Molecular analysis of the swallow gene of *Drosophila melanogaster*

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We report the cloning and molecular characterization of the *swallow* gene, a maternal-effect gene of *Drosophila melanogaster*. Maternal-effect genes are transcribed only during oogenesis; one such gene, *bicoid*, encodes a message that is localized at the anterior end of oocytes, eggs, and early embryos. *swallow*⁺ activity is necessary during oogenesis for *bicoid* message localization. We show here that a fragment of a previously identified clone, introduced into the genome by P-element-mediated transformation, is able to rescue the maternal-effect lethality of *swallow* mutants, confirming the identity of this clone as *swallow*. By in situ hybridization, we show that *swallow* message is synthesized in nurse cells during oogenesis but is not localized along either the anterior-posterior or dorsal-ventral axes. We have confirmed an earlier finding that the localization of *bicoid* message is defective in *swallow*⁻ oocytes and eggs.

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In *Drosophila*, the specification of the embryonic axes utilizes information packaged into the egg during oogenesis (Anderson 1987; Nüsslein-Volhard et al. 1987). Anterior development relies on the product of the maternal-effect gene *bicoid*. *bicoid*⁻ embryos (i.e., embryos whose mothers were homozygous for *bicoid* mutant alleles) lack most segmented head and thoracic structures, and the unsegmented portion of the head, the acron, develops as its posterior equivalent, the telson (Frohnhofer and Nüsslein-Volhard 1986; Nüsslein-Volhard et al. 1987). *bicoid*⁻ embryos can be rescued by injecting cytoplasm from a wild-type embryo. However, only cytoplasm from the extreme anterior tip of the embryo is effective, suggesting that the *bicoid* gene product is highly localized (Frohnhofer and Nüsslein-Volhard 1986). The localization of *bicoid* activity results from the localization of *bicoid* mRNA at the anterior end of oocytes and embryos (Frigerio et al. 1986; Berleth et al. 1988). Two other maternal-effect genes, *swallow* and *exuperantia*, are necessary for the localization of *bicoid* activity; in *swallow*⁻ or *exu*⁻ embryos, *bicoid* activity is not localized at the anterior end of the egg, as assayed in cytoplasmic transfer experiments (Frohnhofer and Nüsslein-Volhard 1987), and *bicoid* message is distributed more or less uniformly (Berleth et al. 1988; this paper).

In this paper we report the cloning and initial characterization of *swallow*, one of the genes necessary for *bi-*

coid message localization. We previously described a molecular screen for clones of maternal-effect genes (Stephenson and Mahowald 1987) and presented evidence based on genomic proximity that one of these clones was the *swallow* gene. Here, we provide direct evidence that the clone identified previously contains the *swallow* gene and investigate the temporal and spatial expression of *swallow* RNA in oogenesis and embryogenesis. Also, we have examined the requirement for *swallow*⁺ activity in *bicoid* message localization.

Results

Mapping the position of the maternally restricted transcript

In an earlier publication (Stephenson and Mahowald 1987) we described the isolation of genomic clone B70, which encodes part of a 2.1-kb RNA that appears to be restricted to the maternal RNA population. We also showed that both clone B70 and the maternal-effect locus *swallow* are located within the small deficiency *Df(1)JF5*, suggesting that they represent the same gene. As a next step in the characterization of the maternal RNA encoded by clone B70, we isolated genomic clones adjacent to B70. The restriction map of a part of this walk is shown in Figure 1. Using probes from various parts of the walk for in situ hybridizations to the polytene chromosomes of larvae heterozygous for *Df(1)JF5*, we determined that the 28 kb of DNA represented in Figure 1 lies entirely within deficiency *Df(1)JF5* (data not shown).

To determine the position of the maternally restricted transcript, fragments of these clones were employed as

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Figure 2. Mapping the position of the maternally restricted RNA. (a) Northern blot analysis of the maternal-specific gene and environs. The restriction fragments indicated in Fig. 1 were used as probes in Northern blot analysis to nucleic acids extracted from the developmental stages indicated. Sizes of RNAs detected by these probes are given by the numbers to the left of panels 1, 5, and 8. The heterogeneous material near the top of some lanes is DNA. Probes: (1) *SalI*–*HindIII* 2.2-kb fragment from B70; (2) *HindIII*–*SalI* 3.4-kb fragment from B70; (3) *SalI*–*SstI* 2.55-kb fragment from B70; (4) *SstI*–*HindIII* 1.65-kb fragment from B70; (5) *HindIII*–*HindIII* 1.3-kb fragment from 782; (6) *HindIII*–*HindIII* 1.5-kb fragment from 782; (7) *HindIII*–*HindIII* 1.2-kb fragment from 782; (8) *HindIII*–*SstI* 3.8-kb fragment from 782. (b) Northern analysis of the 2.1-kb RNA, using asymmetric probes. ³²P-labeled T7 polymerase transcripts of probe 5 (as in Fig. 1) were hybridized to duplicate Northern blots. From the orientation of the insert in these clones, we conclude that the direction of transcription is left to right as the map is drawn in Fig. 1.

above to encode the maternally restricted 2.1-kb RNA and ~0.3 kb of downstream DNA. It also contains 1.6 kb of sequence upstream from the leftmost *HindIII* site and includes a portion of the DNA that encodes the 3.8/3.4-kb RNA.

A mixture of this construct and the transposase helper plasmid pII25.7wc (Karess and Rubin 1984) was injected into *ry*[−] embryos, and *ry*⁺ flies in the G2 generation selected to begin lines. Lines in which *ry*⁺ segregated as an autosomal character were crossed into a *sww*[−] *ry*[−] background, and stocks of the genotype *sww*¹⁴⁹⁷/*sww*¹⁴⁹⁷; *ry*⁵⁰⁶/*ry*⁵⁰⁶; P[*ry*⁺, *sww*⁺] *t528.11* were constructed and maintained. *ry*⁺ inserts on the X chromosome were crossed onto a *sww*¹¹⁻⁹⁹⁹ X chromosome and maintained in a *ry*⁵⁰⁶/*ry*⁵⁰⁶ third chromosome background. The chromosomal positions of the P-element

inserts were determined by in situ hybridization to polytene chromosomes and are shown in Table 1.

Restoration of *swallow*⁺ function is scored easily as the survival of progeny past the first larval instar: Almost all progeny from *swallow*[−] females die as unhatched embryos, and the rest die as small first instar larvae (Stephenson and Mahowald 1987). For each of the seven lines containing a P-element-mediated insertion, the introduced 5.9-kb fragment rescued the maternal-effect lethality of *swallow*. For five lines in which the P-element transposon was inserted on an autosome, we compared the development of embryos whose mothers were sisters of the genotypes *sww*/*sww*; *ry*/*ry* and *sww*/*sww*; *ry*/*ry*; P[*ry*⁺, *sww*⁺] *t528.11*. All progeny produced by *ry*[−] females died with the lethal *swallow* phenotype. However, *ry*⁺ females produced viable embryos

Table 1. *P*-element transformation rescues swallow

Stock	Chromosomal position	Survival (%)
bb154	73BC	98.1
bb275	46CD	97.0
bb344	53BC	81.0
bb421	7C	90.1
bb524	1F	94.1
bb635	45CD	93.5
bb854	94EF	97.4

Stock numbers for seven lines of flies transformed with *P*-element construct t528.11 are given in the first column. The chromosomal site of insertion, as determined by in situ hybridization to polytene chromosomes, is given in the second column. The third column presents measurements of the survival to the second larval instar of progeny from females of the genotype *sww*¹⁴⁹⁷/*sww*¹¹⁻⁹⁹⁹; *ry*⁵⁰⁶/*ry*⁵⁰⁶; *P*[(*ry*⁺, *sww*⁺) t528.11]. Each of seven different sites of insertion rescues the maternal-effect lethality of flies whose resident *swallow* genes are both mutant.

at a frequency equivalent to that of wild-type flies (Table 1). For the two lines in which the *ry*⁺ construct was inserted on the X chromosome, we recombined the *P*-element insert onto a *sww*⁻ X chromosome and found that the *P*-element insert was able to rescue the maternal-effect lethality of *swallow*. For each of these X chromosome lines, we subsequently removed the *ry*⁺ *sww*⁺ *P* element by recombination to confirm that the resident copy of *swallow* was a mutant allele.

For six of the seven lines, >90% of the embryos produced by transformed females survived to the second larval instar (Table 1). This value is similar to the survival of the progeny of wild-type flies tested under the same conditions. Surviving larvae were morphologically normal, and virtually all developed into normal adults. Of the progeny that failed to reach the second larval instar, none developed with a *swallow*⁻ phenotype, and most appear to have been unfertilized eggs. For the seventh line, *bb344*, only ~80% of the progeny developed normally. However, the chromosome containing this insert is homozygous lethal and confers a weak dominant Minute phenotype; both phenotypes are apparently associated with the *swallow*⁺ *P*-element-mediated insert. We believe that the lower survival rate of these embryos resulted from a generally lower viability in this stock and is not related to *swallow* function. As was true for the other lines, none of the offspring that died before the second larval instar had a *swallow*⁻ phenotype.

Synthesis and accumulation of swallow RNA during oogenesis and embryogenesis

We determined the spatial pattern of expression of *swallow* message in oogenesis and embryogenesis by in situ hybridization to sectioned ovaries and embryos (Fig. 3; see Methods). Oogenesis begins with four incomplete divisions of a cytotblast that generates a cyst of 16 interconnected cells, one of which becomes the oocyte, and the other 15 of which become nurse cells. The 15 nurse cells, the oocyte, and a surrounding layer of follicle cells of somatic origin are known as the egg chamber. We are able to detect *swallow* message reliably in stage-3 egg chambers; younger stages have lower autoradiographic signals that are only slightly above background levels [Fig. 3a; for a description of oogenesis and its morphological stages, see Mahowald and Kambyzellis (1980)]. The amount of *swallow* message increases in previtellogenic stages, reaching its highest concentration in the nurse cell complex of stage-10 egg chambers. In early stages, *swallow* message appears to be distributed uniformly in the oocyte–nurse cell complex and is absent from the surrounding follicle cells. As the egg chamber grows during these previtellogenic stages, the concentration of *swallow* message increases substantially, but its level remains approximately equivalent in nurse cells and in the oocyte proper. In vitellogenic stages of *Drosophila* oogenesis, the oocyte grows through the accumulation of yolk, synthesized in fat bodies and in the follicle cells. By stage 10 (Fig. 3b) when the oocyte occupies about half of the egg chamber, the concentration of *swallow* message is clearly higher in nurse cells than in the oocyte. Within nurse cells, the concentration of *swallow* message is uniform except for the nurse cell nuclei, which have substantially lower levels. In stages of oogenesis following that shown in Figure 3b, the nurse cells empty their cytoplasmic contents into the oocyte. The end product of this process, the stage-14 oocyte shown in Figure 3c, has a uniform distribution of *swallow* message.

Early embryogenesis involves the rapid cleavage of zygotic nuclei and their migration to the periphery of the embryo. In cleavage-stage embryos, *swallow* message is distributed uniformly along anterior–posterior and dorsal–ventral axes (Fig. 3d). As the nuclei migrate to the periphery of the embryo, *swallow* message becomes most highly concentrated in the noncytoly peripheral cytoplasm that surrounds the nuclear layer (Fig. 3e). The amount of *swallow* message in the embryo is reduced during the transition from the cleavage stage to the blas-

Figure 3. *swallow* message accumulation in ovaries and embryos. *swallow* mRNAs were detected by in situ hybridization to sections of wild-type ovaries and embryos. Bright-field (left) and dark-field (right) micrographs are shown. The bar in *a* represents 100 μ m. Exposures for *a* and *b* were 8 days and for *c–f* 6 days. (*a*) A cluster of previtellogenic egg chambers. Stages of oogenesis, as described in Mahowald and Kambyzellis (1980), are indicated by the numbers. *swallow* message concentration increases in these early stages, reaching its highest level in the nurse cells of stage-10 egg chambers (lower left and lower right; see also *b*). (*b*) Stage-10 egg chamber. Stage-10 nurse cells contain the highest concentration of *swallow* message, which is several times higher than in stage-10 oocytes. *swallow* RNA is absent from follicle cells at this and all other stages of oogenesis. (N) Nurse cell complex; (O) oocyte; (F) follicle cells. (*c*) Stage-14 oocyte. *swallow* message is uniform in its distribution. (*d*) Preblastoderm embryo. *swallow* message is uniformly distributed. (*e*) Syncytial blastoderm embryo. *swallow* message is more highly concentrated at the embryonic periphery. (*f*) Cellular blastoderm embryo. The small amount of *swallow* message still detectable is in the cellularized periphery of the embryo.

totoderm stage (cf. Fig. 3d and e). The micrographs shown in Figure 3, d–f, are from the same experiment and are the same autoradiographic exposure, so the decrease in signal observed at successive embryonic stages roughly

represents the actual decrease in *swallow* message levels. During cellularization of the blastoderm (Fig. 3f), the concentration of *swallow* message is reduced further, although that remaining is concentrated in the pe-

Figure 3. (See facing page for legend.)

ripheral cytoplasm. *swallow* message can no longer be detected by the beginning of gastrulation (not shown).

The uniform distribution of *swallow* message levels in oocytes and early embryos raises the concern that these hybridization results might be due to a nonspecific association of the probe with the tissue. As a control for the specificity of *swallow* message hybridization, we hybridized sense strand RNA probes to early embryos; there is no detectable signal using such a probe (not shown). In addition, although *swallow* message levels are uniform in oocytes and early embryos, the absence of a hybridization signal in other tissues (follicle cells, nurse cell nuclei, postblastoderm embryos) argues against such a nonspecific association.

bicoid message localization in wild-type and *swallow*⁻ egg chambers

As reported previously (Frigerio et al. 1986; Berleth et al. 1988), *bicoid* message is localized to the anterior end of oocytes and early embryos. Examples of the localization pattern in wild-type egg chambers are shown in Figure 4. Figure 4a shows the distribution of *bicoid* message in a stage-10 wild-type egg chamber, where it is present in the nurse cell complex and localized to the anterior margin of the oocyte, the part of the oocyte closest to the nurse cells. *bicoid* message is clearly nonuniform, or 'patchy,' in its distribution in nurse cell cytoplasm, in contrast to the relatively uniform distribution of *swallow* message in nurse cell cytoplasm (cf. Figs. 3b and 4a). *bicoid* message remains restricted to the anterior oocyte margin in subsequent stages, as in the full-grown stage-14 oocyte shown in Figure 4b.

bicoid message distribution in *swallow*⁻ oocytes is abnormal, with defects that become apparent during vitellogenic stages of oogenesis. In stage-10 egg chambers (Fig. 4c) *bicoid* message is restricted to the oocyte margin, as in wild-type oocytes at this stage. However, in contrast to the strict anterior localization observed in wild-type oocytes, *bicoid* message is most highly concentrated in lateral regions of *swallow*⁻ oocytes (arrows in Fig. 4c). Older oocytes show a progressive degeneration of the localization pattern, as in the stage-12 *swallow*⁻ oocyte shown in Figure 4d: *bicoid* message is still highly concentrated in the oocyte periphery, but this peripheral localization includes the anterior two-thirds of the oocyte surface. In addition, there are significant levels of *bicoid* message in internal parts of the oocyte. Finally, in mature *swallow*⁻ oocytes (Fig. 4e), *bicoid* message is only slightly more concentrated at the oocyte periphery than in internal regions and is distributed in a shallow anterior-posterior gradient, with the anterior end of the oocyte having the highest *bicoid* message levels. The micrographs shown in Figure 4, c-e, are from a different experiment and a shorter autoradiographic exposure than those shown in Figure 4, a and b, so differences in signal intensity in Figure 4 do not indicate differences in *bicoid* message levels in wild-type and *swallow* egg chambers. On the contrary, we believe that *bicoid* message levels in *swallow* egg

chambers are approximately the same as in wild-type egg chambers, based on visual comparison of in situ hybridization signals from an experiment where wild-type and *swallow* slides had the same exposure time (data not shown). We have also examined *bicoid* message levels by dot blot analysis and found that message levels are approximately the same in wild-type and *swallow* ovaries (N.J. Pokrywka and E.C. Stephenson, unpubl.).

Discussion

We report here the initial molecular characterization of the maternal-effect gene *swallow* of *Drosophila melanogaster*. We show that a cloned fragment is able to rescue the maternal-effect lethality of *swallow* alleles, proving that a 2.1-kb maternally restricted transcript encoded therein is the transcript of the *swallow* gene. *swallow* was identified originally in genetic screens for female sterile and maternal-effect mutations (Gans et al. 1975; Mohler and Carroll 1984; Stephenson and Mahowald 1987). All known alleles of *swallow* are homozygous and hemizygous viable, and males have normal fertility. However, females produce only defective embryos, most of which die at the end of embryogenesis with defects in the cephalopharyngeal apparatus, the anterior-most part of the embryonic body plan. From its genetic behavior, *swallow* has been classified as a maternal-effect lethal, a gene whose expression should be limited to oogenesis. We showed previously by Northern blot analysis that *swallow* message is detectable only in the ovaries of adult females and in very early embryos (Stephenson and Mahowald 1987). In situ hybridization experiments presented here extend these observations, showing that nurse cells and the oocyte contain high levels of *swallow* message but that *swallow* message is undetectable in follicle cells. These results are consistent with genetic experiments showing that *swallow*⁺ activity is necessary in the germ-line portion of the ovary (i.e., nurse cells and the oocyte) but not necessary in ovarian tissues of somatic origin (i.e., follicle cells) (Perrimon and Gans 1983). Furthermore, in situ hybridization experiments shown here indicate that high levels of *swallow* message are present in the newly fertilized egg but decrease rapidly during the blastoderm stages of early embryogenesis. Because *swallow* message levels begin to decrease at the time when the zygotic genome first becomes active (the syncytial blastoderm stage; Zalkar 1976; Edgar and Schubiger 1986), we think it unlikely that transcription from the zygotic genome contributes significantly to *swallow* message levels in the early embryo, once again as would be expected for a strict maternal-effect gene.

Data presented here show that a 5.9-kb cloned fragment rescues the maternal effect lethality of *swallow*. Seven independent lines, each with a different genomic site of insertion for *swallow*⁺, were effective; in this small sample we did not find a genomic position that allowed *rosy*⁺ expression but failed to permit expression of *swallow*⁺ sufficient to rescue the maternal-effect lethality of *swallow* mutants. Although these data indi-

Figure 4. *bicoid* mRNAs in wild-type and *swallow*⁻ egg chambers. ³⁵S-Labeled *bicoid* RNA probes were hybridized to sectioned ovaries as described in Methods. Bright-field (left) and dark-field (right) micrographs are shown. The bar in *a* represents 100 μ m. Exposure time for *a* was 13 days and for *b–e* 6 days. (*a*) Stage-10 wild-type egg chamber. *bicoid* message accumulates in the oocyte at its nurse cell-proximal margin. (*b*) Stage-14 wild-type oocyte. *bicoid* message is prominent at the anterior margin of the mature oocyte. (*c*) Stage-10 *swallow*⁻ egg chambers. *bicoid* message is predominantly localized to the oocyte margin, but to the lateral rather than the anterior margin of the oocyte (arrows). (*d*) Stage-12 *swallow*⁻ oocyte. Much of the *bicoid* message is localized at the periphery of the oocyte, although some is internal as well. Degenerating nurse cells (N) contain no *bicoid* message. (*e*) Stage-14 *swallow*⁻ oocyte. The peripheral concentration of *bicoid* message seen in earlier stages is reduced, and *bicoid* message is distributed more or less uniformly on the anterior-posterior axis, with a slightly higher concentration at the anterior end of the egg.

cate the molecular identity of the *swallow* gene, they do not address the question of whether the P-element-inserted copies of *swallow* are expressed correctly. We have not attempted to quantitate the level or timing of expression from the P-element-inserted copies, nor do we know what level of *swallow*⁺ expression is required

to rescue the maternal-effect lethality of the *swallow* mutants we employed in these studies.

Despite its role in the *bicoid* message localization process, *swallow* message is not itself localized along the anterior-posterior axis. The distribution of *swallow* message is notable, however, in a few respects. First, in

vitellogenic stages of oogenesis, there are higher concentrations of *swallow* message in the nurse cell complex than in the oocyte itself. This accumulation in nurse cells may reflect a requirement that most of the *swallow* protein be translated in nurse cells and not in the oocyte. As an alternative, *swallow* message accumulation may reflect the kinetics of synthesis and export: Nurse cell nuclei may be synthesizing *swallow* message at a rate that exceeds the rate of transport into the oocyte. Second, in blastoderm embryos *swallow* message distribution changes from a uniform pattern to one in which it is more highly concentrated at the embryonic periphery. Because this redistribution is accompanied by a reduction in the quantity of *swallow* message in the embryo, we do not know whether this change reflects the movement of *swallow* message, its selective degradation in the center of the embryo, or a combination of both mechanisms.

One of the roles of *swallow* is to localize or maintain the localization of *bicoid* message. Frohnhofer and Nüsslein-Volhard (1987) inferred such a role for *swallow* and *exuperantia* because they were unable to find high concentrations of *bicoid*⁺ rescuing activity at the anterior tip of *sww*⁻ and *exu*⁻ early embryos. This observation has since been confirmed at the molecular level by Berleth et al. (1988) and for *swallow* in the experiments reported in this paper. One can envision two general classes of models for the mechanism of *bicoid* message localization during oogenesis. In the first, cytoplasm at the anterior end of the egg is different from cytoplasm elsewhere, and *bicoid* message is localized by a homing or transport mechanism that depends on this preexisting molecular inhomogeneity. In the second model, oocyte cytoplasm is uniform, with the capacity to localize *bicoid* message everywhere: *bicoid* message is 'trapped' as it enters the oocyte from the nurse cells, and the anterior localization of *bicoid* message results simply from the anterior location of nurse cells. We favor the second model for the simple way in which the inherent polarity of the egg chamber is translated into embryonic polarity. In support of the second model, the studies reported here indicate that *swallow* message is not itself localized or concentrated along the anterior-posterior axis. Of course, the observations on *swallow* do not directly invalidate the first model described above: *swallow* protein may be localized even though its message is not, or another component of the *bicoid* message localization machinery may be localized, whereas the *swallow* RNA and protein are distributed uniformly.

We show here that the failure to localize *bicoid* message in *swallow*⁻ oocytes is not absolute: In early vitellogenic stages, *bicoid* message is localized, but as oogenesis proceeds, the localization deteriorates. The significance of the transient *bicoid* message localization is not clear, but it is interesting to speculate that it reflects the role of the *swallow* gene product in this process. The *swallow*⁺ protein may, for example, cross-link or stabilize the cytoskeletal elements to which *bicoid* message is presumably attached. In this case, one might imagine that the initial localization of *bicoid* message in

swallow⁻ oocytes results from the near-normal assembly of a cytoskeletal system and that the localization deteriorates later in the absence of a *swallow*⁺-dependent stabilization of this cytoskeletal network. As noted by others (Frohnhofer and Nüsslein-Volhard 1987; Berleth et al. 1988), a role for *swallow* in cytoskeletal function is attractive because some of the embryos from *swallow*⁻ mothers are abnormal with respect to nuclear migration and cellularization of the blastoderm, processes that probably require cytoskeletal functions. On the other hand, it is conceivable that the partial loss of *bicoid* message localization may result from partial loss of *swallow*⁺ function in the mutants employed in the in situ hybridization experiments. The animals used here were heterozygous for a strong *swallow* allele (*sww*¹¹⁻⁹⁹⁹) and a deficiency for the locus, *Df(1)JF5*, the strongest phenotypic combination available to us. We have observed the same gradual loss of *bicoid* message localization, using flies heterozygous for another strong allele, *sww*¹⁴⁹⁷, and the deficiency (E.C. Stephenson, unpubl.). However, we are not certain that these genotypic combinations represent the amorphic condition and cannot rule out the possibility that the transient localization of *bicoid* message in these oocytes reflects residual *sww*⁺ function.

The eggs of many animals are nonhomogeneous, with distinctive cytoplasmic domains or organelles localized in particular regions. These inhomogeneities may exist prior to fertilization or may come about through a cytoplasmic rearrangement shortly after fertilization. In some cases, it has been shown that the segregation of a particular cytoplasmic domain into a subset of blastomeres during the early embryonic cleavages is necessary and sufficient for the development of particular embryonic tissues. The pole plasm in *Drosophila*, the polar granules in *Caenorhabditis elegans*, the yellow crescent in ascidians, and the polar lobe cytoplasm of mollusk and annelid eggs are well-characterized examples (for review, see Davidson 1986). Some of these systems have been studied for many years, yet there are only a few studies on the cellular mechanisms of cytoplasmic localization in eggs and cytoplasmic reorganization in embryos. In ascidian embryos, the yellow pigment granules appear to be associated with a cortical actin-rich cytoskeleton (Jeffrey and Meier 1983), and the concentration of these granules into the yellow crescent requires actin filament function (Zalokar 1974). In *C. elegans*, the P granules coalesce and segregate into the posterior P1 cell at the first cleavage; this movement requires actin function (Strome and Wood 1983). In *Xenopus* embryos, specification of the dorsoventral axis involves a signal that emanates from the vegetal pole, and its transport to or utilization by the equatorial mesoderm requires microtubule function (Scharf and Gerhart 1983). The conventional view has been that molecules or organelles necessary for particular developmental decisions are bound in the specialized cortical cytoplasm of the egg, a region devoid of yolk granules and lipid droplets and relatively rich in cytoskeletal elements (for review, see Davidson 1986). However, this cortical cytoskeleton is not

well characterized, and the mechanisms by which molecules or organelles might be segregated into an asymmetric position in the egg and embryo and maintained there are not well understood. The specification of anterior development in *Drosophila* can be added to the list of systems in which an asymmetrically localized cytoplasmic domain specifies the development of embryonic pattern. The molecular and genetic analysis of *swallow* and other genes that participate in the *bicoid* message localization process should bring us closer to a general understanding of the mechanism of cytoplasmic localization.

Methods

Mutants

Alleles of *swallow* and *Df(1)JF5* are described in Gans et al. (1975), Mohler and Carroll (1984), and Stephenson and Mahowald (1987). *ry⁵⁰⁶* is described in Coté et al. (1986). Other mutations are described in Lindsley and Grell (1966).

Genomic walking

Genomic clone B70 was isolated from the Canton S library of Maniatis et al. (1978), as described in Stephenson and Mahowald (1987). Other clones were isolated by homology to B70. λ 782 was isolated from the Canton S library, and λ 528 was isolated from a genomic library constructed by Robert Boswell from a strain of flies that he isogenized for a *cn bw* second chromosome. Library screening, phage growth, and clone characterization were carried out according to standard procedures (Maniatis et al. 1982).

Northern blots

Total nucleic acids were extracted from staged embryos, denatured with glyoxal, electrophoresed, blotted, and hybridized, all as described previously (Stephenson and Mahowald 1987). Most Northern blots employed nick-translated restriction fragments as probes. To determine the direction of transcription of the 2.1-kb maternally restricted RNA, the 1.3-kb *Hind*III fragment from clone B70 was cloned into pGEM2 (Promega). Clones with the insert in each orientation were identified by an asymmetrically positioned restriction site. Each was digested with *Eco*RI, which cuts only within the multiple cloning site, and ³²P-labeled transcripts were produced using T7 RNA polymerase (Promega), using the reaction conditions suggested by the enzyme supplier. The two probes were hybridized to Northern blot filters using the same procedures as for DNA probes.

P-element transformation

The 5.9-kb *Sst*I–*Eco*RI fragment from λ 528 (see Fig. 1) was subcloned into the *Sst*I–*Eco*RI sites of pUC18. The resulting plasmid, p528.1, has a *Sal*I site at one end of the insert, because there is a *Sal*I site in the multiple cloning site of pUC18. To create a *Sal*I site at the other end of the insert, p528.1 was digested with *Eco*RI and treated with Klenow DNA polymerase to fill in the overhanging ends, and *Sal*I linkers were added by ligation with T4 DNA ligase. The reaction was partially digested with *Sal*I and electrophoresed, and an 8.6-kb band was collected and eluted. This fragment was ligated at low DNA concentrations to promote recircularization and transformed into competent bacteria. The resulting plasmid, p528.11, con-

tains the 5.9-kb fragment of λ 528 with *Sal*I sites at each end. This fragment was recloned into the *Sal*I site of the P-element cloning vector Carnegie 20 (Rubin and Spradling 1983), and transformants containing the desired fragment were identified by colony hybridization. The λ 528-P-element construct, t528.11, was purified using a protocol that included banding in a CsCl–ethidium bromide gradient. Earlier attempts at P-element transformation using DNA that had not been purified by CsCl banding were unsuccessful.

Plasmids t528.11 and p125.7wc (Karess and Rubin 1984) were mixed, ethanol-precipitated, and resuspended in 5 mM KCl, 0.1 mM NaPO₄ (pH 6.9), at a final concentration of 300 μ g/ml for t528.11 and 50 μ g/ml for p125.7wc. Injection of preblastoderm *ry⁵⁰⁶* embryos and selection of *ry⁺* transformants were as described (Rubin and Spradling 1982; Spradling and Rubin 1982; Spradling 1986).

Lines of flies with *ry⁺* inserts were established and maintained initially by manual selection for *ry⁺* at each generation. Outcrosses of *ry⁵⁰⁶/ry⁵⁰⁶*; P[(*ry⁺*, *sww⁺*) t528.11] males to *ry⁵⁰⁶/ry⁵⁰⁶* females revealed lines with P-element inserts on the X chromosome. For the autosomal inserts, we crossed the P-element construct-bearing stock to *sww¹⁴⁹⁷ v*; *ry⁵⁰⁶/ry⁵⁰⁶*, to produce, in two generations, stable stocks of the genotype *sww¹⁴⁹⁷ v/sww¹⁴⁹⁷ v*; *ry⁵⁰⁶/ry⁵⁰⁶*; P[(*ry⁺*, *sww⁺*) t528.11]. *ry⁻* females in these lines produce only progeny with the *sww⁻* phenotype, whereas *ry⁺* females produce normal progeny (see text). For the two transformed lines in which the P-element construct was inserted on the X chromosome, we crossed the P-element insert onto a *sww¹¹⁻⁹⁹⁹*, *y cv v f* X chromosome in a *ry⁵⁰⁶/ry⁵⁰⁶* background, selecting for *ry⁺*, *cv⁻* recombinants (*cv* is only 4 cM from *sww* and is thus a reasonably good visible marker for *swallow*), creating a stable stock of the genotype *sww¹¹⁻⁹⁹⁹*, P[(*ry⁺*, *sww⁺*) t528.11]; *ry⁵⁰⁶/ry⁵⁰⁶*. For each of these two lines, we subsequently removed the P-element insert from the X chromosome by recombination, selecting for *ry⁻*, *cv⁻* recombinants, to confirm that the resident *swallow* allele was mutant.

Embryonic development was measured using females of the genotype *sww¹⁴⁹⁷/sww¹¹⁻⁹⁹⁹*; *ry⁵⁰⁶/ry⁵⁰⁶*; P[(*ry⁺*, *sww⁺*) t528.11], mated to an excess of Oregon R males. Eggs were collected on lightly yeasted grape juice/agar plates overnight. The eggs were counted and allowed to develop at 25°C for 48 hr. Rescue of the *swallow⁻* phenotype was calculated as the percentage of eggs that develop to second larval instar, avoiding the complication that some embryos of *swallow⁻* mothers hatch but die during the first larval instar.

In situ hybridization to polytene chromosomes to determine the site of insertion of the P-element construct was carried out as described (Stephenson and Mahowald 1987), using a p528.1 probe.

In situ hybridizations to oocytes and embryos

swallow probe was synthesized from plasmid g528.4, a 2.1-kb genomic fragment in the vector pGEM3-blue (Promega). The plasmid contains a *Hind*III–*Pst*I fragment between coordinates 6.0 and 8.1 in Figure 1. The plasmid was digested with *Hind*III and purified by phenol–chloroform extraction and ethanol precipitation. Antisense RNA was synthesized from the T7 promoter. *bicoid* probe was synthesized from plasmid pC'3-2:2, a 1.5-kb cDNA clone inserted in Bluescribe+ (Stratagene) and provided by Mark Seeger and Thom Kaufman. The plasmid was truncated by digestion with *Xba*I and purified as above, and the probe was synthesized from the T7 promoter. Transcription reactions were carried out as described (Ingham et al. 1985), using a mixture of ³⁵S-labeled thio-UTP (Amersham) and cold thio-UTP to give a final concentration of 75 nM. Specific activity of

the resulting probe was 2.2×10^8 dpm/ μ g. T7 RNA polymerase was obtained from Promega or was the generous gift of Paul Kingsley. After the transcription reaction, nucleic acids were purified by phenol–chloroform extraction and ethanol-precipitation, digested with DNase I (Promega RQI DNase), phenol–chloroform-extracted, and ethanol-precipitated, and the RNA probe degraded with 0.1 M sodium carbonate (pH 10.2) to give a mean fragment length of about 150 nucleotides (Angerer et al. 1987). The RNA was precipitated with 2-propanol and resuspended at 0.2 μ g/ml in the hybridization buffer described in Ingham et al. (1985).

Wild-type ovaries and embryos were from the Oregon R strain. *swallow*⁻ ovaries were from females of the genotype *sww¹¹⁻⁹⁹⁹/Df(1)JF5*.

Embryos at 0–3 hr were collected on lightly yeasted grape juice/agar plates, dechorionated in 50% commercial bleach for 5 min, and washed in PBS [130 mM NaCl, 10 mM NaPO₄ (pH 6.5)]. Embryos were fixed for 10 min in a two-phase mixture of 4% formaldehyde in PBS (freshly prepared from paraformaldehyde) and heptane. The aqueous solution was removed and replaced with an equal volume of methanol. The tube was shaken vigorously, and embryos that had lost their vitelline membrane were removed from the bottom of the methanol layer and transferred to pure methanol. Embryos were rehydrated through methanol, methanol/PBS (3 : 1), methanol/PBS (1 : 1), methanol/PBS (1 : 3), and, finally, in pure PBS. Embryos were fixed for an additional 30 min in 4% formaldehyde in PBS and dehydrated, embedded, and sectioned as described below for ovaries.

Ovaries were dissected from females that had been kept for 1–2 days in an uncrowded vial with fresh yeast present. Large ovaries were teased into two or three fragments, fixed for 30 min in 4% formaldehyde in PBS, and washed for 1 hr in several changes of PBS. Ovaries and embryos were dehydrated and embedded using 50%, 70%, 95%, and 99% ethanol, 99% ethanol/xylene (1 : 1), xylene, xylene/paraffin (1 : 1) at 60°C, and three changes in pure paraffin (Paraplast) at 60°C. In our hands, ovaries become brittle if left at any stage for too long a time, and each step was typically 30–60 min, except for the final embedding step, which was usually allowed to proceed overnight.

Five-micron sections were cut and mounted on poly-L-lysine-treated slides, and the slides were deparaffinized and treated with acetic anhydride as described (Angerer et al. 1987). We obtain adequate signals without protease treatment and have omitted this step. The RNA probes in hybridization buffer were heated at 65°C for 5 min before application to the slides, and the tissue covered with a siliconized coverslip and sealed with rubber cement. Hybridization was allowed to proceed overnight at 50°C, and slides were washed as described in Ingham et al. (1985), a protocol that includes an RNase A treatment in 0.5 M NaCl. Dipping in autoradiographic emulsion and developing followed published procedures (Angerer et al. 1987). Slides were stained in 1% Giemsa stock (Banco) in 10 mM NaPO₄ (pH 6.5) for 5 min.

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