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Multiple steps in the localization of *bicoid* RNA to the anterior pole of the *Drosophila* oocyte

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Summary

The anterior region of the *Drosophila* embryonic pattern is determined by a gradient of the bicoid (bcd) protein. The correct formation of this gradient requires the localization of *bcd* RNA to the anterior pole of the egg. Here we use a wholemount *in situ* technique to examine the process of *bcd* RNA localization during oogenesis and embryogenesis. While bcd protein becomes distributed in a gradient that extends throughout the anterior two thirds of the early embryo, *bcd* RNA remains restricted to a much smaller region at the anterior pole. The difference between these distributions indicates that the shape of the protein gradient must depend to some extent on the posterior movement of the protein after it has been synthesized.

Four distinct phases of *bcd* RNA localization can be distinguished during oogenesis. Between stages 6 and 9 of oogenesis, the RNA accumulates in a ring at the anterior end of the oocyte. During the second phase, in stage 9–10a follicles, the RNA also localizes to the apical regions of the nurse cells, demonstrating that the nurse cells possess an intrinsic polarity. As the nurse cells contract during stages 10b–11, all of the *bcd* RNA becomes localized to the cortex at the anterior end of the oocyte. During a final phase that must occur between stage 12 of oogenesis and egg deposition, the RNA becomes localized to a spherical region that occupies a slightly dorsal position at the anterior pole.

Mutations in the maternal-effect genes, *exuperantia* (*exu*) and *swallow* (*sww*), lead to an almost uniform distribution of *bcd* RNA in the early embryo, while *staufen* (*stau*) mutations produce a gradient of RNA at the anterior pole. *exu* mutations disrupt the second stage of *bcd* RNA localization during oogenesis, *sww* mutations disrupt the third, and *stau* mutations affect the fourth phase.

Key words: *Drosophila*, *bicoid*, RNA localization, oogenesis, *exuperantia*, *swallow*, *staufen*, gradient formation.

Introduction

The anterior–posterior pattern of the *Drosophila* embryo is determined in response to maternal factors that are deposited in the egg during oogenesis. Three groups of maternal genes are required to specify distinct regions of this pattern; the head and thorax (the anterior group), the abdomen (the posterior group) and the acron and telson (the terminal group) (reviewed in Nüsslein-Volhard and Roth, 1989; Nüsslein-Volhard *et al.* 1987). The anterior portion of the body plan depends on the product of the *bicoid* (*bcd*) gene (Frohnhöfer and Nüsslein-Volhard, 1986). In *bicoid*$^{-}$ embryos, the head and thorax are absent, and the abdominal fate map expands anteriorly. In addition, a duplicated telson forms at the anterior end of the embryo. Transplantation experiments have shown that *bcd*$^{+}$ activity is localized to the anterior pole of the egg, and when transplanted to more posterior positions, can induce the formation of anterior structures at ectopic sites (Frohnhöfer and Nüsslein-Volhard, 1986; Frohnhöfer *et al.* 1986).

In agreement with the results of the transplantation experiments, *bcd* RNA is localized to the anterior pole of the egg (Frigerio *et al.* 1986; Berleth *et al.* 1988). The protein that is translated from this RNA forms a concentration gradient which extends throughout the anterior two thirds of the embryo (Driever and Nüsslein-Volhard, 1988*a*). This protein gradient appears to determine anterior positional values in a concentration-dependent manner, since changes in the maternal *bcd*$^{+}$ gene dosage produce complementary shifts in both the protein gradient and the fate map (Driever and Nüsslein-Volhard, 1988*b*). Further support for the role of bcd protein as the anterior morphogen comes from the results of experiments in which *bcd* RNA synthesized *in vitro* is injected into early embryos. When the RNA is injected into the middle of a recipient embryo, it can induce the development of ectopic head and thoracic structures (Driever, Siegel and Nüsslein-Volhard, in

preparation). The most anterior pattern elements form closest to the site of injection, with more posterior (thoracic) structures developing on either side. This result indicates not only that the bcd protein determines the anterior quality of the structures that develop, but also that the slope of the protein gradient specifies the polarity of the pattern. In addition, the ability of *bcd* RNA to organize anterior pattern in the middle of the embryo shows that no other anteriorly localized molecules are required for this process.

The bicoid protein contains a homeodomain, suggesting that *bicoid* encodes a DNA-binding protein (Frigerio *et al.* 1986). This makes it attractive to suppose that bcd protein specifies anterior positional values by activating or repressing zygotic target genes in a concentration-dependent manner. Genetic and molecular evidence indicates that *bcd* regulates the anterior zygotic expression of the gap gene, *hunchback* (Lehmann and Nüsslein-Volhard, 1987; Schröder *et al.* 1988; Tautz, 1988). bcd protein binds to several sites in the *hunchback* promoter, and acts as a transcriptional activator of *hunchback* (Driever and Nüsslein-Volhard, 1989). Driever *et al.* (in press) have made constructs containing synthetic-bcd-binding sites fused to the hsp70 promoter and a reporter gene. When transformed into flies, the bcd-binding sites drive the anterior expression of the reporter gene in the embryo. The size of this anterior domain of expression is reduced when binding sites with lower affinities for the bcd protein are used. The observation that promoters with low-affinity bcd-binding sites are only activated at high protein concentrations suggests a model for how the bcd protein gradient could activate several zygotic target genes in distinct anterior domains, and thereby determine several levels of anterior development.

The formation of the wild-type protein gradient, and thus the determination of a normal anterior pattern, depends upon the localization of *bcd* RNA to the anterior pole. Mutations in the maternal genes *exuperantia* (*exu*) and *swallow* (*sww*) disrupt this localization during oogenesis, and lead to an almost uniform distribution of *bcd* RNA in the early embryo (Berleth *et al.* 1988; Stephenson *et al.* 1988). Both of these mutations result in phenotypes in which anterior structures of the head are deleted, and the thoracic region is expanded (Frohnhöfer and Nüsslein-Volhard, 1987). Embryos derived from females mutant for the posterior group gene *staufen* show similar but weaker head defects, in addition to the abdominal deletions characteristic of all mutations in this class (Schüpbach and Wieschaus, 1986; Nüsslein-Volhard *et al.* 1987; Lehmann, 1988). These embryos also show an anterior shift in the fate map, which can be seen in a shift in the position of the cephalic furrow and the first *fushi tarazu* stripe (Schüpbach and Wieschaus, 1986; Carroll *et al.* 1986; Lehmann, 1988). The observation that the bcd protein gradient is shallower in the mutant embryos suggests that *staufen* mutations may also alter the distribution of *bcd* RNA (Driever and Nüsslein-Volhard, 1988*b*).

In this report, we use a non-radioactive, enzyme-linked *in situ* technique developed by Tautz and Pfeifle (1989) to examine the process of *bcd* RNA localization during oogenesis and early embryogenesis. This technique has the advantage that one can perform hybridizations on wholemount preparations. We have used these wholemount stainings to make direct comparisons between the *bcd* RNA distribution and the protein distribution, as revealed by antibody stainings.

Results

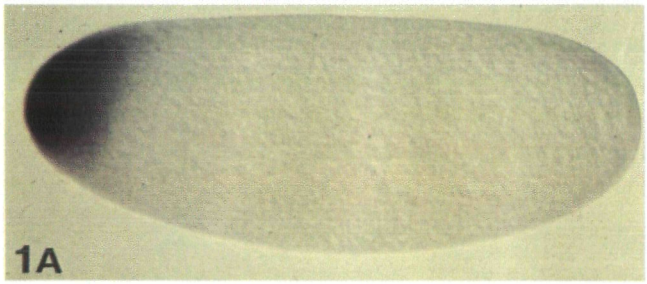
bcd *RNA localization in wild-type embryos*

The localization of *bcd* RNA in wild-type embryos has previously been described by Frigerio *et al.* (1986) and Berleth *et al.* (1988). We have repeated these investigations in order to gain a clearer understanding of the three-dimensional distribution of *bcd* RNA during the first stages of embryogenesis. In very early (stage 1) embryos, *bcd* RNA staining resembles a flattened ball which is closely apposed to the anterior pole, and which frequently occupies a slightly dorsal position (Fig. 1A). The RNA does not seem to be specifically bound to the cortex of the egg, since much of it is in the interior. As development proceeds through pole cell formation (Fig. 1B) to syncytial blastoderm (Fig. 1C), most of the RNA becomes localized to the periphery, in the clear cytoplasm that surrounds each nucleus. This movement to the cortex sometimes results in a slight posteriorwards shift in the RNA distribution. By early nuclear cycle 14, *bcd* RNA begins to disappear (Fig. 1D) and midway through cellularization the signal is no longer detectable (Fig. 1E).

Fig. 1. The distribution of *bcd* RNA in wild-type embryos. The embryos were fixed, and hybridized with a random-primed probe synthesized from a *bicoid* cDNA clone, following the procedure of Tautz and Pfeifle (1989). In all figures, anterior is to the left and dorsal is uppermost. (A) Early cleavage stage. (B) Late cleavage stage, after pole cell formation. (C) Syncytial blastoderm. (D) Late syncytial blastoderm, after the 13th nuclear division. (E) Early cellularization. These embryos have been overstained in order to clearly show the posterior extent of the *bcd* RNA distribution. This overstaining partially obscures the cortical localization of the RNA in syncytial blastoderm embryos (C), which can be clearly seen in understained embryos or in later embryos as the RNA starts to disappear (D).

Fig. 2. The distributions of *bicoid* RNA, bicoid protein and *hunchback* RNA in syncytial blastoderm embryos. (A) *bcd* RNA (B) bcd protein. The embryos were stained with a polyclonal anti-bicoid antibody, as described by Driever and Nüsslein-Volhard (1988*a*). (C) *hunchback* RNA (Tautz *et al.* 1987).

Fig. 4. The *bcd* RNA distribution in mutant embryos. (A) Wild-type cleavage stage. (B) Cleavage-stage embryo derived from an exu^{PJ}/exu^{PJ} mother, showing a uniform distribution of *bcd* RNA. (C) Syncytial blastoderm embryo derived from an exu^{PJ}/exu^{PJ} mother. By this stage a shallow anterior–posterior gradient of RNA has formed. (D) Cleavage-stage embryo derived from a sww^{14}/sww^{14} mother, showing the early weak RNA gradient. (E) Cleavage-stage embryo derived from a $stau^{D3}/Df(2R)$ *PC4* mother. The RNA is distributed in a steep gradient at the anterior end of the embryo.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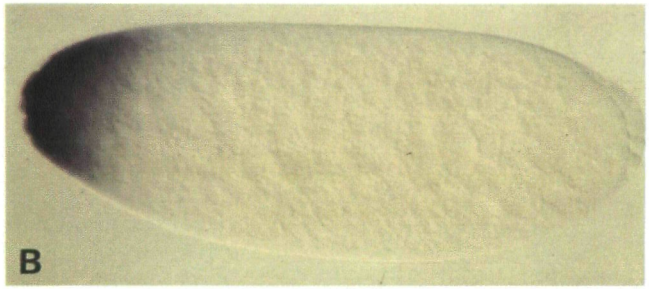
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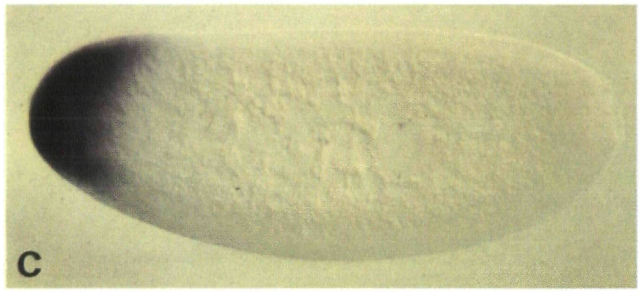
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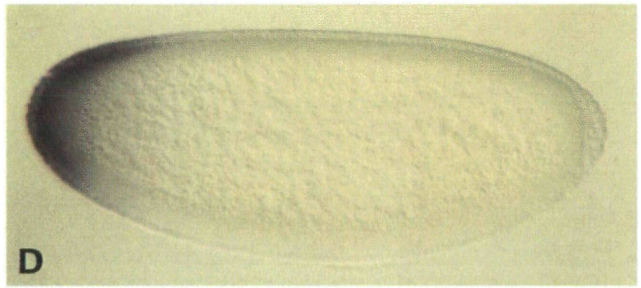
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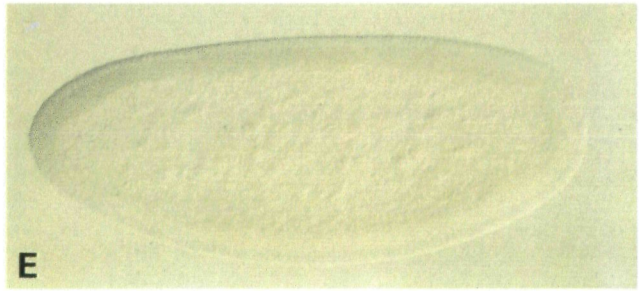
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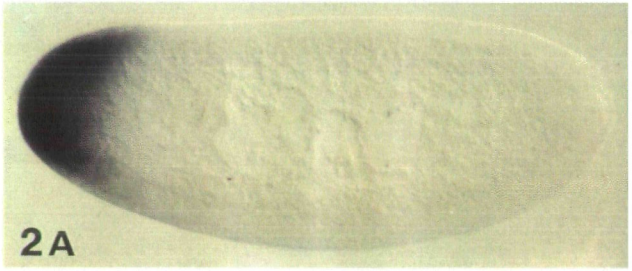
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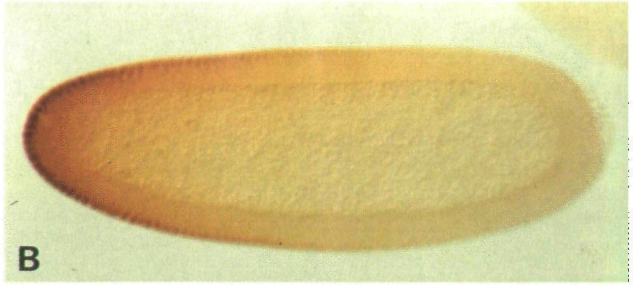
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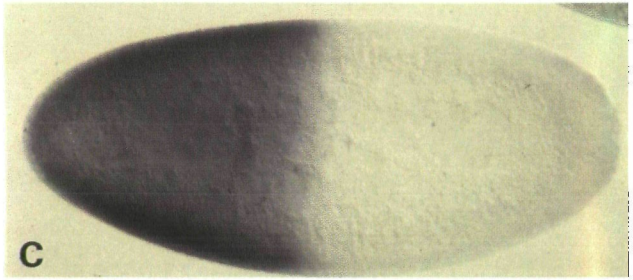
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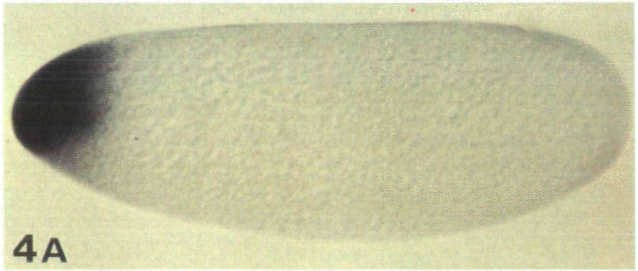
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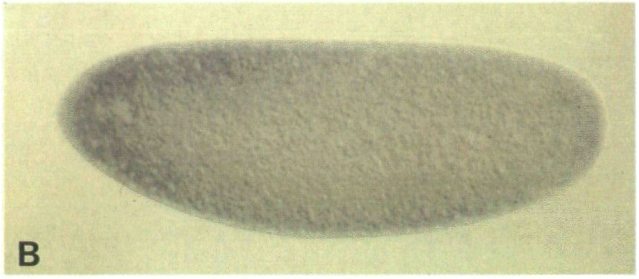
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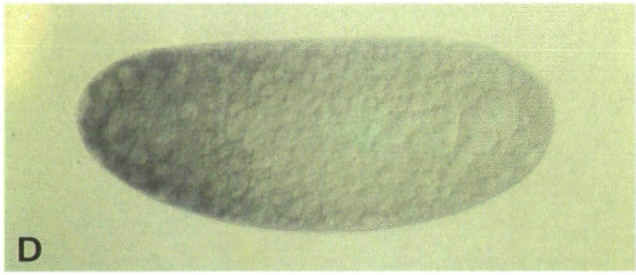
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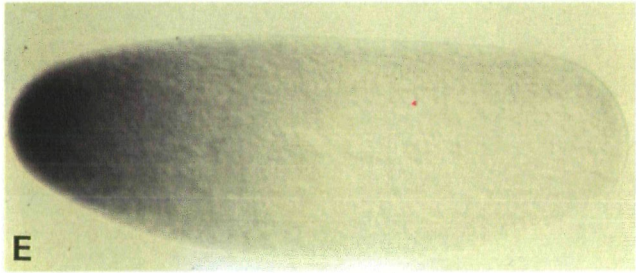
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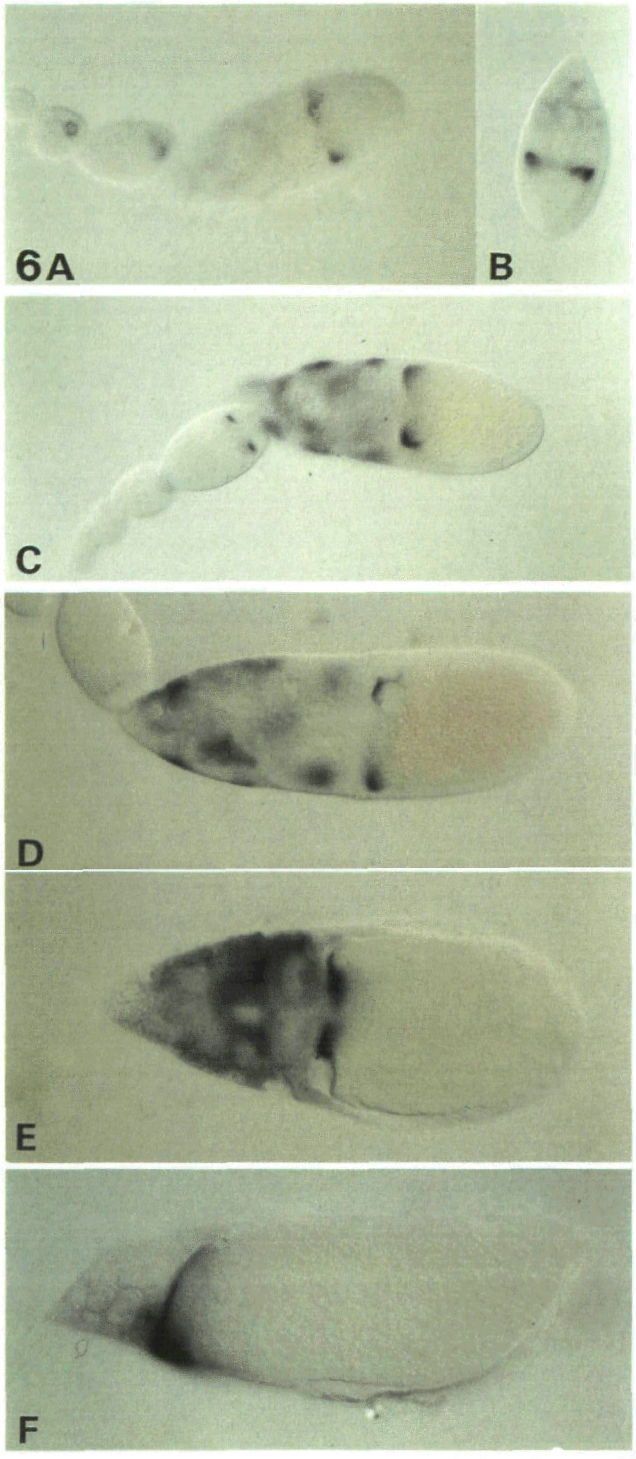

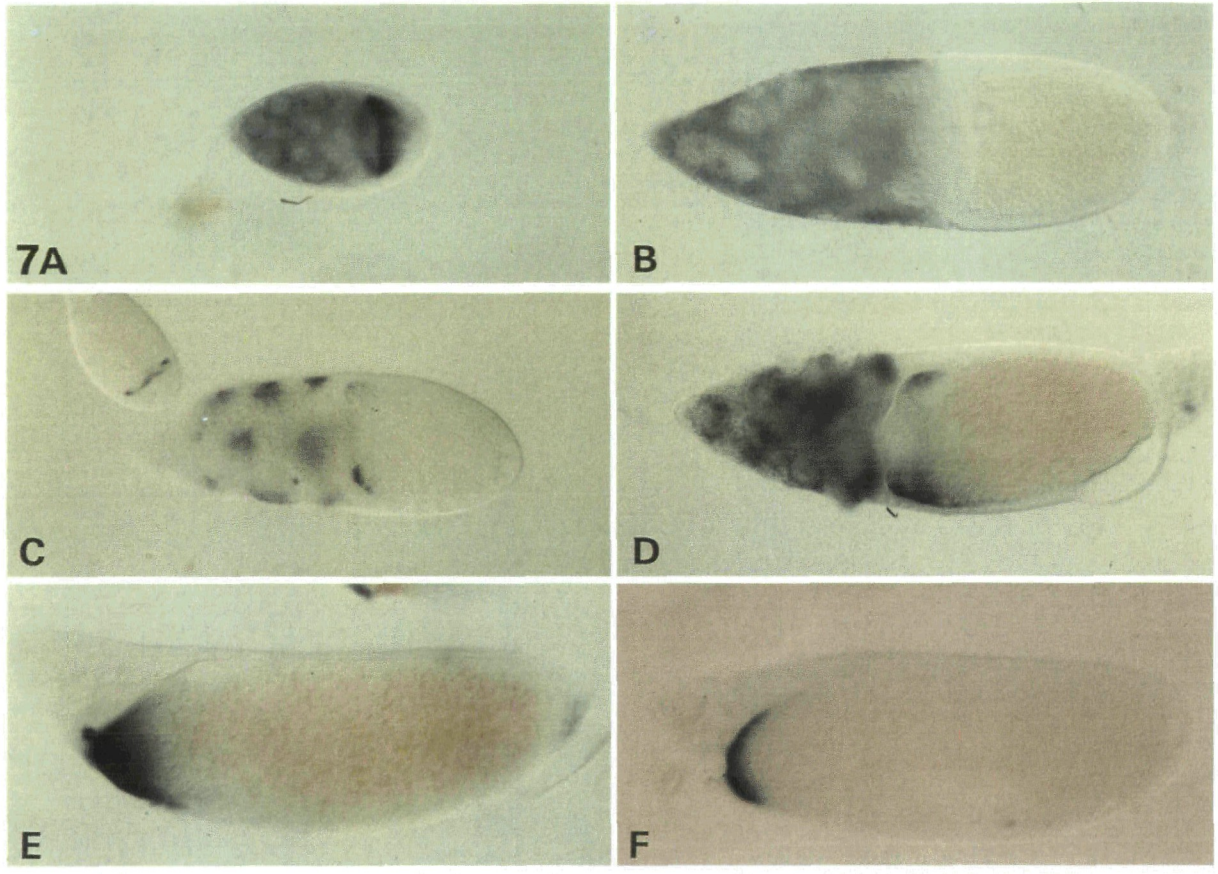

Fig. 6. The distribution of *bcd* RNA during wild-type oogenesis. (A) Part of an ovariole containing a stage-6 follicle with a ring of *bcd* RNA in the presumptive oocyte. The adjacent stage 7 and stage 9 follicles also show *bcd* RNA staining. (B) A stage 8 follicle with *bcd* RNA localized to the anterior margins of the oocyte. A weak signal can also be seen in the nurse cells. (C) A stage 9 follicle showing the apical localization in the 15 nurse cells, in addition to the anterior signal in the oocyte. The stage 8 follicle to the left also shows the anterior ring of RNA. (D) A stage 10a follicle. (E) A stage 10b follicle. The nurse cell localization is disappearing, as the nurse cells contract and the *bcd* RNA accumulates at the anterior pole of the oocyte. (F) A stage 12 follicle. The nurse cells are degenerating, and all *bcd* RNA is now localized to the cortex at the anterior of the oocyte.

Fig. 7. The distribution of *bcd* RNA in mutant ovaries. (A) exu^{PJ}, stage 7–8. The RNA forms a ring at the anterior end of the oocyte, but this ring appears more diffuse. (B) exu^{PJ}, stage 10a. The RNA shows a uniform distribution in the cytoplasm of the nurse cells. (C) sww^{14}, stage 9. The RNA localizes normally in the nurse cells and the oocyte. (D) sww^{14}, stage 10b. The cortical *bcd* RNA is no longer localized to the most anterior end of the oocyte but instead extends posteriorly. Much of the RNA entering the oocyte at this stage is not localized to the cortex, and leads to weak staining in the anterior third of the oocyte. (E) $stau^{79}$, stage 12. (F) $stau^{D3}$, stage 12. The *bcd* RNA localization is completely normal at this stage. We have also examined exu^{QR} and exu^{VL} ovaries which show the same phenotypes as exu^{PJ}.

Fig. 2 compares the distributions of *bcd* RNA and protein at the syncytial blastoderm stage. The levels of RNA and protein were quantified using the procedure described by Driever and Nüsslein-Volhard (1988*a*), and these results are presented graphically in Fig. 3. These measurements clearly demonstrate that the RNA is more tightly localized to the anterior end of the embryo, than is the protein. *bcd* RNA forms a steep gradient at the anterior end of the embryo, in which 90% of the RNA is restricted to the region anterior to 82% egg length (0% EL is the posterior pole). In contrast, bcd protein is distributed in a much shallower gradient, in which only 57% of the protein falls within this anterior region. The protein must therefore move posteriorly after it has been synthesized. One of the functions of bcd protein is to activate the anterior zygotic expression of hunchback (Schröder *et al.* 1988; Tautz, 1988; Driever and Nüsslein-Volhard, 1989). This anterior hunchback domain is shown in Fig. 2C, and illustrates that the bcd protein gradient must extend to at least 55% egg length, a position at which *bcd* RNA is not detectable above background.

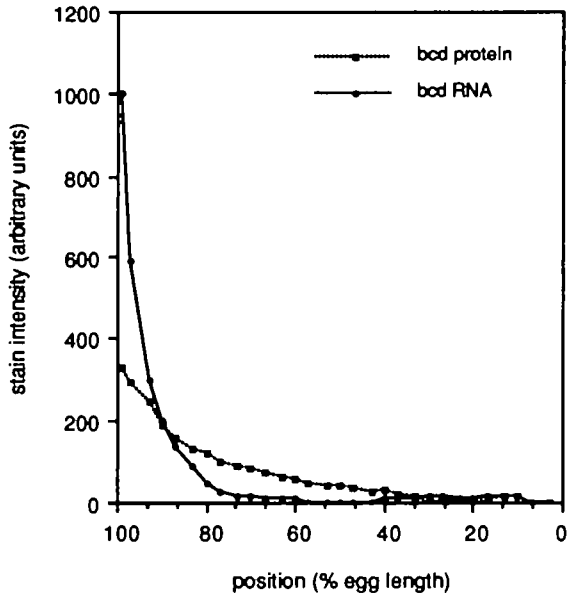

Fig. 3. The distribution of *bcd* mRNA (solid line) and bcd protein (dotted line) in the embryo. Whole wild-type embryos were stained for bcd protein, using an immunohistochemical method (Driever and Nüsslein-Volhard, 1988*a*), or for *bcd* mRNA, using the enzyme linked *in situ* detection technique. Video images of whole-mount embryos were taken and a background image subtracted. The distribution of the stain intensities along the anterior posterior midline was recorded for five embryos (nuclear cycle 13) and the average values at 30 equidistant positions calculated. Signal linearity of the *in situ* detection method was tested by performing the enzymatic colour reaction for 3 and a half or 16 minutes, respectively. Values in the anteriormost 15% of the embryos appear to be nonlinear in the 16 min reaction and were corrected accordingly. The areas under the RNA and protein curves have been made equal in order to allow a comparison of the two distribution profiles. Anterior is to the left.

bcd *RNA localization in mutant embryos*

In early (stage 1) embryos derived from *exu* homozygous mothers, *bcd* RNA is uniformly distributed throughout the egg cytoplasm (Fig. 4B). However, by syncytial blastoderm a shallow anterior–posterior gradient has formed (Fig. 4C). Berleth *et al.* (1987) have found that *bcd* RNA remains uniformly distributed in eggs laid by mothers which are mutant for both *exu* and the posterior group gene *vasa*. This suggests that the late *bcd* RNA gradient is created by the degradation of the RNA in the posterior region of the embryo, due to the activity of the posterior organizing centre (Nüsslein-Volhard *et al.* 1987).

Embryos laid by *sww* homozygotes have a more variable distribution of *bcd* RNA. While some embryos show no localization, most contain a weak anterior-to-posterior gradient (Fig. 4D). As is the case for *exu* mutant embryos, the gradient becomes more pronounced as development proceeds, due to the posterior degradation of the RNA. The variability of the *bcd* RNA distribution in *sww* mutant embryos is reflected in the variability in the final cuticular phenotype (Frohnhöfer and Nüsslein-Volhard, 1986).

In embryos derived from *staufen* mutant mothers, *bcd* RNA forms a gradient in the anterior region of the embryo (Fig. 4E). This phenotype is clearly distinct from that produced by *exu* or *sww* mutations, and cannot depend upon the posterior degradation of the RNA, since there is no localized posterior activity in *stau* mutant embryos (Nüsslein-Volhard *et al.* 1987; Lehmann and Nüsslein-Volhard, unpublished results). This partial mislocalization of *bcd* RNA leads to a shallower protein gradient (Driever and Nüsslein-Volhard, 1988*b*). The *bcd* RNA distributions in *exu*, *sww*, and *stau* mutant embryos are compared to the wild-type distribution in Fig. 5. Since these comparisons were performed using syncytial blastoderm embryos, *exu* and *sww* both show a shallow anterior-to-posterior RNA gradient.

The process of bcd *RNA localization during wild-type oogenesis*

bcd RNA can first be detected in late previtellogenic follicles (stages 5–6 of King (1970)) accumulating in a single, posteriorly located cell of the germ cell cluster (Fig. 6A). As oogenesis proceeds and the oocyte grows larger than the fifteen nurse cells, it becomes clear that this cell is the oocyte. At these stages, very little RNA can be seen in the nurse cells, and the RNA forms a ring around the anterior margin of the developing oocyte (Fig. 6B). This ring increases in size as the follicle grows. During stages 9–10a, large amounts of *bcd* RNA accumulate in the nurse cells (Fig. 6C,D). Unlike other maternal RNAs, *bcd* RNA is not uniformly distributed within the nurse cell cytoplasm, but is concentrated in a peripheral region adjacent to each nurse cell nucleus. During stages 10b and 11, the localization within the nurse cells gradually disappears, as the nurse cells contract and transfer their cytoplasm to the oocyte (Fig. 6E). Upon entering the oocyte, the *bcd* RNA becomes localized to the cortex of the anterior pole. At

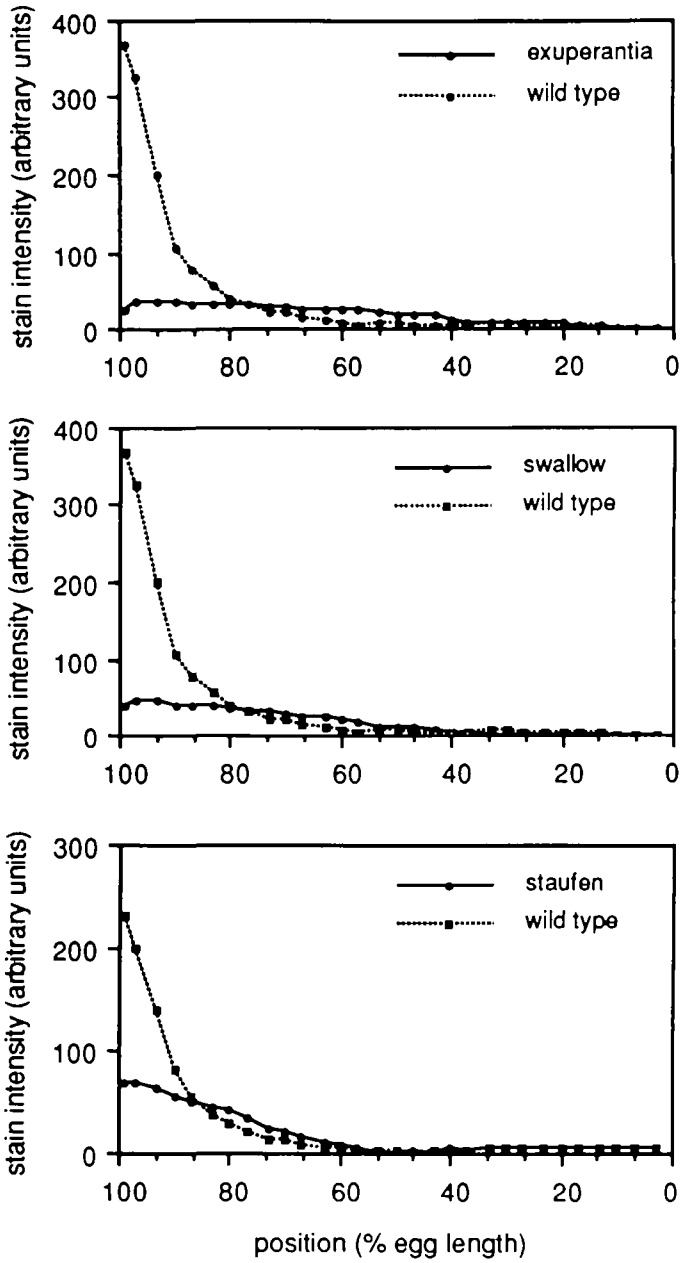

Fig. 5. Distribution of *bcd* mRNA in wild-type embryos and in embryos from females mutant for *exu*, *sww* and *stau*. The distribution of *bcd* mRNA in early nuclear cycle 13 embryos was visualized and measured as described in the legend to Fig. 3. The RNA distribution profiles of embryos derived from mutant females (solid lines; (A) exu^{PJ}/exu^{PJ}; (B) sww^{14}/sww^{14}; (C) $stau^{D3}/Df(2R)$ *PC4*) were plotted together with those of wild-type embryos (dotted lines; (A and B) wild-type controls stained in parallel to the mutant embryos; (C) wild-type embryos stained in the same batch as the *stau* mutant embryos, which were identified by the lack of polecells).

this stage, the RNA is no longer restricted to a ring around the anterior pole, and instead covers most of the anterior end of the oocyte. By stage 12, when the nurse cells are degenerating, *bcd* RNA is localized in a cap at the anterior end of the egg, with more of the RNA being found ventrally than dorsally (Fig. 6F). During the final few hours of oogenesis, stages 13 and 14, the follicle cells that surround the oocyte secrete the chorion (King, 1970). We have been unable to analyze *bcd* RNA localization during these stages because the vitelline membrane and chorion prevent the entry of the probe into the oocyte. However, the distribution of the RNA in stage 12 oocytes is different from that observed in very young eggs. In the oocytes, the RNA is localized to the cortex and is more concentrated ventrally, whereas in the early embryo, the RNA is found in a spherical region that extends into the interior of the egg and which is often located slightly dorsally. These differences suggest that there is a redistribution of *bcd* RNA, either during stages 13 and 14 of oogenesis, or immediately after fertilization.

bcd *RNA localization in mutant ovaries*

In order to understand how the mutations that alter the *bcd* RNA distribution in the embryo affect the four phases of RNA localization during oogenesis, we have performed *in situ* hybridizations on *exu*, *sww* and *stau* mutant ovaries. The earliest differences from wild-type *bcd* RNA localization are observed in *exu* mutant ovaries. The initial accumulation of *bcd* RNA during stages 5–7 occurs normally. As the oocyte increases in size, the RNA still forms a ring at the anterior end, but this ring often appears more diffuse (Fig. 7A). The first major deviation from normal oogenesis becomes apparent during stage 10a, when *bcd* RNA is not localized to the apical regions of the nurse cells, and instead shows a uniform distribution in the nurse cell cytoplasm (Fig. 7B). When this RNA enters the oocyte, it is not retained at the anterior pole, and from stage 10 onwards no localization within the oocyte is visible.

In the ovaries of *sww* homozygous females, the process of *bcd* RNA localization appears entirely normal up to stage 10a. The ring of RNA forms at the anterior end of the oocyte, and RNA accumulates in the apical regions of the nurse cells (Fig. 7C). During stages 10b and 11, the anterior ring of RNA seems to slip posteriorly and become more diffuse (Fig. 7D). In addition, much of the RNA that enters the oocyte during this time is not localized to the cortex. By stage 12, all *bcd* RNA appears to have been released from the cortex and forms a shallow anterior-to-posterior gradient.

In homozygous *stau* ovaries, the process of *bcd* RNA localization appears completely normal up until stage 12 (Fig. 7E,F), the latest stage that we have examined. Since the RNA is distributed in a gradient in early embryos, it must be released from the anterior pole after stage 12 of oogenesis.

Discussion

The experiments presented in this report use a whole-mount *in situ* technique to provide a detailed picture of the process of *bcd* RNA localization during oogenesis and embryogenesis. These results confirm and extend the previous analyses of *bcd* RNA localization, which

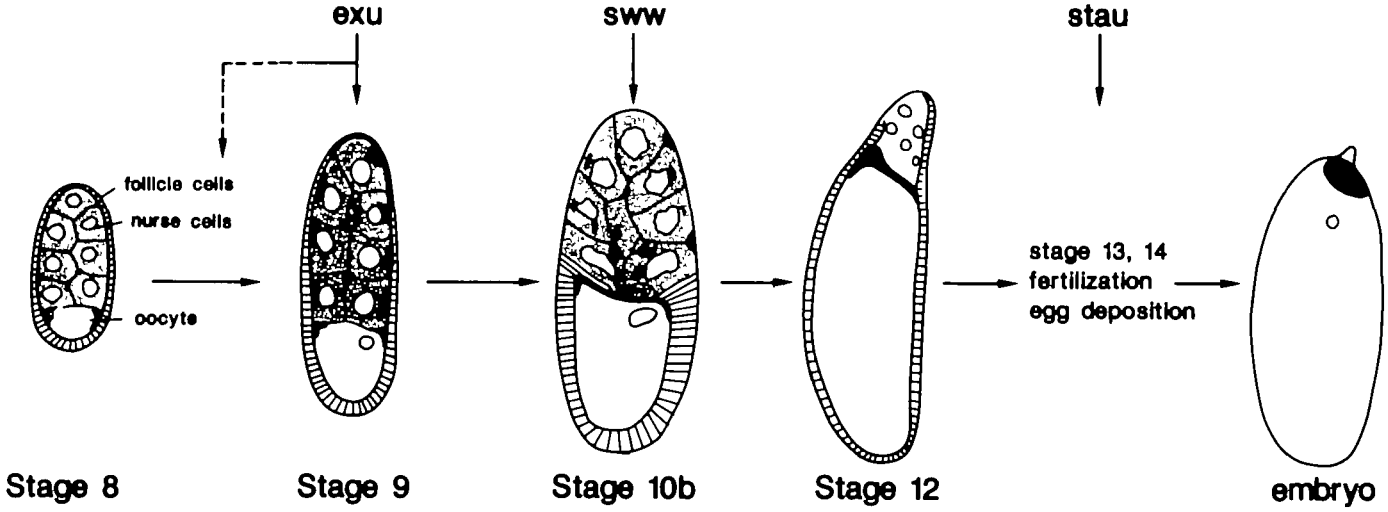

Fig. 8. A drawing showing the four phases of *bcd* RNA localization during oogenesis and the points at which *exu*, *sww*, and *stau* are required. The drawings are based on King (1972).

were performed using radioactive probes on sectioned material (Frigerio *et al.* 1986; Berleth *et al.* 1987; Stephenson *et al.* 1988). The wholemount procedure allows direct comparisons between the *bcd* RNA and protein distributions. As shown in Figs 2 and 3, the two distributions are quite different. Given that no RNA can be detected posterior to 60% egg length even in overstained embryos, whereas the protein gradient extends at least to 30% EL, the shape of the protein gradient must depend to some extent on the movement of protein molecules towards the posterior after they have been translated. Simple diffusion of the protein can account for this distribution (Driever and Nüsslein-Volhard, 1988*a*).

The *in situ* hybridizations to embryos derived from *staufen* mutant females reveal that, like *exuperantia* and *swallow*, *staufen* is required for the correct localization of *bcd* RNA to the anterior pole. However, *staufen* mutations only cause a partial mislocalization of *bcd* RNA. The anterior RNA gradient produced by these mutations results in a shallower bcd protein distribution, in which the anterior levels of bcd protein are strongly reduced compared to wild-type, and more posterior levels are slightly increased (Driever and Nüsslein-Volhard, 1988*b*). The anterior reduction in bcd protein concentration accounts for the loss of anterior head structures observed in *staufen* embryos (Schüpbach and Wieschaus, 1986). In addition to the head defects, *staufen* mutations produce a typical posterior group phenotype in which the abdomen is deleted and pole cells do not form. The phenotypes produced in double mutant combinations between *staufen* and other maternal effect mutations strongly suggest that the posterior effects of *staufen* are due to a failure to transport pole plasm constituents to the posterior pole (R. Lehmann, personal communication). Thus the *staufen* gene product is implicated in the localization of maternal factors to both the anterior and posterior poles of the egg.

Based on the geometry of the follicle, Frohnhöfer and Nüsslein-Volhard (1987) have proposed a simple model for *bcd* RNA localization, in which RNA entering at the anterior end of the oocyte is trapped and attached to the cytoskeleton by factors that are uniformly distributed in the egg. The present data suggest that the process of localization is likely to be more complex. The *in situ* hybridizations to wild-type ovaries reveal at least four phases of *bcd* RNA localization. In the first phase, which extends from stage 6 to early stage 9 of oogenesis, *bcd* RNA is found in a ring at the anterior end of the oocyte. During stages 9–10a, RNA is still found in this ring but *bcd* RNA also accumulates to high levels in the apical regions of the nurse cells. Stephenson *et al.* (1988) have also noticed the nonuniform distribution of *bcd* RNA in the nurse cells of sectioned ovaries. The apical localization of *bcd* RNA within the nurse cells is unusual since all other maternal RNAs that have been examined show a uniform distribution in the nurse cell cytoplasm, and no specialized cytological structures have been observed in these regions (for example Kobayashi *et al.* 1988; Sprenger *et al.* 1989). In the third phase, during stages 10b–12, the nurse cell localization disappears and all *bcd* RNA becomes localized to the cortex at the anterior pole of the egg. A final phase of RNA redistribution must occur after stage 12, to produce the spherical pattern of *bcd* staining seen in early embryos. The *bcd* RNA distributions during these four phases are presented schematically in Fig. 8. The second and fourth phases of *bcd* RNA localization cannot be explained by the simple model, since, during the second phase, the RNA is localized in the nurse cells before it enters the egg, and, in the fourth phase, the RNA is redistributed within the egg.

Since *exu* mutations lead to a uniform distribution of *bcd* RNA in the nurse cell cytoplasm as well as in the oocyte, it seems likely that in wild-type ovaries both localizations occur by similar mechanisms. The apical region of a nurse cell can be considered to be the anterior end of the cell, since it lies on the opposite side

of the cell from the ring canals which connect to the other nurse cells and the oocyte. Thus *bcd* RNA may be transiently localized to the anterior ends of the nurse cells in a similar fashion to its localization within the oocyte, suggesting that the nurse cells also possess an intrinsic anterior–posterior polarity. The molecules that are required for *bcd* RNA localization in the oocyte are most probably synthesized in the nurse cells. These molecules could therefore mediate the transient RNA localization within the nurse cells, before they themselves are transported into the oocyte. Since *bcd* RNA is synthesized within the nurse cells, this localization cannot depend upon the polar entry of the RNA into one side of the cell. Thus the localization mechanism can function, at least within the nurse cells, in the absence of an asymmetric source of the RNA. If the process of localization within the egg is similar to that in nurse cells, the anterior accumulation of *bcd* RNA within the oocyte may involve a more active mechanism than the simple trapping of the RNA as it enters at the anterior pole.

None of the mutations examined in this study completely disrupts all phases of *bcd* RNA localization. The earliest phenotypes are seen in *exu* mutant ovaries, in which the RNA does not become restricted to the apical regions of the nurse cells during stages 9–10a. *exu* mutations also seem to have a weak effect on the first phase of *bcd* RNA localization, since the anterior ring often appears more diffuse. Although we have used three different strong *exu* alleles, which all produce the same phenotype, it is possible that none of these is an amorphic mutation. In an *exu* null genotype, even the earliest phase of *bcd* RNA localization might be abolished. Berleth *et al.* (1988) have proposed that the *exu* gene product binds directly to *bcd* RNA, and mediates its attachment to the cytoskeleton. Since *exu* mutations abolish the localization in nurse cells, if *exu* does bind the RNA, it must first do so within the nurse cells. This raises the possibility that *bcd* RNA enters the oocyte as part of a ribonucleoprotein complex that also contains *exu* protein. If this is the case, one might expect *exu* protein to colocalize with *bcd* RNA at the anterior pole of the embryo.

In *sww* mutant ovaries, the third phase of *bcd* RNA localization is disrupted. As Berleth *et al.* (1988) and Stephenson *et al.* (1988) have previously noted, the RNA is gradually released from the cortex of the oocyte during stages 10b–11 of oogenesis. Since *sww* mutations also cause defects in nuclear migration and cellularization during embryogenesis, Frohnhöfer and Nüsslein-Volhard (1987) and Stephenson *et al.* (1988) have suggested that the *swallow* protein is a component of the cytoskeleton. In *sww* mutants, the lack of this protein would cause a destabilization of the cytoskeletal elements that anchor *bcd* RNA to the cortex of the oocyte and lead to a gradual release of the RNA. Our observation that *sww* mutations have no effect on the apical localization of the RNA in nurse cells provide support for the idea that *swallow* encodes an oocyte-specific component of the cytoskeleton.

In *staufen* homozygotes, the process of *bcd* RNA localization appears completely normal up until stage 12 of oogenesis, yet the RNA is not correctly localized in early embryos. The difference between these distributions indicates that the RNA must be released from the anterior pole sometime between stage 12 and egg deposition. This suggests that the *staufen* gene product may be required for the movement of *bcd* RNA from the anterior/ventral cortex to the more dorsally located spherical region observed in early embryos, and provides further evidence for a fourth phase of *bcd* RNA localization. The anterior gradient of RNA observed in *staufen* mutant embryos most probably results from the diffusion of the RNA during a short period of time between its release from the anterior pole and the start of embryogenesis. In general, there is a correlation between the stage at which a mutation affects *bcd* RNA during oogenesis and the distribution of the RNA in embryos. *exu* mutations show the earliest phenotype (stage 9) and result in a uniform distribution in the embryo, *sww* mutations disrupt localization during stages 10–11 and lead to a very shallow embryonic RNA gradient, while *staufen* alleles seem to cause a release of the RNA after stage 12, producing a much steeper gradient at the anterior pole.

This study has identified four phases of *bcd* RNA localization during oogenesis, and has demonstrated that *exu*, *sww*, and *stau* mutations each affect a different stage of this process. At present, we still lack any information on how this localization is achieved at a biochemical or cell biological level. Macdonald and Struhl (1988) have identified a 3′ untranslated region of of the *bcd* RNA that is sufficient for localization to the anterior half of the embryo. As the molecular analysis of the *trans*-acting factors involved in this process advances, we may discover how this region of the *bcd* RNA is recognized, and what components of the cytoarchitecture of the oocyte participate in each phase of the localization of *bcd* RNA to the anterior pole.

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