Development 128, 2823-2832 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
DEV5922

aubergine encodes a *Drosophila* polar granule component required for pole cell formation and related to eIF2C

Adam N. Harris[1] and Paul M. Macdonald[2]

[1]Department of Biological Sciences, Stanford University, Stanford, CA 94305, USA
[2]Institute for Cellular and Molecular Biology, University of Texas, Austin, TX 78712, USA

Accepted 26 April 2001

SUMMARY

In *Drosophila* oocytes, activation of Oskar translation from a transcript localized to the posterior pole is an essential step in the organization of the pole plasm, specialized cytoplasm that contains germline and abdominal body patterning determinants. Oskar is a component of polar granules, large particles associated with the pole plasm and the germline precursor pole cells of the embryo. *aubergine* mutants fail to translate *oskar* mRNA efficiently and are thus defective in posterior body patterning and pole cell formation. We have found that Aubergine protein is related to eukaryotic translation initiation factor 2C and suggest how it may activate translation. In addition, we found that Aubergine was recruited to the posterior pole in a *vas*-dependent manner and is itself a polar granule component. Consistent with its presence in these structures, Aubergine is required for pole cell formation independently of its initial role in *oskar* translation. Unlike two other known polar granule components, Vasa and Oskar, Aubergine remains cytoplasmic after pole cell formation, suggesting that the roles of these proteins diverge during embryogenesis.

Key words: Aubergine, Oskar, eIF2C, Translation, Pole cell, Polar granule, *Drosophila*

INTRODUCTION

One way in which daughter cells may adopt different cell fates is through the asymmetric localization of cytoplasmic determinants before cell division. A prominent example of this phenomenon is found in early *Drosophila* development, where determinants localized to different regions of the oocyte specify the fates of cells formed at those regions in the developing embryo. During oogenesis a specialized cytoplasm, called pole plasm, is assembled at the posterior of the oocyte (for a review, see Rongo and Lehmann, 1996). Late in oogenesis, electron-dense organelles called polar granules can be found in the pole plasm, and these persist in the embryonic pole cells, which are germline precursor cells (for a review, see Mahowald, 2001). Pole plasm is necessary for induction of abdominal segmentation and germline formation during embryogenesis, and is sufficient to induce both features when transplanted ectopically to the anterior of the embryo (Illmensee and Mahowald, 1974; Frohnhöfer et al., 1986).

Mutations in several genes affect the assembly of pole plasm and thus germline formation and abdominal patterning. Among these, *oskar* (*osk*) has a central role. Both *osk* mRNA and the protein it encodes are localized in the pole plasm, and Osk protein is a component of polar granules (Kim-Ha et al., 1991; Ephrussi et al., 1991; Smith et al., 1992; Ephrussi and Lehmann, 1992; Breitwieser et al., 1996). The level of other pole plasm components and the number of pole cells are sensitive to *osk* gene dose, and misexpression of Osk protein induces ectopic pole plasm, pole cells and abdominal body patterning (Ephrussi and Lehmann, 1992; Smith et al., 1992). The distribution of Osk protein is normally spatially and temporally restricted through the combinatorial action of mRNA localization, translational regulation and the subsequent anchoring of Osk protein at the cortex of the oocyte. *osk* mRNA localizes to the posterior pole at around stage 8 of oogenesis (Kim-Ha et al., 1991; Ephrussi et al., 1991). Before localization, the mRNA is translationally repressed by Bruno (Bru; Aret – FlyBase) protein (Kim-Ha et al., 1995; Webster et al., 1997). Additional factors, including Bicaudal C (BicC; Saffman et al., 1998), Apontic (Lie and Macdonald, 1999) and possibly a factor named p50 (Gunkel et al., 1998) also contribute to repression of *osk* translation.

In contrast, products of the *vasa* (*vas*), *staufen* (*stau*) and *aubergine* (*aub*) genes are required for the accumulation of high levels of Osk protein at the posterior pole (Kim-Ha et al., 1995; Markussen et al., 1995; Rongo et al., 1995; Wilson et al., 1996). The *vas* and *stau* genes have been previously identified. *vas* encodes an RNA helicase related to the translation initiation factor eIF-4A (Hay et al., 1988b; Lasko and Ashburner, 1988; Liang et al., 1994). Stau is a double-stranded RNA-binding protein (St Johnston et al., 1992). Both Vas and Stau proteins have been shown to localize to the posterior pole of the oocyte, and Vas, like Osk, is a polar granule component (Hay et al., 1988a; Hay et al., 1988b; St Johnston et al., 1991). The identities and distribution of these gene products are consistent with models in which they interact directly with *osk* mRNA to promote its translation.

We now report that Aub is a cytoplasmic protein related to

the translation initiation factor eIF2C. Using a fully functional Aub protein tagged with GFP, we show that Aub accumulates in pole plasm in a manner dependent on *vas* and is itself a polar granule component. Several lines of evidence strongly suggest that the posterior localization of Aub is not required for its role in translational activation. Instead, the posterior concentration is likely to be required for two additional functions of Aub. The first is a previously defined role in posterior localization of *nanos* (*nos*) mRNA (Wilson et al., 1996), whose restricted expression is required for correct abdominal patterning (Gavis and Lehmann, 1994). The second is in pole cell formation, as we demonstrate that Aub is required for this process independently of its function in activation of *osk* mRNA translation.

MATERIALS AND METHODS

Fly stocks and transgenes

uas-gfp-aub was injected by standard methods (Spradling and Rubin, 1982) using w^{1118} flies as recipients. The transgene is derived from the UASp vector of Rorth (Rorth, 1998) and was made by inserting mGFP6 (Siemering et al., 1996) fused in frame to the start codon of an *aub* cDNA (LD23087 of BDGP; Rubin et al., 2000). Transgenic lines carrying the *aub* genomic rescue constructs MR-1 and MR-3 (Schmidt et al., 1999) were provided by Ulrich Schäfer. The *osk-bcd* transgene used was the *short osk-bcd* version (Breitwieser et al., 1996) and was jumped onto the third chromosome from a stock provided by Anne Ephrussi. *vas* mutants used were vas^{AS}/vas^{PD23} transheterozygotes. Similar results were also obtained with a vas^{PD23}/vas^{O11} combination. *vas-gfp* (Breitwieser et al., 1996) flies were provided by Anne Ephrussi. *stau* mutants were homozygous $stau^{D3}$. *aub* mutants were aub^{N11}/aub^{HN2}. The P insertion in region 31E (P31E) used for RFLP analysis came from the stock w^{1118}; P{w^{+mC}=NM}31E P{$ry^{+t7.2}$=neoFRT}40A (Xu and Rubin, 1993). This stock also carries a $ry^{+t7.2}$ insertion at position 40A, which was ignored. The P insertion in 33A (P33A) was believed to be P{lacW}crol^{s2346} (Spradling et al., 1995; Spradling et al., 1999). However, subsequent analysis led us to conclude that our flies contained a different insertion that is unexpectedly complemented by Df(2)Prl (Lindsley and Zimm, 1992) and probably lies proximal to 33A. Nevertheless, this line enabled us to generate RFLPs and successfully locate the *aub* gene.

RFLP analysis

Recombinants were generated between a *Sp*-marked aub^{N11} chromosome and the P33A line. A single recombinant line was selected, isogenized and crossed to an isogenized P31E stock to generate recombinants between the P insertions. Genomic DNA was prepared according to Protocol 47 (Ashburner, 1989) from the *Sp* aub^{N11} P33A and aub^{+} P31E parental lines and aub^{+} recombinants lacking both P elements. Approximately 5 μg of DNA from each parental line was digested with numerous restriction enzymes, electrophoresed on agarose gels, and Southern blotted. Probes were made by random priming of subcloned BDGP P1 DNA or by transcription using viral promoters flanking P1 inserts, when present. Enzyme/probe combinations that identified polymorphisms between the parental chromosomes were then used to test digested DNA from the recombinant flies. The most informative polymorphisms flanked the closest recombination event distal to the aub^{N11} mutation and were generated from P1 DS04172 (Kimmerly et al., 1996), which was partially restriction mapped. While this mapping was being carried out, sequence information from the overlapping P1 DS01831 became available and was used to identify candidate genes in the region, including *sting*.

Sequencing of mutant alleles

The entire *aub*-coding region was amplified by PCR (Expand^{tm} High Fidelity kit, Boehringer Mannheim; primers: sense 5′-cactctagagcatttgtgacgagtagatcgtaaga-3′ and antisense 5′-cacggtaccgttaacgcatttaatgcaagtcatagt-3′) from genomic DNA obtained from single aub^{N11}/CyO, aub^{N11}/aub^{HN2}, and aub^{N11}/aub^{K86} male flies (Turelli and Hoffmann, 1995). The product from the aub^{N11} allele was recognized to contain a small deletion, which enabled identification of the correct PCR product for each of the other mutant chromosomes in *trans*. The PCR products were cloned into pBluescript SK(+) via *Xba*I and Asp718 sites in the primers. Two clones from independent PCR reactions were sequenced for each allele using pairs of primers approximately 1 kb apart and between them covering the entire coding region. Nucleotide differences from the wild-type *aub* sequence (GenBank Accession Number, X94613) were distinguished from PCR errors by their appearance in both of the independent clones. GenBank accession numbers for the coding regions of exons with nucleotide differences from the wild-type are as follows: aub^{HN2} exon 9, AF334408; aub^{K86} exon 4, AF334409; aub^{K86} exon 6, AF334410; aub^{N11} exon 4, AF334411; and aub^{N11} exon 9, AF334412.

Aub antibody and western blot

Polyclonal antibodies were raised in a rat by Josman, LLC, against the C-terminal region of Aub protein (amino acids 562-866) encoded by the *Bgl*II-*Xho*I fragment of EST LD23087 (Rubin et al., 2000) and expressed in inclusion bodies in *Escherichia coli* using the pET21a vector (Novagen). Crude ovary extracts were prepared by homogenizing hand-dissected ovaries from wild-type or aub^{N11}/aub^{HN2} transheterozygous females in SDS-loading buffer and boiling for 5 minutes. Approximately one ovary pair equivalent was loaded on each lane of an 8% SDS-polyacrylamide gel. Proteins from the gel were transferred to nitrocellulose by submerged electroblotting at 20V for 45 minutes in transfer buffer (25 mM Tris base, 200 mM glycine, 20% methanol). The membrane was allowed to air dry. For detection, we used the Tropix Western-Light kit according to the manufacturer's instructions, except that the incubation time with the primary antibody (at 1:1000) was increased to 2 hours and the number of washes was increased to a total of eight.

In situ hybridization, antibody staining and confocal microscopy

Ovaries were hand-dissected in PBS and disrupted with flame-drawn Pasteur pipettes. For in situ hybridization, ovaries were fixed and processed as described (Kim-Ha et al., 1991), using an RNA probe prepared from the full-length *aub* cDNA and labeled with digoxigenin-11-UTP (Boehringer Mannheim). For antibody staining, ovaries were fixed by rotating for 20 minutes in PBS + 6% formaldehyde under heptane, and embryos were fixed as described (Smith et al., 1992). Secondary antibodies coupled to Cy5 (Jackson Immunoresearch) or AlexaFluor^{tm} 488 (Molecular Probes) were used as suggested by the manufacturer. Rabbit anti-Osk antibodies were used at 1:3000 final concentration. Microscopy was performed with a Leica SP-TCS using Leica Confocal Software version 2.00. Identical laser power and photomultiplier settings were used when direct comparisons of protein levels were made.

RESULTS

aub encodes a protein related to eIF2C

To investigate the molecular function of *aub*, we devised a strategy to positionally clone the locus. Previously, *aub* had been mapped by meiotic recombination to genetic position 39 on the second chromosome (Schüpbach and Wieschaus, 1991). We subsequently mapped *aub* between P elements reported to

be inserted in polytene regions 31E and 33A (see Materials and Methods). We used RFLP mapping as well as clones and sequence information from the Berkeley *Drosophila* Genome Project (BDGP; Kimmerly et al., 1996; Adams et al., 2000) to narrow the location of the *aub* gene to a small region at the boundary of polytene divisions 32C and 32D (Fig. 1A, Materials and Methods).

This region contains *sting* (Schmidt et al., 1999), a member of an ancient gene family that includes the gene for the eukaryotic translation initiation factor eIF2C (Zou et al., 1998). With support from three lines of evidence, we were able to identify *aub* and conclude that *aub* and *sting* are the same gene. First, in each of three *aub* mutant chromosomes, we identified mutations predicted to truncate the Aub/Sting protein (Fig. 1B). Second, antibodies raised against a bacterially expressed C-terminal portion of Aub/Sting recognize a band of approximately 105 kDa in extracts from wild-type ovaries but not ovaries from *aub* mutants (Fig. 1C). A band corresponding to a similar molecular weight is produced by in vitro transcription and translation of an *aub/sting* cDNA (data not shown). Third, two genomic DNA fragments (Fig. 1A; Schmidt et al., 1999) which include the *aub/sting* gene rescue *aub* mutants: *aub*$^{-}$ mothers carrying a single copy of either transgene are fertile and produce viable offspring. Following tradition, we use the original name, *aubergine*, for this locus.

The *Drosophila* genome contains four other members of the *eIF2C*-like gene family. One of these is *piwi*, a close chromosomal neighbor of *aub* (Fig. 1A) that acts in germline stem cell maintenance (Cox et al., 1998). Two additional members, *CG7439* and *dAGO1*, are reported in the genome annotation (Adams et al., 2000). The latter is the closest known relative of *eIF2C* in flies and is presumably the *Drosophila eIF2C* homolog. We identified the fifth family member, corresponding to the genomic sequence AE003107 (Adams et al., 2000) and EST clot 2083 (Rubin et al., 2000), by tBLASTn searches of the BDGP databases using parts of Aub protein as the query sequence.

Aub protein is cytoplasmic and concentrates in the pole plasm

The *aub* transcript is expressed at relatively high levels in the germarium, at lower levels during mid-oogenesis, and again at high levels in the nurse cells and oocyte from about stage 6 of oogenesis (Fig. 2A; data not shown). Of greater interest is the distribution of Aub protein. Our antibodies, which detect Aub on western blots (Fig. 1C), fail to detect Aub protein in whole-mount ovaries. In both wild-type and *aub* mutant ovaries, we detect a similar low level of uniform staining (data not shown). As an alternate approach to determine the distribution of Aub protein, we used the UAS/GAL4 system to express a GFP-tagged version of Aub in vivo. Flies carrying a *uas-gfp-aub* transgene were crossed to those that express a *nos-gal4-vp16* transgene which drives expression of GAL4-VP16 in the female germline (Van Doren et al., 1998).

The temporal expression of the *nos-gal4-vp16* driver parallels that of *aub* (Van Doren et al., 1998; Fig. 2A), and *uas-gfp-aub* driven by *nos-gal4-vp16* is sufficient to fully rescue *aub* mutant defects, indicating both that the fusion protein is functional and that its distribution includes the pattern required of native Aub protein. GFP-Aub protein can be visualized throughout oogenesis. In the nurse cells, the protein is distributed evenly in the cytoplasm and in concentrated foci surrounding the nuclei (Fig. 2B). These presumptive perinuclear particles remain until around stage 10. In the oocyte, GFP-Aub is at first dispersed evenly in the cytoplasm. Later, beginning at stage 8/9, it becomes strikingly concentrated in the posterior pole plasm, where it largely overlaps the distribution of Osk protein (Fig. 2C-E). This posterior concentration appears to occur by recruitment of the GFP-Aub protein, as the *aub* mRNA is not itself localized within the oocyte (Fig. 2A).

Aub is a polar granule component

The localization of Aub to the posterior pole of the oocyte raises the possibility that it is a component of polar granules. Polar granules are large, electron-dense structures found in the posterior pole plasm of the oocyte (for a review, see Mahowald, 2001). In the embryo, these granules remain localized to the posterior pole where they are largely incorporated into the

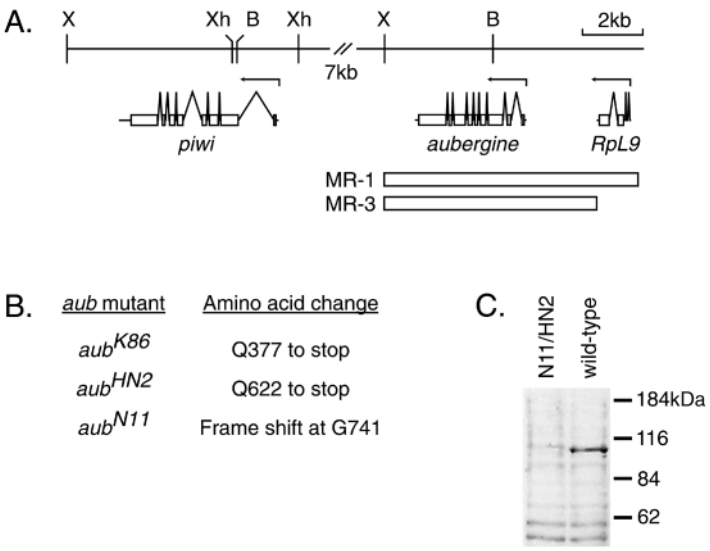

Fig. 1. Identification of *aubergine*. (A) Schematic of genomic region 32C4-D1 on arm 2L. Left is distal. The positions of restriction sites are shown above the line (B, *Bam*HI; X, *Xba*I; Xh, *Xho*I). Arrows indicate start positions and directions of transcription. In the transcripts, the coding regions are shown by boxes. The MR-1 and MR-3 genomic fragments (Schmidt et al., 1999) rescue *aub* mutant defects. (B) Molecular defects of *aub* mutant alleles, with the affected amino acids indicated. The frameshift in *aub*^{N11} is caused by a 154 bp deletion which is predicted to add 16 novel amino acids after E740. Two mutants, *aub*^{K86} and *aub*^{N11}, share an additional change relative to the wild-type sequence (GenBank Accession Number, X94613), 5549 G to A, which does not affect the protein sequence. Both alleles came from a single mutagenesis (Wilson et al., 1996), and this change presumably represents a polymorphism in the common parental chromosome. The third mutant, *aub*^{HN2}, which was obtained in a separate screen using a different parental chromosome (Schüpbach and Wieschaus, 1991) includes three other changes, all of which are 3′ to the stop codon mutation: 7304 A to C, 7326 T to A, and 7337 T to C. (C) A Western blot of ovary extracts. A band of approximately 105kDa is recognized in ovary extracts from flies wild-type at the *aub* locus (*w*^{1118}), but not from transheterozygotes for the *aub*^{N11} and *aub*^{HN2} alleles.

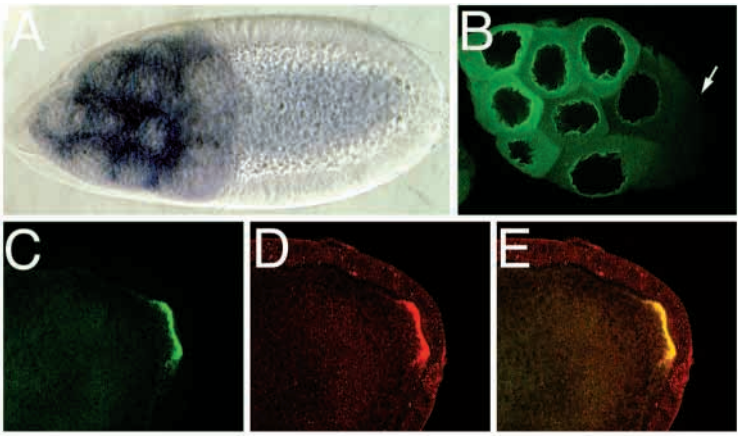

Fig. 2. *aub* mRNA and GFP-Aub expression pattern during oogenesis. (A) In situ hybridization for *aub* mRNA in a stage 10 egg chamber, showing expression in the nurse cells and oocyte but no localization to the posterior ooplasm. (B) A stage 8 egg chamber displaying cytoplasmic GFP-Aub in the nurse cells, identifiable by their large unstained nuclei and oocyte (arrow). Note that the nurse cell nuclei are closely surrounded by concentrated GFP-Aub (see also Fig. 5). Levels of GFP-Aub are commonly unequal in different nurse cells, as seen here. (C,D) GFP-Aub (green) and Osk (red) at the posterior pole of the oocyte in a stage 10 egg chamber. (E) Merged image of green and red channels showing colocalization (yellow).

budding pole cells. Several other posteriorly localized proteins, including Tudor (Tud), Vas and Osk, have been shown by immunoelectron microscopy to be components of the polar granules (Hay et al., 1988a; Hay et al., 1988b; Bardsley et al., 1993; Breitwieser et al., 1996). With fluorescence-based detection methods, polar granule components appear in particles, which have been presumed to be the polar granules. Colocalization of Osk and Vas (fused to GFP) in these particles supports this conclusion (Breitwieser et al., 1996).

To determine if Aub is a polar granule component, we used confocal microscopy to characterize the subcellular distribution of GFP-Aub in embryos. Before pole cell formation, GFP-Aub is found throughout the cytoplasm but strongly concentrated at the posterior pole in distinct particles (Fig. 3A), most of which also contain Osk protein (Fig. 3D,G). The particles are predominantly incorporated into the pole buds, which form at the posterior pole of the embryo and initiate pole cell formation (Fig. 3B,E,H). At this time, the particles increase in size and decrease in number, suggesting that individual particles are fusing. Simultaneously, the level of dispersed, non-particulate GFP-Aub increases in the pole cell cytoplasm. Before cellularization, between nuclear division cycles 11 and 13, the particles often cluster in zones at opposite sides of the pole cell nuclei (Fig. 3C, arrowheads). Most of the particles continue to display colocalization of GFP-Aub and Osk (Fig. 3C,F,I). However, some Osk-containing particles now begin to appear in the pole cell nuclei, while GFP-Aub is not detectable in these particles and consistently remains excluded from the nuclei. By the time of cellularization, following nuclear division cycle 14, only a few large cytoplasmic particles persist in each pole cell, with much of the GFP-Aub distributed evenly throughout the cytoplasm (Fig. 4A). Osk continues to colocalize strongly with GFP-Aub in the particles, but most Osk staining is now detected in the nuclear particles and dispersed in the nucleoplasm (Fig. 4B,C). Both cytoplasmic and nuclear particles often appear in striking hollow spherical shapes (donut-like in optical sections), which are characteristic of some polar granule clusters as well as similar particles that are present in nuclei, called nuclear bodies (Mahowald, 1962; Mahowald, 1968; Fig. 4A-C). In comparable colocalization experiments, we determined that Vas-GFP behaves very similarly to Osk: there is always a high level of colocalization in particles, both nuclear and cytoplasmic (Fig. 4D-F and data not shown).

Our data demonstrate that GFP-Aub is found in particles that (1) also contain Osk and are inferred to contain Vas, both known polar granule components; (2) are located, like polar granules, at the posterior pole of the oocyte and embryo and are incorporated into pole cells; (3) increase in size within the pole cells with similar timing to that previously described for polar granules; and (4) frequently appear, as do polar granules, in spherical structures that are donut-shaped in sections. From these results, we conclude that Aub is a polar granule component. However, unlike Osk and Vas, which are largely

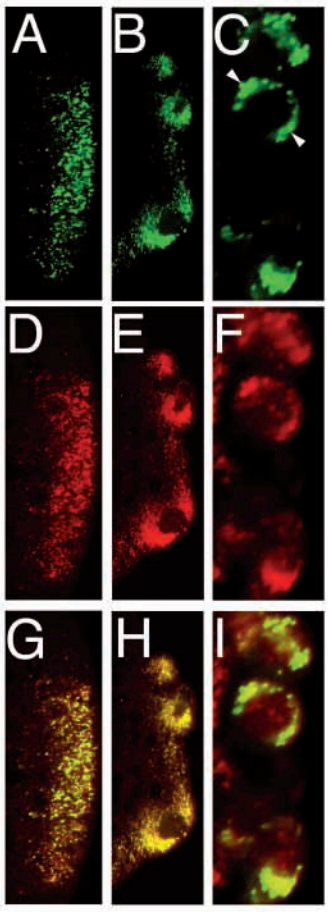

Fig. 3. Comparison of GFP-Aub and Osk protein distributions in embryogenesis. Each column shows GFP-Aub (A-C), Osk (D-F) and overlap (G-I) at the posterior pole of embryos of increasing age. (A,D,G) Embryo before pole bud formation. GFP-Aub and Osk are found in overlapping sets of particles. Some particles are labeled uniquely with GFP-Aub or Osk, but most show colocalization. (B,E,H) Embryo with pole buds forming. Both GFP-Aub and Osk are cytoplasmic, and continue to colocalize. (C,F,I) Pole cells in a precellularization embryo. GFP-Aub remains cytoplasmic, but some Osk now appears in the nuclei. Within the cytoplasm GFP-Aub and Osk are colocalized in particles of increased size. These particles are frequently concentrated on opposing faces of the nuclei, as indicated by arrowheads. All aspects of Osk protein localization are virtually identical to that displayed by a Vas-GFP fusion protein, with both proteins colocalizing in particles at all stages shown, as well as at later stages of embryogenesis (not shown).

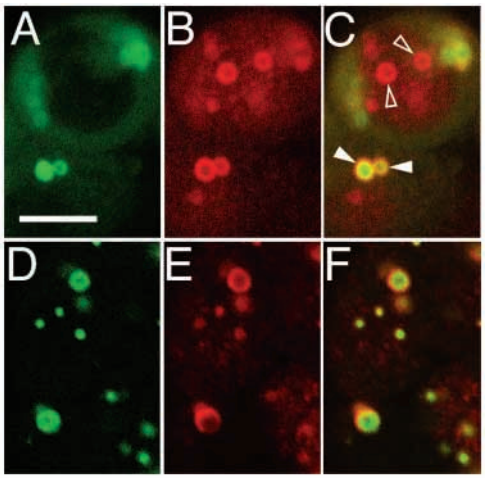

Fig. 4. Aub, Osk and Vas in structures characteristic of polar granule material. Donut-shaped structures in pole cells of cellularized embryos contain GFP-Aub (A), Osk (B,E) and Vas-GFP (D). Each of the structures containing GFP-Aub also contains Osk, as shown in the overlap of the two signals (C). Note that GFP-Aub is limited to the cytoplasm (filled arrowheads), with the nuclear Osk-containing donut-shaped structures lacking Aub (open arrowheads). All Osk-containing structures also include Vas-GFP, as seen in the overlap of the Osk and Vas-GFP signals (F). Scale bar: 5 μm. Most of the donut-shaped structures have diameters of approximately 0.5 to 1.0 μm, consistent with a diameter of 0.75 μm to 1.0 μm for polar granules and nuclear bodies as reported by Mahowald (Mahowald et al., 1976).

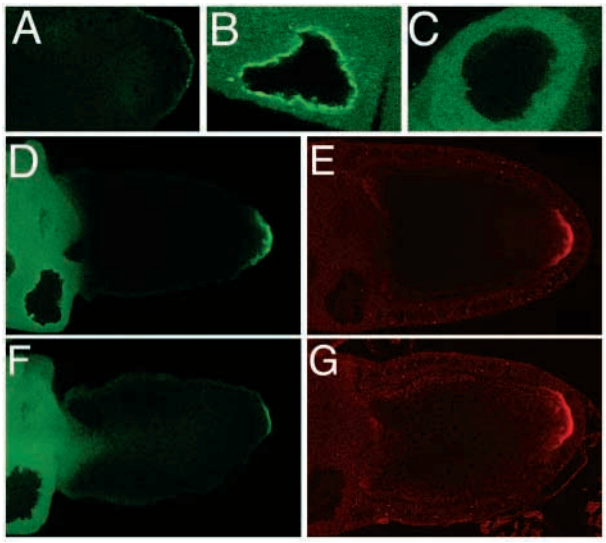

Fig. 5. Differential effects of *stau* and *vas* mutants on GFP-Aub localization. (A) Posterior portion of a stage 10 *stau* mutant oocyte. GFP-Aub localization at the posterior pole is virtually eliminated. (B) *stau* mutant nurse cell. The appearance of GFP-Aub in perinuclear particles is unaffected. (C) *vas* mutant nurse cell. The perinuclear clustering of GFP-Aub particles is largely abolished. Occasionally, one or two particles may be seen. (D-E) *vas* heterozygous stage 10 oocyte. Both GFP-Aub (D) and Osk (E) are concentrated at the posterior pole as in wild-type. (F-G) *vas* mutant late stage 10 oocyte. GFP-Aub (F) localization to the posterior pole is dramatically reduced, while the level of posteriorly localized Osk (G) remains essentially normal. Although this result differs from published reports of a substantial decrease in the level of Osk protein in *vas* mutant ovaries, the earlier studies compared average protein levels throughout the ovary and did not differentiate between different stages of oogenesis (see text).

nuclear after the cellular blastoderm stage, Aub is restricted to the cytoplasmic class of particles, indicating that the nuclear bodies and cytoplasmic polar granules do not have identical compositions (see Discussion).

Because GFP-Aub concentrates at the posterior pole of the oocyte and is found in polar granules, we reasoned that Aub localization should depend on the activity of genes required for polar granule assembly. *stau* and *vas* are two such genes with somewhat different roles in this process. Stau acts primarily in the localization and translation of *osk* mRNA, prerequisites for polar granule formation (Kim-Ha et al., 1991; Ephrussi et al., 1991; Kim-Ha et al., 1995; Micklem et al., 2000). Although Stau is concentrated at the posterior pole of the oocyte, it is not incorporated into the polar granules early in embryogenesis (Breitwieser et al., 1996). By contrast, Vas is a polar granule component and appears to be more directly involved in the formation and integrity of these structures (Schüpbach and Wieschaus, 1986; Hay et al., 1988a; Hay et al., 1988b; Hay et al., 1990; Lasko and Ashburner, 1990; Liang et al., 1994).

Not surprisingly, we find that GFP-Aub posterior localization is almost completely defective in *stau* mutants (Fig. 5A) and greatly reduced in *vas* mutants (compare Fig. 5D with 5F), confirming that Aub is localized by the same general mechanism implicated in the localization of other polar granule components. In addition, a second and equally striking defect occurs in only the *vas* mutants: perinuclear localization of GFP-Aub in the nurse cells is almost entirely eliminated (compare Fig. 5B with 5C). Taken in conjunction with the known perinuclear concentration of Vas protein (Hay et al., 1988a; Liang et al., 1994), this result raises the possibility that *vas*-dependent localization of Aub initiates in the nurse cells and involves a complex already containing both Vas and Aub proteins (see Discussion).

aub is required for pole cell formation

Our identification of Aub as a polar granule component suggests that it, like other such components, may be required for pole cell formation. Although *aub* mutants lack pole cells, this could be attributed to their deficiency in synthesis of Osk protein, a prerequisite for pole cell formation. Therefore, we used an *osk-bcd* transgene to bypass the requirement for *aub* in *osk* mRNA translation and assess the role of *aub* in pole cell formation. In this transgene, the *osk* 3′UTR is replaced with the *bicoid* (*bcd*) localization signal, and the encoded mRNA is localized to the anterior of the oocyte (Ephrussi and Lehmann, 1992). Eggs produced by aub^{+} females carrying an *osk-bcd* transgene synthesize Osk protein at both poles (Wilson et al., 1996; Fig. 6A,B). In embryos produced by *aub* mutants carrying the *osk-bcd* transgene, Osk protein is absent from the posterior pole but efficiently translated at the anterior, reflecting a difference in the requirement for *aub* for translation of the transgenic and endogenous mRNAs (Wilson et al., 1996; Fig. 6E,F). However, while most of the Osk protein at the anterior pole is taken up in ectopic pole cells in embryos from *aub* heterozygous mothers (Fig. 6C), Osk protein particles become dispersed over the cortex in the anterior part of embryos from homozygous *aub* mutant mothers (Fig. 6G) and

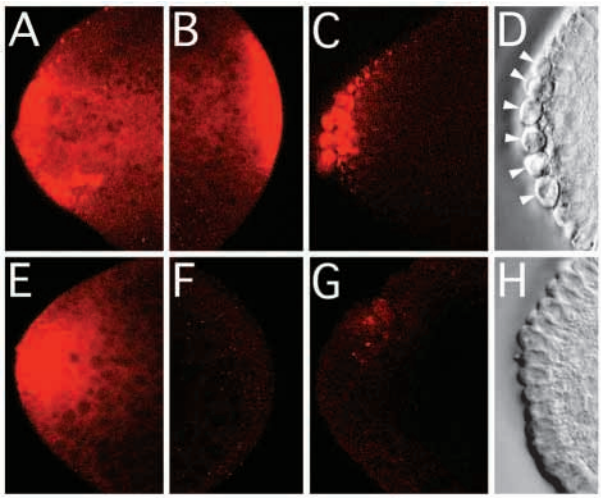

Fig. 6. *aub* is required for normal pole cell formation. Embryos produced by *aub* heterozygous (A-D) or *aub* mutant (E-H) females expressing an *osk-bcd* transgene. (A-C,E-G) Projections of multiple *z*-sections showing the location of Osk (fluorescence). (A-C) Transgenic Osk protein can be found at the anterior (A) in addition to the endogenous Osk at the posterior pole (B) of precellularized embryos. After cellularization, Osk can be found in pole cells at the anterior (C) and posterior (data not shown). (D) Pole cells (arrowheads) can be identified by their round cell bodies and nuclei while those of other cells at the surface of the blastoderm have become elongated. (E,F) In early stage embryos maternally mutant for *aub*, only the transgenic, anterior Osk (E) accumulates to high levels. Posterior Osk (F) is absent or greatly reduced. Note that anterior localization of *nos* mRNA in such early stage embryos is defective, despite the anterior accumulation of Osk protein (Wilson et al., 1996). Thus, the *nos* mRNA localization defect cannot be attributed to the subsequent dispersal of Osk protein seen in G. (G) By the time of cellularization in embryos from *aub* mutant females, local concentrations of Osk at the anterior are greatly diminished, and Osk often appears dispersed from the anterior pole. Osk occasionally is found in cells along the cortex, but only rarely do these have the morphological characteristics of pole cells. Osk protein is not maintained in these cells, as we never see Osk in late embryos from *aub* mutants, while it persists in both ectopic and native pole cells in embryos from *aub* heterozygotes (not shown). (H) No obvious pole cells are found at the anterior of an embryo maternally mutant for *aub*.

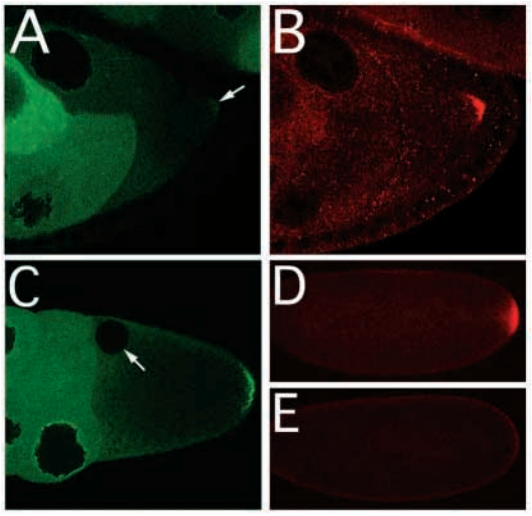

Fig. 7. GFP-Aub localization is unlikely to be required for its role in translation. (A,B) Stage 8/9 egg chamber. Only a low level of GFP-Aub (A) is localized at the posterior pole of the oocyte (arrow), even though significant Osk protein (B) has already been translated. Note that the initial appearance of GFP-Aub at the posterior pole is not delayed by a time lag in acquiring fluorescence, as the *aub* mRNA is not itself localized and thus the protein is not synthesized at the posterior but is likely transported from existing pools elsewhere in the egg chamber. (C) Stage 9 egg chamber, with GFP-Aub concentrated at posterior of oocyte (right). Although Grk protein (not shown) is produced in the anterodorsal corner near the oocyte nucleus (arrow) at this stage, no localization of GFP-Aub is evident. The focal plane was chosen to emphasize the nucleus; the highest concentration of GFP-Aub at the posterior lies in a different plane. (D,E) Early embryos. Compared with control embryos produced by *vas* heterozygous females (D), little or no Osk protein remains at the posterior pole (right) of embryos from *vas* mutant females (E). No difference in Osk levels is detected in stage 10 oocytes (see Fig. 4), indicating that the defect of *vas* mutants in translation of Osk begins only late in oogenesis.

fail to direct pole cell formation at the anterior pole (Fig. 6H). Thus, *aub* is required for the development of ectopic pole cells at the anterior of the embryo, and we infer that, like genes for other polar granule components, *aub* is involved in pole cell formation at the posterior as well.

Aub posterior localization appears to be distinct from its initial role in translation

Although the posterior concentration of Aub in oocytes might seem to contribute to its role in translation of *osk* mRNA, which occurs predominantly at that site, three observations are at odds with this inference.

First, in examination of more than 20 stage 8/9 egg chambers, we discovered that the posterior accumulation of GFP-Aub is slightly delayed from the appearance of Osk protein in the same region (Fig. 7A,B). In the oocytes, we often detected significant Osk protein when very little GFP-Aub concentration at the posterior had occurred. In contrast, we never observed an egg chamber that had localized GFP-Aub but only low levels of Osk protein. Thus, it appears that Aub is not concentrated at the posterior before the onset of *osk* translation.

Second, we do not detect any concentration of Aub in the anterodorsal region of the oocyte where *gurken* (*grk*) mRNA is localized (Fig. 7C) even though *aub* has also been implicated in the activation of *grk* translation (Wilson et al., 1996).

Finally, it is possible to greatly reduce the posterior concentration of GFP-Aub and still initiate translation of Osk at normal levels. This situation occurs in *vas* mutants. Although previous reports have shown that *vas* mutant ovaries accumulate reduced levels of Osk protein (Markussen et al., 1995; Rongo et al., 1995), we find that Osk initially appears at normal levels in *vas* mutants. The previous analyses relied on western blots of total ovarian protein, and so were unable to reveal any temporal specificity to the reduction. We used confocal microscopy to compare Osk protein levels in ovaries and embryos from *vas* mutant and heterozygous females. Up to stage 11 of oogenesis, before deposition of the vitelline membrane interferes with whole-mount antibody staining, *vas* mutants accumulate normal amounts of Osk (compare Fig. 5E

with 5G). By contrast, early embryos from *vas* mutant mothers display dramatic reductions in Osk protein (compare Fig. 7D with 7E). Thus, Osk protein levels are affected in *vas* mutants only in late stages of oogenesis, either through reduced translation or stability. The fact that normal levels of Osk appear in stage 9-11 *vas* mutant oocytes despite greatly lowered levels of GFP-Aub posterior accumulation, together with our other observations, strongly suggest that the posterior concentration is not required for *aub*-dependent activation of *osk* translation.

Thus, the uniform low level of Aub found throughout the ooplasm appears to be sufficient for its action in *osk* and *grk* translation at the posterior pole and anterodorsal corner of the oocyte, respectively. The higher levels of Aub resulting from posterior recruitment presumably reflect other roles for Aub. These include two roles Aub shares with Vas: the posterior localization of *nos* mRNA (Wilson et al., 1996) and pole cell formation.

DISCUSSION

Previous analysis of *aub* implicated it in two processes: the translational activation of a narrowly restricted subset of ovarian mRNAs, including *osk* and *grk*, and the subsequent localization of *nos* mRNA (Wilson et al., 1996). The latter function could be indirect, with Aub activating translation of a factor directly involved in *nos* mRNA localization, or Aub might itself play a more direct role in the process. Our demonstration that Aub is related to the translation initiation factor eIF2C lends further support for a direct role of Aub in translational activation. Evidence that Aub is a component of polar granules and is required for pole cell formation adds an additional function and also suggests that its later role in localization of *nos* mRNA may be relatively direct.

aub is a member of a family of genes that includes *eIF2C* and *piwi*

The central and C-terminal portions of Aub contain two conserved regions, designated the PAZ and Piwi domains (Cerutti et al., 2000), which are encoded by a group of genes from organisms as diverse as plants, fungi and metazoans (including vertebrates). Recently, several of these genes have been characterized genetically and have been found to play essential roles in development. Both *argonaute* (*ago1*) and *pinhead/zwille* are required for maintenance of the axillary shoot meristem in *Arabidopsis thaliana* (Bohmert et al., 1998; Moussian et al., 1998; Lynn et al., 1999). In *Drosophila*, *piwi* has a demonstrated role in germline stem cell maintenance (Cox et al., 1998; Cox et al., 2000). Similarly, two *Caenorhabditis elegans* genes closely related to *aub* and *piwi*, *prg-1* and *prg-2*, are also likely to be involved in germline proliferation (Cox et al., 1998). Other genes in the *eIF2C*/*piwi* family are implicated in mediating double-stranded RNA interference (RNAi) in *C. elegans* (*rde-1*; Tabara et al., 1999; Grishok et al., 2000) or the potentially related phenomena of post transcriptional gene silencing (PTGS) in *Arabidopsis* (*ago1*; Fagard et al., 2000) and quelling in *Neurospora* (*qde-2*; Catalanotto et al., 2000). The roles for *ago1* in both PTGS and a cell fate decision reveal that a single gene in the family can carry out two functions, but it is not known if these functions are mechanistically distinct.

Although the genetic pathways in which these family members act have been identified, the direct activity through which any plays a role in development or RNAi has not been determined to date. The only gene product in the family for which a specific biochemical activity has been demonstrated is the translation initiation factor eIF2C (formerly Co-eIF-2A) (Zou et al., 1998). eIF2C purified from rabbit reticulocytes has two related activities that affect the ternary complex, which is composed of initiator methionine tRNA, GTP and eIF-2. The ternary complex binds the 40S ribosomal subunit to allow scanning for AUG codons in mRNA (for a review, see Hinnebusch, 2000). Purified eIF2C stimulates formation of the ternary complex from components present at physiological levels, and it stabilizes the complex against dissociation in the presence of natural mRNAs (Roy et al., 1981; Bagchi et al., 1985; Chakravarty et al., 1985; Roy et al., 1988). The known activity of eIF2C offers a trivial explanation of the roles of other family members in multiple processes: each could simply enhance the translation of a protein required for that particular process. This seems unlikely, in large part because Piwi has been shown to be nuclear in both the germline and somatic cells in which its activity is required (Cox et al., 2000). Furthermore, we argue here that Aub may play a direct role in *nos* mRNA localization. Nevertheless, the conserved domains that define this protein family should be expected to perform similar functions in the different proteins, and so some similarity to eIF2C function among other family members would not be surprising.

Translational enhancement by Aub

The *Drosophila* genome contains five members of the *eIF2C*/*piwi* family. One of these, *dAGO1*, is most closely related to *eIF2C* and is likely to be the *Drosophila eIF2C* homolog. Our identification of another family member as *aub*, a gene already implicated in translation, suggests that Aub protein may have biochemical activities similar to those of eIF2C. While eIF2C is predicted to play a global role in translation, mutants of *aub* only detectably affect the translation of a limited number of transcripts, revealing specificity in Aub function. We propose two broad classes of models for the role of Aub in message-specific translational activation. First, Aub may simply elevate the level of eIF2C-like activity in the ovary, leading to enhanced translation of mRNAs that depend most on this initiation factor. Differential dependence would presumably reflect some feature of mRNA structure, such as folding or AUG sequence context, or association of the mRNA with other proteins or regulatory factors. A variation of this model invokes association of Aub with dependent mRNAs, either directly through an RNA-binding activity or indirectly via binding to other factors, effectively elevating the local concentration of the eIF2C-like activity. Second, Aub may perform an activity distinct from that of eIF2C but still be involved in some aspect of translation. As with the first model, the activity could be concentrated on dependent mRNAs though direct or indirect binding. We have shown that a GFP-tagged form of Aub is not concentrated at the site of *grk* mRNA localization, and we have argued that posterior concentration of Aub is not required for *osk* mRNA translation. Thus, if Aub does associate with dependent

mRNAs as part of its role in translation, the interaction is likely to be transient.

Efforts to define the feature of transcripts that makes them dependent on Aub are constrained by the limited number of such mRNAs which have been identified: at present only *osk* and *grk*. For *osk* mRNA, *aub* dependence is conferred in part by the 3′ UTR (Wilson et al., 1996), a region known to bind multiple proteins and mediate both mRNA localization and translational repression (Kim-Ha et al., 1993; Kim-Ha et al., 1995; Gunkel et al., 1998; Lie and Macdonald, 1999). Therefore, Aub-dependence may be conferred by some aspect of translational repression or mRNA localization. The features of *grk* mRNA that confer *aub*-dependence are unknown.

osk mRNA is repressed by Bru and BicC, probably in concert with other factors (Kim-Ha et al., 1995; Webster et al., 1997; Saffman et al., 1998; Gunkel et al., 1998; Lie and Macdonald, 1999). Bru also binds to *grk* (Kim-Ha et al., 1995), and this interaction has been suggested to mediate repression (Nilson and Schupbach, 1999). If repression by Bru is indeed a shared feature of the *osk* and *grk* mRNAs, then Aub might inhibit or override this process. However, elimination of Bru-mediated repression by mutation of the BREs, the Bru-binding sites in the *osk* mRNA 3′ UTR, does not abrogate the requirement for *aub* in translation of posteriorly localized *osk* mRNA (Wilson et al., 1996). Thus, Aub does not simply alleviate Bru-dependent repression at the posterior pole of the oocyte (although it may contribute to this process).

One feature that is unquestionably shared by the *osk* and *grk* mRNAs is localization to specific domains within the oocyte. Perhaps tenacious binding of localization factors places unusual constraints on the translational apparatus that can only be overcome through the action of Aub. Supporting this possibility is the curious requirement for Aub in translation of posteriorly localized *oskBRE*$^{-}$ mRNA, as noted above, but not in the precocious translation that occurs prior to localization, when *oskBRE*$^{-}$ mRNA is present throughout much of the oocyte (Wilson et al., 1996). Thus, posterior localization of *osk* mRNA correlates with the Aub-dependent phase of its translation. However, anterior localization of *osk* mRNA, achieved by exchanging the *osk* 3′ UTR with the *bcd* mRNA localization signal, obviates the need for Aub. Therefore, mRNA localization in and of itself need not impose the requirement for Aub, although different localization signals may have unique properties that affect the relative translatability of their transcripts.

Assembly and function of polar granules

We have shown that GFP-Aub colocalizes with Osk in polar granules in the pole plasm and pole cells. Moreover, as Osk and Vas are also largely colocalized in polar granules, we infer that the Aub-containing particles also include Vas. GFP-Aub is also highly enriched in the perinuclear zones of the nurse cells, a distribution shared by Vas (Hay et al., 1988a; Hay et al., 1988b; Liang et al., 1994) and Tud, another polar granule component (Bardsley et al., 1993) whose distribution we did not monitor in our experiments. Notably, *vas* is required for the presence of normal levels of GFP-Aub in both the polar granules and the perinuclear zones. Pole plasm components have been suggested to be transported in particles from the nurse cells to the posterior ooplasm (Mahowald, 1971; Hay et al., 1988a; Liang et al., 1994), and our results are consistent with such a scheme. The defect in GFP-Aub accumulation at the posterior in *vas* mutants may be a direct result of the initial failure to recruit Aub to the periphery of the nurse cell nuclei, a process that may well involve the assembly of complexes containing both Vas and Aub. In contrast, Stau is not required for the perinuclear localization of either Vas (Lasko and Ashburner, 1990) or GFP-Aub, and thus may only act later in the recruitment of particles to the posterior pole of the oocyte.

In the embryo, polar granules are present in the cytoplasm of pole cells, and nuclear bodies that are similar in appearance form in the nuclei of these cells (Mahowald, 1968; Mahowald et al., 1976). Evidence supporting a structural relationship between the two classes of particle has come from the demonstration that Vas is present in both (Hay et al., 1988a; Hay et al., 1988b). Our work provides additional supporting evidence of such a relationship: further indication of structural similarity comes from our demonstration that Osk and Vas are colocalized in both nuclear and cytoplasmic aggregates, some of which have the peculiar donut-like shape that is characteristic of a subset of polar granules and nuclear bodies. However, our data also reveal that the cytoplasmic and nuclear particles are not identical in composition, as the nuclear bodies are distinguished from the polar granules by the absence of GFP-Aub. Nevertheless, Aub, like Osk and Vas, is involved in pole cell formation, and all are predominantly or exclusively cytoplasmic during that process. It is possible that later aspects of germ cell development require certain polar granule components, such as Osk or Vas, to act in the nucleus. Future experiments in which the selective partitioning of the various polar granule and nuclear body components is perturbed may provide insight into how pole cell fates are specified and maintained or lost during development.

We thank Joanne Connell for the preliminary mapping of *aub*, and Ulrich Schäfer for transgenic flies, mutants and information provided prior to publication. We are grateful to Anne Ephrussi for providing anti-Osk antibodies, and *vas-gfp* and *osk-bcd* flies; Andrea Brand for the mGFP6 construct; Pernile Rorth for UASp; and Mark van Doren and Ruth Lehmann for *nos-gal4-vp16* transgenic flies. We thank Eric Arn, Koen Braat and Jennifer Harris for critical comments on the manuscript. This work was supported by NIH Grant GM54409 to P. M. M.

REFERENCES

Adams, M. D., Celniker, S. E., Holt, R. A., Evans, C. A., Gocayne, J. D., Amanatides, P. G., Scherer, S. E., Li, P. W., Hoskins, R. A., Galle, R. F., George, R. A. et al. (2000). The genome sequence of Drosophila melanogaster. *Science* **287**, 2185-2195.

Ashburner, M. (1989). Drosophila: *A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Bagchi, M. K., Chakravarty, I., Ahmad, M. F., Nasrin, N., Banerjee, A. C., Olson, C. and Gupta, N. K. (1985). Protein synthesis in rabbit reticulocytes. A study of the roles of Co- eIF-2, Co-eIF-2A^{80}, and GDP in peptide chain initiation. *J. Biol. Chem.* **260**, 6950-6954.

Bardsley, A., McDonald, K. and Boswell, R. E. (1993). Distribution of tudor protein in the *Drosophila* embryo suggests separation of functions based on site of localization. *Development* **119**, 207-219.

Bohmert, K., Camus, I., Bellini, C., Bouchez, D., Caboche, M. and Benning, C. (1998). AGO1 defines a novel locus of Arabidopsis controlling leaf development. *EMBO J.* **17**, 170-180.

Breitwieser, W., Markussen, F.-H., Horstmann, H. and Ephrussi, A. (1996). Oskar protein interaction with vasa represents an essential step in polar granule assembly. *Genes Dev.* **10**, 2179-2188.

Catalanotto, C., Azzalin, G., Macino, G. and Cogoni, C. (2000). Gene silencing in worms and fungi. *Nature* **404**, 245.

Cerutti, L., Mian, N. and Bateman, A. (2000). Domains in gene silencing and cell differentiation proteins: the novel PAZ domain and redefinition of the piwi domain. *Trends Biochem. Sci.* **25**, 481-482.

Chakravarty, I., Bagchi, M. K., Roy, R., Banerjee, A. C. and Gupta, N. K. (1985). Protein synthesis in rabbit reticulocytes. Purification and properties of an Mr 80,000 polypeptide (Co-eIF-2A80) with Co-eIF-2A activity. *J. Biol. Chem.* **260**, 6945-6949.

Cox, D. N., Chao, A., Baker, J., Chang, L., Qiao, D. and Lin, H. (1998). A novel class of evolutionarily conserved genes defined by piwi are essential for stem cell self-renewal. *Genes Dev.* **12**, 3715-3727.

Cox, D. N., Chao, A. and Lin, H. (2000). piwi encodes a nucleoplasmic factor whose activity modulates the number and division rate of germline stem cells. *Development* **127**, 503-514.

Ephrussi, A., Dickinson, L. K. and Lehmann, R. (1991). *oskar* organizes the germ plasm and directs localization of the posterior determinant *nanos*. *Cell* **66**, 37-50.

Ephrussi, A. and Lehmann, R. (1992). Induction of germ cell formation by *oskar*. *Nature* **358**, 387-392.

Fagard, M., Boutet, S., Morel, J. B., Bellini, C. and Vaucheret, H. (2000). AGO1, QDE-2, and RDE-1 are related proteins required for post-transcriptional gene silencing in plants, quelling in fungi, and RNA interference in animals. *Proc. Natl. Acad. Sci. USA* **97**, 11650-11654.

Frohnhöfer, H. G., Lehmann, R. and Nüsslein-Volhard, C. (1986). Manipulating the anteroposterior pattern of the *Drosophila* embryo. *J. Embryol. Exp. Morphol.* **97**, **Suppl.**, 169-179.

Gavis, E. R. and Lehmann, R. (1994). Translational regulation of *nanos* by RNA localization. *Nature* **369**, 315-318.

Grishok, A., Tabara, H. and Mello, C. C. (2000). Genetic requirements for inheritance of RNAi in *C. elegans*. *Science* **287**, 2494-2497.

Gunkel, N., Yano, T., Markussen, F. H., Olsen, L. C. and Ephrussi, A. (1998). Localization-dependent translation requires a functional interaction between the 5′ and 3′ ends of oskar mRNA. *Genes Dev.* **12**, 1652-1664.

Hay, B., Ackerman, L., Barbel, S., Jan, L. and Jan, Y. N. (1988a). Identification of a component of *Drosophila* polar granules. *Development* **103**, 625-640.

Hay, B., Jan, L. Y. and Jan, Y. N. (1988b). A protein component of *Drosophila* polar granules is encoded by *vasa* and has extensive sequence similarity to ATP-dependent helicases. *Cell* **55**, 577-587.

Hay, B., Jan, L. Y. and Jan, Y. N. (1990). Localization of vasa, a component of *Drosophila* polar granules, in maternal-effect mutants that alter embryonic anteroposterior polarity. *Development* **109**, 425-433.

Hinnebusch, A. G. (2000). Mechanism and regulation of initiator methionyl-tRNA binding to ribosomes. In *Translational Control of Gene Exression* (ed. N. Sonenberg, J. W. B. Hershey and M. B. Matthews), pp. 185-243. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Illmensee, K. and Mahowald, A. P. (1974). Transplantation of posterior polar plasm in *Drosophila*. Induction of germ cells at the anterior pole of the egg. *Proc. Natl. Acad. Sci. USA* **71**, 1016-1020.

Kim-Ha, J., Smith, J. L. and Macdonald, P. M. (1991). *oskar* mRNA is localized to the posterior pole of the Drosophila ooctye. *Cell* **66**, 23-35.

Kim-Ha, J., Webster, P. J., Smith, J. L. and Macdonald, P. M. (1993). Multiple RNA regulatory elements mediate distinct steps in localization of *oskar* mRNA. *Development* **119**, 169-178.

Kim-Ha, J., Kerr, K. and Macdonald, P. M. (1995). Translational regulation of *oskar* mRNA by bruno, an ovarian RNA-binding protein, is essential. *Cell* **81**, 403-412.

Kimmerly, W., Stultz, K., Lewis, S., Lewis, K., Lustre, V., Romero, R., Benke, J., Sun, D., Shirley, G., Martin, C. and Palazzolo, M. (1996). A P1-based physical map of the Drosophila euchromatic genome. *Genome Res.* **6**, 414-430.

Lasko, P. F. and Ashburner, M. (1988). The product of the *Drosophila* gene *vasa* is very similar to eukaryotic initiation factor-4A. *Nature* **335**, 611-617.

Lasko, P. F. and Ashburner, M. (1990). Posterior localization of *vasa* protein correlates with, but is not sufficient for, pole cell development. *Genes Dev.* **4**, 905-921.

Liang, L., Diehl-Jones, W. and Lasko, P. (1994). Localization of vasa protein to the *Drosophila* pole plasm is independent of its RNA-binding and helicase activities. *Development* **120**, 1201-1211.

Lie, Y. S. and Macdonald, P. M. (1999). Apontic binds the translational repressor Bruno and is implicated in regulation of *oskar* mRNA translation. *Development* **126**, 1129-1138.

Lindsley, D. L. and Zimm, G. G. (1992). *The Genome of* Drosophila melanogaster. San Diego, CA: Academic Press.

Lynn, K., Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P. and Barton, M. K. (1999). The PINHEAD/ZWILLE gene acts pleiotropically in Arabidopsis development and has overlapping functions with the ARGONAUTE1 gene. *Development* **126**, 469-481.

Mahowald, A. P. (1962). Fine structure of pole cells and polar granules in *Drosophila melanogaster*. *J. Exp. Zool.* **151**, 201-216.

Mahowald, A. P. (1968). Polar granules of *Drosophila*. II. Ultrastructural changes during early embryogenesis. *J. Exp. Zool.* **167**, 237-262.

Mahowald, A. P. (1971). Polar granules of *Drosophila*. III. The continuity of polar granules during the life cycle of *Drosophila*. *J. Exp. Zool.* **176**, 329-344.

Mahowald, A. P. (2001). Assembly of the Drosophila germ plasm. *Int. Rev. Cytol.* **203**, 187-213.

Mahowald, A. P., Illmensee, K. and Turner, F. R. (1976). Interspecific transplantation of polar plasm between Drosophila embryos. *J. Cell Biol.* **70**, 358-373.

Markussen, F.-H., Michon, A.-M., Breitwieser, W. and Ephrussi, A. (1995). Translational control of *oskar* generates Short OSK, the isoform that induces pole plasm assembly. *Development* **121**, 3723-3732.

Micklem, D. R., Adams, J., Grunert, S. and St Johnston, D. (2000). Distinct roles of two conserved Staufen domains in oskar mRNA localization and translation. *EMBO J.* **19**, 1366-1377.

Moussian, B., Schoof, H., Haecker, A., Jurgens, G. and Laux, T. (1998). Role of the ZWILLE gene in the regulation of central shoot meristem cell fate during Arabidopsis embryogenesis. *EMBO J.* **17**, 1799-1809.

Nilson, L. A. and Schüpbach, T. (1999). EGF receptor signaling in Drosophila oogenesis. *Curr. Top. Dev. Biol.* **44**, 203-243.

Rongo, C. and Lehmann, R. (1996). Regulated synthesis, transport and assembly of the Drosophila germ plasm. *Trends Genet.* **12**, 102-109.

Rongo, C., Gavis, E. R. and Lehmann, R. (1995). Localization of *oskar* RNA regulates *oskar* translation and requires Oskar protein. *Development* **121**, 2737-2746.

Rorth, P. (1998). Gal4 in the Drosophila female germline. *Mech. Dev.* **78**, 113-118.

Roy, A. L., Chakrabarti, D., Datta, B., Hileman, R. E. and Gupta, N. K. (1988). Natural mRNA is required for directing Met-tRNA(f) binding to 40S ribosomal subunits in animal cells: involvement of Co-eIF-2A in natural mRNA-directed initiation complex formation. *Biochemistry* **27**, 8203-8209.

Roy, R., Ghosh-Dastidar, P., Das, A., Yaghmai, B. and Gupta, N. K. (1981). Protein synthesis in rabbit reticulocytes. Co-eIF-2A reverses mRNA inhibition of ternary complex (Met-tRNAf.eIF-2.GTP) formation by eIF-2. *J. Biol. Chem.* **256**, 4719-4722.

Rubin, G. M., Hong, L., Brokstein, P., Evans-Holm, M., Frise, E., Stapleton, M. and Harvey, D. A. (2000). A Drosophila complementary DNA resource. *Science* **287**, 2222-2224.

Saffman, E. E., Styhler, S., Rother, K., Li, W., Richard, S. and Lasko, P. (1998). Premature translation of oskar in oocytes lacking the RNA-binding protein bicaudal-C. *Mol. Cell. Biol.* **18**, 4855-4862.

Schmidt, A., Palumbo, G., Bozzetti, M. P., Tritto, P., Pimpinelli, S. and Schäfer, U. (1999). Genetic and molecular characterization of *sting*, a gene involved in crystal formation and meiotic drive in the male germ line of *Drosophila melanogaster*. *Genetics* **151**, 749-760.

Schüpbach, T. and Wieschaus, E. (1986). Maternal-effect mutations altering the anterior-posterior pattern of the *Drosophila* embryo. *Roux's Arch. Dev. Biol.* **195**, 302-317.

Schüpbach, T. and Wieschaus, E. (1991). Female sterile mutations on the second chromosome of *Drosophila melanogaster*. II. Mutations blocking oogenesis or altering egg morphology. *Genetics* **129**, 1119-1136.

Siemering, K. R., Golbik, R., Sever, R. and Haseloff, J. (1996). Mutations that suppress the thermosensitivity of green fluorescent protein. *Curr. Biol.* **6**, 1653-1663.

Smith, J. L., Wilson, J. E. and Macdonald, P. M. (1992). Overexpression of *oskar* directs ectopic activaton of *nanos* and presumptive pole cell formation in Drosophila embryos. *Cell* **70**, 849-859.

Spradling, A. C. and Rubin, G. M. (1982). Transposition of cloned P elements into Drosophila germ line chromosomes. *Science* **218**, 341-347.

Spradling, A. C., Stern, D. M., Kiss, I., Roote, J., Laverty, T. and Rubin, G. M. (1995). Gene disruptions using P transposable elements: an integral component of the Drosophila genome project. *Proc. Natl. Acad. Sci. USA* **92**, 10824-10830.

Spradling, A. C., Stern, D., Beaton, A., Rhem, E. J., Laverty, T., Mozden, N., Misra, S. and Rubin, G. M. (1999). The Berkeley Drosophila Genome

Project gene disruption project: single P-element insertions mutating 25% of vital Drosophila genes. *Genetics* **153**, 135-177.

St Johnston, D., Beuchle, D. and Nüsslein-Volhard, C. (1991). *staufen*, a gene required to localize maternal RNAs in the Drosophila egg. *Cell* **66**, 51-63.

St Johnston, D., Brown, N. H., Gall, J. G. and Jantsch, M. (1992). A conserved double-stranded RNA-binding domain. *Proc. Natl. Acad. Sci. USA* **89**, 10979-10983.

Tabara, H., Sarkissian, M., Kelly, W. G., Fleenor, J., Grishok, A., Timmons, L., Fire, A. and Mello, C. C. (1999). The rde-1 gene, RNA interference, and transposon silencing in C. elegans. *Cell* **99**, 123-132.

Turelli, M. and Hoffmann, A. A. (1995). Cytoplasmic incompatibility in Drosophila simulans: dynamics and parameter estimates from natural populations. *Genetics* **140**, 1319-1338.

Van Doren, M., Williamson, A. L. and Lehmann, R. (1998). Regulation of zygotic gene expression in Drosophila primordial germ cells. *Curr. Biol.* **8**, 243-246.

Webster, P. J., Liang, L., Berg, C. A., lasko, P. and Macdonald, P. M. (1997). Translational repressor bruno plays multiple roles in development and is widely conserved. *Genes Dev.* **11**, 2510-2521.

Wilson, J. E., Connell, J. E. and Macdonald, P. M. (1996). *aubergine* enhances *oskar* translation in the *Drosophila* ovary. *Development* **122**, 1631-1639.

Xu, T. and Rubin, G. M. (1993). Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development* **117**, 1223-1237.

Zou, C., Zhang, Z., Wu, S. and Osterman, J. C. (1998). Molecular cloning and characterization of a rabbit eIF2C protein. *Gene* **211**, 187-194.