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The *mago nashi* locus encodes an essential product required for germ plasm assembly in *Drosophila*

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SUMMARY

In *Drosophila*, the localization of maternal determinants to the posterior pole of the oocyte is required for abdominal segmentation and germ cell formation. These processes are disrupted by maternal effect mutations in ten genes that constitute the posterior group. Here, the molecular analysis of one posterior group gene, *mago nashi*, is presented. Restriction fragment length polymorphisms and transcript alterations associated with *mago nashi* mutations were used to identify the *mago nashi* locus within a chromosomal walk. The *mago nashi* locus was sequenced and found to encode a 147 amino acid protein with no similarity to proteins of known or suspected function. The identification of the *mago nashi* locus was confirmed by sequencing mutant alleles and by P element-mediated transformation. Nonsense mutations in *mago nashi*, as well as a deletion of the 5′ coding sequences, result in zygotic lethality. The original *mago nashi* allele disrupts the localization of *oskar* mRNA and staufen protein to the posterior pole of the oocyte during oogenesis; anterior localization of *bicoid* mRNA is unaffected by the mutation. These results demonstrate that *mago nashi* encodes an essential product necessary for the localization of germ plasm components to the posterior pole of the oocyte.

Key words: *mago nashi*, *Drosophila*, posterior group genes, germ plasm assembly, mRNA/protein localization

INTRODUCTION

For over a century developmental biologists have sought to understand the mechanisms that specify cell fates in the developing embryo. Utilizing cell lineage analysis and experimental manipulations of embryos, classical embryologists demonstrated that the fate of a given blastomere could be correlated with the region of cytoplasm that it inherited (Wilson, 1925; Davidson, 1986; Slack, 1991). The cytoplasm was therefore postulated to contain localized factors (determinants) capable of specifying cell fates. One of the best characterized examples of localized determinants occurs in the germ plasm of various metazoans. Studying chrysomelid beetles at the beginning of this century, Hegner observed granules at the posterior pole of the egg that were incorporated into the cytoplasm of primordial germ cells as they formed. By disrupting the posterior pole plasm experimentally, Hegner impaired the ability of the embryo to form germ cells (Hegner, 1908, 1909, 1911).

Subsequent work on *Drosophila melanogaster* embryos showed that the posterior pole cytoplasm contains determinants specifying germ cell fate. The primordial germ cells (pole cells) form when cleavage nuclei migrate into specialized yolk-free cytoplasm (the pole plasm or germ plasm) at the posterior pole of the embryo (Counce, 1973). This posterior pole plasm contains polar granules, organelles composed of RNA and protein, that appear as electron-dense structures lacking a limiting membrane when viewed by electron microscopy (Mahowald, 1962; Counce, 1963). Similar structures are associated with the germ plasms of diverse organisms ranging from nematodes to amphibians (Beams and Kessel, 1974; Eddy, 1975; Wolf et al., 1983). By transferring pole plasm (containing polar granules) to the anterior tip of recipient embryos, Illmensee and Mahowald (1974) demonstrated that functional pole cells could be induced at this ectopic location. The pole plasm was thus shown to contain localized determinants that can autonomously specify germ cell fate.

In addition to containing determinants for the germ cell lineage, the posterior pole plasm is the site of localization for determinants specifying somatic development. By removing cytoplasm from the posterior pole, Frohnhöfer et al. (1986) showed that factors localized to the posterior pole of the embryo are required for abdominal segmentation. Cytoplasm transfer experiments have demonstrated that both germ cell and abdominal determinants are synthesized and/or assembled during the later stages of oogenesis (Illmensee et al., 1976; Sander and Lehmann, 1988). It has been possible, therefore, to isolate maternal effect mutations that disrupt the localization, synthesis, and/or assembly of these determinants (St. Johnston and Nüsslein-Volhard, 1992). To date, maternal effect mutations that disrupt either abdominal segmentation alone (in the genes *nanos* and *pumilio*) or abdominal segmentation as well as germ cell determination (in the genes *cappuccino*, *mago nashi*, *oskar*, *spire*, *staufen*, *tudor*, *valois* and *vasa*) have been identified (Boswell and Mahowald, 1985; Lehmann and Nüsslein-Volhard, 1986; Schüpbach and Wieschaus, 1986; Lehmann and Nüsslein-Volhard, 1987; Manseau and Schüpbach, 1989; Boswell et al., 1991; Lehmann and Nüsslein-Volhard, 1991).

The abdominal defects observed in embryos derived from females carrying mutations in these genes resemble defects seen when posterior cytoplasm is removed from wild-type embryos (Frohnhöfer et al., 1986), suggesting that these mutations result in the inactivation of the posterior determinant(s). These genes are, therefore, referred to as the posterior group genes. The inability to form pole cells can be attributed to defects in the pole plasm; ultrastructural analysis of the pole plasm of embryos derived from females carrying mutations at *cappuccino*, *mago nashi*, *oskar*, *spire*, *staufen*, *tudor*, *valois* or *vasa* revealed that polar granules are absent or severely reduced in size and amount (Boswell and Mahowald, 1985; Lehmann and Nüsslein-Volhard, 1986; Schüpbach and Wieschaus, 1986; Manseau and Schüpbach, 1989; Boswell et al., 1991). In contrast, the pole plasm of embryos derived from *nanos* or *pumilio* mutant mothers appears normal, consistent with the fact that these mutations do not disrupt germ cell formation (Lehmann and Nüsslein-Volhard, 1987; Lehmann and Nüsslein-Volhard, 1991).

Thus far, the molecular analysis of several posterior group genes (*nanos*, *oskar*, *pumilio*, *staufen* and *tudor*) has not demonstrated the biochemical functions of their gene products (Ephrussi et al., 1991; Golumbeski et al., 1991; Kim-Ha et al., 1991; St. Johnston et al., 1991; Wang and Lehmann, 1991; Barker et al., 1992; Macdonald, 1992). Of the posterior group, only the vasa protein, which shares sequence similarity with the translation factor eIF4A, may be tentatively assigned a biochemical role (Hay et al., 1988b; Lasko and Ashburner, 1988). In spite of this lack of informative sequence similarity in the posterior group genes, some important details are beginning to emerge about the role these genes play in determinative events in the early embryo. For example, injection of *nanos* mRNA synthesized in vitro can alleviate the abdominal defects of most posterior group mutants (Wang and Lehmann, 1991), suggesting that the abdominal segmentation defects observed in posterior group mutants are largely the result of their effects on *nanos* function or localization.

To dissect genetically the process of germ cell determination, screens for maternal effect mutations resulting in the sterility of the F_1 progeny of homozygous females (the *grandchildless*-like phenotype) were undertaken; the *mago nashi* (*mago*) locus was identified in one such screen (Boswell et al., 1991). Hypomorphic (reduced function) mutations in *mago* result in ~99% inviability in the offspring of mutant females. These inviable progeny display abdominal segmentation defects similar to those observed in the progeny of females carrying mutations in other posterior group genes. Ultrastructural analysis demonstrated that the pole plasm of embryos derived from *mago* mutant females (which, for simplicity, will be referred to as *mago* embryos) is defective; polar granules are either absent or severely reduced. The abdominal defects in *mago* embryos can be alleviated by transplantation of posterior pole plasm from wild-type embryos. Only posterior pole plasm is capable of alleviating the abdominal segmentation defects; cytoplasm from other regions of wild-type embryos is incapable of restoring abdominal segmentation to *mago* embryos. These data indicate that $mago^+$ function is necessary for proper germ plasm assembly (Boswell et al., 1991). To examine the role of the $mago^+$ product in the assembly of the germ plasm we have begun a molecular analysis of the *mago* locus; the initial molecular characterization of the *mago* gene is presented here.

MATERIALS AND METHODS

Fly stocks and culturing

The *mago* mutations and deletions used in this work were described initially in Boswell et al. (1991; $mago^1$ and $mago^3$) and in O'Donnell et al. (1989; *SHL-1*, *RE2*, *RE7*, *E19A*, *WE7*) and were balanced by an isogenic *In(2LR)SM5* (*SM5*) balancer chromosome. The wild-type stock used in all experiments was Oregon-R, unless otherwise noted. *Df(1)w, y* w^{67c23} embryos were used for P element-mediated transformation. Flies were cultured on standard *Drosophila* medium in half-pint milk bottles or in 8-dram vials. Embryos were collected on molasses agar plates from females fed on wet yeast.

Nucleic acid analysis and sequencing

Drosophila genomic DNA was prepared either by cenrifugation in a CsCl gradient (Bingham et al., 1981) or by homogenizing flies in the presence of diethyl pyrocarbonate, EDTA and SDS (Golic and Lindquist, 1989), omitting the phenol-chloroform extractions. DNA blot analysis was performed using either ^{32}P- or digoxigenin-labelled probes. Hybridization using radiolabelled probes was performed as described by Golumbeski et al. (1991). When digoxigenin-labelled probes were used, DNA was transferred to neutral Tropilon membrane (Tropix, Inc., Bedford, MA), cross-linked to the membrane using ultraviolet light and prehybridized in hybridization solution (5× SSC; 0.5% blocking reagent [Boehringer Mannheim]; 0.1% N-lauroylsarcosine; 0.02% SDS) at 68˚C for at least 1 hour. Hybridization solution containing digoxigenin-labelled probe was then added and the hybridization proceeded overnight at 68˚C. The membrane was then washed and processed for chemiluminescent detection using an anti-digoxigenin-alkaline phosphatase conjugate (Boehringer Mannheim) and a luminescent substrate for alkaline phophatase, AMPPD (Tropix, Inc.).

Total RNA was isolated from appropriately staged embryos, larvae and adults by the procedure of Chomczynski and Sacchi (1987). For RNA blot analysis ~10 μg of total RNA from the appropriate stage of the life cycle was electrophoresed in 1.8% agarose MOPS/formaldehyde gels and capillary transferred to Zetabind membranes overnight in 20× SSC. Hybridization was performed as described in Schauer and Wood (1990) and the membranes were washed twice for 10 minutes at room temperature in 2× SSC; 0.1% SDS and twice for 30 minutes at 65˚C in 0.1× SSC; 0.1% SDS. Following exposure to X-ray film, the filters were stripped and rehybridized with a probe for the constitutively expressed rp49 (O'Connell and Rosbash, 1984) to control for the quantity and quality of RNA loaded in each lane.

Standard techniques (Sambrook et al., 1989) were used to isolate *mago* cDNAs from a 0-24 hour embryonic cDNA library (Tamkun et al., 1991). The largest of these cDNAs all terminated prematurely at the internal *Eco*RI site present at postition 170 in the sequence depicted in Fig. 3A. Consequently, a cDNA library constructed without the use of nucleases (Brown and Kafatos, 1988) was screened to obtain full-length *mago* cDNAs. Both strands of two independent cDNAs representing the 0.7 kb and the 1.1 kb mRNAs were sequenced using the dideoxy method (Sanger et al., 1977) with Sequenase (U.S. Biochemical Corp.) and synthetic primers (Operon). These cDNA sequences all begin with a G residue not present in the genomic sequence. This noncoded G is apparently inserted by reverse transcriptase when it is attempting to copy an mRNA cap (Brown et al., 1989); these clones are therefore likely to represent full-length *mago* cDNAs.

The BLAST algorithm (Altschul et al., 1990) was used to search the non-redundant databases at the National Center for Biotechnology Information at the National Library of Medicine. The BLOCKS protein motif database (Henikoff and Henikoff, 1991) was also searched to identify domains shared between mago and other known proteins. Significant similarity was obtained to an open reading frame encoded by the *C. elegans* expressed sequence tag, CEESH75, isolated from an early embryonic cDNA library (GenBank accession

number T00677). The sequence of CEESH75 in the database was obtained from the 3′ end of the cDNA using a single primer. Anthony Kerlavage (Institute for Genomic Research, Gaithersberg) kindly sent us CEESH75 and we obtained sequence from both ends of the clone. The corrected sequence of CEESH75 encodes additional amino terminal amino acids that extend the region of similarity between CEESH75 and the mago protein. CEESH75 hybridizes to two overlapping yeast artificial chromosomes that map to *C. elegans* linkage group II, in a region where there is no correspondence between the physical and genetic maps.

Primers flanking the *mago* coding region were used in the Polymerase Chain Reaction (PCR) to amplify *mago* sequences from genomic DNA isolated from *mago* mutant alleles and from their parental stocks. The amplified DNA was purified by electrophoresis through low melting point agarose. Internal primers were then used to sequence the PCR products directly in the low melting point agarose using Sequenase (Kretz et al., 1989).

P element-mediated transformation

The 2.2 kbp *Bam*HI-*Pst*I fragment illustrated in Fig. 2 was cloned into the transformation vector pCaSpeR 4. This construct was then co-injected with pπ25.7wc helper plasmid into *Df(1)w, y w*^{67c23} embryos. Two independent transformant strains were obtained and both insertions mapped to the second chromosome. These insertions were then mobilized using Δ2-3 as a genomic source of transposase (Robertson et al., 1988) and 11 independent insertions on the X chromosome were obtained. All of these insertions complement the *grandchildless*-like phenotype of *mago*^{1}. Two of these X chromosome insertions were tested further and shown to rescue the zygotic lethality of *mago*^{3}, *SHL-1* and *RE7*.

Whole-mount in situ hybridization and immunofluorescence

For both in situ hybridization and whole-mount immunofluorescence, ovaries were dissected from females fed on wet yeast for ~4 days. For in situ hybridization, ovaries were dissected into PBS; 0.1% Tween-20 (PBT) and fixed for 20 minutes in 0.1 M Hepes (pH 6.9); 2 mM $MgSO_4$; 1 mM EGTA; 4% paraformaldehyde. Following fixation, the ovaries were placed at –80˚C in 90% methanol; 10% dimethyl sulfoxide as described in St. Johnston et al. (1991). After proteinase K digestion, the ovaries were processed for in situ hybridization by the procedure of Tautz and Pfeifle (1989). A 2.15 kbp *Sac*I fragment derived from an *oskar* cDNA clone (kindly provided by Anne Ephrussi and Ruth Lehmann) was gel purified and labelled with digoxigenin using standard techniques. For immunofluorescence, ovaries were dissected and fixed as described by Xue and Cooley (1993). The primary α-staufen antiserum (kindly provided by Daniel St. Johnston) was used at dilutions of 1:2000-1:4000 and the secondary goat α-rabbit IgG-Texas Red conjugate (Amersham) was used at a dilution of 1:100. In all experiments, the ovaries were double-labelled with a primary mouse α-histone monoclonal antibody (Chemicon) and a secondary goat α-mouse IgG-fluorescein conjugate (Tago Immunochemicals, Inc.). These antibodies were both used at 1:500 dilutions. Co-labelling with the α-histone control ensured that the tissue had been properly fixed and that the antibodies had access to the tissue.

RESULTS

Summary of the genetics of the *mago nashi* locus

The name *mago nashi* (Japanese for 'without grandchildren') reflects the basis for identification of the gene, a screen for *grandchildless*-like maternal effect mutations resulting in sterility of the F_1 progeny of homozygous females. Allelic interactions at the *mago* locus are complex and will be summarized briefly. Females homozygous or hemizygous for *mago*^{1} produce offspring that fail to make pole cells. In addition to defects in germ cell formation, *mago*^{1} embryos exhibit temperature-sensitive defects in abdominal segmentation and the embryonic body plan (Boswell et al., 1991). The *mago*^{3} allele was isolated by its failure to complement the *grandchildless*-like phenotype of *mago*^{1}. This allele is homozygous inviable and in *trans* to *mago*^{1} produces phenotypes distinct from those observed when chromosomal deletions of the *mago* region are examined in *trans* to *mago*^{1} (Boswell et al., 1991).

The chromosomal interval to which *mago* maps contains five zygotic lethal mutations that represent a single complementation group (O'Donnell et al., 1989; see Table 1 for the Lindsley and Zimm nomenclature of these mutations). The mutation *SHL-1* fails to complement *mago*^{1} and produces similar phenotypes in *trans* to *mago*^{1} as do chromosomal deletions of the *mago* locus. The mutations *RE2* and *RE7* fail to complement the *grandchildless*-like phenotype and the maternal effect lethality of *mago*^{1}. The mutations *E19A* and *WE7* complement each other for zygotic lethality, and also complement the *grandchildless*-like phenotype of *mago*^{1}.

Table 1. The complementation pattern of *mago* alleles and their effects on the 17×10^3 M_r mago protein

Allele	Lindsley and Zimm nomenclature[a]	Complementation[b] *mago*^{1}	*mago*^{3}	*SHL-1*	*RE7*	*WE7*	*E19A*	Lesion	Result
mago^1			–	–	–	+	+	GGG → AGG	GLY^{19} → ARG
mago^3		–		–	–	–	–	CAG → TAG	GLN^{87} → STOP
SHL-1	*l(2)57Ca*^{2}	–	–		–	–	–	202 bp deletion	N-terminal truncation
RE7[c]	*l(2)57Ca*^{4}	–	–	–		–	–	CAG → TAG	GLN^{128} → STOP
WE7	*l(2)57Ca*^{5}	+	–	–	–		+	ATC → ACC	ILE^{91} → THR
E19A	*l(2)57Ca*^{1}	+	–	–	–	+		none detected[d]	

[a]Lindsley and Zimm (1992).

[b]+ represents complementation, – represents non-complementation (with respect to *mago*^{1}, refers to both maternal effect embryonic lethality and *grandchildless*-like phenotype; all others refer to zygotic lethality).

[c]*RE7* and *RE2* (*l(2)57Ca*^{3}) contain identical lesions, so only *RE7* is reported here.

[d]No lesions were detected in the coding sequence of the 17×10^3 M_r mago protein, the intron, or the untranslated regions shown in Fig. 3A.

Unlike the other lethal mutations in this region, the lethality of *E19A* is leaky and homozygotes are occasionally obtained. Both *E19A* and *WE7* fail to complement the zygotic lethality of the point mutants *mago*^{3}, *RE2* and *RE7* as well as the deletion *SHL-1* (see below). These mutations therefore constitute a single complementation group. This complementation pattern (summarized in Table 1) demonstrates interallelic complementation among mutations at the *mago* locus and that the *mago* locus can mutate to zygotic lethality.

Molecular characterization of *mago nashi*

The *mago* locus has been mapped cytologically to polytene region 57B20; 57C2 on the right arm of chromosome 2, within chromosomal deletions *Df(2R)F36* and *Df(2R)PL3* and centromere proximal to the *Punch-tudor* (*Pu-tud*) region (Boswell et al., 1991). A chromosomal walk through the *Pu-tud* region (Mclean et al., 1990; Golumbeski et al., 1991) was extended beyond the proximal *Df(2R)F36* breakpoint (data not shown) to ensure the isolation of genomic DNA containing the *mago* locus. To delimit the *mago* locus within the chromosome walk, genomic DNA from mutations disrupting *mago* function was analyzed by DNA blotting to identify restriction fragment length polymorphisms (RFLPs). When probed with DNA from phage λC3 or λC4, RFLPs are observed in *SHL-1* and *mago*^{3} genomic DNA. These RFLPs are not detected in the genomic DNA of the parental strain in which these mutations were induced. Both of these lesions map to the region of overlap between λC3 and λC4; *SHL-1* appears to be a deletion of ~200 bp (Fig. 1A) and *mago*^{3} alters a *Sal*I restriction endonuclease recognition site very close to and distal to the *SHL-1* lesion (see Fig. 2 and below).

mago^1 was isolated on the basis of its maternal effect on germ cell formation, so it was expected that the mRNA(s) encoded by the locus would be expressed maternally. RNA blot analysis demonstrates that four transcripts encoded within λC3 and λC4 are detected in early embryos (prior to the onset of zygotic transcription), with sizes of 0.7, 1.1, 3.4 and 5.2 kilobases (kb). The two smallest transcripts map to the region altered by both *SHL-1* and *mago*^{3}, whereas the 3.4 kb transcript maps to proximal λC4 and the 5.2 kb transcript maps to distal λC3. Furthermore, the two smallest transcripts are altered in *SHL-1*/+ heterozygous flies as revealed by RNA blot analysis; novel transcripts ~200 bases smaller than the corresponding wild-type mRNAs are observed (Fig. 1B). The 3.4 kb and the 5.2 kb transcripts are unaltered in *SHL-1*/+ heterozygotes (data not shown). The 0.7 and 1.1 kb mRNAs were tentatively identified as transcripts derived from the *mago* locus on the basis of the alterations observed in *SHL-1* heterozygotes and the mapping of the *mago*^{3} RFLP to the genomic sequence encoding these transcripts.

cDNAs corresponding to the 0.7 and 1.1 kb mRNAs were isolated from embryonic cDNA libraries (Brown and Kafatos, 1988; Tamkun et al., 1991) and mapped to the genomic sequences in λC4 (Fig. 2). Three independent cDNA clones representative of each transcript were sequenced, as was the genomic DNA encoding the mRNAs (Fig. 3A). A single 58 bp intron was found in the genomic sequence; all of the isolated cDNAs represent spliced forms of the transcripts. The largest cDNA begins at position 0 in the genomic sequence shown in Fig. 4A while the 0.7 kb cDNAs begin at position 31. Two polyadenylation signals are present at positions 738 and 1117, and the use of these alternative polyadenylation signals appears to account for the size difference between the two mRNAs. All of the cDNA isolates end in a poly(A) tract not encoded by the genomic DNA.

Both the 0.7 and the 1.1 kb transcripts potentially encode a protein of 147 amino acid residues with a predicted M_r of 17×10^3 (Fig. 3A). This protein is slightly acidic (estimated pI 5.7) and contains a high percentage of charged residues (16% acidic and 16% basic). The carboxy terminus of the protein is hydrophobic, but in the absence of biochemical and histochemical data, it is unclear whether this region serves as a transmembrane domain. Searches of several databases revealed no similarities with proteins of known or suspected function. However, a striking similarity was observed with an open reading frame encoded by an expressed sequence tag isolated from early *C. elegans* embryos. These proteins share 78% sequence identity and 86% conservation over a region of 101 amino acids (Fig. 3B). This striking similarity indicates

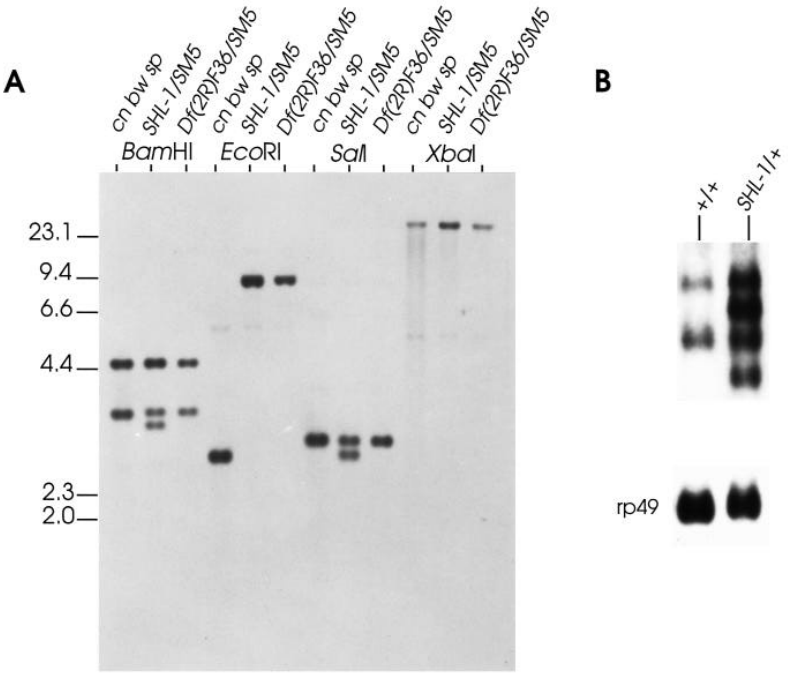

Fig. 1. The lesion in *SHL-1* is an ~200bp deletion that results in the alteration of two transcripts. (A) DNA blot of genomic DNA from flies of the indicated genotypes digested with the indicated restriction endonuclease. The filter was hybridized with a digoxigenin-labelled 2.6 kbp *Eco*RI fragment isolated from λC4 (indicated in Fig. 2). *cn bw sp* is the parental strain in which *SHL-1* was induced. *Df(2R)F36* is a large deletion removing this region of the genome and serves as a control for the migration of DNA from the *SM5* balancer chromosome. Novel bands ~200 bp smaller than the parental DNA can be seen in both *Bam*HI- and *Sal*I-digested *SHL-1* genomic DNA. (B) RNA blot analysis of RNA isolated from wild-type and *SHL-1* heterozygous females. In RNA from *SHL-I* heterozygotes, two novel bands ~200 bases smaller than the wild-type bands are observed. The constitutively expressed rp49 (O'Connell and Rosbash, 1984) serves as a control for the loading and quality of the RNA.

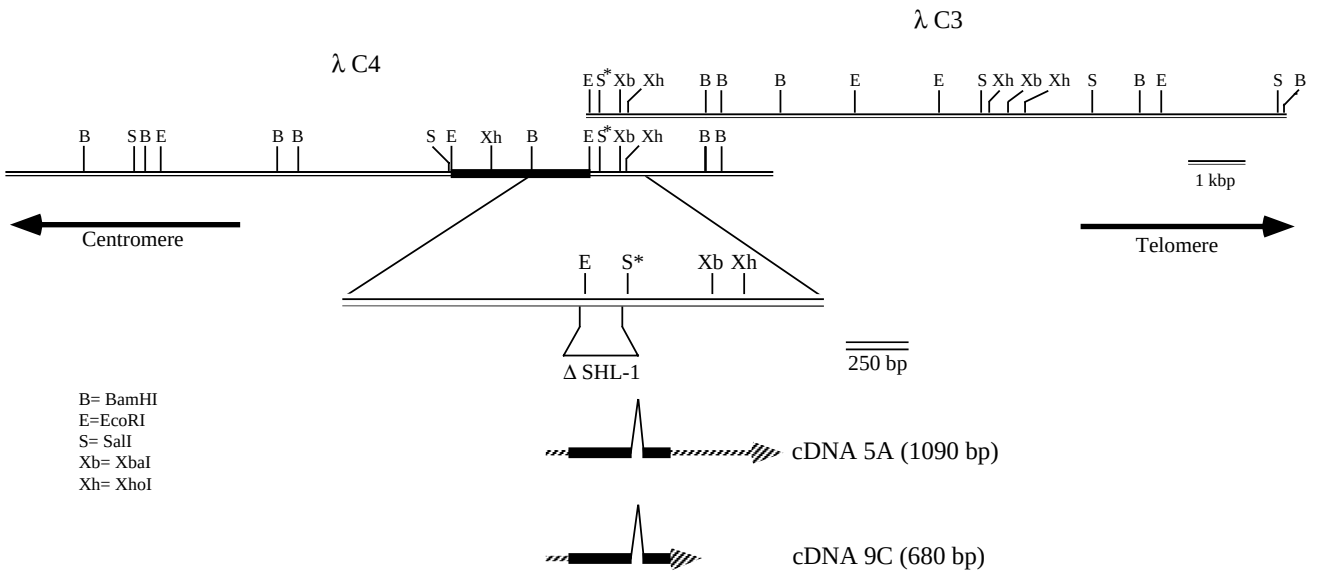

Fig. 2. Restriction map of the *mago* genomic region. The 2.6 kbp *Eco*RI fragment used as the probe in Fig. 1A is blackened. The region deleted by the *SHL-I* lesion is shown and the *Sal*I site altered in *mago*^{3} genomic DNA is indicated with an asterisk. Both of these lesions map to sequences encoding the two transcripts indicated on the map. The arrowhead indicates the 3′ end of the transcripts, the blackened area of the transcripts represents the open reading frame, and the hatched regions represent the untranslated regions. The 2.2 kbp *Bam*HI-*Pst*I fragment that rescues the *grandchildless*-like phenotype of *mago*^{1} and the zygotic lethality of *mago*^{3}, *RE7* and *SHL-1* is magnified.

that the mago protein has been conserved over large evolutionary distances and suggests an important function for the mago protein.

Sequencing of *mago* mutant alleles

The polymerase chain reaction (PCR) was used to amplify *mago* genomic sequences from *mago* mutant alleles. The amplified sequences were then sequenced directly to determine the effects of the mutations on the *mago* product. Six mutations alter the coding sequence of the 17×10^3 M_r product of the *mago* locus (Table 1), whereas lesions are not detected in the parental strains in which the mutations were induced. The lesion in *SHL-1* is a 202 base pair deletion of the 5′ coding sequences, in agreement with the mapping data described earlier. This deletion results in a predicted product containing the first 14 amino acids of the 17×10^3 M_r protein followed by 28 novel amino acids introduced by a shift in reading frame. The *mago*^{3} mutation results from a C→T transition in codon 87 that introduces a premature stop codon in place of glutamine. This mutation alters a *Sal*I recognition site, allowing it to be detected as an RFLP. The *mago*^{1} allele contains a G→A transition in codon 19 resulting in the replacement of glycine by arginine. *RE2* and *RE7* contain an identical C→T transition in codon 128, resulting in a premature stop codon in place of glutamine. Because *RE2* and *RE7* contain the same lesion, they will be referred to as a single allele, *RE7*. *WE7* genomic DNA contains a T→C transition in codon 91 of the 17×10^3 M_r mago protein that results in the substitution of a threonine residue for isoleucine. No mutations have been found in the coding region, the intron, or the untranslated sequences of *mago* in *E19A*.

The sequencing data confirm the identification of the *mago* locus; all of the mutations that disrupt the oogenetic function of the *mago* product (*mago*^{1} and the lethal alleles that fail to complement *mago*^{1}) result in alterations of the coding sequence of the 17×10^3 M_r mago protein. Those mutations predicted to produce truncated mago protein result in zygotic lethality, demonstrating that the wild-type function of the gene is required for viability. One zygotic lethal mutation (*WE7*) results from a missense mutation in the coding sequence of the 17×10^3 M_r mago protein. This mutation complements the *mago*^{1} mutation, demonstrating interallelic complementation between mutations at the *mago* locus. Interallelic complementation has been observed between mutations in many multimeric proteins (Zabin and Villarejo, 1975) and suggests that the mago protein may function as a multimer. Alternatively, the mago protein may contain discrete domains involved in maternal and zygotic functions.

In addition to demonstrating specific lesions in the *mago* locus, a 2.2 kilobasepair (kbp) *Bam*HI-*Pst*I fragment (Fig. 2) encompassing the locus has been introduced into flies utilizing P element-mediated transformation. This 2.2 kbp fragment contains ~0.9 kbp of DNA upstream of the predicted transcription start site and an additional 115 bp following the polyadenylation signal at 1117 in Fig. 3A. This construct rescues the *grandchildless*-like phenotype of *mago*^{1} as well as the zygotic lethality of *SHL-1*, *mago*^{3} and *RE7*.

Expression of transcripts from the *mago* locus

To determine the temporal expression pattern of the *mago* gene, RNA blot analysis was performed using RNAs isolated from different stages of the *Drosophila* life cycle. The two mRNAs are detected throughout the life cycle (Fig. 4). During the first four hours of embryonic development the 0.7 kb transcript appears to be more abundant than the 1.1 kb transcript. The untranslated region of the 1.1 kb transcript contains three

AUUUA sites not included in the 0.7 kb transcript. These sites have been implicated in mRNA instability (Shaw and Kamen, 1986); thus, this difference in abundance may reflect differential stabilities of these mRNAs. Both mRNAs are detected at similar abundance in larvae, adult males and adult females, and are in low abundance in late embryos (Fig. 4, 14-20 hours). The expression of *mago* mRNAs in developmental stages beyond oogenesis and early embryogenesis is consistent with a function for the *mago*$^+$ product in later developmental events, as suggested by the zygotic lethality of *SHL-1*, *mago*^{3} and other lethal *mago* alleles.

The products of most posterior group genes are localized to the posterior pole during oogenesis. The protein products of *staufen*, *tudor* and *vasa* are localized to the posterior pole of the developing oocyte (Hay et al., 1988a; Lasko and Ashburner, 1990; St. Johnston et al., 1991; Bardsley et al., 1993). Although the protein products of these genes are localized posteriorly in the oocyte, the mRNAs encoded by these genes are either undetectable in the oocyte or distributed uniformly throughout the oocyte (Hay et al., 1988b; Lasko and Ashburner, 1988; Golumbeski et al., 1991; St. Johnston et al., 1991). In contrast, *nanos* and *oskar* mRNAs are localized to the posterior pole of the oocyte and the embryo (Ephrussi et al., 1991; Kim-Ha et al., 1991; Wang and Lehmann, 1991). We were interested in determining whether the *mago* mRNAs are also posteriorly localized. To examine the spatial expression of *mago* transcripts, digoxigenin-labelled *mago* cDNA was hybridized in situ to wild-type ovaries and embryos. *mago* mRNAs appear to be uniformly expressed throughout the nurse cell-oocyte complex during early oogenesis, are abundant in nurse cells at stage 10, and appear to be uniformly distributed throughout the embryo by the time of egg deposition (data not shown). A similar spatial expression pattern has been observed for the transcript encoded by the posterior group gene *vasa* (Hay et al., 1988b; Lasko and Ashburner, 1988).

Effects of *mago*^{1} on the localization of posterior group gene products

Of the identified posterior group gene products, the first to be localized to the oocyte posterior are *oskar* (*osk*) mRNA and staufen (stau) protein. These products accumulate at both the anterior and posterior poles of the developing oocyte at stage 8. During stage 9, the anterior localization of both products diminishes and, by stage 10, they are both highly concentrated at the posterior pole of the oocyte (Ephrussi et al., 1991; Kim-Ha et al., 1991; St. Johnston et al., 1991). Mutations in *stau* disrupt *osk* mRNA localization (Ephrussi et al., 1991; Kim-Ha et al., 1991) and nonsense mutations in *osk* result in failure to maintain stau protein localization (St. Johnston et al., 1991); thus the localization of these gene products is interdependent upon the function of both genes. Mislocalization of *osk* mRNA to the anterior pole is sufficient for recruitment of germ plasm components and assembly of functional germ plasm at the anterior pole, demonstrating that *osk*$^+$ product plays a critical role in germ plasm assembly (Ephrussi and Lehmann, 1992). Because stau protein and *osk* mRNA are the earliest identified germ plasm components localized to the posterior pole and, because some *mago* alleles disrupt germ plasm function, it was of interest to determine whether the *mago*^{1} mutation has an effect upon the posterior localization of these gene products.

A

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-200                                                  GCCCTGTCGT
-190  TCACATACTTGTTGAGGTAGTTCCATGGCATCCAAAACTGAATTACATTTTAATAAAAAT
-130  CAGTGCCAAATGCGCGACTTGTACGTGGATTATTGTTGTGTTATCGATACGCCGATAGGC
 -70  TGGCTTCTCTTCCTCATCCCTGCCAGCCATTCGCAGCGAACACTTATCGATACGCGGTGA
 -10  TAGTTATTCCgTGTGTTTTCCCATCTCTAACGCGTTGTAGTaAAAGGAAAAAGAGTGGGAA
  50  ACCAAAAATTGATATTTTTCTGAAAGTCTTTCAAAGTTACGCTTTAAACTAGAAGCAATC
 110  ATGTCCACGGAGGACTTTTACCTACGCTACTACGTCGGACACAAGGGCAAGTTCGGGCAC
      M  S  T  E  D  F  Y  L  R  Y  Y  V  G  H  K  G  K  F  G  H    20
 170  GAATTCTTGGAGTTCGAGTTCCGGCCGGATGGCAAGCTGCGGTACGCCAACAACTCCAAC
      E  F  L  E  F  E  F  R  P  D  G  K  L  R  Y  A  N  N  S  N    40
 230  TACAAGAACGACACCATGATCCGCAAGGAGGCCTTCGTCCACCAGTCCGTGATGGAAGAA
      Y  K  N  D  T  M  I  R  K  E  A  F  V  H  Q  S  V  M  E  E    60
 290  CTGAAGCGAATCATCATCGACTCGGAGATCATGCAGGAGGACGATCTGCCCTGGCCGCCA
      L  K  R  I  I  I  D  S  E  I  M  Q  E  D  D  L  P  W  P  P    80
 350  CCAGATCGCGTGGGTCGACAGgtcggtttgccgcactccgtaactagagctcaactaaac
      P  D  R  V  G  R  Q                                           87
 410  aaccatgtgtctttttcagGAACTGGAGATCGTCATCGGAGACGAGCACATCTCGTTCAC
                         E  L  E  I  V  I  G  D  E  H  I  S  F  T   101
 470  CACCTCGAAAACGGGATCATTGGTGGACGTGAACCGGTCAAAAGATCCCGAGGGCCTGCG
       T  S  K  T  G  S  L  V  D  V  N  R  S  K  D  P  E  G  L  R   121
 530  ATGCTTTTACTACCTGGTGCAGGATCTCAAGTGCCTGGTCTTCTCACTCATCGGCCTGCA
       C  F  Y  Y  L  V  Q  D  L  K  C  L  V  F  S  L  I  G  L  H   141
 590  TTTCAAGATCAAGCCCATATAAGCCGTAGCCACCACCTCAAGCATAGCCTATTTATCGGA
       F  K  I  K  P  I  *                                          147
 650  CGGACACATCGTATTTTATAATGGAACTTCTACGATACTTGTAATTGTATAAGCTATACC
                                                        ↓
 710  TTTGTACTTTTTGGTACAGTTTGCAGTGAATAAAAAAGGATTGGAAGATACTTTGTTTTC
 770  AAAGTCTTGTGTACAGTCTAGATTGTGCTATAGAAGTAAATAGCAAATCTTCGATGTAGT
 830  AGCAGACAACAAGTTTAACAATAATAGCAAACACAATAGTAAACCATTTAACTTTTGATG
 890  TTATAATGTGTAGGTATATTTGAAAAATACAATTTAATATCTATTGTGTTTGAATAATAT
 950  ACATACTCGAGATAAAAATTTAAAGTCCTTTACATTTGTACAATTCGTATATGTCTAAAA
1010  CAGCTTAAGCTAAGTGATTAATATCTCACAAACTGGCAAATTGTCTTAACGTTTAAGTAC
1070  AAGCAGTAACTTAGCCTAAGATAATGATCGGTATCTTAAGAAACTGGAATAAAATACGCA
        ↓
1130  CTCACATACGGTTTTGAGACTTGCAAAGGGTTTGGCTTACATATGCTACTAAATTCAGTC
1190  TTTGGGGTGA
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B

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mago IRKEAfVhQS VMeELKRIIi DSEIMQEDDl pWPpPDRVGR QELEIVigDE HISFTTsKtG 106
     ||||| | *| |* |||||| |||||||||   || ||**|| |||||*  *| |||||| | |
cest IRKEAtVsES VLsELKRIIe DSEIMQEDDd nWPePDKIGR QELEILykNE HISFTTgKiG  60

mago sLvDVNrSKD PEGLRcFYYL VQDLKCLVFS LIGLHFKIKP I•                    147
      | ||| ||| |*||| |||| |||||||||| |||||||||| |
cest aLaDVNnSKD PDGLRsFYYL VQDLKCLVFS LIGLHFKIKP I•                    101
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Fig. 3. (A) The sequence of the *mago* locus and the predicted mago protein. The intron sequences are printed in lower case letters and the polyadenylation signals are underlined. The italicized and underlined residues at positions 0 and 31 represent the 5′ ends of the 1.1 and 0.7 kbp cDNAs depicted in Fig. 2 (respectively). The arrows indicate the site of poly(A) addition. The *mago* sequence presented here may be obtained from GenBank, accession number U03559. (B) Sequence similarity between the predicted proteins encoded by *mago* and the *C. elegans* expressed sequence tag (cest), CEESH75. Identical residues are indicated by a vertical line, conserved residues by an asterisk and non-conserved residues are printed in lower case.

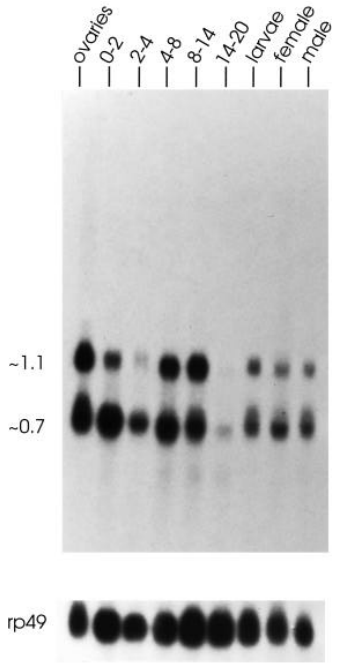

Fig. 4. Developmental time course of *mago* mRNA expression. Approximately 10 μg of total RNA from the indicated developmental stages (or hours after egg deposition) was loaded per lane, separated electrophoretically, transferred to a nylon membrane and hybridized with ^{32}P-labelled *mago* cDNA. The same filter was stripped and rehybridized with an rp49 probe to control for RNA loading.

In oocytes of *mago*^{1} homozygous females, the posterior localization of *osk* mRNA and stau protein is abolished. Rather, *osk* mRNA accumulates at the anterior pole during stage 8 and does not appear to be localized at the posterior pole (compare Fig. 5B to wild type in Fig. 5A). *osk* mRNA remains detectable in the anterior of the oocyte through stage 9 and is occasionally observed at the anterior of stage 10 oocytes (compare Fig. 5D to wild type in Fig. 5C). Expression of *osk* mRNA in the germarium and through the first seven stages of oogenesis appears normal, suggesting that mutations in *mago* do not affect the transcription of *osk* mRNA. A similar pattern of *osk* mRNA distribution has been reported in the ovaries of females homozygous for *staufen* mutations. In oocytes from *stau* mutant females, *osk* mRNA is correctly localized to the anterior pole but does not become localized to the posterior pole, demonstrating that *stau*$^+$ function is required for the proper posterior localization of *osk* mRNA (Ephrussi et al., 1991; Kim-Ha et al., 1991).

The disruption of posterior *osk* mRNA localization in oocytes of *mago*^{1} females may be due to the effects of the mutation upon the localization of stau protein to the oocyte posterior. In *mago* mutant oocytes, stau protein does not accumulate specifically at the posterior pole; rather, it appears to be uniformly distributed throughout the oocyte in stages 8 and 9 (Fig. 6B). Stau protein remains distributed uniformly in stage 10 *mago* mutant oocytes (Fig. 6D), when normally it would be tightly localized to the posterior pole (Fig. 6C). Because stau protein can be detected immunologically at all

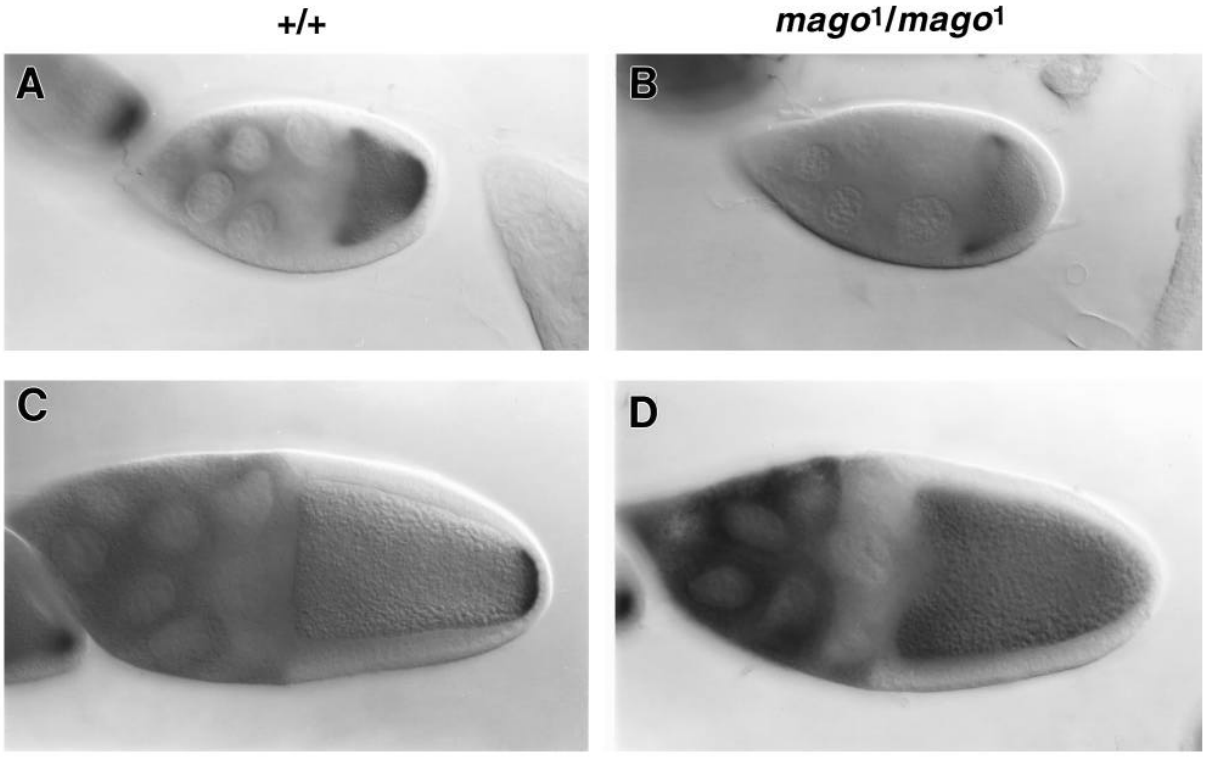

Fig. 5. Disruption of *osk* mRNA localization in *mago* mutant ovaries. (A) Localization of *osk* mRNA in stage 8 wild-type oocytes; note the accumulation of *osk* mRNA at both the anterior and posterior poles. (B) Localization of *osk* mRNA in stage 8 *mago*^{1}/*mago*^{1} oocytes; *osk* mRNA accumulates only at the anterior pole. (C) Stage 10 oocyte from wild-type female; *osk* mRNA is localized to the posterior pole. (D) Stage 10 oocyte from *mago*^{1}/*mago*^{1} female showing detectable *osk* mRNA remaining at anterior pole; no posterior localization of *osk* mRNA is observed.

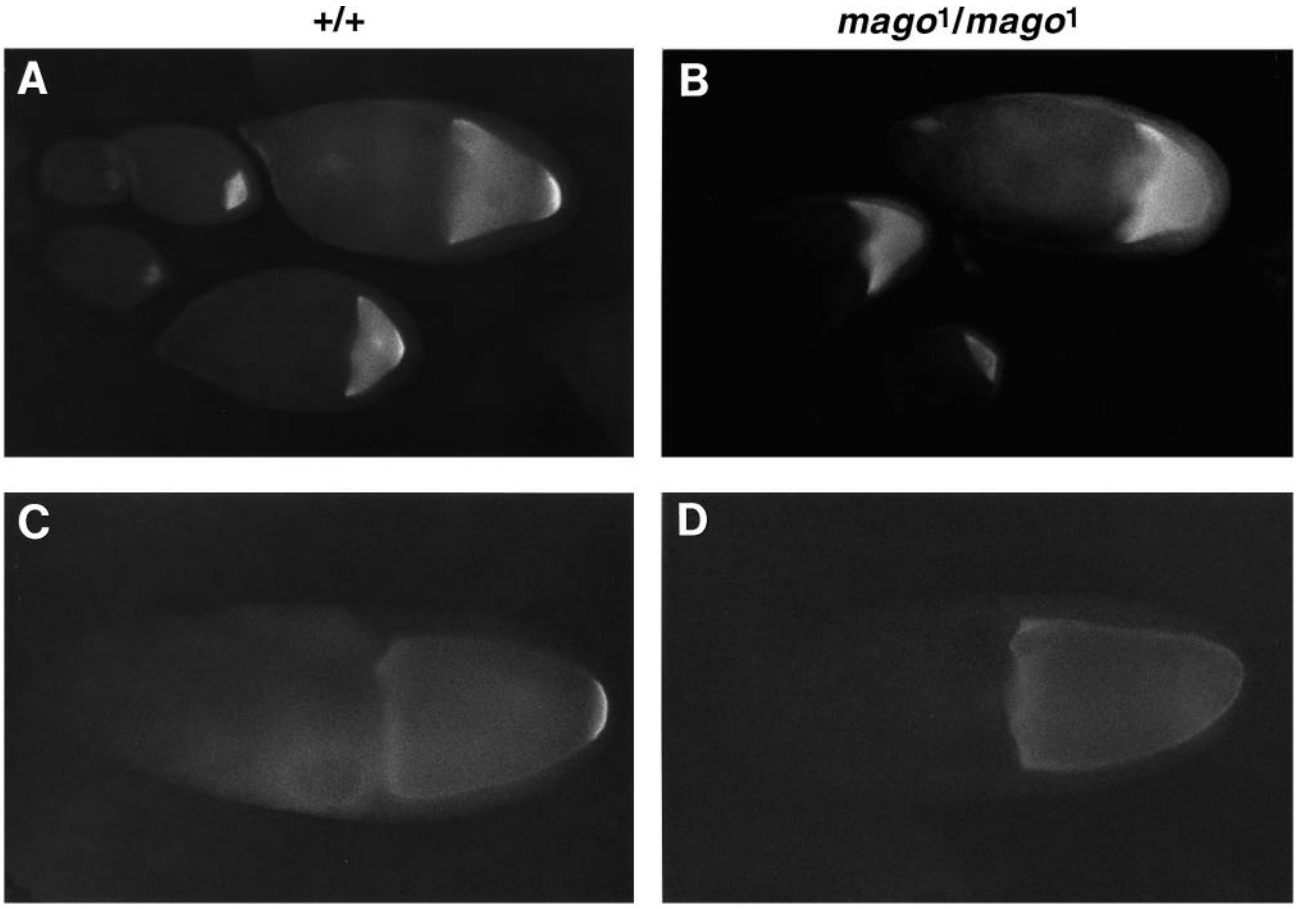

Fig. 6. Disruption of stau protein localization in *mago* mutant ovaries. (A) Stau protein localization in stage 6, 8 and 9 wild-type oocytes. (B) Stau protein in *mago*^{1}/*mago*^{1} oocytes at similar stages to those in A. Note distribution of stau protein throughout the oocyte. (C) Stau protein localization in a wild-type stage 10 oocyte and (D) in a *mago*^{1}/*mago*^{1} stage 10 oocyte. Again, note lack of posterior localization in the *mago* mutant oocyte.

of these stages, it is unlikely that *mago* mutations affect the synthesis of stau protein. Furthermore, the mRNA of the anterior determinant *bicoid* is localized properly in both *mago* mutant ovaries and embryos (data not shown), suggesting that stau function at the anterior pole, which is required for *bicoid* mRNA localization (St. Johnston et al., 1989), is unperturbed by *mago* mutations. These data indicate that mutations in *mago* can result in specific defects in the localization of germ plasm components to the posterior of the oocyte, demonstrating that *mago*$^+$ function is required for proper assembly of the germ plasm.

DISCUSSION

The molecular characterization of the *mago* locus described above can be summarized as follows. Restriction fragment length polymorphisms and transcript alterations associated with *mago* alleles were used to identify the *mago* locus within a chromosomal walk. *mago* cDNAs were isolated, sequenced and predicted to encode a 17×10^3 M_r protein. To confirm the identification of the locus, genomic DNAs from mutant alleles were sequenced and shown to result in alterations of the coding sequence of the predicted 17×10^3 M_r mago protein. P element-mediated transformation was used to demonstrate that a 2.2 kbp *Bam*HI/*Pst*I fragment containing the *mago* locus rescues the grandchildless-like phenotype of *mago*^{1} as well as the zygotic lethality of *SHL-1*, *mago*^{3} and *RE7*. The original *mago* isolate, *mago*^{1}, is the result of a missense mutation changing a glycine to arginine at codon 19 of the predicted mago protein. We have shown that the posterior localization of *osk* mRNA and stau protein is disrupted in *mago*^{1} mutant ovaries. Disruption of the posterior localization of these germ plasm components is consistent with the observed sterility of F_1 progeny of *mago*^{1} mutant mothers (Boswell et al., 1991).

In oocytes of *mago*^{1} homozygous females, *osk* mRNA accumulates at the anterior pole and does not become localized to the posterior pole, reminiscent of the effects of *stau* mutations on *osk* mRNA localization (Ephrussi et al., 1991; Kim-Ha et al., 1991). The disruption of posterior *osk* mRNA localization in *stau* mutants suggested a role for the stau protein in the transport of *osk* mRNA to the posterior pole, perhaps as part of a complex containing stau protein and *osk* mRNA (St. Johnston et al., 1991). The defect in *osk* mRNA localization in *mago* mutant oocytes is therefore likely to result from the failure to localize stau protein (or a stau protein/*osk* mRNA complex) to the posterior pole and provides further evidence

that *mago*$^+$ product is required in the process that assembles and localizes the germ plasm at the oocyte posterior.

Ephrussi and Lehmann (1992) have recently demonstrated that mislocalization of *osk* mRNA to the anterior pole of the oocyte results in the formation of double abdomen embryos that assemble germ plasm and make functional pole cells at the anterior pole. By examining the effects of mislocalized *osk* mRNA in various posterior group mutants, it was shown that the effects on pole cell formation of mutations in *cappuccino* (*capu*), *mago*, *spire*, *stau* and *valois* could be bypassed. In embryos derived from females carrying the *osk* mislocalization construct and mutations in these genes, pole cells formed at the anterior, but not the posterior pole (Ephrussi and Lehmann, 1992). These results suggest that the products of these genes (with the exception of *valois*, see Ephrussi and Lehmann, 1992) play an early role in the localization of the germ plasm to the posterior pole and that this role may be bypassed by the inappropriate localization of *osk*. Our results are consistent with this view; *mago* mutations disrupt the localization of *osk* mRNA to the posterior pole, as do mutations in *capu*, *spire* and *stau* (Ephrussi et al., 1991; Kim-Ha et al., 1991), indicating that these genes are involved in early events assembling the germ plasm at the posterior pole.

Because *osk* mRNA mislocalized to the anterior pole can recruit the assembly of pole plasm components (*nanos* mRNA and vasa and tudor proteins) at the anterior pole (Ephrussi and Lehmann, 1992; Bardsley et al., 1993), it seems likely that the phenotypic effects of mutations at *mago* are the result of their effects on *osk* mRNA localization. As a result of this failure to localize *osk* mRNA, other germ plasm components are not assembled at the posterior pole. We point out, however, that, in spite of the absence of localized *osk* mRNA in *mago* mutant ovaries, the abdominal determinant (*nanos*) can, in some cases, function to induce proper abdominal segmentation. Perhaps when *osk* mRNA is evenly distributed throughout the oocyte, the reduced amount of *osk*$^+$ product present at the posterior pole is sufficient for localization of the abdominal determinant but cannot assemble functional germ plasm. The polar granule remnants occasionally observed in embryos derived from *mago* mutant mothers (Boswell et al., 1991) may reflect residual activity of unlocalized *osk*$^+$ product.

In addition to its role in the assembly of the germ plasm, we have established that nonsense mutations in *mago* result in zygotic lethality, indicating that *mago*$^+$ function is required at other developmental stage(s) for viability. Inviable *SHL-1*, *mago*^{3} or *RE7* homozygous larvae do not have any detectable gross morphological defects, making it difficult to ascertain what the later developmental function(s) of the *mago*$^+$ product may be. This lack of a distinctive terminal phenotype in inviable *mago* alleles may be due to the perdurance of maternal *mago*$^+$ product supplied during oogenesis. Germ line clones homozygous for the lethal *mago* alleles arrest during oogenesis and fail to produce any eggs (A. K. Sotelo, R. E. B., unpublished data), making it extremely difficult to examine the effects of complete removal of *mago* product on embryonic development. These results demonstrate that the *mago* gene encodes an essential product required for the progression through oogenesis and, later, in the assembly of the germ plasm at the posterior of the oocyte.

Many of the maternal effect mutations disrupting embryonic development exhibit a high degree of pleiotropy. In the posterior group alone, very few of the genes have roles limited specifically to abdominal segmentation and/or germ cell determination. Mutations in *capu* and *spire* result in defects in the dorsoventral polarity of the embryo (Manseau and Schüpbach, 1989); mutations in *stau* disrupt the anterior localization of *bicoid* mRNA (St. Johnston et al., 1989); mutations in *valois* produce defects in cellularization (Schüpbach and Wieschaus, 1986); strong alleles of *nanos* and overlapping deficiencies removing *vasa* disrupt progression through oogenesis (Lasko and Ashburner, 1988; Lehmann and Nüsslein-Volhard, 1991); and mutations at *pumilio* result in a decrease in viability (Barker et al., 1992) and true nulls are zygotic lethals (St. Johnston and Nüsslein-Volhard, 1992). No amorphic alleles of *tudor* are available, but based upon the expression of *tud* mRNA throughout the life cycle (Golumbeski et al., 1991) and the complex subcellular localization of the protein (Bardsley et al., 1993), it would not be surprising if *tud*$^+$ had another developmental role. Therefore, of the posterior group genes, only *oskar* appears to be involved specifically in the assembly of the pole plasm. However, no *oskar* allele completely eliminates *oskar* product, so even this conclusion should be viewed with caution. In addition, the maternal effect genes *Toll*, *cactus* and *torpedo* (involved in dorsoventral patterning) and the *Drosophila raf* homologue, *l(1)polehole* (involved in establishing the embryonic termini) all mutate to zygotic lethality (Gerttula et al., 1988; Ambrosio et al., 1989; Price et al., 1989; Roth et al., 1991). Therefore, many of the gene products utilized maternally in the establishment of embryonic polarity play later (or earlier) developmental roles.

St. Johnston and Nüsslein-Volhard (1992) have suggested that the most likely class of unidentified genes with maternal roles in development are those genes that also have a zygotic function. If the behavior of germ line mosaics homozygous for lethal *mago* alleles (failure to progress through oogenesis) is indicative of the behavior of germ line clones of mutations at other essential genes, it may be difficult to identify these potential components of early developmental pathways. Some of these genes may only be identifiable by hypomorphic mutations that yield an interesting phenotype, much like *mago*.

Because the sequence of the predicted mago protein does not provide an indication of a potential biochemical role, one can only hypothesize about what function this protein may be playing in the assembly of the germ plasm. One possibility is that mago protein is a component of the cortical cytoskeleton along which transport of stau protein/*osk* mRNA to the posterior pole may occur. Alternatively, the mago protein may serve to anchor stau protein/*osk* mRNA to the posterior pole. Work in progress to generate antibodies against the mago protein should allow us to distinguish between these (and other) possibilities by providing reagents to examine the distribution and subcellular localization of mago protein in ovaries and embryos. The fact that the mago gene product appears to have been highly conserved between such divergent organisms as flies and nematodes, and that these organisms have similar structures associated with their germ plasms, raises the intriguing possibility that the processes involved in the localization and assembly of germ plasm components have been conserved over large evolutionary distances.

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