein was eluted with 3 column volumes of elution buffer. Elution volumes were immediately neutralized with 1 M Tris base and brought to a final concentration of 1% BSA, 0.02% sodium azide.

Immunocytochemistry

Antibody staining of different stages of oogenesis and embryogenesis with monoclonal antibody Mab46F11, which recognizes the product of the *vasa* locus, was carried out as described in Hay *et al.* (1988a). For immunocytochemistry using the polyclonal antisera, tissue was incubated for 1 h at room temperature with the affinity-purified anti-*vasa* antibodies at a dilution of 1:1000 in antibody buffer (0.1 M sodium phosphate, pH 7.5, 0.3% Triton X-100, 0.3% sodium deoxycholate, 2% bovine serum albumin (Miles, fraction V)). Three, 15 min washes in antibody-binding buffer were followed by incubation of the tissue for 1 h at room temperature with horseradish peroxidase-conjugated goat anti-rabbit IgG (Biorad, EIA grade) at a dilution of 1:1000. Following three 15 min washes as above, the diaminobenzidine reaction product was developed as described in Hay *et al.* (1988a). DNA was visualized by incubating embryos with 0.5 µg ml⁻¹ 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, Boehringer Mannheim Biochemicals) in 0.1 M Tris-HCl for 10 min at room temperature and then washing for 10 min in Tris buffer alone. The embryos were then mounted in 80% glycerol, 0.1 M Tris-HCl, pH 9.0, 5% (wt/vol) *n*-propyl gallate.

Western blotting

For Western blotting, 1 h collections of embryos were dechorionated in bleach: water (1:1), rinsed extensively with water and staged under a dissecting microscope. Cleavage stage embryos were then processed as described in Hay *et al.* (1988a) for Western blotting using the monoclonal antibody Mab46F11.

Oocyte activation

Oocyte activation was carried out according to Mahowald

et al. (1983). Ovaries from well-fed virgin females were hand dissected and activated by placing them in 50% Robb's medium for up to several hours. Removal of unactivated oocytes and dechorionation was carried out by removing the Robb's medium and adding a 30% solution of commercial bleach for several minutes. The surviving activated oocytes were then fixed as above for embryos and devitellinized by hand. Antibody staining was as above.

Results

Embryos derived from females homozygous for strong alleles of the five posterior group genes, *vasa*, *staufer*, *oskar*, *valois* and *tudor*, all have abdominal deletions, fail to form germ cells and lack polar granules at the posterior embryo pole. To examine the effects of these mutations on granule localization, we used monoclonal and polyclonal antibodies against the *vasa* product, a component of polar granules, and carried out Western (immunoblot) analysis and immunocytochemical staining of oocytes and embryos derived from homozygous mutant mothers. Null, as well as weaker alleles of *vasa*, were first analyzed to verify the specificity of the antibodies, before the studies of the other mutations. In order to assess the severity of these mutations, we compared the phenotypes generated by homozygous mutant females with those arising from females carrying the same mutation on one chromosome and a deletion of the locus on the other chromosome.

vasa distribution in embryos and oocytes of the wild-type and of *vasa* mutants

In wild-type embryos, *vasa* immunoreactivity, as seen

with monoclonal antibody Mab46F11, is concentrated in the form of polar granules at the posterior pole (Hay *et al.* 1988a,b). Lower levels of immunoreactivity are also found throughout the embryo during cleavage stages (Hay *et al.* 1988b). Because the *vasa* protein appears to have many different cellular associations: as a part of polar granules during oogenesis and early embryogenesis, as a part of the nuage or dense bodies in the adult germline, and distributed throughout the somatic region of the preblastoderm embryo, we were concerned that in some situations the epitope of *vasa* recognized by monoclonal antibody Mab46F11 might be masked, and thus we would be missing some populations of *vasa* proteins in our immunocytochemical analysis. To avoid this potential difficulty in the interpretation of our immunocytochemical data, we immunized rabbits with a full-length *vasa* protein made as a fusion with the bacterial protein trpE (see Materials and methods) in order to generate polyclonal anti-*vasa* antibodies. *Vasa* immunoreactivity, as seen with the polyclonal antiserum, shows a similar pattern during early embryogenesis (Fig. 1A). The immunoreactivity seen with monoclonal or polyclonal antibodies, localized to the posterior pole and present at lower levels throughout the embryo, is absent in embryos from homozygous *vasa*^{PD} females (a hypomorphic allele) (see Hay *et al.* 1988b, Fig. 4) (Fig. 1B). Thus, both antibodies appear specific for the *vasa* product during early embryogenesis. The specificity of these antibodies for the *vasa* product is further demonstrated by Western analysis of proteins extracted from *vasa* cleavage stage embryos (Hay *et al.* 1988a, Fig. 7) (Fig. 2).

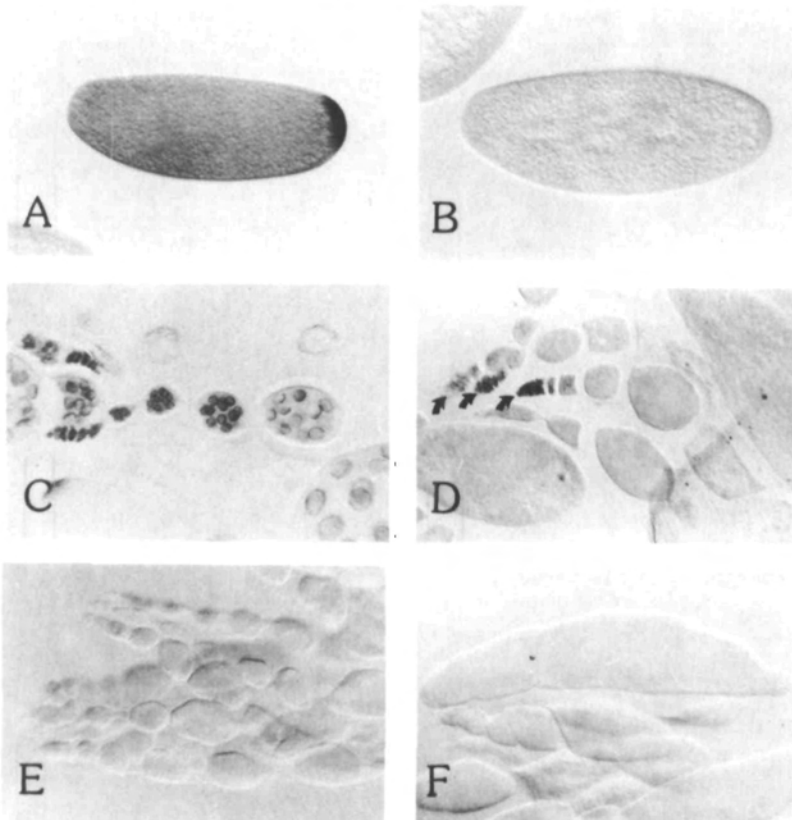


Fig. 1. Immunoreactivity in wild-type and *vasa* mutant ovaries as visualized using polyclonal rabbit anti-*vasa* antibodies. Younger stages of oogenesis, and the anterior of any given egg chamber are located to the left. (A) Cleavage stage wild-type embryo. Label is concentrated at the posterior embryo pole. A general background level of staining is present throughout the somatic regions of the embryo. (B) *vasa*^{PD} cleavage stage embryo. No staining is present, at the posterior pole or throughout the rest of the embryo. (C) Wild-type ovary. (D) Homozygous *vasa*^{PD} ovary. Label is primarily present in the germarium and early vitellarium stages (indicated by the arrows). (E) A *vasa* null ovary, which does not show any late stage oocytes. Label is undetectable. (F) A *vasa* null ovary, which does show a later stage oocyte (approximately stage 10). Label is not present.

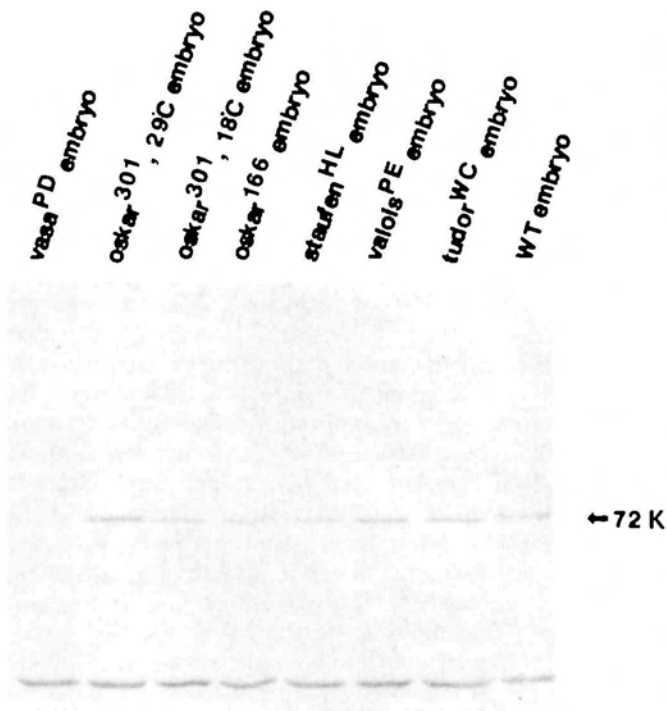


Fig. 2. Western blot of cleavage stage embryos from wild-type and homozygous mutant mothers for the mutations *vasa*, *tudor*, *oskar*, *staufer*, and *valois*. A $72 \times 10^3 M_r$ band is absent only in the *vasa* embryo lane. There is a lower molecular weight band present in all lanes whose identity is unknown. This band provides a convenient marker for relative gel loading (see also Hay *et al.* 1988a). Protein from cleavage stage embryos was prepared as in Hay *et al.* (1988a) and $10 \mu\text{g}$ loaded per lane.

In ovaries of homozygous *vasa*^{PD} females, vasa immunoreactivity is restricted to early stages, primarily the germarium, in which the geometry of the oocyte-nurse cell complex is set up. Occasionally, low levels of vasa immunoreactivity are also found in early stages of the vitellarium, in which the bulk of oocyte and nurse cell growth occurs (Fig. 1D; compare with wild type in Fig. 1C). In a female null for *vasa* function (see experimental methods for details), oogenesis is usually not completed. In ovaries of many such females, egg chambers do not develop beyond the early stages of the vitellarium (Fig. 1E), whereas in other ovaries egg chambers at later stages of oogenesis are present (up through stage 14) (Fig. 1F). None of these show any vasa immunoreactivity with either the monoclonal or polyclonal antibody. These observations show that our

anti-vasa antibodies recognize only the vasa protein during oogenesis and early embryogenesis.

oskar and *staufer* mutations eliminate or reduce vasa localization during oogenesis

The *oskar* and *staufer* mutations affect vasa localization during oogenesis. Ovaries from strong (strength being defined relative to the amount of abdominal segment deletion seen) alleles of *oskar* and *staufer*, *oskar*³³⁶ and *staufer*^{D3} or these mutations in *trans* to deficiencies uncovering the respective loci, show a normal pattern of vasa expression in the nurse cells (Fig. 3A,B). Immunoreactivity appears in the oocyte around stage 10, but never becomes localized to the posterior pole of stage 14 oocytes (Fig. 3C,D). Localization is also not seen in early cleavage stage embryos (Fig. 3E,F).

Western analysis reveals that normal levels of vasa product are present in early embryos of *oskar*¹⁶⁶ (and *oskar*³³⁶, data not shown) or *staufer*^{D3} mothers (Fig. 2). This may derive from vasa synthesized by nurse cells and transported into the oocyte, or it may come from translation of vasa from the large amount of maternally derived *vasa* RNA present in the early embryo (Hay *et al.* 1988b). Although it remains possible that these mutations affected the stability of just the population of vasa that ends up at the posterior pole, from the fact that the absolute levels of vasa are not affected in these mutations, and in the absence of any information suggesting the existence of populations of vasa that differ in their stability, we favour the hypothesis that these mutations directly affect vasa localization. This phenotype is likely due to a reduction or loss of function, because a similar pattern of expression is seen immunocytochemically in *oskar*³³⁶ and *staufer*^{D3} females which carry the mutation on one chromosome and a deficiency for the region on the other chromosome.

A correlation between vasa localization and mutant phenotype is evident in a temperature-sensitive *oskar* mutant, *osk*³⁰¹. At 18°C, the permissive temperature, many embryos show normal abdominal segmentation and hatch, though pole cells do not form. At 29°C, they have strong abdominal deletions, in addition to the absence of pole cells (Lehmann and Nüsslein-Volhard, 1986). The degree of vasa localization in these embryos is correlated with the severity of the abdominal defect. At 18°C, some vasa is found localized to the posterior pole of stage 10 oocytes, following oviposition weak localized staining is sometimes seen in cleavage stage embryos (Fig. 3G,H). Occasionally structures that resemble polar buds are formed, but these do not develop into pole cells and by blastoderm formation localized vasa immunoreactivity is undetectable. At the nonpermissive temperature, *oskar*³⁰¹ resembles *oskar*³³⁶ with respect to its vasa localization phenotype (Fig. 3I,J).

valois and *tudor* mutations affect vasa localization during embryogenesis

The *valois* and *tudor* mutations tested affect vasa localization during early embryogenesis. Oocytes of homozygous *valois*^{RB71} or *valois*^{PE}, or *valois*^{PE}/

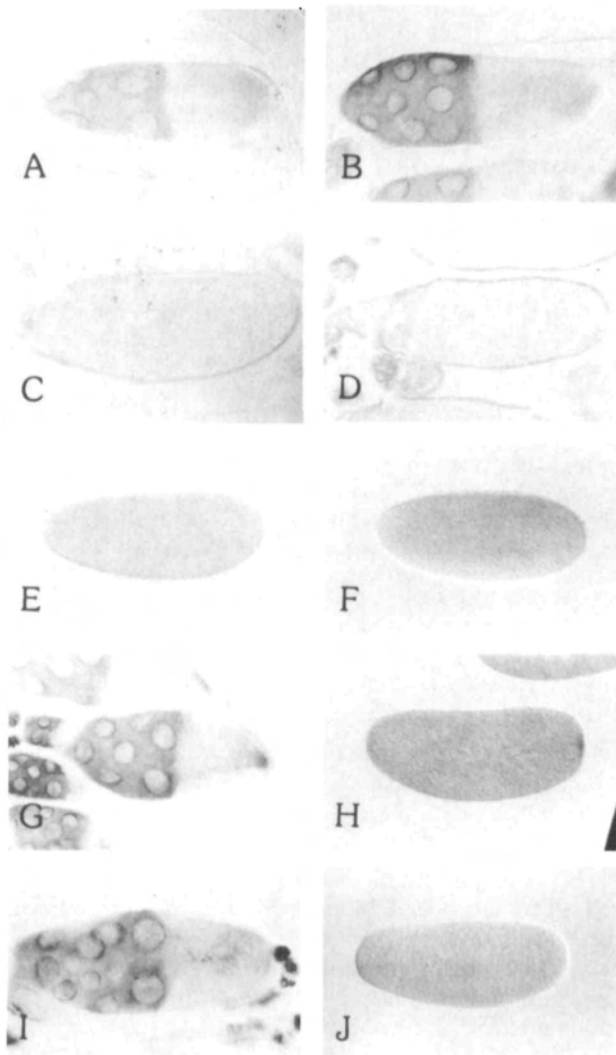


Fig. 3. *vasa* localization in stages of oogenesis and early embryos from homozygous *oskar* and *staufen* mothers. All antibody stainings were carried out with polyclonal anti-*vasa* antibodies. (A) Stage 10 *oskar*³³⁶ egg chamber. Label is present in nurse cells and in the oocyte but is not localized to the posterior oocyte pole. (B) Stage 10 *staufen*^{D3} egg chamber. Label is present in nurse cells and oocyte but not localized to the posterior oocyte pole. (C) Cryostat section of an *oskar*³³⁶ stage 14 oocyte. Posterior label is not present. (D) Cryostat section of a *staufen*^{D3} stage 14 oocyte. Posterior label is not present. (E) *oskar*³³⁶ early cleavage stage embryo. Localized label is not present. (F) *staufen*^{D3} early cleavage stage embryo. Localized label is not present. (G) Stage 10 oocyte from *oskar*³⁰¹ female raised at 18°C. Some localized label is present at the posterior oocyte pole. (H) Early cleavage stage embryo from *oskar*³⁰¹ female raised at 18°C. A small amount of label is localized to the posterior embryo pole. (I) Stage 10 oocyte from *oskar*³⁰¹ female raised at 29°C. Label is not detectably localized to the posterior oocyte pole. (J) Early cleavage stage embryo from *oskar*³⁰¹ female raised at 29°C. Label is not detectably localized to the embryo posterior pole.

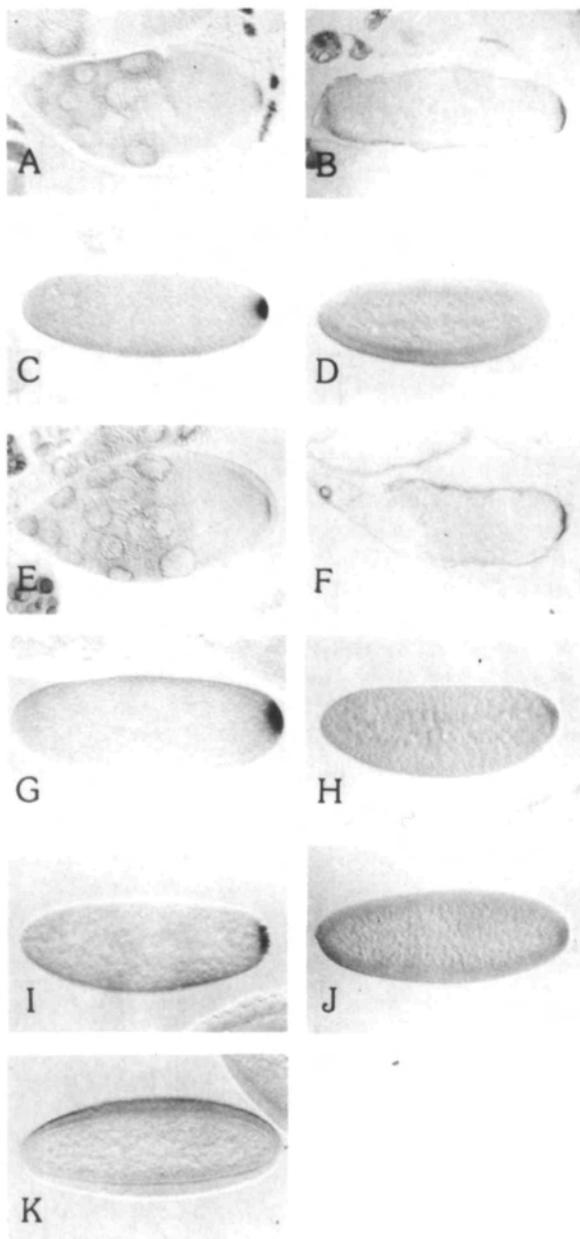
Df(2L)TW2 females, have *vasa* localized to their posterior pole at stage 10 through stage 14, as in the wildtype (Fig. 4A,B). However, following oviposition localized *vasa* immunoreactivity rapidly disappears, such that by the time of cellular blastoderm formation no localized immunoreactivity is present (Fig. 4C,D). Wild-type amounts of *vasa* immunoreactivity are present, however, by Western blotting (Fig. 2). When mutant embryos are also stained with DAPI to visualize their DNA, embryos with localized *vasa* show no more than several cleavage nuclei, whereas embryos that have proceeded through further cleavage divisions show no signs of localized *vasa*. This indicates that *vasa* localization is lost very rapidly following fertilization, long before pole cell formation would have occurred.

Embryos from homozygous *tudor*^{WC8} mothers have a very variable segmentation defect, with some embryos showing normal segmentation and others having abdominal deletions of varying size (Schüpbach and Wieschaus, 1986). Females carrying *tudor*^{WC8} on one chromosome and a deficiency for the region on the other consistently give rise to embryos with a stronger phenotype, indicating that *tudor*^{WC8} (as with other published *tudor* alleles) is probably a hypomorphic allele (Schüpbach and Wieschaus, 1986). In both the homozygous mutant and hemizygous situation, normal *vasa* localization and accumulation at the posterior pole is observed during the second half of oogenesis (Fig. 4E,F). Following oviposition, early cleavage stage embryos still have localized *vasa* immunoreactivity (Fig. 4G), whereas in later cleavage stage embryos, it becomes progressively weaker (Fig. 4H). In some embryos, pole cell buds are observed to form (Fig. 4I), but these buds disappear by blastoderm formation (Fig. 4J,K). Localized *vasa* immunoreactivity is always absent by blastoderm formation (Fig. 4K), as is the general background seen during cleavage stages in wild-type and mutant embryos.

tudor^{WC8} embryos have some small electron-dense structures associated with mitochondria. These structures may be some form of polar granule. In a phenotypically weaker allele, such as *tudor*², more of these structures can be found and a more normal abdomen develops in the embryo (Boswell and Mahowald, 1985). Correspondingly, in these embryos localized *vasa* immunoreactivity is more intense and frequent, though this immunoreactivity still disappears prior to blastoderm formation.

vasa localization in Bicaudal-D embryos

In some of the embryos from mothers carrying the dominant bicaudal mutation *Bicaudal-D*, anterior structures are reduced or absent, and in extreme cases a posterior abdomen is duplicated in mirror-image symmetry to the normal abdomen, at the anterior end (Mohler and Wieschaus, 1986). At the transformed anterior end polar granules are not apparent and pole cells do not form, though in wild-type embryos a source of abdomen-inducing activity is colocalized at the posterior pole along with the germline determinants (Frohnhofer *et al.* 1986; Lehmann and Nüsslein-Vol-



hard, 1986). The maternally encoded product of the *nanos* locus is likely to be the active component in the abdomen-inducing activity (Sander and Lehmann, 1988; Hülkamp *et al.* 1989; Irish *et al.* 1989; Struhl, 1989). Genetic and cytoplasmic transplantation experiments suggest that in *Bicaudal-D*, but not in wild-type embryos, *nanos* activity is localized at the anterior pole of the egg, as well as at the posterior pole (Lehmann and Nüsslein-Volhard, 1986; Wharton and Struhl, 1989). Interestingly, in *Bicaudal-D* females that are also heterozygous for *vasa*, the bicaudal phenotype is suppressed (Mohler and Wieschaus, 1986). The *vasa*-dependent suppression of the bicaudal phenotype indicates that *vasa* is required for the ectopic function of *nanos* activity. *vasa*-dependent suppression may occur during oogenesis or in the egg following fertilization. One possibility is that *vasa* is required for ectopic localization of the *nanos* product, for instance by

Fig. 4. vasa localization in stages of oogenesis and early embryogenesis from *valois*^{PE}/*Df*(2L)TW2 and *tudor*^{WC8}/*Df*(2R)PL3 mothers. All antibody stainings were carried out with polyclonal anti-vasa antibodies. Similar observations were made with monoclonal antibody Mab46F11. (A) Stage 10 *valois*^{PE}/*Df*(2L)TW2 oocyte. Some label is localized to the oocyte posterior pole. (B) Cryostat section of a stage 14 *valois*^{PE}/*Df*(2L)TW2 oocyte. A dense cortical band of label is present at the oocyte posterior pole. (C) Early cleavage stage *valois*^{PE}/*Df*(2L)TW2 embryo, which shows some label at the posterior embryo pole. (D) Later cleavage stage *valois*^{PE}/*Df*(2L)TW2 embryo with no localized posterior label. (E) Stage 10 *tudor*^{WC8}/*Df*(2R)PL3 oocyte. Localized label is present at the posterior oocyte pole. (F) Stage 14 *tudor*^{WC8}/*Df*(2R)PL3 oocyte. A dense band of label is present at the posterior oocyte pole. (G) Early cleavage stage *tudor*^{WC8}/*Df*(2R)PL3 embryo. Large amounts of label are localized to the embryo posterior pole. (H) Later cleavage stage *tudor*^{WC8}/*Df*(2R)PL3 embryo. Label is becoming more diffuse. (I) *tudor*^{WC8}/*Df*(2R)PL3 embryo in the process of forming pole buds. Some localized label is present. (J) Slightly later stage *tudor*^{WC8}/*Df*(2R)PL3 embryo than in (I). The blastoderm is about to form. Pole buds are not present. A very faint localized label is present at the posterior embryo pole. (K) *tudor*^{WC8}/*Df*(2R)PL3 embryo beginning gastrulation. Pole cells are not present. Localized posterior label and the general background staining are absent.

forming a complex with *nanos* which becomes localized to the anterior end in a *Bicaudal-D* embryo. If this is correct, *vasa* should be localized to the anterior pole as well as to the posterior pole in *Bicaudal-D* embryos. This hypothesis has not been borne out; under egg-laying conditions in which *Bicaudal-D* females produce large numbers of embryos with a bicaudal phenotype, we see no localization of the *vasa* protein to the anterior pole of oocytes, *in vitro* activated eggs or early cleavage stage embryos. A failure to see *vasa* localization to the anterior pole of *Bicaudal-D* embryos has been independently noted by Wharton and Struhl (1989). Since *vasa* distribution in a *Bicaudal-D* embryo is normal, it is not the case that the formation of the anterior abdomen is initiated via a mislocalization of *vasa*, and the bicaudal egg must increase the concentration of posterior rescuing activity (*nanos* product) at the anterior end independent of *vasa* localization. Given that formation of the anterior end still requires *vasa* activity, *vasa* must have a function other than simply localizing *nanos* (see discussion).

Discussion

Polar granules are concentrated at the posterior pole of late stage oocytes and early embryos, and may be involved in the posterior localization and function of germ cell determinants and activities that specify abdominal segmentation. We have shown previously that the *vasa* protein is an essential component of the polar granule (Hay *et al.* 1988a,b). In an attempt to study the mechanism and function of polar granule localization,

we have followed the fate of the vasa product in six maternal effect mutants, *vasa*, *staufer*, *oskar*, *valois*, *tudor*, and *Bicaudal-D*, because these mutations alter localization of cytoplasmic determinants required for development of the germ line and for embryonic abdomen formation.

Functions of vasa

The phenotype of *vasa* is complex. A hypomorphic allele, *vasa*^{PD}, has vasa immunoreactivity restricted largely to the germarium, and fails to localize (or perhaps even assemble) polar granules. In contrast, null mutants are usually blocked during early oogenesis at roughly the stages in which vasa seems to be produced in *vasa*^{PD}. These results suggest a requirement for vasa at two distinct phases of oogenesis. Early during oogenesis vasa is necessary for the development of the oocyte and nurse cells following their formation, whereas in the second half of oogenesis vasa is required for the localization of cytoplasmic determinants required for germ line formation and abdomen specification. During embryogenesis, there may be additional requirements for vasa. At the stage of the somatic blastoderm formation, *vasa*^{PD} and *vasa*^{DL} embryos show frequent cellularization defects (data not shown). This may reflect an essential function of the low level of vasa seen throughout the wildtype embryo, as revealed in immunocytochemical experiments.

The vasa protein has a high degree of homology with members of the helicase class of RNA-binding proteins (Hay *et al.* 1988; Lasko and Ashburner, 1988), suggesting that it functions in some context as an RNA-binding protein. Three possible roles that vasa as an RNA-binding protein might be playing in the function of the posterior determinant system are (1) to regulate the translation of localized transcripts required for determination of posterior structures; (2) to participate in the localization of the posterior determinants by providing a permissive environment for this localization (its helicase activity stabilizing a particular transcript conformation that allows binding by specific RNA-binding proteins) or (3) to localize the determinants directly. The last possibility is unlikely in light of the vasa localization phenotype in *Bicaudal-D* embryos: females *transheterozygous* for *vasa* and *Bicaudal-D* suppress the bicaudal phenotype (anterior function of abdominal determinants) though vasa is not localized anteriorly in *Bicaudal-D* embryos. We cannot completely rule out the possibility that vasa is required for directly targeting determinants to the posterior pole in the normal embryo, but that it plays a different role in localization via a *Bicaudal-D*-dependent mechanism. The first two possibilities can be distinguished on the basis of the localization of the *nanos* product in *Bicaudal-D vasa transheterozygote* embryos. Since embryos from heterozygous *vasa* females, which contain large amounts of vasa localized to the posterior pole, give rise to normal posterior structures while the anterior formation of abdominal structures is suppressed in *transheterozygotes* of *vasa* and *Bicaudal-D*, it is possible that the vasa protein present throughout

the somatic region of the cleavage stage embryo provides the *vasa*-dependent activity present in *Bicaudal-D* embryos. Decreasing the level of vasa in *transheterozygotes* reduces the amount of available anterior vasa below a critical threshold and thus prevents anterior abdomen formation.

Localization of vasa

Following fertilization or egg activation polar granules undergo rapid changes in their associations with other structures and in their morphology. During oogenesis polar granules are associated with mitochondria and appear to contain large amounts of RNA. Following either egg activation or fertilization, they lose this association with mitochondria, fragment and become associated with polysomes (Mahowald, 1962, 1968, 1971b). These observations raise the possibility that different mediators of localization, including perhaps cytoskeletal elements, may be used during oogenesis and early embryogenesis.

The process of vasa localization is altered at an early stage by *oskar* and *staufer* mutations. Females homozygous for strong alleles of *staufer* and *oskar* synthesize roughly normal amounts of vasa though it does not become localized during oogenesis. Thus both *oskar* and *staufer* gene products are required for some aspect of the initial vasa localization event. Further evidence for this hypothesis derives from studies of *oskar*³⁰¹, the temperature-sensitive allele. The temperature-sensitive period for the *oskar*-dependent development of the abdominal deletion phenotype corresponds to the last half of oogenesis (stages 10–14), the same time period during which polar granules become localized to the posterior oocyte pole (Lehmann and Nüsslein-Volhard, 1986). Indeed, some vasa is localized at permissive, but not at nonpermissive, temperatures during oogenesis. Thus the strength of the *oskar* phenotype is related to the amount of vasa, and probably polar granules, that become localized to the posterior pole. The *oskar* activity in *oskar*³⁰¹ mutants is not normal even at the permissive temperature since pole cells do not form in these embryos and the vasa that is localized to the posterior pole during oogenesis does not remain localized following oviposition. The latter observation suggests that *oskar*-dependent activity (which may be either quantitatively or qualitatively insufficient in the mutant) is also required during cleavage stages of embryogenesis to maintain vasa localization.

Though mutations of *oskar*, *staufer*, *cappuccino* and *spire* have a similar vasa localization phenotype (this paper; Manseau and Schüpbach, 1989) they differ in their interactions with *Bicaudal-D*. *Bicaudal-D* in a homozygous *oskar* background suppresses the bicaudal phenotype (Lehmann and Nüsslein-Volhard, 1986), while *Bicaudal-D* in a heterozygous *staufer* background (Mohler and Wieschaus, 1986) or a homozygous *cappuccino* or *spire* background (Manseau and Schüpbach, 1989) results in an enhancement of the bicaudal phenotype.

If the product of the *oskar* locus is a polar granule component having a similar distribution to that of vasa

and this product is required for some aspect of abdominal determinant (*nanos*) function, the block in anterior function of the abdominal determinants could be similarly explained. *Oskar* product function may be required for *nanos* function either indirectly, through interactions with *vasa*, or directly through association with the products of the *nanos* or *Bicaudal-D* loci. In the case of *staufer*, *cappuccino* and *spire*, a likely possibility is that these gene functions are required for transport of the abdomen-specifying signal (*nanos*) to the posterior pole. In the absence of any of these gene functions, posterior localization of the abdomen-inducing signal does not occur. In *Bicaudal-D* embryos, some of this activity becomes localized to the anterior pole under the influence of the *Bicaudal-D*-dependent localization mechanism. In the double mutant combinations of *Bicaudal-D* with *staufer*, *cappuccino* or *spire* described above, more of this activity becomes localized to the anterior pole because the action of the *Bicaudal-D*-dependent localization mechanism is not competing as much or at all (for *nanos*-binding perhaps) with elements required for normal posterior-dependent localization.

The phenotype of *tudor* is more difficult to interpret, since *tudor*^{WC8}, a strong allele of *tudor*, is almost certainly hypomorphic. Boswell and Mahowald (1985) have shown in a series of *tudor* mutants that the number and size of structures tentatively identified as polar granules, based on their electron-dense appearance and association with mitochondria, decreases as the severity of the abdominal deletion phenotype increases. Polar granules are not apparent in *tudor*^{WC8}/Df(2L)PL3 heterozygotes (Boswell and Mahowald, 1985), yet we see good *vasa* localization during oogenesis and during early cleavage stages following fertilization. On the other hand, consistent with this phenotypic series, we find that in *tudor*², a weaker *tudor* allele, the localized *vasa* immunoreactivity typically is more abundant than in *tudor*^{WC8}/Df(2L)PL3 heterozygotes. It is not clear from this phenotypic series, however, whether *vasa* would be localized in oocytes from mothers totally deficient in *tudor* function. One possibility is that the strong phenotype (if viable) is a complete lack of localization. Alternatively, or perhaps in addition to this aspect of *tudor* function, *tudor* may play some other role in polar granule function following fertilization. This seems likely because, though *vasa* localization is quite good in early cleavage stage *tudor* embryos, and *vasa* immunoreactivity remains somewhat localized until about the time the pole cells would normally be forming, pole cells do not form and abdominal segmentation is disrupted to a variable extent. The fact that *vasa* is well localized in oocytes and early embryos from *tudor*^{WC8}/Df(2L)PL3 females, but that localized polar granules are not visible with electron microscopy suggests that the *tudor* product is a polar granule component required for interactions between granule components necessary for assembly of a functional granule structure. The observation that *transheterozygotes* of *tudor* and *Bicaudal-D* suppress the bicaudal phenotype, as is the case with *vasa*, is also consistent

with the idea that *tudor* encodes a polar granule component required for the function of the posterior determinants. The observation that the abdominal segment deletion phenotype is variable in *tudor*^{WC8}, whereas pole cells never form, may simply reflect a higher threshold requirement for *tudor*-dependent functions for pole cell formation, or it may indicate a qualitative difference in the mutant's ability to carry out functions required for abdomen and pole cell development.

valois^{PE} appears to be a strong allele of *valois*, based on the consistency of both the abdominal deletion and *vasa* localization phenotypes, either as a homozygote or in heterozygous combination with a deficiency for the region. It shows good localization during oogenesis, but this disappears very rapidly following fertilization, even though normal amounts of *vasa* protein can be detected on Western blots. This suggests that the maternal *valois* product is required for some aspect of stabilization of *vasa* localization following fertilization. Interestingly, many *valois* embryos do not cellularize, or cellularize incompletely. This phenotype is consistent with the possibility that *valois* might be important for some aspects of cellular function (the cytoskeleton perhaps) in the embryo which are required for both somatic cellularization and maintenance of polar granule localization. We cannot completely rule out a role for *valois* during the initial localization process.

Summary

In summary, it seems likely that, based on *vasa*'s homology with RNA helicases, its localization phenotype in *Bicaudal-D* and its suppression of the bicaudal phenotype, a primary role of *vasa* in the function of the posterior determinant system is to regulate the translation of mRNA important for determination of posterior structures and/or to provide a permissive environment (RNA secondary structure) for RNA localization via other RNA-binding proteins. The fact that localized polar granules are absent in *vasa* mutants indicates that *vasa* is also a necessary component of the granules. *Vasa*'s role during early oogenesis is mysterious.

The *vasa* localization phenotypes described for the other six posterior group genes show that function of these genes is required for posterior *vasa* localization during oogenesis (*oskar*, *staufer*, *cappuccino* and *spire*), its maintenance following fertilization (*valois*), or events occurring after fertilization required for macroscopic polar granule structure and posterior determinant functions (*tudor*). Mutations in *staufer*, *cappuccino*, *spire* and *oskar* all affect early steps of *vasa* localization in oocytes. Their different interactions with *Bicaudal-D*, however, suggest that *staufer*, *cappuccino* and *spire* gene functions are likely to be required for transport of determinants to the posterior pole or their localization there, while *oskar* activity, perhaps in the form of another polar granule component or a molecule required for posterior-specific localization of the gran-

ules, is required for proper function of the determinants. In addition, *oskar*-dependent activity may also be required for maintenance of posterior *vasa* localization following fertilization. The observation that *valois* mutations affect somatic cellularization and the maintenance of *vasa* localization following fertilization suggest that the *valois* product functions as a cytoskeletal component required throughout the embryo following fertilization. In contrast, *tudor* function may not be required for *vasa* localization during oogenesis or the maintenance of this localization following fertilization since *vasa* remains localized through late cleavage stages of embryogenesis in *tudor* embryos. This observation and the lack of macroscopic polar granules seen in *tudor* embryos suggest that the *tudor* product is a polar granule component that is required for the assembly of that structure. Since *tudor* embryos have abdominal segment deletions and lack pole cells though *vasa* is localized relatively normally, and *tudor* suppresses *Bicaudal-D*, *tudor* may be required for the activation of the posterior determinant activity or to provide an environment permissive for the localization of this activity.

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