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Distribution of swallow protein in egg chambers and embryos of *Drosophila melanogaster*

Jay Hegdé[1,*] and Edwin C. Stephenson[2,†]

[1]Department of Biology, University of Rochester, Rochester, NY 14627, USA
[2]Department of Biological Sciences, University of Alabama, Tuscaloosa, AL 35487-0344, USA

*Present address: Department of Neurobiology and Anatomy, University of Texas Health Sciences Center, 6431 Fannin, Houston TX 77030, USA
†Author for correspondence

SUMMARY

The *Drosophila* maternal effect gene *swallow* has a role in localizing *bicoid* mRNA at the anterior margin of the oocyte during oogenesis, and a poorly characterized role in nuclear divisions in early embryogenesis. We have examined the distribution of swallow protein during oogenesis and embryogenesis using anti-swallow antibodies. During oogenesis, high levels of swallow protein are present in basal nurse cell cytoplasm, although small amounts are also present at the anterior oocyte margin, the site of *bicoid* RNA localization. Only a small fraction of swallow protein is in a position to interact directly with *bicoid* RNA during localization. The asymmetric distribution of swallow protein is disrupted in *swallow* ovaries, in which *bicoid* RNA becomes unlocalized late in oogenesis. swallow protein is uniformly distributed in eggs, but becomes localized to nuclei during early mitotic divisions in early embryogenesis. swallow protein enters each nucleus at the beginning of mitosis, occupies a position complementary to that of condensed chromatin, and leaves each nucleus at the end of mitosis. We show examples of nuclear division defects in *swallow* mutant embryos, and suggest that the abnormal nuclear divisions in early *swallow* embryos reflect a second function for swallow protein that contributes to abdominal segmentation defects common in *swallow* embryos.

Key words: maternal effect gene, oogenesis, RNA localization, *bicoid* RNA, nuclear divisions, mitosis, nuclear matrix

INTRODUCTION

Many of the proteins necessary for the early embryogenesis of *Drosophila melanogaster* are encoded by maternal effect genes, genes expressed during oogenesis whose function is required for proper embryonic development. One of these genes, *swallow*, is required for the normal development of the anterior embryo, and for other aspects of embryonic development (Gans et al., 1975; Zalokar et al., 1975; Frohnhöfer and Nüsslein-Volhard, 1987; Stephenson and Mahowald, 1987). The underlying cause of defective anterior development is the failure of *swallow* oocytes and eggs to localize *bicoid* messenger RNA, the anterior morphogen (Berleth et al., 1988; Stephenson et al., 1988). In addition, mitotic divisions in early *swallow* embryos are often defective (Zalokar et al., 1975), and abdominal segmentation shows variable degrees of disruption (Frohnhöfer and Nüsslein-Volhard, 1987; Stephenson and Mahowald, 1987).

bicoid mRNA is localized to the anterior part of the oocyte during oogenesis (Frigerio et al., 1986; Berleth et al., 1988; Stephenson et al., 1988), and remains localized after fertilization, leading to the specification of the anterior embryo. During oogenesis, each egg develops from an egg chamber, which is composed of one oocyte, 15 nurse cells, and several hundred follicle cells. Nurse cells and the oocyte are sister cells, generated during four mitotic divisions early in oogenesis, and remaining connected by cytoplasmic bridges. *bicoid* mRNA is synthesized in nurse cells, moves to the oocyte through cytoplasmic bridges and becomes localized at the anterior oocyte margin, the nurse cell-proximal part. Early descriptions of the process suggested *bicoid* RNA might be localized by selective trapping; receptors anchored in the oocyte might capture *bicoid* RNA as it enters the oocyte from its site of synthesis in nurse cells (Berleth et al., 1988; Stephenson et al., 1988). However this simple model does not account for some of the details of *bicoid* RNA localization, suggesting the existence of a more complex mechanism (St. Johnston et al., 1989; Stephenson and Pokrywka, 1992). For instance, *bicoid* RNA is localized around nurse cell nuclei, suggesting it becomes associated with the localization machinery before it enters the oocyte. Also, some *bicoid* RNA is localized during the rapid influx of nurse cell cytoplasm into the oocyte at the end of vitellogenesis, an observation difficult to reconcile with the idea of a receptor-ligand mechanism.

Two genes have been identified whose products participate in *bicoid* RNA localization during oogenesis. *exuper* -

antia mutants fail to localize *bicoid* RNA around nurse cell nuclei and in the oocyte (St. Johnston et al., 1989). Exuperantia protein is concentrated around nurse cell nuclei, but not at the anterior oocyte margin, consistent with the idea that *exuperantia*$^+$ plays an early, nurse cell-limited role in the process, but is not required for the maintenance of localization in the oocyte (Marcey et al., 1991; Macdonald et al., 1991). *swallow* mutants localize *bicoid* RNA around nurse cell nuclei, and at the anterior oocyte margin, but localization in the oocyte deteriorates in late vitellogenic stages (St. Johnston et al., 1989). These observations suggest that *swallow*$^+$ might be required for a late step in localization, or for the maintenance of localization.

Developmentally regulated mitotic divisions play an important role in the early embryogenesis of *Drosophila melanogaster*. *Drosophila* embryogenesis begins with 13 rounds of rapid synchronous nuclear divisions, occurring approximately every 9 minutes (Campos-Ortega and Hartenstein, 1985). Subsequent cell divisions are slower and asynchronous and occur in spatially regulated patterns (Foe, 1989). Early cleavage divisions are syncytial; cellular membranes form during the 14th cell cycle. Most embryonic cells except neuroblasts stop dividing after mitotic division cycle 16 or 17 (Hartenstein et al., 1987). Early cleavage divisions employ maternally provided gene products, since embryonic nuclei are transcriptionally quiescent for most of the early cleavage cycles (Edgar and Schubiger, 1986).

The genetic requirements for mitotic events in the early *Drosophila* embryo have been studied extensively in recent years (for reviews, see Glover, 1989, 1991). Some of these genes, such as *abnormal chromatin* (Vessey et al., 1991), *BJ1* (Frasch, 1991), *lodestar* (Girdham and Glover, 1991) and *polo* (Llamazares et al., 1991), regulate chromatin behavior during the cell cycle. Mutations in these genes cause a variety of aberrations in chromosomal behavior, ultimately leading to a reduction in the number of nuclei (Glover, 1989). Gastrulation occurs in a reasonably normal fashion despite the reduced number of nuclei (Edgar and O'Farrell, 1989; O'Farrell et al., 1989), but embryos usually die before hatching (Edgar and O'Farrell, 1989; Yasuda et al., 1991).

In addition to its role in localizing *bicoid* RNA in oogenesis, *swallow* appears to be required for another process during early embryogenesis. *swallow* embryos, (i.e., embryos produced by females homozygous for a *swallow* mutation) have defects in anterior development and in thoracic and abdominal segmentation. (Zalokar et al., 1975; Frohnhöfer and Nüsslein-Volhard, 1987; Stephenson and Mahowald, 1987). However, *exuperantia* embryos, which also fail to localize *bicoid* RNA, do not have thoracic and abdominal defects (Frohnhöfer and Nüsslein-Volhard, 1987; Schüpbach and Wieschaus, 1989), suggesting the thoracic and abdominal defects of *swallow* embryos are not due to the failure to localize *bicoid* mRNA. Early *swallow* embryos show defects in the synchrony of nuclear division, nuclear migration to the embryonic periphery and in the timing of early embryonic processes (Zalokar et al., 1975).

In this report we study the protein product of the *swallow* gene during oogenesis and embryogenesis. We show here that swallow protein is concentrated in the early oocyte, and is prominent in basal nurse cell cytoplasm later in oogenesis, but never accumulates to high levels at the anterior oocyte margin. Our results suggest that there is not a simple relationship between the position of swallow protein and the site of its action in *bicoid* RNA localization. In early embryos we show that swallow protein is associated with the nucleus in a cell cycle-dependent fashion. We examine the mitotic defects of *swallow* embryos, and suggest that these contribute to segmentation defects in *swallow* embryos.

MATERIALS AND METHODS

Antibody production and purification

To prepare swallow protein, we constructed the chimeric cDNA clone 8480.93 by joining *swallow* partial cDNAs 8480.1 and 8493.1 at the *Hin*dIII site at nucleotide position 2835 (see Chao et al., 1991). This chimeric cDNA codes for the carboxy-terminal 496 amino acids, of the 548 amino acids in the *swallow* open reading frame. This construct was inserted into the pEV expression vector (Crowl et al., 1985) and the expression of swallow polypeptide was induced by incubating the cells at 42°C. Whole-cell protein extract from induced cells was separated on a polyacrylamide gel and the band corresponding to the induced swallow polypeptide was excised and was used for immunizing rats in a commercial facility (Pocono Rabbit Farms, Canadensis, PA). Immune sera were purified using the affinity purification procedure of Olmsted (1981) or by the tissue adsorption procedure of Ashburner (1989). To affinity purify the antibodies, we reacted the polyclonal serum to trpE-swallow fusion protein bound to nitrocellulose, washed the blot extensively to remove non-specific antibodies and eluted the anti-swallow antibodies with 5 M KI, 20 mM Tris-HCl pH 8.0, 0.1% Tween-20. To purify the antibodies by tissue adsorption, we diluted the serum 1:20 in PBS and incubated with 0.1 volume of fixed wild-type embryos (10-20 hours) at 4°C overnight. Sera purified by either procedure give similar results. Before use, affinity purified serum was diluted 1:300 (for immunocytochemistry) or 1:600 (for western analysis) relative to crude sera. Secondary antibodies were purified by adsorbing against older embryos (Ashburner, 1989) and used at a concentration of 0.5 µg/ml. trpE-swallow fusion protein used to test the specificity of anti-swallow antibodies was constructed by inserting a 0.8 kb *Bam*HI/*Hin*dIII fragment of the *swallow* cDNA in pATH2 vector (Harlow and Lane, 1988). Similarly, the GST-swallow fusion protein was constructed by inserting all but one codon of the *swallow* open reading frame into the pGEX3X vector (Smith and Johnson, 1988).

Immunoblotting

Western blotting was carried out as described by Driever and Nüsslein-Volhard (1988a), except that the chemiluminescent substrate Lumiphos 530 (Boehringer-Mannheim) was sometimes used as a staining substrate. Protein concentrations were determined by silver staining SDS-PAGE gels containing aliquots of the protein samples; approximately equal amounts of protein were loaded into each lane of the blots shown here.

Immunocytochemical analysis

Egg chambers were dissected in PBS and fixed in a 2:8 mixture of methanol:*n*-heptane for 10 minutes at −20°C followed by permeabilization by incubating in a 9:1 mixture of methanol:dimethyl sulfoxide at −20°C for 1 hour. The egg chambers were then rehydrated, washed in PBS and extracted in 1% Triton X-100 in PBS at room temperature for 2 hours. Following several washes in PBT (PBS + 0.3% Triton X-100), the egg chambers were blocked at room temperature for 1 hour in 5% normal rabbit serum, 5% normal goat serum, 0.2% bovine serum albumin in PBT. The egg chambers

were incubated in 1:500 dilution of affinity purified rat polyclonal antibody at room temperature overnight. Following extensive washes in PBT, the egg chambers were incubated with biotinylated anti-rat secondary antibody followed by BODIPY-conjugated streptavidin (Molecular Probes, Eugene, OR). Following the streptavidin incubation, the egg chambers were washed in PBT and viewed using confocal laser scanning microscopy.

Immunocytochemistry on whole-mount embryos was performed using a formaldehyde fixation protocol (Karr and Alberts, 1986) or using the heat fixation protocol of Sullivan et al. (1990). swallow staining of the nuclear dot (nucleolus) is evident in embryos fixed in formaldehyde in the presence of 0.5 μM taxol, or in embryos fixed by heat, but not in embryos fixed in formaldehyde without taxol. Other aspects of swallow staining do not depend on the fixation method. Rhodamine- or BODIPY-conjugated secondary antibodies (Molecular Probes) were used to visualize primary antibody binding. DNA was stained with Hoechst 33258 as described by Karr and Alberts (1986). The embryos were viewed by conventional immunofluorescence microscopy or by confocal laser scanning microscopy. For double staining of embryos, secondary antibodies purified for dual labeling (Chemicon) were used. Anti-lamin antibodies were obtained from Dr P. Fisher (SUNY Stony Brook) and from Dr H. Saumweber (U. Cologne, FRG); and anti-nuclear pore complex (p62) antibodies from Dr D. Goldfarb (U. Rochester).

For aphidicolin treatment, embryos were permeabilized with octane as described by Ashburner (1989) and incubated in *Drosophila* culture medium (Ashburner, 1989) at room temperature with 5 μg/ml aphidicolin for 15 minutes. Following aphidicolin treatment, embryos were fixed and stained with anti-swallow antibodies. The *string* mutant stock used in these experiments was obtained from Dr B. Edgar (UCSF), and the *giant nuclei (gnu)* strain from Dr M. Wolfner (Cornell).

Fuchsin staining of embryos

Embryos from wild-type or *swallow* females were fixed and stained with basic fuchsin as described by Ashburner (1989).

Identification of peptide motifs

We used the University of Wisconsin Genetics Computer Group software (Devereux et al., 1984) to analyze sequences and search for homologies. Homologies to nucleolar proteins were first uncovered using the BLAST program of Altschul et al. (1990). Similarities were also discovered in database searches using the FASTA program.

RESULTS

Specificity of anti-swallow antibodies

Sequence analysis of the *swallow* gene predicts a protein of 548 amino acids and a relative molecular mass of 62×10^3 (Chao et al., 1991). The predicted amino acid sequence contains a heptad repeat (amino acids 206-269) and a region similar to the RNA recognition motif of RNA binding proteins (amino acids 110-188). To analyze swallow protein, we raised rat polyclonal antibodies against a portion of swallow protein expressed in *E. coli*. This polypeptide contains the carboxy-terminal 496 amino acids of the *swallow* open reading frame, including the heptad repeat and RNA recognition motifs, plus three amino acids from the vector. This protein was isolated and used to raise antibodies, and the resulting sera were purified as described in Materials and methods. To establish the specificity of the antibodies, we performed western blot analyses on fly and *E. coli* extracts (Fig. 1A).

The antibodies recognize a single 65×10^3 M_r band corresponding to trpE-sww fusion protein (lane 1) and a single 93×10^3 M_r band corresponding to GST-sww fusion protein (lane 2); these are the sizes expected for the respective fusion proteins. The antibodies recognize a single band of 60×10^3 M_r in extracts from *Drosophila* early embryos (lane 3) and ovaries (lane 6). swallow protein is not detectable in males (lane 4) or in female bodies excluding ovaries (lane 5). swallow protein is present at detectable levels throughout oogenesis (data not shown), and after fertilization, swallow protein is present in 0-2 hour embryos (Fig. 1B, lane 1), and persists at approximately constant levels until 4 hours after fertilization (Fig. 1B, lane 2). 4-6 hour embryos have lower levels of swallow protein (lane 3), and the protein is not detectable in embryos older than 6 hours (lanes 4-7) or in first and second instar larvae (lane 8). These observations are consistent with genetic and molecular studies which showed that *swallow* transcription is restricted to the female germline (Perrimon and Gans, 1983; Stephenson et al, 1988).

We see a small difference in the mobility of swallow protein in egg chambers and embryos (data not shown).

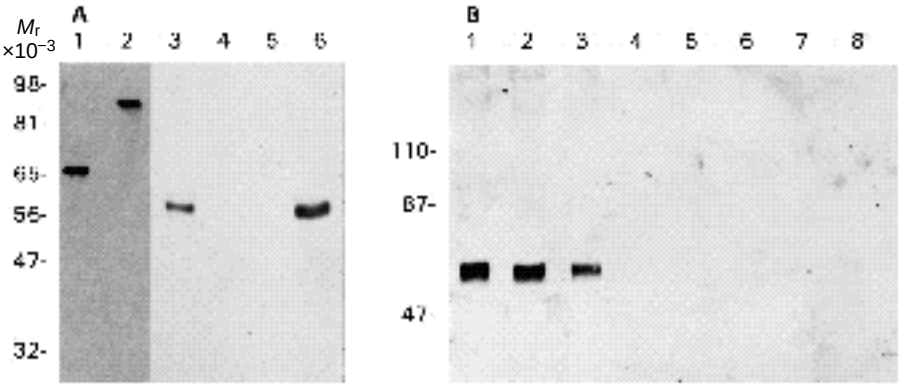

Fig. 1. Characterization of anti-swallow antibodies, and expression of swallow protein. (A) Whole cell protein extracts were prepared from *E. coli* expressing the appropriate swallow fusion protein or from wild-type flies. The extracts were probed with purified anti-swallow serum as described in Materials and methods. Lanes: 1, trpE-sww fusion protein; 2, GST-sww fusion protein; 3, 0-3 hour embryos; 4, males; 5, female body excluding ovaries; 6, ovaries. The predicted relative molecular masses of trpE-sww and GST-sww fusion proteins are 65×10^3 and 93×10^3, respectively. The expected relative molecular mass of swallow protein, based on the DNA sequence, is 62×10^3. (B) swallow protein during development. Each lane contains approximately equal amounts of protein, as determined by staining aliquots of these samples run on a previous gel. Lane 1, 0-2 hour embryos; lane 2, 2-4 hour; lane 3, 4-6 hour; lane 4, 6-8 hour; lane 5, 8-10 hour; lane 6, 10-18 hour; lane 7, 18-24 hour; lane 8, 24-72 hour.

swallow protein from egg chambers is slightly larger and more heterogeneous in size than that from embryos (this small difference is not detectable in Fig. 1). We do not know the cause of this mobility difference, nor do we know whether it is significant with respect to the different subcellular distributions of swallow protein in oogenesis and embryogenesis.

Distribution of swallow protein in oogenesis

In order to determine the distribution of the protein in egg chambers during oogenesis, we stained egg chambers with anti-swallow antibodies and viewed swallow protein distribution by confocal laser scanning microscopy (Fig. 2). swallow protein is first easily detectable in stage 5 egg chambers (Fig. 2A), and is present at very low levels or is absent altogether from earlier stages (not shown). swallow protein is strictly cytoplasmic in egg chambers, in contrast to embryos, in which it is localized to the nucleus during part of the cell cycle (Figs 3, 4). In addition, swallow protein is absent from follicle cells, as expected from earlier genetic studies (Perrimon and Gans, 1983) and studies of *swallow* mRNA expression (Stephenson et al., 1988). In early stages swallow protein accumulates strongly in the oocyte, as in the stage 5 and 6 egg chambers in Fig. 2A. At stage 7, swallow protein begins to accumulate in the nurse cells while the level of swallow staining in the oocyte remains approximately constant (Fig. 2A). In stage 8 egg chambers, the level of swallow staining in the nurse cells continues to increase while the oocyte signal is reduced in intensity.

swallow protein continues to accumulate in nurse cells throughout vitellogenic stages (stages 8 and 9, Fig. 2A; stage 10, Fig. 2B). There is more swallow protein near the interior of the nurse cell complex than near the periphery, that is, swallow protein occupies a basal position in each nurse cell. Some swallow protein seems to enter the oocyte throughout these stages. However, most swallow protein remains in nurse cells until the nurse cell contents are dumped into the oocyte at the end of vitellogenesis. The oocyte staining pattern is difficult to detect in postvitellogenic egg chambers, due to the impermeability of the vitelline membrane and the chorion. However, we know maturing oocytes contain swallow protein, since swallow protein is detectable by western blot analysis throughout oogenesis (not shown), and because swallow protein is present in unfertilized eggs (Fig. 3).

While most swallow protein is present in nurse cells, small amounts of swallow protein are present within the oocyte itself. We consistently observe low but significant levels of swallow protein within the anterior oocyte (Fig. 2A,B). Some of this protein is coincident in position with *bicoid* RNA, which is localized at the anterior oocyte margin. However, it should be noted that swallow protein in the anterior oocyte is not restricted to the anterior margin, but is present in deeper oocyte cytoplasm as well.

We examined the distribution of mutant swallow protein in egg chambers from females homozygous for two strong alleles of *swallow*, *1497* and *11-999*. The ovaries of homozygous females contain *swallow* mRNA of normal size and abundance (Chao and Stephenson, unpublished data), and immunoreactive swallow protein (Hegdé and Stephenson, unpublished data) of normal abundance and approximately the same size as wild-type ovaries. The distribution of swallow protein in these egg chambers is somewhat variable. Fig. 2C,D show the typical staining pattern in mutant egg chambers, in this case homozygous for *1497*. The major features of wild-type swallow protein expression are disrupted in mutant egg chambers. The oocyte accumulation of swallow protein in stage 5 and 6 egg chambers is almost entirely absent (Fig. 2C), and the localization of swallow protein in basal nurse cell cytoplasm is lost. Instead, protein is distributed more or less uniformly throughout the nurse cells. swallow protein is detectable in the oocyte of stage 10 egg chambers (Fig. 2D), although it is not as concentrated at the anterior end of the oocyte as in wild-type stage 10 egg chambers. The distribution of swallow protein in $swallow^{11\text{-}999}$ egg chambers is similar to that of $swallow^{1497}$ egg chambers (data not shown).

swallow is a nuclear protein in early embryos

After fertilization, the zygotic nucleus undergoes a series of rapid asynchronous divisions without cytokinesis (for reviews, see Foe and Alberts, 1983; Campos-Ortega and Hartenstein, 1985). During cycles 8 through 13, nuclei form a monolayer at the periphery of the embryo. Cellularization occurs during cycle 14. The mitosis that ends cycle 14 is asynchronous; cells enter cycle 14 in small local groups called mitotic domains, while other cells remain in interphase. Most cells do not divide after the germ band shortening stage (Foe, 1989).

We stained embryos with anti-swallow antibodies to examine the distribution of swallow protein in embryogenesis (Fig. 3). swallow protein is present in unfertilized eggs (Fig. 3A) and newly fertilized embryos (Fig. 3B), and is uniformly distributed. In embryos in mitotic cycle 5, most swallow staining is cytoplasmic (Fig. 3C). As embryogenesis progresses, the cytoplasmic swallow staining decreases rapidly, and by cycle 8 most swallow protein is clearly localized to dividing nuclei (Fig. 3D). Confocal microscopic analysis shows that swallow protein is present in dividing nuclei in earlier stages of embryogenesis as well (data not shown), although this is not obvious from the conventional microscopic images in Fig. 3B because the nuclei are in the interior of the embryo and are obscured by the heavier cytoplasmic staining. At the syncytial blastoderm stages, swallow staining is almost exclusively nuclear, with little or no cytoplasmic staining (Fig. 3E,F). In cycles 13 and 14, interphase nuclei contain stained ‘dots’, while mitotic nuclei are stained in the general nuclear pattern. Fig. 3G shows an embryo with both mitotic and interphase nuclei, with the boundary between the dot-like and general staining patterns indicated by arrows. A higher magnification of the dot-like structures from the center of this embryo is shown in Fig. 3H. During the long interphase of cycle 14, swallow protein is confined to a small dot within each nucleus, similar to those shown in 3H. As the nuclei enter the mitotic phase of cycle 14, the dot-like swallow staining broadens to cover the entire nucleus, resembling the mitotic nuclei of preblastoderm embryos. Gastrulating embryos with nuclei in mitosis 14 are shown in Fig. 3I,J. Beginning at the germ band elongation stage, swallow protein gradually disappears from all dividing and non-dividing cells, and can no longer be detected by the germ band shortening stage (not shown).

Cell cycle-dependent changes in the nuclear distribution of swallow protein

We stained embryos with anti-swallow antibodies and examined the nuclei during mitosis by confocal laser scanning microscopy (Fig. 4). In early interphase nuclei (Fig. 4A,B, arrow), in which chromatin is completely dispersed, swallow protein is absent from nuclei. In late interphase or early prophase nuclei, at the beginning of chromatin condensation, anti-swallow antibodies stain one or two dot-like structures within each nucleus (Fig. 4C,D),

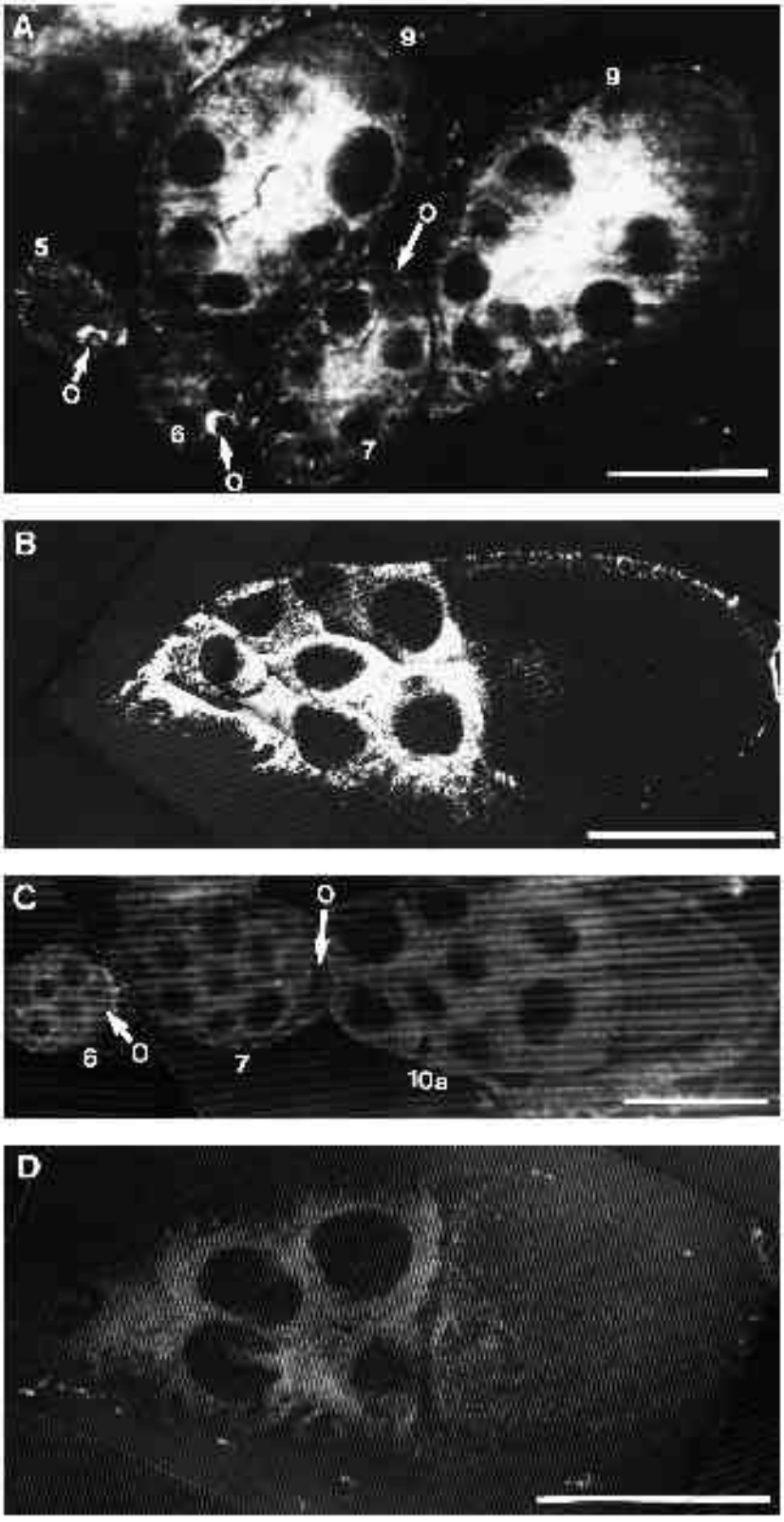

Fig. 2. Distribution of swallow protein in egg chambers. Wild-type egg chambers were stained with anti-swallow antibodies and viewed using confocal laser scanning microscopy. The developmental stage of each egg chamber is denoted by the number next to it. O, oocyte. Bars, 100 μm. Stages of egg chambers are designated after King (1970). (A) Wild-type egg chambers, stages 5 through 9. swallow protein accumulates in the oocyte of younger stages. In later stages, the highest levels of swallow protein in the egg chamber are in basal (internal) nurse cell cytoplasm, although small amounts are also present within the anterior oocyte. The large black areas in nurse cells are nurse cell nuclei. Sharp discontinuities in staining within the nurse cell complex, and between nurse cells and the oocyte, are presumably the cellular membranes. (B) Wild-type stage 10 egg chamber. High levels of swallow protein are present in nurse cells, although small amounts are present in what we presume to be the anterior oocyte. (C) sww^{1497} / sww^{1497} egg chambers, stages 6 through 10a. Little swallow protein accumulates in young oocytes, and swallow protein in nurse cells is uniformly distributed. (D) sww^{1497} / sww^{1497} egg chambers, stage 10b. swallow protein in the oocyte is more uniform in its distribution.

about 2 μm in diameter. As the nuclei enter prophase, swallow protein becomes distributed throughout the entire nucleus (Fig. 4E,F). As nuclei enter metaphase, two masses of swallow protein sandwich the condensed chromatin (Fig. 4G,H). At late metaphase and anaphase, the swallow-staining structure elongates into two cones that appear to represent the interior of each half-spindle (Fig. 4I-L). In telophase these cones appear to compact into two solid spheres (Fig. 4M,N). These changes in the swallow-staining structure are similar to the changes in nuclear morphology described by Stafstrom and Staehelin (1984).

The late interphase/early prophase dots (Fig. 4C) are first visible during cycle 13; at earlier stages the swallow staining pattern progresses from exclusively cytoplasmic in interphase (Fig. 4A) directly to general nuclear in mitosis (Fig. 4E-M). To determine whether these dots correspond to nucleoli, we double stained embryos with antibodies against swallow protein and against the nucleolar protein Aj1 (Frasch et al., 1985) as described in Materials and methods. The dot structure that stains with anti-swallow antibodies also stains with this anti-nucleolar antibody (Fig. 5A,B). Similar results (not shown) were obtained when we used antibodies to the nucleolar protein fibrillarin (Aris and Blobel, 1988). We also expressed swallow protein in the *Drosophila* cell line S2, and find staining of the nucleoli, which are clearly visible by phase contrast microscopy

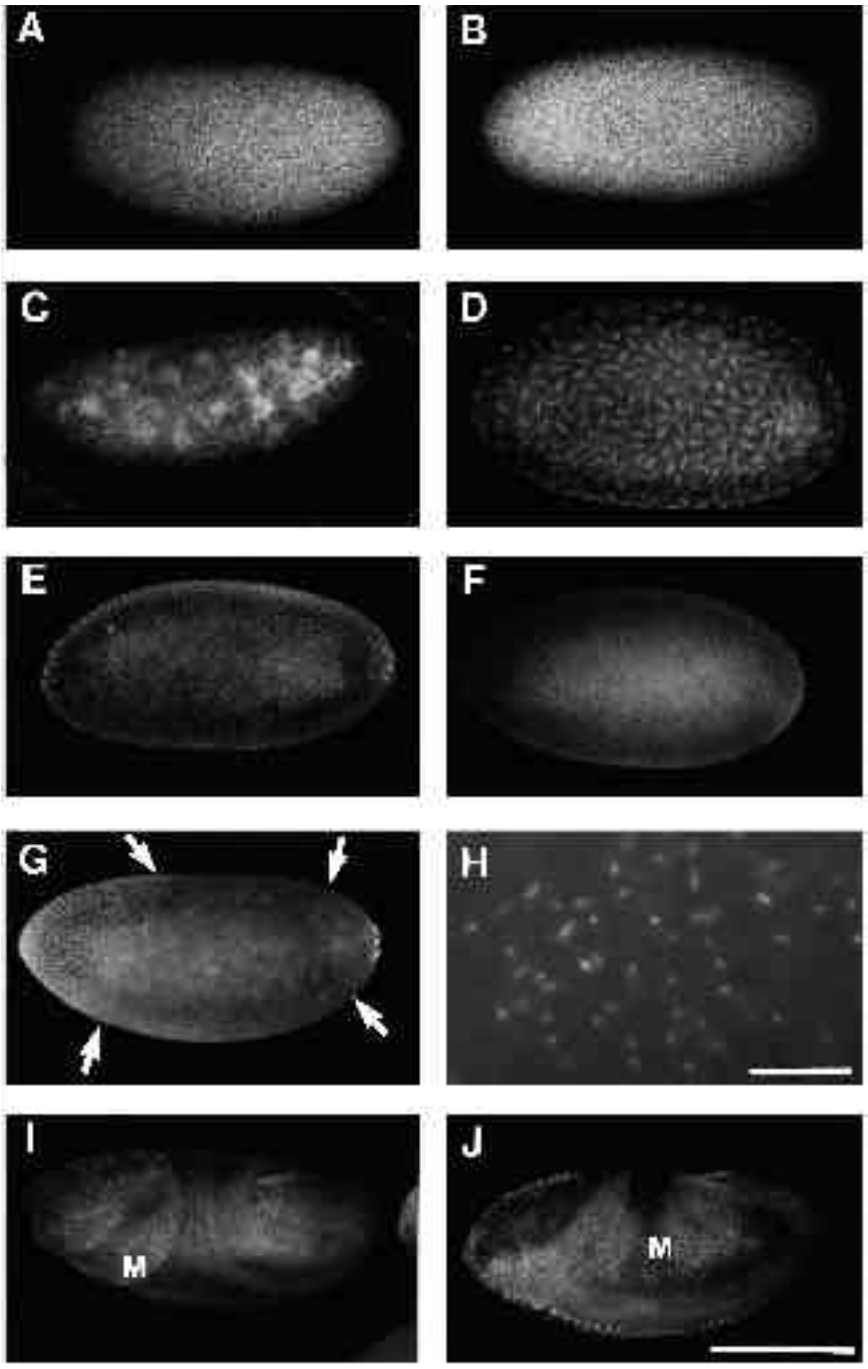

Fig. 3. Distribution of swallow protein in wild-type embryos. Wild-type embryos were probed with anti-swallow antibodies and examined by conventional immunofluorescence microscopy. Each embryo is shown with its anterior end to the left and dorsal side to the top, except I, which is a dorsolateral view of a gastrula embryo. (A) Unfertilized egg. (B) Newly fertilized embryo. (C) Embryo in mitotic cycle 5. (D) Embryo in mitotic cycle 8. (E) Embryo in mitotic cycle 10. (F) Embryo in mitotic cycle 12. (G) An embryo in which nuclei near both poles are in mitotic cycle 13. The nuclei in the center are in the interphase between cycles 12 and 13. The points at which nuclei are entering mitosis are indicated by arrows. (H) A higher magnification view of nuclei in the interphase of cycle 14. (I,J) Gastrulating embryos. M, mitotic domain. Bars, 100 μm (A-G,I and J) and 20 μm (H).

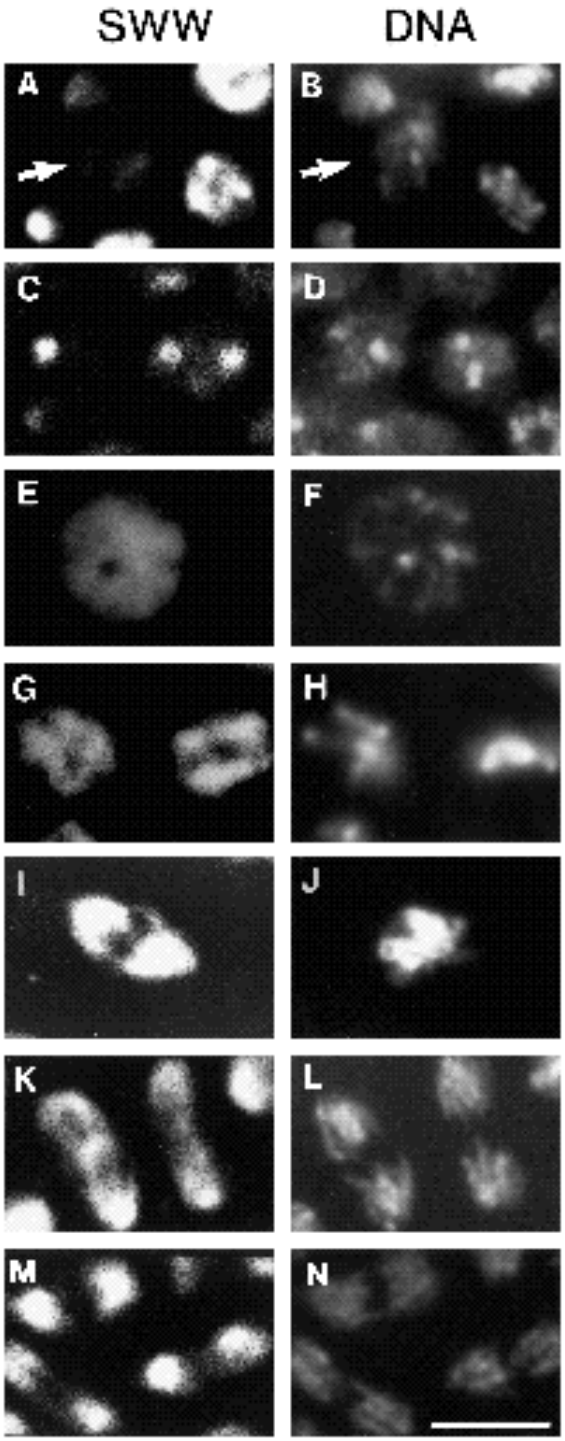

Fig. 4. Cell cycle-dependent changes in the nuclear distribution of swallow protein. Blastoderm embryos were stained with anti-swallow antibodies and the DNA stain Hoechst 33258 as described in Materials and methods. swallow protein staining (left panels) was then imaged by confocal laser scanning microscopy. DNA staining (right panels) was imaged by conventional fluorescence microscopy. (A,B) Nucleus in early interphase (arrows). (C,D) Nuclei in late interphase. Note that anti-swallow antibodies stain one or two dots in these nuclei. (E,F) Early prophase. (G,H) Late prophase. (I,J) Metaphase. (K,L) Anaphase. (M,N) Telophase. Bar, 10 μm.

(Hegdé, unpublished data). We conclude that the dot structures that stain with anti-swallow antibodies in late interphase or early prophase nuclei are nucleoli.

The nuclear distribution of swallow protein during mitosis is complementary to that of condensed chromatin, that is, swallow is absent from portions of the nucleus occupied by condensed chromatin. Furthermore, swallow protein is present throughout the nucleus, and not just at the nuclear periphery, as is evident by comparing the staining pattern of nuclear lamin proteins (Fisher et al., 1982; Smith et al., 1987), and nuclear envelope proteins (Fig. 5C,D,E). The structure containing swallow protein occupies the interior of the nucleus, and is similar to nuclear matrins described in mammalian cells (Berezny, 1991).

To determine cell cycle requirements for swallow protein entry into the nucleus, we examined embryos arrested at various points in the cell cycle (Fig. 5F-M). Embryos treated with the DNA synthesis inhibitor aphidicolin arrest in early S-phase (Raff and Glover, 1988). swallow protein is absent from nuclei in aphidicolin treated embryos (Fig. 5F,G), in contrast to control embryos in which swallow is nuclear (Fig. 5H,I). Early gastrula embryos that lack string protein (a homologue of the cell cycle regulator *cdc25)* arrest in late G2 (Edgar and O'Farrell, 1989). swallow protein is excluded from nuclei in *string* embryos and accumulates in surrounding cytoplasm (Fig. 6J,K). In *giant nuclei* embryos, in which nuclei undergo successive rounds of DNA replication without undergoing mitosis (Freeman and Glover, 1987), swallow is present in the resulting large polyploid nuclei (Fig. 6L,M). Taken together, these results suggest that entry of swallow protein into embryonic nuclei requires the completion of S and G2, but does not require entry into mitosis.

Nuclear defects in *swallow* embryos

Pregastrula and gastrula *swallow* embryos exhibit a variety of anomalies in nuclear behavior during early embryogenesis. (*swallow* embryos are the progeny of females homozygous for *swallow* mutations). Zalokar et al. (1975) described allele *1497* as showing irregular population of the blastoderm, uneven blastoderm nuclear sizes, and fewer than normal nuclei at the beginning of gastrulation. These authors also examined allele *1502*, but do not describe any early embryonic defects. We have examined five *swallow* alleles in detail, including *1497* and *1502*, and find that each mutant shows the early nuclear defects described by Zalokar et al. (1975) for allele *1497*. *swallow* alleles can be ranked according to the relative numbers of strongly and weakly affected embryos, but even the weakest alleles produce some strongly affected embryos, and strong alleles produce some weakly affected embryos. More embryos exhibit the strong phenotype at 18°C than at 25°C. Typical defects are shown in Fig. 6. In wild-type embryos (A and B), nuclear density is uniform and internal 'yolk' nuclei are evenly distributed and regularly shaped. While the peripheral blastoderm nuclei of weakly affected *swallow* embryos appear normal (Fig. 6C), all *swallow* embryos have an excess of irregularly shaped internal chromatin masses (Fig. 6D). In many embryos, blastoderm nuclei are non-uniform in size and chromatin condensation, and asynchronous in the cell cycle (Fig. 6E,F,I). Some embryos begin gastrulation with

neighboring nuclei of diverse sizes and degrees of chromatin condensation (Fig. 6E). The most strongly affected embryos are filled with disorganized chromatin masses (Fig. 6F), and have too few cortical nuclei. In rare cases, all chromatin is present in the amorphous internal masses, and there are no detectable nuclei with normal organization (not shown). Since these nuclear defects do not occur in *exuperantia* embryos, they probably represent a separate function for *swallow* that is unrelated to *bicoid* RNA localization in oogenesis.

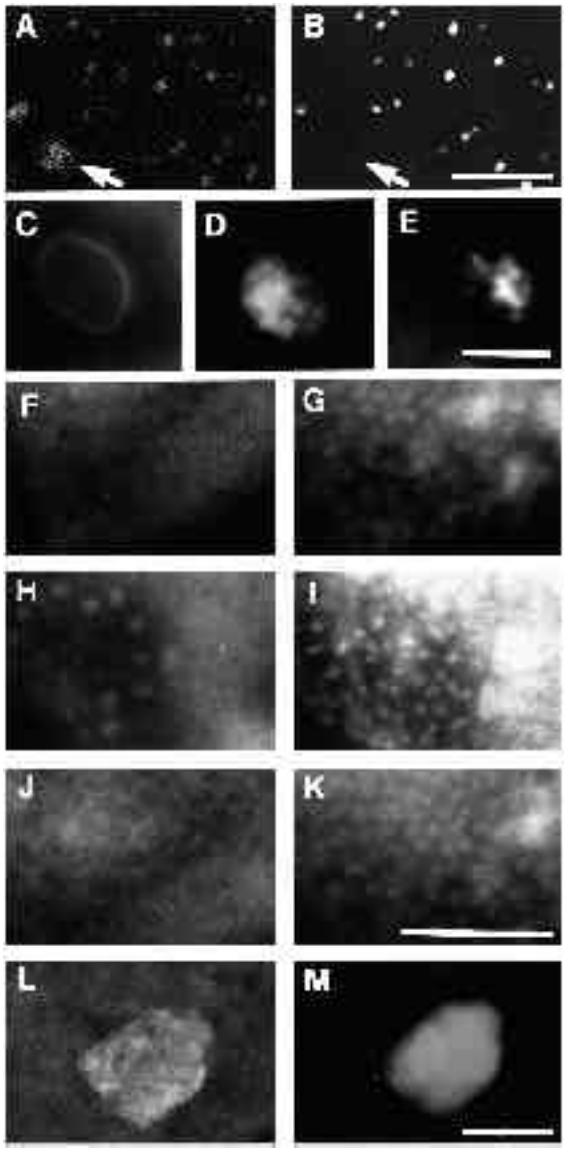

Fig. 5. Cell cycle behavior of swallow protein. (A,B) Gastrulating embryos were double stained with anti-swallow (A) and anti-Aj1 antibodies (B). Arrow indicates the position of a nucleus in mitosis, which stains for swallow protein but not for the nucleolar antigen Aj1. Other nuclei are in interphase. (C-E) Embryos were stained simultaneously for nuclear lamin (C), swallow (D) and for DNA (E). (F-G) Wild-type embryo treated with aphidicolin. (H-I) Untreated control. (J,K) A *string* embryo. (L,M) A nucleus of a *giant nuclei* embryo. F,H,J and L show swallow staining pattern, and G,I,K and M show DNA staining. Bars: in B, 20 μm (A,B); in E, 10 μm (C-E); in K, 50 μm (F-K); in M, 40 μm (L,M). A and B are confocal images, the rest are conventional fluorescence images.

Nuclear defects in early *swallow* embryos appear to result from misregulation of the cell cycle, chromosome segregation in mitosis, or migration of nuclei to the embryonic periphery. During preblastoderm and early blastoderm cleavages, lagging chromosomes during anaphase are common (Fig. 6G). Chromatin threads connect daughter nuclei at telophase and into the succeeding interphase (Fig. 6H). In contrast to the strong mitotic synchrony in wild-type embryos (Foe and Alberts, 1983), nuclear divisions in *swallow* embryos are frequently asynchronous (Fig. 6I). Neighboring nuclei are often different in size, stage of the cell cycle and degree of chromatin condensation, and have a morphology inappropriate for the developmental stage (Fig. 6E,F,I). In mitotic cycles 10 through 13, the blastoderm layer frequently contains patches that lack nuclei. *swallow* embryos are similar in this respect to *daughterless-abo-like*. Like *swallow*, *dal* blastoderm embryos contain nuclear 'holes'. Sullivan et al. (1990) found that these 'holes' resulted when abnormal nuclei drop from the embryo cortex into the central embryo. In *swallow* embryos, as in *dal* embryos, these holes overlie nuclei that are just under the cortical nuclear layer (Fig. 6J). It is likely that these subcortical nuclei dropped from the cortex in the preceding cell cycle, but another possibility is that they simply failed to complete their migration to the cortex.

We stained early embryos from females homozygous for *swallow* alleles *1497, 11-999, 1502* and *384* with swallow antibodies to determine whether the mutant protein behaves normally in embryos. Mutant swallow protein is present within mitotic nuclei of all mutant embryos (data not shown), suggesting the ability of swallow protein to enter embryonic nuclei is not altered by these mutations. The pattern of swallow protein within nuclei is not normal, but we cannot determine whether this is due to abnormal associations or positions of the mutant swallow protein, or to the abnormal morphology of nuclei in *swallow* embryos.

Peptide motifs in the swallow protein

Chao et al. (1991) determined the sequence of the *swallow* open reading frame and identified a likely amphipathic α-helix and a putative RNA recognition motif. We examined the *swallow* sequence for additional peptide motifs (see Materials and methods) and found two putative bipartite nuclear localization motifs. Bipartite nuclear localization motifs consist of two short clusters of two to four basic amino acids each, separated by a spacer of 10 or more non-specific residues (see Dingwall and Laskey, 1991). Such motifs have been shown to be necessary and sufficient for nuclear localization (Picard et al., 1990; Addison et al., 1990; Picard and Yamamato, 1987). The two putative bipartite motifs in swallow are located at amino acids 123-148 and amino acids 243-274 and have the structure 123 KRR (20 X) KRR, and 243 RK (23 X) KLRR, where X is any amino acid. The spacers in swallow protein are relatively long at 20 and 23 amino acids respectively, but bipartite motifs with up to 22 amino acid-long spacers have been to shown to localize proteins efficiently (Robbins et al., 1991; see also Dingwall and Laskey, 1991).

A 40 amino acid region in the amino terminus of swallow

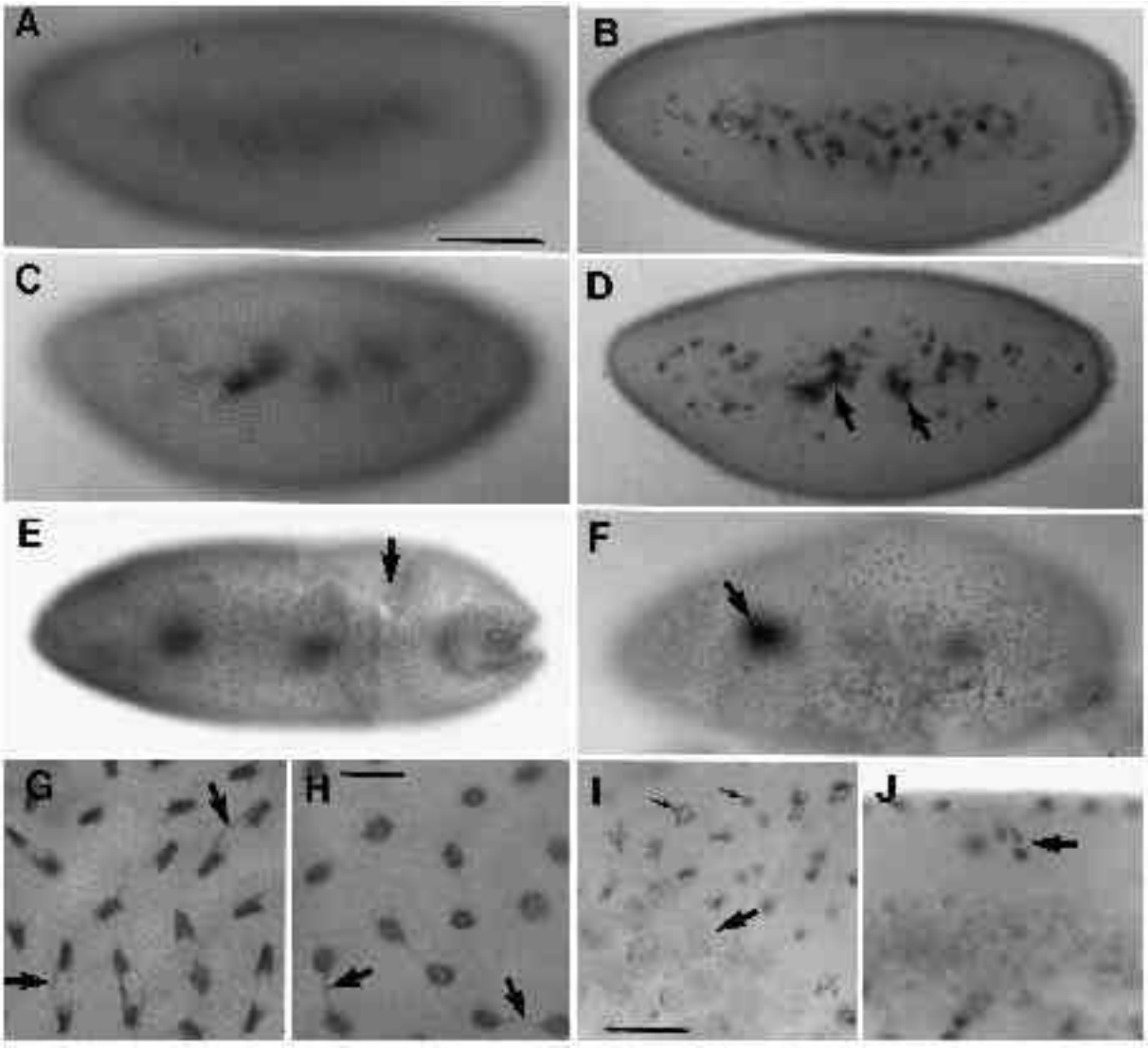

Fig. 6. Nuclear defects in early *swallow* embryos. Embryos were stained with basic fuchsin and examined by conventional microscopy. (A) Wild-type embryo, cycle 14, surface focal plane. Blastoderm nuclei have uniform size, spacing and chromatin density, and are at the same stage of the cell cycle. (B) Wild-type embryo, cycle 14, medial focal plane of the same embryo shown in A. Internal nuclei ('yolk' nuclei) of unknown function are confined to the centre of the embryo. These nuclei are slightly polyploid at this stage, but are fairly regular in shape, size and spacing. (C) *swallow* embryo, cycle 14, surface focal plane. In this relatively normal embryo, blastoderm nuclei are normal in size, spacing, and degree of chromatin condensation. (D) *swallow* embryo, cycle 14, medial focal plane of the same embryo shown in C. Large amorphous chromatin masses (arrows) are present within the embryo. This chromatin is not organized into typical nuclei or confined to the centre of the embryo. (E) *swallow* embryo, early gastrula, dorsal surface. Wild-type gastrula embryos have uniform nuclear density and spacing, similar to that in A. This *swallow* embryo has begun gastrulation with two discrete populations of nuclei at different nuclear densities. In the posterior third of the embryo, to the right of the arrow, nuclei are slightly larger and less densely spaced than in a wild-type gastrulating embryo, and in the anterior two-thirds of the embryo, nuclei are smaller and about twice as densely spaced as they should be. (F) *swallow* embryo. This embryo is at least 2 hours old and thus should be at approximately the same stage as those in A-E. There are too few cortical nuclei, and they are not uniform in either size or stage of the cell cycle. Several large masses of internal chromatin are visible as out of focus images (arrow) below the layer of cortical nuclei. (G) *swallow* embryo, anaphase of cycle 13. Lagging chromosomes or chromatin bridges (arrows) are common. (H) *swallow* embryo, telophase of cycle 12. Chromatin threads (arrows), presumably the remnants of lagging chromosomes, connect most newly separated daughter nuclei. (I) *swallow* embryo, high magnification of the surface of the embryo in F. Some nuclei are in mitosis (small arrows), while others are in interphase (large arrow). The large diffuse nuclei at the bottom of the panel (large arrow) have a unique morphology; their size is typical of pre-blastoderm nuclei, but the degree of chromatin condensation is typical of late cycle 14 nuclei. Similarly, the broad spindle morphology of mitotic nuclei at the top of the panel (small arrow) is atypical for blastoderm mitoses. (J) *swallow* embryo, medial focal plane. Apparently normal cortical nuclei in prophase or metaphase are within a few microns of the cell surface. A group of nuclei just beneath the cortical layer (arrow) are in interphase and thus have lost mitotic synchrony with cortical nuclei. Wild-type embryos are shown in A and B. *swallow* embryos are the progeny of sww^{1502} / *Df(1)JF5* females and wild-type males (C-E,G,H,J), or sww^{1497} / *Df(1)JF5* females and wild-type males (F,I). All embryos were raised at 25°C. Bars: 100 μm in A (A-F); 10 μm in H (G,H); 20 μm in I (I,J).

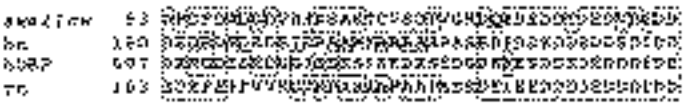

Fig. 7. Homology between swallow and nucleolar proteins. Amino acids are denoted by single letter codes. Similarities between a 41 amino acid stretch of swallow protein and several nucleolar proteins recovered from a BLAST database search are shown. The regions of similarity among these four proteins are boxed. For simplicity, aspartic acid (D) and glutamic acid (E) are boxed as though they were the same amino acid. swallow, (Chao et al.,1991); hn, human nucleolin (Srivastava et al., 1989); hUBF, human nucleolar transcription factor UBF (Jantzen et al., 1990); and rn, rat nucleolin (Bourbon et al., 1990) are shown.

protein shares significant homology with several nucleolar proteins (Fig. 7). This homology is especially strong in a 16 amino acid long hyperacidic region. 75% of amino acids (12/16) in this region of swallow protein are either glutamic acid or aspartic acid residues. Similar hyperacidic regions are found in nucleolar proteins (Lapeyre et al., 1987; Schmidt-Zachmann, et al., 1987; Jantzen et al., 1990), and a number of other nuclear proteins (Earnshaw, 1987).

DISCUSSION

swallow protein in oogenesis

swallow protein shows three temporally distinct patterns of expression and accumulation in egg chambers. First, swallow protein accumulates strongly in the late previtellogenic oocyte (stages 5 and 6). The early accumulation of swallow protein in oocytes is similar to that of *bicoid* RNA, which like swallow protein, is first detectable in stage 5 oocytes and accumulates in the oocyte through stage 6. We do not know whether swallow protein is synthesized in the nurse cells and transported rapidly to the oocyte or is synthesized in the oocyte itself. In any case, at previtellogenic stages of oogenesis the positions of *bicoid* RNA and swallow protein are coincident. It is not clear, however, whether the swallow protein participates in the early accumulation of *bicoid* RNA at this stage, since the preferential oocyte accumulation of *bicoid* RNA is normal in *swallow* mutants (St. Johnston et al., 1989). Protein encoded by the *exuperantia* gene, which also participates in *bicoid* RNA localization, accumulates preferentially in the oocyte (Marcey et al., 1991; Macdonald et al., 1991), although oocyte accumulation is evident as early as stage 1 and thus precedes the expression of *bicoid* RNA (Macdonald et al., 1991).

Other molecules important in oocyte determination and polarity accumulate in the oocyte in early oogenesis, although their time of accumulation is first evident at stages earlier than that of *bicoid* RNA and swallow protein. This group of molecules includes the *oskar* (Ephrussi et al., 1991) and *Bicaudal-D* mRNAs (Suter et al., 1988) and the Bicaudal-D protein (Wharton and Struhl, 1989; Suter and Steward, 1991), which accumulate in the oocyte while the egg chamber is still in the germarium, and the *fs(1)K10* RNA, which accumulates in the oocyte beginning at stage 2 (Cheung et al., 1992). Thus, of the molecules described that show preferential oocyte accumulation, most accumulate either in the germarium or in stages 1 or 2; *bicoid* RNA and swallow protein are distinct from this group in exhibiting initial expression and oocyte accumulation beginning at stage 5.

A second phase of swallow protein distribution in egg chambers begins at stage 6 and continues through stage 10a. Within each nurse cell, swallow protein is concentrated near the nucleus and in the internal or basal aspect of the cytoplasm. Some swallow protein seems to enter the oocyte from the nurse cells throughout this period, but does not accumulate strongly within the oocyte as it does at earlier stages. In the oocyte, swallow protein is most concentrated in anterior oocyte cytoplasm. Some, but not all swallow protein is present at the anterior oocyte cortex. These observations stand in strong contrast to the distribution of *bicoid* RNA and exuperantia protein at the same stages. *bicoid* RNA (St. Johnston et al., 1989) and exuperantia protein (Macdonald et al., 1991; Marcey et al., 1991) are localized around nurse cell nuclei. swallow protein near nurse cell nuclei may slightly overlap the distribution of *bicoid* RNA and exuperantia protein. However most swallow protein in nurse cells is in basal nurse cell cytoplasm, a portion of the cell that lacks significant levels of *bicoid* RNA or exuperantia protein. In the oocyte, *bicoid* RNA and exuperantia protein are restricted to the anterior margin (St. Johnston et al., 1989; Marcey et al., 1991; Macdonald et al., 1991). Some swallow protein is present at the anterior margin, but most is more generally distributed, occupying deeper positions within the oocyte.

A third phase of swallow protein distribution occurs in late oogenesis, when swallow protein is dumped into the oocyte with the remainder of nurse cell cytoplasm. Unfertilized eggs and early embryos contain uniform levels of swallow protein. After fertilization this maternally stored swallow protein is a nuclear protein in early embryonic cleavage divisions.

We believe the pattern of swallow protein distribution in oogenesis is functionally relevant, since mutant swallow protein from two alleles shows none of the patterns of localization in oocyte or nurse cells. swallow protein fails to accumulate in stage 5 and 6 oocytes in swallow egg chambers, and later in oogenesis the protein is distributed more or less uniformly throughout the nurse cell complex and in the oocyte. It is possible that the *swallow* mutant phenotype for these two alleles, and perhaps others, results from the abnormal subcellular localization of swallow protein.

Roles for swallow in *bicoid* RNA localization

On the basis of the late deterioration of *bicoid* RNA localization in *swallow* oocytes, we and others (Berleth et al., 1988; Stephenson et al., 1988; St. Johnston et al., 1989; Stephenson and Pokrywka, 1992) suggested that the likely role of the swallow protein is to maintain localization. It is easiest to imagine swallow protein 'anchoring' *bicoid* RNA at the anterior oocyte margin, or stabilizing the cytoskeletal assemblies to which *bicoid* RNA is attached. Some swallow protein is present at the anterior oocyte margin, the site at which it would be expected to act most directly in stabiliz-

ing the position of *bicoid* RNA. However, most is located in basal nurse cell cytoplasm, or in deep anterior oocyte cytoplasm, where its role in *bicoid* RNA localization is not obvious.

There are three ways in which we might reconcile the presence of swallow protein in these unexpected locations with its suspected function in *bicoid* RNA localization. First, the small amount of swallow protein at the anterior oocyte cortex may anchor *bicoid* RNA, as envisioned above, while swallow protein elsewhere does not participate in *bicoid* RNA localization at all. For instance, swallow protein in nurse cell cytoplasm might simply represent the protein that is dumped into the oocyte at the end of oogenesis and that has a nuclear function in early embryos. However, since swallow protein is localized in nurse cell cytoplasm, rather than being uniformly distributed, this idea is less than satisfying.

A second possibility is that all swallow protein, including that in unexpected locations, participates in *bicoid* RNA localization, but the significance is not obvious because the process is more complex than has been previously imagined. For instance, swallow protein and *bicoid* RNA may interact in some way in basal nurse cell cytoplasm, and this interaction may be required for the later stability of localization. Cytoplasmic bridges, which connect nurse cells to each other and to the oocyte, are located on the basal sides of nurse cells, so *bicoid* RNA must pass through this swallow-rich cytoplasmic domain on its way to the oocyte. Based on the course of *bicoid* RNA localization in *swallow* mutants, these putative interactions should be required for the stability of localization, but not for the initial localization event itself. We showed that *bicoid* RNA localization in the oocyte is more resistant to colchicine disruption than is localization around nurse cell nuclei (Pokrywka and Stephenson, 1991); perhaps swallow protein in basal nurse cell cytoplasm ensures that *bicoid* RNA is targeted to a population of very stable microtubules in the anterior oocyte cortex.

A third possibility is that our suspicions concerning the role of the *swallow* gene product in *bicoid* RNA localization are incorrect. The idea that swallow protein anchors *bicoid* RNA at the anterior cortex comes from the late deterioration of *bicoid* RNA localization in existing *swallow* mutants. However these mutants may not be amorphic, and earlier defects in *bicoid* RNA localization might be evident in the complete absence of *swallow* function. The alleles examined for swallow protein distribution (*1497* and *11-999*) are the strongest available, and produce the same range of embryonic phenotypes when homozygous as when heterozygous with a deficiency (Stephenson, unpublished data). However each allele produces immunoreactive protein of normal abundance and approximately normal size (Hegdé, unpublished data) so we cannot exclude the possibility that a low level of *swallow* function is present. This residual function might be sufficient to localize *bicoid* RNA early in oogenesis, but not to maintain it later in oogenesis.

swallow protein in early embryos

swallow protein is present in early embryos and cells of the female germline, but absent from other developmental stages. Older embryos, which contain some dividing cells such as neuroblasts (Hartenstein et al., 1987), and larvae, which contain dividing imaginal disk cells (Campos-Ortega and Hartenstein, 1985), lack detectable *swallow* RNA (Stephenson and Mahowald, 1987) and swallow protein (this report; Hegdé, unpublished data), and do not require *swallow*$^+$ function (Perrimon et al., 1983). These results suggest that the function provided by swallow protein in early embryos is not necessary in these later stages, or is provided by another protein. Mitotic divisions in the early embryo are unusual in their rapidity and synchrony, and are not followed by cytokinesis (Glover, 1989, 1991). Perhaps swallow protein provides a function specifically required only in these early nuclear divisions. swallow protein is associated with the nucleolus during late interphase/early prophase, and may perform the same nucleolar function during late interphase/early prophase as it performs in the nucleus as a whole during mitosis.

One difficulty with these observations is that most *swallow* embryos do not arrest in early development, as might be expected if *swallow*$^+$ function were essential at this stage. We know that all five existing *swallow* alleles produce protein of apparently normal abundance and approximately normal size (Hegdé, unpublished data), allowing for the possibility that *swallow* mutations are hypomorphic for this function. As an alternative, the function carried out by *swallow* in embryogenesis may not be absolutely essential for development, or may be partially complemented by another protein with a similar function.

Nuclear cycling

Newly laid eggs contain significant quantities of swallow protein. During early cleavage divisions, swallow protein enters nuclei at mitosis and disappears at interphase. At these early stages, there appears to be excess swallow protein, so that significant quantities of the protein are present in the cytoplasm, even during mitosis. Therefore, nuclear localization of swallow protein in early embryos is probably regulated by a cell cycle-dependent nuclear import mechanism rather than by the availability of swallow protein. The level of cytoplasmic staining decreases during cleavage stages as the number of nuclei increase, and less and less of the protein is detectable in the cytoplasm during mitosis. In late blastoderm and gastrula embryos, little or no swallow protein is detectable in the cytoplasm, even during interphase. The apparent absence of a cytoplasmic pool suggests that nuclear localization of swallow protein at these stages must be at least partially regulated at the level of de novo synthesis. Substantial quantities of *swallow* mRNA are present in embryos until the cellular blastoderm stage (Stephenson et al., 1988). However, even at late blastoderm and early gastrula stages, nuclear import of swallow protein is a cell cycle-dependent event, since in *string* embryos, swallow protein fails to enter the nucleus and accumulates in the cytoplasm.

The mechanism by which swallow protein enters and leaves the nucleus is not clear. swallow enters the nucleus at late interphase or early prophase, well before the poles of the nuclear envelope rupture during prometaphase (Stafstrom and Staehelin, 1984). Therefore, it seems likely that import of swallow protein is through nuclear pores and is a regulated process. We find that the predicted swallow

protein contains two putative bipartite nuclear localization signals which may mediate its selective transport into the nucleus.

Nuclear defects and the *swallow* segmentation phenotype

Anterior defects are common to *swallow* and *exuperantia* embryos, and result from the failure to localize *bicoid* RNA (Frohnhöfer and Nüsslein-Volhard, 1987; Stephenson et al., 1988; Driever and Nüsslein-Volhard, 1988b). In addition, *swallow* embryos exhibit frequent defects in thoracic and abdominal segmentation (Stephenson and Mahowald, 1987; Stephenson, unpublished data). However since segmentation defects are uncommon in *exuperantia* embryos (Schüpbach and Wieschaus, 1986; Stephenson, unpublished data), segmentation defects in *swallow* embryos probably do not result from the failure to localize *bicoid* RNA. *swallow* mutants are cold sensitive for the segmentation phenotype, and the temperature sensitive period is in oogenesis, not embryogenesis (Frohnhöfer and Nüsslein-Volhard, 1987). That is, larger numbers of strongly affected embryos are produced by *swallow* females kept at 18°C than at 25°C, regardless of the temperature at which the embryos develop. Since each of the five *swallow* alleles examined to date show this temperature sensitivity (Stephenson, unpublished data), it is likely that the process in which *swallow* functions is itself cold sensitive in some way. The alternative, that five independent alleles of *swallow* coincidently produce protein with lower activity or lower stability at lower temperature, is unlikely.

swallow blastoderm and early gastrula embryos are often disorganized; nuclei have incorrect or nonuniform size and spacing, and are often out of mitotic synchrony with their neighbors (Zalokar et al., 1975; this report). Fate maps of early *swallow* embryos using molecular markers show invariant anterior defects and variable defects in thoracic and abdominal segmentation: stripes of fushi tarazu protein expression in the abdomen are missing, reduced in size and have abnormal spacing (Frohnhöfer and Nüsslein-Volhard, 1987), and expression of the gap gene *hunchback* shows variable levels of reduction in the posterior domain (Schröder et al., 1988).

No simple model of *swallow* function during embryogenesis accounts for all of these data. The simplest idea is that the embryonic nuclear division defects and larval segmentation defects are both related to embryonic functions of the *swallow*$^+$ gene product. In the absence of the regulatory and/or structural function provided by *swallow*$^+$, some nuclear divisions are abnormal, resulting in a disorganized blastoderm with nuclei of incorrect spacing and size, and which are not in mitotic synchrony. The precise pattern of segmentation gene expression is abnormal in these disorganized blastoderm embryos, leading to larval segmentation defects. The model fails to explain adequately the cold sensitivity of the *swallow* segmentation phenotype, and the fact that the temperature sensitive period is in oogenesis, not embryogenesis when swallow would seem most likely to play a direct role. Perhaps one or more molecular interactions during oogenesis occur with lower efficiency at lower temperatures, and the presence of the *swallow*$^+$ gene product in embryogenesis in some way ameliorates this effect. Of molecular interactions common to both oogenesis and early embryogenesis, the most prominent cold-sensitive process is microtubule assembly. Since *swallow* is involved in *bicoid* RNA localization, and since *bicoid* RNA localization requires microtubule function (Pokrywka and Stephenson, 1991), one possibility is that swallow interacts with microtubules both during oogenesis in the course of *bicoid* RNA localization, and again with microtubules in regulating nuclear divisions during early embryogenesis. The obvious problem with this model is that it fails to account for the presence of swallow protein within embryonic nuclei.

An alternative is that *swallow* has an unidentified, cold-sensitive role in oogenesis, the failure of which causes segmentation defects. One possibility might be that the functions of *swallow* in *bicoid* RNA localization and in this hypothetical cold-sensitive process are the same, modifying or stabilizing the egg cytoskeleton, for instance. Failure to carry out this function leads to the loss of *bicoid* RNA localization during oogenesis, and to aberrant nuclear divisions in embryogenesis. Again, this model fails to account for the presence of swallow protein within embryonic nuclei.

A final possibility is that swallow protein is modified or becomes associated with other molecules during oogenesis, and that this modification/association is essential for swallow embryonic function in regulating nuclear divisions. The modification process may itself be cold sensitive, and its partial failure at low temperatures may limit the effectiveness of mutant swallow protein in carrying out its role in embryogenesis.

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