**mago nashi mediates the posterior follicle cell-to-oocyte signal to organize axis formation in Drosophila**

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**SUMMARY**

Establishment of the anteroposterior and dorsoventral axes in the Drosophila egg chamber requires reciprocal signaling between the germ line and soma. Upon activation of the Drosophila EGF receptor in the posterior follicle cells, these cells signal back to the oocyte, resulting in a reorganization of the oocyte cytoplasm and anterodorsal migration of the oocyte nucleus. We demonstrate that the gene *mago nashi* (*mago*) encodes an evolutionarily conserved protein that must be localized within the posterior pole plasm for germ-plasm assembly and *Caenorhabditis elegans mago* is a functional homologue of *Drosophila mago*. In the absence of *mago* function during oogenesis, the anteroposterior and dorsoventral coordinates of the oocyte are not specified and the germ plasm fails to assemble.

Key words: *Drosophila*, axis formation, germ plasm, *mago nashi*, follicle, oocyte, signaling

**INTRODUCTION**

Formation of the anteroposterior and dorsoventral axes of *Drosophila* requires inductive interactions between the germ-line-derived oocyte and surrounding somatic follicle cells during oogenesis (Ray and Schüpbach, 1996). These inductive interactions require *gurken* (*grk*) function in the germ line and *torpedo* (*top*) function in the somatic follicle cells (González-Reyes et al., 1995; Roth et al., 1995). During early oogenesis, when the germinal vesicle is located at the posterior end of the developing egg chamber, Grk protein (a member of the transforming growth factor α family) is necessary to induce the localized activation of the *top*-encoded *Drosophila* epidermal growth factor receptor (top/DER) in a subset of posterior follicle cells (González-Reyes et al., 1995; Roth et al., 1995). Grk-induced activation of the top/DER tyrosine kinase is likely to lead to Ras signaling in these follicles, causing them to adopt a posterior fate. It has been proposed that these posterior follicle cells in turn send a signal back to the oocyte. Neither the ligand produced by the posterior follicle cells nor the receptor on the oocyte have been identified, but it appears that protein kinase A is necessary in the oocyte for the signal to be transduced (Lane and Kalderon, 1994). Reception of the posterior follicle cell signal by the oocyte induces a reorganization of the oocyte microtubule cytoskeleton (stages 6-7), movement of the oocyte nucleus to an anterodorsal position (stage 8), and localization of germ cell and abdominal determinants within the posterior pole plasm (stages 8-14) (González-Reyes et al., 1995; Roth et al., 1995). This reorganization of cytoplasmic determinants leads to cellular differentiation during embryogenesis.

To study the localization and function of these determinants, maternal effect mutations that impair the formation and function of the posterior pole plasm (germ plasm) have been isolated. Females carrying mutations in the posterior group genes, *cappuccino*, *mago nashi*, *oskar*, *spire*, *staufen*, *tudor*, *valois* and *vasa*, produce embryos with defects in abdominal segmentation and germ cell formation (Boswell and Mahowald, 1985; Lehmann and Nüsslein-Volhard, 1986; Schüpbach and Wieschaus, 1986; Manseau and Schüpbach, 1989; Boswell et al., 1991). In each of these cases, polar granules (germ-plasm-specific organelles) fail to assemble during oogenesis and the progeny of mutant females lack germ cells.

Detailed analysis of the posterior group genes reveals that the posterior pole plasm is assembled in a step-wise manner, with earlier acting components required for the localization of later components (for review, see St. Johnston, 1993). For example, mislocalization of *oskar* (*osk*) mRNA to the anterior pole of the oocyte is sufficient to direct the assembly of a functional ectopic posterior pole plasm (Ephrussi and Lehmann, 1992). This leads to the formation of polar granules at the anterior pole, anterior recruitment of Vasa and Tudor proteins, anterior localization of the abdominal segmentation determinant (*nanos* mRNA; Wang and Lehmann, 1991), and induction of ectopic germ cell formation and abdominal segmentation (Ephrussi and Lehmann, 1992; Bardsley et al., 1993). Females carrying the *osk* mislocalization construct and mutations in the genes *cappuccino*, *mago nashi*, *spire* or *staufen* assemble functional germ plasm at the anterior pole (Ephrussi and Lehmann, 1992). Based on these studies, it appears that the *cappuccino*,

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mago nashi, spire, and staufen gene products play a role in the posterior localization of osk mRNA. Once localized, osk mRNA directs the assembly of the germ-plasm components and the localization of the abdominal segmentation determinant nanos.

As with most posterior group genes, the sequence of mago nashi (mago) does not immediately suggest a biochemical role for the protein (Newkarm and Boswell, 1994). To help clarify the role of mago in development, we have extended the phenotypic analysis of mago mutants and isolated homologues of Drosophila mago. Our extended phenotypic characterization demonstrates that, by mediating the response of the oocyte to the grk-top/DER-induced signal transmitted by the posterior follicle cells, mago is involved in the establishment of antero-posterior and dorsoventral polarity as well as assembly of a functional posterior pole plasm. We also show that Mago protein is transiently localized within the posterior pole during two distinct stages of oogenesis. This posterior pole localization is dependent on the wild-type functions of cappuccino, spire and grk. In addition, we have identified homologues of Drosophila mago in Caenorhabditis elegans, Xenopus laevis and Mus musculus. We demonstrate that the most distantly related gene, C. elegans mago, is a functional homologue of Drosophila mago, suggesting that components of germ-plasm assembly have been conserved over large evolutionary time frames.

**MATERIALS AND METHODS**

**Fly stocks and culturing**

The mago alleles used in this work (mago1, mago3, WE7, RE7 and SHL-1), the deletion Df(2R)F36, as well as the mutations capit1, spireP4, varP3 and grk108 were maintained in stocks containing Dr(1)w,y,w6£23 X chromosome(s) and an In(2LR)SM5 (SM5) balancer chromosome (Lindsley and Zimm, 1992). Flies were cultured in half-pint milk bottles or in 8-dram vials on standard Drosophila medium.

Eggs were collected for egg counts and observation of egg shells on 60x15 mm plates containing apple juice agar medium (Wieschaus and Nüsslein-Volhard, 1986). Females were reared at 25°C. eggs were collected at 12 hour intervals for 2-3 days, chorions were initially viewed with a Leica MZ6 dissecting microscope and subsequently mounted for observation using a Zeiss axiophot microscope as described by Wieschaus and Nüsslein-Volhard (1986). After rearing females for 2-3 days at 25°C, females were shifted to 17°C, eggs were collected at 12 hour intervals and egg shells were observed as described above. The production of ventralized eggs increases 4 days after shifting females from 25°C to 17°C. Females reared continuously at 17°C did not produce ventralized eggs but ~30-40% of stage 10 egg chambers contained an oocyte nucleus localized within the posterior pole.

Germ-line and follicle cell mosaics were produced as described by Chou and Perrimon (1996).

**Construction and characterization of transgenes encoding N-terminal-tagged Mago proteins**

Synthetic oligonucleotides (Operon, Inc.) encoding a myc-epitope (Dsalmyc: 5′-CACGATGGAGAAACCATTTAGCGAGGAAGATCTGGAATT-3′) and Dsal/AS-myc: 5′-CTGGAAATTCA-GATCTTTCTCGATAAAGCTTTTCCTCCAT-3′) were annealed and cloned into a unique Dsal recognition site in the mago cDNA, p5A (Newmark and Boswell, 1994), resulting in pMyc5A. An Mial-Stul fragment derived from pMyc5A was used to replace the corresponding fragment of the mago transformation vector pCaS4-B/P2.2 (Newmark and Boswell, 1994). DNA sequencing was performed to confirm the incorporation of myc-encoding sequences into mago genomic DNA and the resulting construct, pCaS4-myc-mago, was co-injected into su1118 embryos with pZ25.7cve helper plasmid. One X-linked homozygous viable insertion was obtained, p[myc-mago].

The p[myc-mago] element on the X chromosome was mobilized using D2-3 as a genomic source of transposase activity (Robertson et al., 1988). Fourteen independent autosomal insertions were examined for the production of myc-Mago by immunoblot analysis using affinity-purified, anti-Mago antibodies (see below) and whole-mount immunofluorescence (see below).

The mago1 mutation (GLY 19→ARG; Newmark and Boswell, 1994) was introduced into the corresponding codon in pMyc5A using the oligo 5′-GAATTCGCGCCTGAATTGCCCCC-3′ with the Sculptor in vitro mutagenesis kit (Amersham). Introduction of the mutation was confirmed by DNA sequencing and the construct was subcloned into pCaS4-B/P2.2 as described for pCaS4-myc mago, resulting in pCaS4-myc-mago1. A single second chromosome-linked insertion, p[myc-mago1], was recombined onto a chromosome with the zygotic lethal mago allele SHL-1 and the distribution of myc-Mago1 was determined in ovaries from homozygous p[myc-mago1]-SHL-1 females (see below).

GFp-tagged mago was constructed by inserting blunt-ended GFP sequence into the unique Dsal restriction site in p5A, replacing the Mlu-XbaI fragment of pCaS4-B/P2.2 with the corresponding fragment of pGFP5A and sequencing to confirm the in-frame incorporation of GFP into the mago gene. One X-linked, viable insertion, p(GFP-mago), was obtained by P-element-mediated transformation.

The distribution of GFP-Mago protein in p(GFP-mago); Df(2R)F36/CyO females was determined by dissecting ovaries in PBST (1x PBS, 0.1% Triton X-100) and fixing for 15 minutes in 4% paraformaldehyde, PBST. Samples were examined by epifluorescence microscopy using a Zeiss axiophot and analyzed using a Molecular Dynamics MultiProbe 2001 confocal microscope.

**Construction of Mago fusion proteins for antibody production and purification**

To construct a maltose-binding protein (MBP)-Mago fusion protein, a mago cDNA fragment was cloned into pMAL-c2 (New England Biolabs). This construction (pMal-5A) was confirmed by DNA sequencing and results in an MBP-Mago fusion protein. The pMal-5A vector was transformed into Escherichia coli strains BL21 (DE3) and C43 (DE3), and the fusion protein was purified under denaturing conditions by metal chelate affinity chromatography on an amylose resin following the recommendations of the supplier (NEB). Purified MBP-Mago was then dialyzed against multiple changes of PBS and injected into three rats at a concentration of ~400 μg/mL. Commercial injections and bleeds were performed at the Pocono Rabbit Farm and Laboratories (Canadensis, PA).

**Affinity purification of anti-Mago antibodies using 6xHis-Mago fusion protein**

Antiserum obtained from the rats immunized with the MBP-Mago fusion protein recognize an approximately 17×103 M1 protein in Drosophila ovarian extracts. Anti-Mago antibodies were affinity purified using a 6xHis-Mago fusion protein. The 6xHis-Mago fusion protein was generated by in-frame insertion of mago cDNA sequence into pQE-8 (Qiagen), resulting in pQE-mago. Insoluble 6xHis-Mago protein was purified under denaturing conditions by metal chelate affinity chromatography on Ni-NTA resin as recommended by the supplier (Qiagen). Eluted 6xHis-Mago protein was dialyzed into PBS/0.1% SDS and coupled to AminoLink gel following the recommendations of the supplier (Pierce). Rat anti-sera were diluted 1:10 in PBS and circulated over a column containing the soluble protein fraction from IPTG-induced E. coli cells containing the pMAL-c2 vector alone. The flow-through from this column was then circulated...
over the 6xHis-Mago affinity column, washed and eluted with 0.1 M glycine (pH 2.8) into 1/10 volume of 1 M Tris-Cl (pH 8.0). Protein-containing fractions were pooled, BSA was added to 1 mg/ml and fractions were dialyzed into PBS/0.02% Na-azide. The specificity of affinity-purified material was examined by immunoblot analysis on total protein isolated from Drosophila ovariess.

**Immunoblot analysis**

Total protein isolated from Drosophila ovaries was separated on tricine-SDS-polyacrylamide gels (Slähger and von Jagow, 1987). 10% T, 3% C gels were used for most applications. Ovaries were frozen on dry ice and homogenized in 1× sample buffer (4% SDS, 12% glycerol, 50 mM Tris-Cl (pH 6.8), 2% mercaptoethanol, 0.01% brilliant blue G), refrozen, thawed, passed through a 22-gauge needle and then boiled for 5 minutes prior to gel loading. After electrophoresis, gels were electro-transferred to nitrocellulose (0.2 μm pore size) in a Hoefer SemiPhor semidy blotting apparatus. Primary antibodies were diluted in Blotto/Tween (PBS or TBS, 5% non-fat dry milk, 0.1% Tween-20) and incubated overnight at 4°C. 9E10 monoclonal antibody (courtesy of Robert Cary and Michael Klymkowski) was used at 2 μg/mL and α-Mago antibodies were used at 1:250. HRP-conjugated α-rat IgG secondary antibody (Pierce) was used at 1:25,000 and HRP-conjugated α-mouse IgG (Amersham) was used at 1:1000. Blots were washed and processed for detection using enhanced chemiluminescence (ECL) reagents as recommended by the supplier (Amersham).

**Whole-mount immunodetection and in situ hybridization on ovarian tissue**

Ovaries were dissected in 1× EBR (130 mM NaCl; 10 mM Hepes (pH 6.9); 5 mM KCl; 2 mM CaCl2) or PBST from females fed on wet yeast paste for 2-4 days after eclosion. For detection of myc-tagged proteins, fixation was as described by Peifer et al. (1993) or Xue and Cooley (1993). Similar results were obtained using either fixation protocol, α-myc monoclonal antibody 9E10 was used at 2-4 μg/mL (in PBSTB). Secondary antibodies were used in these concentrations: goat α-mouse-FITC or goat α-mouse-Texas Red (Amersham), 1:100; horse α-mouse-FITC (Vector), 1:250. Samples were examined by epifluorescence microscopy using a Zeiss axiophot and analyzed using a Molecular Dynamics MultiProbe 2001 confocal microscope.

Detection of Gurken protein was as described in Roth et al. (1995). Anti-Gurken antibodies (kindly provided by T. Schüpbach) were pre-absorbed to ovaries from homozygous gurkenK females and used at a final dilution of 1:100. HRP-conjugated goat α-rat secondary antibodies (Pierce) were used at a dilution of 1:100.

Localization of kinesin-βg fusion protein (Clark et al., 1994) was determined in ovaries from mago1/Df(2R)F36; kinesin-LacZ+y and mago1/SMS5; kinesin-LacZ control females. Ovaries were fixed in 4% paraformaldehyde, PBST for 30 minutes. The fusion protein was detected by immunofluorescence staining with mouse α-βg antibodies (Promega) at 1:500 dilution and FITC-conjugated horse α-mouse secondary antibodies (Vector) at 1:250 dilution.

In situ hybridizations were performed as described by Klingler and Gergen (1993).

**Isolation of C. elegans mago and construction of a ce-mago minigene for P-element transformation**

CEESH75, a C. elegans expressed sequence tag with significant similarity to Drosophila mago (Newmark and Boswell, 1994) was provided by Anthony Kerlavage and used to screen a C. elegans embryonic cDNA library (Barstead and Waterston, 1989; provided by Natasha Singh and Min Han). PCR was used to determine insert sizes of the positive plaques and the clone with the largest insert (ce-mago2B) was sequenced. The linker used for cloning this cDNA occupies the likely position of the start codon and creates a unique BspEI site. ce-mago2B was digested with BspEI, filled in with Klenow, digested with XhoI and ligated into Dsul-digested p5A (mago cDNA) that had been blunt-ended with Klenow and then digested with XhoI. This results in the in-frame insertion of ce-mago coding sequences directly behind the first three codons of the mago cDNA. An MluI-XhoI fragment from this clone was then cloned into pCaS4-B/P2.2, resulting in a construct in which ce-mago cDNA is expressed under the control of the Drosophila mago promoter, with the dm-mago 5’ and 3’ untranslated regions left intact. The ce-mago transformation construct was co-injected with pr25.7w helper plasmid into Df[1]w; y6723 embryos. Two homozygous viable transformant strains were obtained. The strain containing an insert on the third chromosome was used for complementation analysis with balanced mutant mago alleles in a Df[1]w; y6723 background.

**Isolation of Xenopus laevis and Mus musculus mago genes**

The coding sequences from dm-mago were amplified by PCR and used to screen a Xenopus laevis cDNA library (kindly provided by Michael Klymkowski). Approximately 106 phage were screened by hybridization with 32P-labelled dm-mago sequences at 50°C in 6× SSC; 0.5% SDS: 5× Denhardt’s solution; 500 μg/mL salmon sperm DNA and washed twice at room temperature with 2× SSC; 0.1% SDS and three times at 50°C in 0.5× SSC; 0.1% SDS (Sambrook et al., 1989). Seven positive plaques were identified; the three largest clones (as determined by PCR) were sequenced.

The Xi-mago coding sequences were amplified by PCR and used to screen a Mus musculus cDNA library (kindly provided by Barbara Knowles). Conditions for screening of approximately 105 colonies were similar to those used to identify Xi-mago, except that hybridization was done at 65°C and washes were done at 37°C and 55°C. Ten positive colonies were identified; a full-length clone was sequenced.

**RESULTS**

**Aberrant dorsoventral axis formation: mislocalization of the oocyte nucleus in egg chambers of hemizygous mago1 females**

Approximately 1% (n=365) of the eggs from hemizygous mago1 females (to be referred to as mago eggs) raised at 25°C are ventralized. When these females are shifted from 25°C to 17°C, however, approximately 38% (n=751) of the eggs produced are ventralized, exhibiting a phenotype similar to that observed when eggs are collected from grk mutant females. In eggs with defective shells, expansion of ventral fates results in an elimination of the dorsal appendages (Fig. 1A,B). Although females mutant for top, grk or cornichon (cni) produce eggs exhibiting a similar ventralized phenotype (González-Reyes et al., 1995; Roth et al., 1995), eggs and embryos from these females display striking differences from those produced by hemizygous mago1 mothers. Eggs from mutant top, grk or cni females may lack anteroposterior polarity, as exemplified by a microtubule that is formed in place of an aeropyle at the posterior pole (González-Reyes et al., 1995; Roth et al., 1995). This is in contrast to ventralized mago eggs which possess an aeropyle indistinguishable from that observed in wild-type eggs (Fig. 1B, right panel), suggesting that anteroposterior patterning of the egg shell is normal. Moreover, embryos derived from ventralized eggs produced by top, grk or cni mutant females have defects in dorsoventral patterning, whereas ventralized mago eggs are not fertilized due to a defective microtubule canal and, thus, do not develop (data not shown).

At stage 7 of oogenesis, the oocyte nucleus migrates from the posterior of the oocyte to an anterodorsal cortical position.
This movement is critical for suppression of ventral and establishment of dorsal follicular cell fates because it allows apical localization of grk mRNA and the spatially restricted synthesis of Grk protein, the likely ligand activating the top/DER signaling pathway in the overlying follicle cells (González-Reyes et al., 1995; Roth et al., 1995). Given the nuclear migration defects observed in mutants disrupting the grk-top/DER signaling pathway, we examined the position of the oocyte nucleus in mago egg chambers collected from females reared at 25°C and females that had been shifted from 25°C to 17°C. Approximately 1% (n=160) of the stage 10 mago egg chambers from females reared at 25°C contained an oocyte nucleus that was mislocalized, whereas 36% (n=216) of the stage 10 mago egg chambers of females shifted from 25°C to 17°C had germinal vesicles within the posterior pole (Fig. 1D,F). The excellent correspondence between the percentage of egg chambers with mislocalized oocyte nuclei and ventralized eggs produced by hemizygous mago1 females suggests that the ventralized phenotype observed in mago eggs results from the failure of nuclear migration to the anterodorsal cortex during oogenesis. Moreover, when the germ line is homozygous for mago1 or mago3 (a lethal allele, GLN87→STOP; Newmark and Boswell, 1994), egg chambers containing oocyte nuclei mislocalized within the posterior pole and eggs with ventralized shells are observed (Table 1). To determine whether grk mRNA and protein are synthesized and/or properly localized, we performed in situ hybridization and immunolocalization on whole-mount egg chambers collected from wild-type, hemizygous mago1, mago1 germ-line mosaic and mago3 germ-line mosaic females. Regardless of the position of the oocyte nucleus in mago egg chambers, grk mRNA and protein are always detected, and accumulate in a perinuclear position between the germinal vesicle and plasma membrane (Fig. 1C-F). This suggests that the synthesis, stability and perinuclear accumulation of grk mRNA and protein are not dependent on wild-type mago function. Clearly, the failure of the oocyte nucleus to migrate anteriorly suggests a defect in antero-posterior axis formation.

**Aberrant anteroposterior axis formation:** mago egg chambers display defects in the organization of the oocyte microtubule cytoskeleton

In the absence of grk-top/DER signaling during early oogenesis (stages 1-6), the fate of the posterior follicle cells is not specified and they behave as anterior follicle cells. Failure to specify posterior follicle cell fates results in an altered polarization of the oocyte microtubule network and the oocyte nucleus fails to migrate to the anterodorsal position during stage 7 of oogenesis (González-Reyes et al., 1995; Roth et al., 1995). Altered polarization of the microtubule cytoskeleton is manifested by the localization of bicoid (bcd, the anterior determinant) mRNA at both poles, and colocalization of osk mRNA and kinesin-β-gal to a central region within the oocyte cytoplasm (González-Reyes et al., 1995; Roth et al., 1995). Thus, in egg chambers from mutant

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**Fig. 1.** The egg shells and egg chambers of mago1/Df(2R)F36 mothers exhibit defects in dorsoventral axis formation and alterations of the oocyte microtubule cytoskeleton. Eggs and egg chambers are oriented with anterior to the left and posterior to the right. Dorsal is up in A, C, E and G. (A,B) Phase-contrast micrographs of egg shells from wild-type and mago1/Df(2R)F36 females, respectively. The arrows indicate the position of the micropyle and arrowheads show the position of the aeropyle. In A and B, the left panel is anterior and the right panel is posterior. (A) Phase-contrast micrograph of a wild-type egg shell. The dorsal appendages and micropyle are clearly visible in the anterior. At the extreme posterior end, a rounded aeropyle is observed. (B) Approximately 38% (285/751) of the egg shells collected from mago1/Df(2R)F36 mothers shifted from 25°C to 17°C are ventralized. Dorsal appendages are not observed. However, a micropyle is visible in the anterior and an aeropyle can be seen at the extreme posterior pole. (C) Localization of the oocyte nucleus and grk mRNA in wild-type stage 10 egg chambers. grk mRNA is clearly detected between the oocyte nucleus and overlying follicle cells, indicating the position of the future dorsal surface of the egg. (D) Mislocalization of the oocyte nucleus is observed in 36% (78/216) of the stage 10 egg chambers from mago1/Df(2R)F36 females shifted from 25°C to 17°C. Perinuclear accumulation of grk mRNA is detected at the posterior pole with the mislocalized oocyte nucleus. grk mRNA accumulates between the oocyte nucleus and overlying follicle cells. (E,G,I) The distribution of Gurken protein, bicoid RNA and kinesin-β-gal in wild-type egg chambers, respectively. (F,H,J) The distribution of Gurken protein, bicoid RNA and kinesin-β-gal in egg chambers of mago1/Df(2R)F36 females shifted from 25°C to 17°C.
In mago egg chambers may be due to the inability of Grk to signal to the oocyte, since posterior follicle cells may be due to the inability of Grk to signal to the oocyte. However, strains containing RA5 and homozygous for mutations in grk produce egg chambers lacking detectable β-galactosidase in dorsal follicle cells (Fig. 2G,H, respectively). In mago egg chambers carrying the RA5 enhancer-trap line and in which the oocyte nucleus is mislocalized within the posterior pole, β-galactosidase is detected in posterior follicle cells overlying the germinal vesicle (Fig. 2I). The ectopic expression of β-galactosidase in the posterior follicle cells of these mago egg chambers indicates that mago1 does not eliminate signaling. These results indicate that mago+ functions within posterior follicle cells to the oocyte. In the absence of wild-type grk function, the reorganization of the microtubule network essential for axis formation and subsequent germ-plasm assembly fails to occur.

Table 1. Germ-line clonal analysis of mago alleles

<table>
<thead>
<tr>
<th>Genotype of green line</th>
<th>Mislocalization of the oocyte nucleus</th>
<th>RNA in egg chambers</th>
<th>Ventralized egg shells</th>
</tr>
</thead>
<tbody>
<tr>
<td>mago+/mago1</td>
<td>4% (n=53)</td>
<td>–</td>
<td>2% (n=701)</td>
</tr>
<tr>
<td>mago+/mago3</td>
<td>29% (n=14)</td>
<td>–</td>
<td>19% (n=37)</td>
</tr>
<tr>
<td>FRT103 (control)</td>
<td>0% (n=75)</td>
<td>+</td>
<td>0% (n=699)</td>
</tr>
</tbody>
</table>

Progeny from females producing mago+/mago1 germ-line clones were sterile.

*Egg chambers were reacted with antisera recognizing Gurken protein or with antisense gurken RNA probes. Regardless of nuclear position Gurken protein and RNA colocalize with the oocyte nucleus.

The (−) indicates no detectable localized oskar RNA within the posterior pole and (+) indicates localization of oskar RNA within the posterior pole.

grk, top and cni females, both poles of the oocyte behave as anterior poles and a central region of the oocyte accumulates components normally localized within the posterior pole plasma. To ascertain whether the microtubule cytoskeleton is altered in mago egg chambers, we examined the distribution of bcd mRNA and kinesin-β-gal in mago egg chambers collected from females that had been shifted from 25˚C to 17˚C. In these mago egg chambers, bcd mRNA accumulates at both poles (Fig. 1G,H) and kinesin-β-gal accumulates in the center of the oocyte (Fig. 1I,J). Although osk mRNA and Staufen protein accumulate within mago egg chambers and transiently localize within the anterior pole, they do not concentrate within the posterior pole plasma or within a central ooplasmic region (Newmark and Boswell, 1994). These results indicate that polarization of the microtubule cytoskeleton is abnormal in egg chambers from hemizygous mago1 females shifted from 25˚C to 17˚C, and provide further evidence that mago+ function is required both for axis formation and assembly of germ-plasm components within the posterior pole.

Defects in the organization of the microtubule cytoskeleton in mago egg chambers may be due to the inability of Grk to signal to the oocyte from the overlying posterior follicle cells during oogenic stages 1-6, or because mago+ functions to mediate a signal received by the oocyte from the overlying posterior follicle cells. If grk signaling fails to occur in the absence of mago+ function, the overlying posterior follicle cells might be expected to behave as anterior follicle cells in mago egg chambers (González-Reyes et al., 1995; Roth et al., 1995). Regardless of the temperature at which hemizygous mago1 females are reared, enhancer-trap lines that mark anterior follicle cells are expressed in a manner indistinguishable from wild type (Fig. 2A-F). This result is consistent with the fact that the posterior aeropyle is formed normally and suggests that the fate of posterior follicle cells is specified properly. To determine whether mutations in mago eliminate grk signaling, an enhancer-trap line, RA5, that expresses β-galactosidase in response to grk signaling (Duffy, personal communication) was utilized. In wild-type egg chambers derived from strains containing the RA5 enhancer-trap line, β-galactosidase is detected in anterior dorsal follicle cells. However, strains containing RA5 and homozygous for mutations in grk produce egg chambers lacking detectable β-galactosidase in dorsal follicle cells (Fig. 2G,H, respectively). In mago egg chambers carrying the RA5 enhancer-trap line and in which the oocyte nucleus is mislocalized within the posterior pole, β-galactosidase is detected in posterior follicle cells overlying the germinal vesicle (Fig. 2I). The ectopic expression of β-galactosidase in the posterior follicle cells of these mago egg chambers indicates that mago1 does not eliminate grk signaling. These results indicate that mago+ functions within the oocyte to mediate the signal(s) sent from the posterior follicle cells to the oocyte. In the absence of wild-type mago function, the reorganization of the microtubule network essential for axis formation and subsequent germ-plasm assembly fails to occur.

![Fig. 2. Expression pattern of enhancer trap lines in egg chambers from wild-type and mutant females. (A-C) Females carrying the enhancer trap line p[w+ lacZ] 5A7, which expresses β-galactosidase in the border cells. (A) Wild type; (B) homozygous grkHK; (C) hemizygous mago1 females shifted from 25˚C to 17˚C. The expression pattern in mago egg chambers is indistinguishable from wild type. (D-F) The p[w+ lacZ] BB127 enhancer trap line is expressed in the centripetal follicle cells and nurse cells. (D) Wild type; (E) hemizygous topQY; (F) hemizygous mago1 females shifted from 25˚C to 17˚C. The arrow in E indicates X-gal staining in a single posterior follicle cell. In mago egg chambers BB127 appears to be expressed in a pattern identical to wild type. (G-I) The enhancer trap line p[ry+ lacZ] RA5 expresses β-galactosidase in the follicle cells overlying the oocyte nucleus in response to grk signaling. (G) Wild type; (H) homozygous grkHK; (I) hemizygous mago1 females shifted from 25˚C to 17˚C. (H,J) The females also carry the enhancer trap insertion ex(3) 79 that serves to mark the nurse cell nuclei and oocyte nucleus with β-galactosidase. (C,F,I) Only mago egg chambers with the oocyte nucleus mislocalized within the posterior pole were counted (in each case n=34). In mago egg chambers, ectopic expression of p[ry+ lacZ] RA5 is detected in posterior follicle cells overlying the mislocalized oocyte nucleus.](Image 171x541 to 571x721)
Dynamic distribution of the Mago protein during oogenesis – nuclear localization and association with the posterior pole

To examine the distribution of Mago protein in oogenesis, we first generated antibodies against Mago protein (see Materials and methods). Although these antibodies are effective for immunoblotting applications, they are less effective at detecting protein in fixed whole-mount tissues. Therefore, sequences encoding a myc-epitope (Evan et al., 1985) and the green fluorescent protein (GFP) of the jellyfish Aequorea victoria (Chalfie et al., 1994) were introduced into the 5′ coding sequences of the mago gene (see Materials and methods). Both the p[myc-mago] and p[GFPMago] constructs were introduced into the Drosophila germ line using P-element-mediated transformation (Rubin and Spradling, 1982). The p[myc-mago] and p[GFPMago] constructs complement the defects in axis formation and germ-plasm assembly of \( \text{mago}^1 \) and the zygotic lethality of the lethal mago allele, \( \text{SHL-1} \) (202 bp deletion of 5′ coding sequences). Immunoblot analysis using affinity-purified antibodies against Mago protein (\( \alpha \)-Mago) was used to confirm the complementation of \( \text{SHL-1} \) lethality by the p[myc-mago] transgene (see Fig. 3). Thus, p[myc-mago] and p[GFPMago] confer wild-type mago function, indicating that the N-terminal tags do not interfere with Mago protein function.

We have used a monoclonal antibody recognizing the myc-epitope (Evan et al., 1985) to monitor the distribution of myc-Mago protein in oogenesis (for simplicity, we will refer to the myc-Mago protein as Mago protein throughout the remainder of the paper, except where it is essential to distinguish between myc-Mago and endogenous Mago protein). Ovaries from the parental strain \( \text{w}^{1118} \) (not containing the p[myc-mago] transgene) reveal no staining above background when processed for immunofluorescence using the \( \alpha \)-myc monoclonal antibody (Fig. 4H).

Whole-mount immunofluorescence on fixed ovarian tissue from p[myc-mago] strains using the \( \alpha \)-myc monoclonal antibody reveals a striking pattern of Mago distribution during oogenesis. In the gerarium, Mago is associated with all germ-line nuclei (data not shown). During stages 1 and 2, after the cyst leaves the gerarium, Mago is detected within the nurse cell nuclei. By oogenetic stages 3-4, Mago is detected in the oocyte, both within the nucleoplasm and in the cytoplasm (Fig. 4A,B). Confocal microscopic analysis reveals that during the early stages of oogenesis (~1-7), the nurse cell nuclei contain Mago concentrated near the nuclear envelope (Fig. 4A-D) whereas the oocyte nucleus contains Mago throughout the nucleoplasm. As oogenesis continues Mago accumulates within the nurse cell nucleoplasm but is not detected within the prominent nucleoli (Fig. 4E). The distribution of myc-Mago and GFPMago throughout oogenesis are indistinguishable (compare Fig. 4E,G), providing evidence that these proteins accurately reflect the distribution of endogenous Mago. Within the oocyte nucleus, there is a region devoid of detectable Mago (Fig. 4A-D). Double-labelling with the DNA-binding stain DAPI reveals that this region is the karyosome, a region of condensed oocyte chromosomes (data not shown). Occasionally, a ‘spot’ containing Mago can be detected within the karyosome (Fig. 4A). This ‘spot’ may correspond to the endobody (originally, Binnenkörper in German), an ultrastructurally defined nuclear domain approximately 1 μm in diameter that is distinct from nucleoli (Bier et al., 1967; Mahowald and Tiefert, 1970).

Confocal microscopic analysis also reveals that Mago is associated with the posterior-most region of the oocyte. This posterior localization is observed during two distinct periods of oogenesis: the first during stages ~3-5, and the second during stages 8 and 9. During stages ~3-5, Mago is observed along the posterior periphery of the oocyte (Fig. 4A-C). At these stages, Mago is detected throughout the oocyte cytoplasm, with an apparent enrichment toward the posterior of the oocyte. During stages 6 and 7, Mago is not detected at the posterior pole, but remains associated with the germ-line nuclei (Fig. 4D). At stages 8 and 9, Mago is again detected at the posterior of the oocyte, at the site of pole plasm formation (Fig. 4E). By stage 10, Mago is no longer detectable at the posterior pole of the oocyte (data not shown).

Mago is also detected in the somatic follicle cell nuclei, but
is not detected within the nucleoli (Fig. 4E; King and Koch, 1963). Germ-line clonal analysis reveals that mago* function is required in the germ line (Table 1); thus, detection of Mago in somatic cells was somewhat unexpected. Egg chambers of strains containing p[GFP-mago] have detectable GFP-Mago within the nuclei of follicle cells. Furthermore, thirteen independent homozygous viable strains containing autosomal insertions of the p[myc-mago] transgene exhibit detectable myc-Mago in the nuclei of somatic follicle cells. Thus, the nuclear distribution of myc-Mago does not appear to be due to the presence of the myc epitope on the Mago protein nor does it appear that Mago localization within follicle nuclei is due to an enhancer element near the insertion site of the transgene.

The viable mago allele, mago1 (GLY19→ARG; Newmark and Boswell, 1994), when homo- or hemizygous disrupts the assembly of the posterior pole plasm during oogenesis. Given that wild-type Mago is localized within the posterior pole and required for the posterior pole localization of germ plasm (Newmark and Boswell, 1994), it was of interest to examine the distribution of Mago1 protein within egg chambers. This was accomplished by introducing a myc-epitope into the 5′ coding sequences of a mago gene containing the mago1 lesion (see Materials and Methods). The p[myc-mago1] construct was introduced into the germ line by P-element-mediated transformation. Consistent with the mago1 phenotype, p[myc-mago1] complements the lethality of the zygotic lethal allele SHL-1, but the progeny of p[myc-mago1]SHL-1 homozygous females are sterile. Whole-mount immunolocalization on fixed ovarian tissues from p[myc-mago1] strains reveals myc-Mago1 within all germ-line nuclei and follicle nuclei of the egg chamber. In contrast to wild-type Mago, myc-Mago1 is not detected within the posterior pole cytoplasm during oogenesis (Fig. 4F), demonstrating that posterior pole localization but not nuclear
localization of Mago is critical for assembly of a functional posterior pole plasm.

**Posterior localization of Mago requires capppuccino*, spire* and gurken* functions**

Mutations in capppuccino (cap), spire (spir) and gurken (grk) disrupt the posterior localization of the products of all previously identified posterior group genes (Manseau and Schüpbach, 1989; Ephrussi et al., 1991; Kim-Ha et al., 1991; St. Johnston et al., 1991; Bardsley et al., 1993; Wang et al., 1994; González-Reyes et al., 1995; Roth et al., 1995). Mutations in cap and spir result in premature commencement of microtubule-dependent cytoplasmic streaming during oogenesis (Theurkauf et al., 1992; Emmons et al., 1995); thus, they likely disrupt some cytoskeletal function required for posterior localization. Examination of Mago distribution in ovaries isolated from cap and spir homozygous females reveals that the posterior localization of Mago during stages 8-9 is disrupted (Fig. 5B, C). In contrast, the posterior localization of Mago protein in vasa homozygous mutant oocytes appears normal (Fig. 5A). Thus, gene products required early in the process of pole plasm assembly and localization (cap and spir) are required for posterior Mago localization, whereas one of the genes required later for pole plasm function (vasa) is not required for Mago localization. Mutations in grk disrupt the microtubule cytoskeleton and the egg chambers have germ-plasm components such as osk mRNA mislocalized to a central region of the cytoplasm (González-Reyes et al., 1995; Roth et al., 1995). In egg chambers from homozygous grkHK mothers, Mago is also localized in a central region of the oocyte cytoplasm (Fig. 5D), indicating that Mago colocalizes with germ-plasm components in grk mutants. These results are consistent with a role for Mago in the posterior localization of maternal determinants, rather than in the function of the determinants per se.

**C. elegans mago is a functional homologue of Drosophila mago**

Homologues of dm-mago (ce-mago), X. laevis (xl-mago) and M. musculus (mm-mago). Database searches revealed a striking similarity between the D. melanogaster mago gene (dm-mago) and an expressed sequence tag isolated from early C. elegans embryos (Newmark and Boswell, 1994). The original cDNA, CEESH75, did not contain the entire coding sequence of C. elegans mago (ce-mago) and, therefore, was used to isolate near full-length ce-mago cDNAs. The predicted protein encoded by the largest ce-mago cDNA is 80% identical to dm-Mago (Fig. 6). If conservative substitutions are taken into account, it is 88% conserved (Fig. 6). Concomitantly, we screened X. laevis and M. musculus cDNA libraries (see Materials and methods) for full-length cDNAs. The predicted proteins encoded by xl-mago and mm-mago are 88% identical to dm-Mago (Fig. 6), indicating that Mago is highly conserved in these organisms.

Utilizing the most divergent homologue (ce-mago), we tested the hypothesis that the identified genes might be functional homologues of dm-mago. The coding sequence of ce-mago cDNA was cloned directly downstream of the promoter, 5’ untranslated region, and the first two codons of the Drosophila mago gene in a P-element transformation vector (see Materials and methods). This construct was then intro-
duced into the Drosophila genome by P-element-mediated transformation (Rubin and Spradling, 1982). A single copy of ce-mago is sufficient to complement the axis defects and sterility of mago1 and the zygotic lethality of the mutant WE7 [both are point mutations in the mago gene (Newmark and Boswell, 1994)]. In contrast, two copies of ce-mago are necessary to complement the lethality of SHL-1, mago3 and RE7 [deletion and two nonsense mutations, respectively (Newmark and Boswell, 1994)]. Whether this dosage-dependent complementation is due to a lower abundance of ce-Mago protein or reduced function of ce-Mago protein relative to dm-Mago protein is unclear. Our α-dm-Mago antibodies do not recognize ce-Mago protein (data not shown), so we are unable to address this question at the present time. In either case, these results clearly indicate that ce-mago is able to provide mago+ function(s) required for both germ-plasm assembly and zygotic viability; thus, ce-mago is a functional homologue of dm-mago.

**DISCUSSION**

Mislocalization of the nucleus within the posterior pole of mago egg chambers suggests that mago+ function is necessary to establish the spatial coordinates of the egg chamber. In ∼36% of the stage 10 egg chambers collected from females hemizygous for the temperature-sensitive allele mago1, shifted from 25°C to 17°C, the oocyte nucleus remains in the posterior pole (Fig. 1D). A similar mislocalization of the oocyte nucleus within the posterior pole is also observed in egg chambers of females mutant for grk, top/DER and cni (González-Reyes et al., 1995; Roth et al., 1995), and is sufficient to explain the occurrence of ventralized mago egg shells. Thus, mago nashi participates in the bidirectional intercellular signaling between the posterior follicle cells and oocyte to establish spatial coordinates that induce axis formation (González-Reyes et al., 1995; Roth et al., 1995).

In the absence of top/DER signaling during oogenesis, the fate of the posterior follicle cells is not specified and these cells take on an anterior fate. The consequence is that, instead of an aeropyle (an egg shell structure formed by the posterior follicle cells), a micropyle (anterior sperm entry site) is formed at the posterior pole; enhancer-trap lines that normally mark only the anterior follicle cells express β-galactosidase in follicle cells at the posterior pole. In contrast, females mutant for mago produce egg shells with an aeropyle that is indistinguishable from wild type. Utilizing enhancer-trap lines that serve as markers, we have established that in mago egg chambers posterior follicle cells are not transformed into anterior follicle cells. Although the oocyte nucleus is mislocalized in mago egg chambers, grk RNA and protein are synthesized and accumulate in a perinuclear position between the oocyte nucleus and plasma membrane, regardless of oocyte nucleus position. These results, and those described in Newmark and Boswell (1994), demonstrate that altering mago+ function does not have a general effect on RNA or protein accumulation and localization.

To test the ability of grk to signal follicle cells in mago egg chambers, we monitored the expression of the enhancer-trap line, RA5, that is expressed in response to grk signaling (Duffy, personal communication). In mago egg chambers, RA5 is expressed in the posterior follicle cells overlying the mislocalized oocyte nucleus. Thus, the presence of an aeropyle in mago egg shells, the failure to detect a transformation of posterior follicle cells into anterior follicle cells and the pattern of expression of RA5 in mago egg chambers suggest that grk-top/DER signaling occurs normally in mago egg chambers. These results suggest that the primary signal from the oocyte to the posterior follicle cells is not disrupted in the absence of wild-type mago function.

Our current working model is that mago+ function is necessary within the oocyte to mediate the response to the secondary signal sent to the oocyte by the posterior follicle cells. This model is supported by the analysis of germ-line clones. Disruption of anteroposterior and dorsoventral axis formation and posterior pole plasm assembly is observed when mago+ function is altered in the germ line. In the absence of mago+ function, the oocyte nucleus fails to migrate to an anterodorsal position and posterior pole components fail to localize.

The posterior localization of Mago during stages 3-5 and stages 8-9 of oogenesis suggests that mago+ functions in at least two developmental processes: establishment of anteroposterior/dorsoventral polarity of the egg chamber, and anchoring of components within the posterior pole critical for anteroposterior polarity and germ cell determination during
embryogenesis. Here we have presented evidence that mago functions within the germ line to mediate grk-top/DER-induced signaling from the posterior follicle cells to regulate reorganization of the cytoskeletal network. This cytoskeletal reorganization is critical for establishment of dorsoventral polarity. Thus, the posterior localization of Mago during stages 3-5 of oogenesis is consistent with a role for mago in mediating the grk-top/DER-induced signal from the posterior follicle cells to establish the anteroposterior/dorsoventral axes. Posterior localization of Mago during stages 8 and 9 of oogenesis corresponds to the stages when osk mRNA and Staufen protein are first localized (Ephrussi et al., 1991; Kim-Ha et al., 1991; St. Johnston et al., 1991). Unlike osk mRNA and Staufen protein, however, Mago is not detected at the anterior pole of the oocyte during stages 8 and 9. In mutant mago egg chambers, osk mRNA and Staufen protein localize within the anterior pole during these stages but are not detected within the posterior pole plasm (Newmark and Boswell, 1994). Although wild-type Mago localizes within the posterior pole, Mago1 is not detected in the posterior pole of the oocyte. Thus, mago function is essential for axis formation and anchoring/localization of components within the posterior pole.

The apparent absence of Mago from the posterior pole during stages 6 and 7 may reflect the reorganization of the oocyte cytoskeleton that takes place at these stages (Theurkauf et al., 1992). During stages 2-6, most microtubules are concentrated at the posterior of the oocyte (Theurkauf et al., 1992). By stage 7, the microtubules have reorganized so that they are concentrated at the anterior pole and an anterior-posterior gradient of microtubules begins to form (Theurkauf et al., 1992). If Mago localization relies on the microtubule cytoskeleton [as the localization of osk mRNA and Staufen protein appears to (Clark et al., 1994)] then this reorganization may temporarily disrupt the posterior localization of Mago.

We have shown that homologues of Drosophila mago exist in C. elegans, X. laevis and M. musculus. The evidence provided here indicates that the least conserved of these homologues, ce-mago, can replace dm-mago function in axis formation and posterior pole plasm assembly. There is already substantial experimental and morphological evidence suggesting that the mechanisms of germ cell determination may be conserved among distantly related animal groups. In both Drosophila and Xenopus, ultraviolet irradiation disrupts germ cell formation and transplantation of non-irradiated germ plasm restores germ-plasm formation (Smith, 1966; Okada et al., 1974; Warn, 1975). Ultrastructural analysis has revealed the presence of electron-dense structures in the germ lines of organisms representative of many different phyla, including Nematoda, Annelida, Arthropoda and Chordata (Eddy, 1975). These structures, termed ‘nuage’ or germinal granules, resemble the polar granules observed in the Drosophila germ line. Similar to polar granules, nuage consist of a fibrous material, lack surrounding membranes and are often observed in association with mitochondria or the nuclear envelopes of germ cells. It has been proposed that these structures play similar roles in specifying the germ cell lineage of highly divergent organisms (Beams and Kessel, 1974; Eddy, 1975). Given the similarities between the germ-line granules observed in Drosophila, C. elegans and X. laevis, as well as their germ-plasm localization and segregation into the germ cells during early embryogenesis, there has been speculation that these species may use similar mechanisms to construct their germ plasms. By demonstrating that ce-mago can functionally replace the dm-mago gene, we provide strong evidence that this speculation may be correct.

Localization of RNAs and/or proteins is critical for the establishment of polarity and germ cell determination in both invertebrates and vertebrates (Gurdon, 1992; St. Johnston and Nüsslein-Volhard, 1992). Asymmetrically distributed proteins have also been shown to act as cell fate determinants during neurogenesis (Lin and Schagat, 1997). In addition, reorganization of cytoskeletal components has been demonstrated to be important for establishment of axial polarity and specification of cell fate during neurogenesis (Gurdon, 1992; Guo and Kemphues, 1996; Lin and Schagat, 1997). Hence, a functional analysis of mago genes in C. elegans, X. laevis and M. musculus will indicate whether Mago is required for cytoskeletal reorganizations and RNA localization necessary to establish polarity, determine the germ line or specify somatic cell fates during development of these organisms.

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