**maelstrom** is required for an early step in the establishment of *Drosophila* oocyte polarity: posterior localization of grk mRNA

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SUMMARY

We describe a mutant, **maelstrom**, that disrupts a previously unobserved step in mRNA localization within the early oocyte, distinct from nurse-cell-to-oocyte RNA transport. Mutations in **maelstrom** disturb the localization of mRNAs for Gurken (a ligand for the *Drosophila* Egf receptor), Oskar and Bicoid at the posterior of the developing (stage 3-6) oocyte. **maelstrom** mutants display phenotypes detected in **gurken** loss-of-function mutants: posterior follicle cells with anterior cell fates, **bicoid** mRNA localization at both poles of the stage 8 oocyte and ventralization of the eggshell. These data are consistent with the suggestion that early posterior localization of **gurken** mRNA is essential for activation of the Egf receptor pathway in posterior follicle cells. Posterior localization of mRNA in stage 3-6 oocytes could therefore be one of the earliest known steps in the establishment of oocyte polarity. The **maelstrom** gene encodes a novel protein that has a punctate distribution in the cytoplasm of the nurse cells and the oocyte until the protein disappears in stage 7 of oogenesis.

Key words: *Drosophila*, axis formation, RNA localization, Egfr signalling, asymmetry, **maelstrom**, **gurken**

INTRODUCTION

Anterior/posterior (A/P) and dorsal/ventral (D/V) polarity in the *Drosophila* oocyte is established through a series of inductive interactions between the oocyte and the adjacent monolayer of somatic follicle cells (reviewed in Anderson, 1995; Grünert and St. Johnston, 1996; Ray and Schüpbach, 1996; Rongo and Lehmann, 1996). The oocyte, which is located at the posterior of a cluster of germline-derived nurse cells, induces the adjacent follicle cells to adopt a posterior fate, rather than a default anterior fate (González-Reyes and St. Johnston, 1995; Roth et al., 1995; Larkin et al., 1996). This induction requires the activity of **gurken** (grk) in the germline and **torpedo** (top) in the follicle cells (González-Reyes et al., 1995; Roth et al., 1995), implicating the Egf receptor (Egrf) signalling pathway in the establishment of posterior cell fates. Grk is a transforming growth factor (TGF)-α-like molecule with an epidermal growth factor (EGF) domain (Neuman-Silberberg and Schüpbach, 1993), while Top is the homologue of the Egfr (Livneh et al., 1985; Price et al., 1989; Wadsworth et al., 1985). Egfr signalling activates genes in the posterior follicle cells, including **pointed** (Morimoto et al., 1996).

Following Egfr signalling, posterior follicle cells signal to the underlying oocyte to establish A/P polarity. This second signal requires the function of **Notch** (N) and **Delta** (Dl) in the follicle cells, and **mago nashi** and the catalytic subunit of protein kinase A in the germline (Ruohola et al., 1991; Larkin et al., 1996; Micklem et al., 1997; Newmark et al., 1997; Lane and Kalderon, 1994). Perturbation of this ill-defined somatic signal leads to the mislocalization of morphogenetic determinants along the A/P axis. **bicoid** (bcd) mRNA, which is normally localized to the anterior pole, becomes mislocalized to both ends of the oocyte and posterior components are mislocalized to the centre (Ruohola et al., 1991; González-Reyes and St. Johnston, 1994; Lane and Kalderon, 1994; González-Reyes et al., 1995; Roth et al., 1995; Larkin et al., 1996). Current models propose that the somatic signal acts to reorganize the oocyte cytoskeleton (Lane and Kalderon, 1994; Ruohola et al., 1994). When it is perturbed, microtubules grow from both poles of the oocyte, creating a bipolar array with the growing ends of microtubules in the centre of the oocyte.

Formation of the D/V axis requires additional inductive events between the germline and follicle cells (reviewed in Grünert and St. Johnston, 1996; Ray and Schüpbach, 1996; Rongo and Lehmann, 1996). Following A/P axis specification, grk mRNA accumulates between the nucleus and the cell membrane at the future dorsal-anterior margin of the oocyte (Neuman-Silberberg and Schüpbach, 1993). Spatially restricted Grk is believed to activate Top in the adjacent epithelium, thereby specifying dorsal follicle cell fate and restrict-
ing the activity of a ventrally localized signal that directs embryonic polarity. Thus, Egrf signalling from the germline to the soma determines both embryonic axes.

Roth et al. (1995) and González-Reyes et al. (1995) have shown that Grk plays a key role in establishing posterior follicle cell fate and therefore the A/P axis of the oocyte. Moreover, González-Reyes et al. (1995) have proposed that grk mRNA localization at the posterior of stage 1-6 oocytes plays an important role in this process. Here we describe the mutant phenotype and molecular characterization of a newly identified gene, maelsstrom (mael), whose function is required in the germline for correct posterior localization of a variety of transcripts in early (stage 3-6) oocytes, including grk mRNA. The majority of later mael mutant phenotypes can be explained by an early defect in Egrf signalling. We infer that the posterior localization of grk mRNA in the early oocyte is essential for proper A/P axis formation. Mutations in mael reveal a novel step in mRNA localization within the oocyte.

MATERIALS AND METHODS

Stocks

Drosophila melanogaster stocks were raised on standard cornmeal-yeast-agar medium at 25°C. Genetic markers and balancers are described in Lindsay and Zimm (1992), grk[DC29] and grk[HK36] are described in Schüpbach (1987) and Neuman-Silberberg and Schüpbach (1993); cap67 is described in Manseau and Schüpbach (1989). Enhancer trap insertion lines P[w + lacZ](SA7 and P[w + lacZ]BB127 are described in Roth et al. (1995). P[w + lacZ](B84 and P[w + lacZ]I144 are enhancer trap insertion lines generated in the laboratory of Y. N. and L. Jan (see Bier et al., 1989). The latter is recessive female sterile. Kinesis heavy chain: lacZ insertion lines KL503 and KL32 (Clark et al., 1994) were used for Kin;β-Gal fusion protein studies.

Hypomorphic alleles of mael were generated by reintroducing A2-3 transposase (Robertson et al., 1988) into a mael[11A4] background. Heterozygous mael[11A4]A2-3, Sb males were crossed to w/v, Ly/TM3 females and white-eyed males were selected to establish stocks. 77% (24/31) of the alleles fully complemented mael[11A4], indicating that the mutation was P-element induced. Two alleles with less severe phenotypes than mael[11A4](mael[15] and mael[20]) were selected for this study. In the assays described here, mael[15]mael[20] and mael[DF1](SL)79E females have the same phenotypes. Both alleles differ in the amount of P-element that remains inserted in the mael gene (data not shown). A strong mael allele, mael[351], was isolated in a similar screen. The ovaries of mael[351] hemizygotes have two independent defects: (1) germline cells are rapidly depleted after eclosion, and (2) egg chambers have A/P axis defects identical to those observed in mael[15] and mael[20] mutants. Both mael[351] phenotypes can be rescued using a mael transgene (see below).

DF1(SL)79E-F, hereafter referred to as DF1, was created by X-ray mutagenesis (4500 rads) of P[w + lacZ]E7-3.52 (Hartenstein and Jan, 1992). Deficiency breakpoints were determined after lacZ-orcein staining of polytene chromosomes from DF1/+ flies.

Mosaic analysis and genetic interactions

Germline clones were induced as described by Chou et al. (1993). First instar larvae (24-48 hours old) from the cross KL32/KL32; mael/TM3, Sb × yw; P[ovoD1]C360/TM3 were gamma-irradiated at a constant dose of 1000 rads (+ indicates either mael[15] or mael[20]). Females were crossed to mael/TM3, Sb males, and those with a mosaic germline were identified by their ability to lay eggs. Because P[ovoD1]C360 is centromere-distal to mael, genotypes were determined by progeny testing. Only the cross-over events that resulted in a homozygous mael germline were relevant to our analysis. A cross-over between ovoD1 and mael (which yields no information about the site of mael action) would result in a 1:1 ratio of sterile and fertile F1 progeny. Germline mosaic analysis was performed three times. Control crosses replacing mael with a wild-type chromosome gave similar proportions of mosaic females (2-3%).

Through a screen for enhancers of the mael mutant phenotype, we observed an interaction between Star, a component of the Egrf pathway, and a deficiency for mael (DF1). 6.5% (67/1022) of the eggs laid by DF1/+ females had fused appendages and 27.5% (327/1190) of the eggs laid by Star[67F]/+ females had fused appendages. In contrast, 62% (550/884) of the eggs laid by Star[67F]/+; DF1/+ trans-heterozygotes had fused appendages. Similar phenotypic enhancement was observed in flies transheterozygous for DF1 and DF2Last2, a deficiency that uncovers Star.

Staining procedures for light microscopy

Immunocytochemistry, in situ hybridization, DAPI-staining and X-Gal staining procedures were performed as described earlier (Ruohola et al., 1991; Larkin et al., 1996). The antibodies used in this study were anti-FasIII (1:15; Ruohola et al., 1991), anti-65F (1:4000; uncharacterized oocyte nuclear protein) and anti-Mael (1:50).

The cDNA fragments used for in situ hybridization were from: bcd (Driever et al., 1990), osk (Ephrussi et al., 1991), grk (Neuman-Silberberg and Schüpbach, 1993) and mael cDNA829 (this study). For in situ hybridization using early egg chambers, we report the strongest region of staining in weakly stained preparations. Longer development times obscure the entire oocyte.

Light microscopy was performed either on a Leitz DMRB with Nomarski differential interference contrast, or a Zeiss Axiohot with epifluorescence.

Staining procedures for confocal microscopy

For microtubule staining, ovaries were dissected in modified Robbs medium (Theurkauf et al., 1992), fixed 10 minutes in -20°C methanol, rinsed 3× in PBS, then extracted in PBS with 1% saponin for 1.5-2 hours. After three washes in PBS (PBS and 0.1% saponin), diluted mouse anti-α-tubulin (1:250; Sigma, clone DM1A) was added and the ovaries were incubated for 48 hours at 4°C. Ovaries were washed 3× 15 minutes in PBS and incubated with diluted Texas Red-conjugated goat anti-mouse secondary antibodies (3:500; Molecular Probes). After 3× 15 minutes in PBS, ovaries were dehydrated with methanol and mounted in Murray’s mounting medium (Theurkauf et al., 1992). Optical sections, 1 μm thick, were collected using an MRC 600 laser scanning confocal microscope (Bio-Rad Microsciences Division).

General molecular techniques

DNA cloning and library screening were performed according to Maniatis et al. (1989). A full-length mael cDNA was recovered from...
an ovarian cDNA library constructed in lambda-ZAP (B. Hays and Y. N. Jan, unpublished). The mael gene was subcloned from P1 clone DS04985 (79E4–79E6; Drosophila Genome Project). The mael cDNA and gene were subcloned in pBlueScript (Stratagene) and sequenced by the dideoxy chain termination method using single-strand primers (Sanger et al., 1977).

Total RNA was isolated using a hot-phenol method (Jowett, 1986), fractionated in formaldehyde gels (30 μg/lane) and transferred to nylon membrane (Amersham).

**Germline transformation**

The plasmid pXP was constructed by ligating a 4.5 kb XbaI/PsrI fragment into the w+ P-element vector pCaSpeR4 (Pirrotta, 1988). P-element-mediated germline transformation into v1118 flies was performed according to Spradling (1986).

Five independent P[XP] transgenic lines were recovered from 1000 injected eggs. Two lines were tested and found to rescue mael mutant phenotypes. The transgene insertion used in this study, XP3, is on the second chromosome; XP3 homozygotes are viable.

**Mael antibody**

A his-tagged mael fusion protein was prepared using the QIAexpressionist system (Qiagen) and injected into rabbits (R & R Rabbitry, Stanwood, WA). cDNA insert was amplified by PCR using primer CR2Bam (GGGGA TCCAACTCAGGCGGCAAGG; see Fig. 10, bp Stanwood, W A). cDNA insert was amplified by PCR using primer A his-tagged Mael antibody were homogenized in 2·DNA fragment was ligated into the pQE series of vectors. Transgenic mice injected eggs. Two lines were tested and found to rescue mael mutant phenotypes. The transgene insertion used in this study, XP3, is on the second chromosome; XP3 homozygotes are viable.

**RESULTS**

**mael mutant oocytes have altered A/P polarity**

The Drosophila ovary is composed of 15-20 ovarioles each of which contains multiple egg chambers composed of 15 nurse cells, an oocyte and a surrounding layer of follicle cells. The development of each egg chamber is divided into 14 morphologically distinct stages (reviewed in Spradling, 1993). By mid-oogenesis (stage 8), A/P polarity within the oocyte is readily detected by assaying a variety of known, asymmetrically localized components.

**maelstrom** was identified as a P-element-induced female-sterile mutant that affects A/P polarity of the oocyte. The effect of mael mutations on A/P axis formation was determined by analyzing the distribution of three asymmetrically localized molecules, bcd and osk mRNAs and a Kinesin-β-Galactosidase fusion protein, Kin:β-Gal, in mutant oocytes. In wild-type stage 8-9 egg chambers, bcd mRNA is localized to the anterior pole of the oocyte (Fig. 1A; Berleth et al., 1988; St. Johnston et al., 1989), while oskar mRNA and Kin:β-Gal accumulate at the posterior pole (Fig. 1C; Ephrussi et al., 1991; Kim-Ha et al., 1991; Clark et al., 1994). All of these markers are mislocalized in mael mutants. In stage 8, bcd mRNA is mislocalized to both poles in 80% (36/45) of mael oocytes (Fig. 1B). In addition, osk mRNA accumulates in the centre of mael oocytes (36/36; Fig. 1D), as does Kin:β-Gal (109/109; Fig. 1F). Thus, mael mutants disrupt the normal asymmetric distribution of markers along the A/P axis, creating a symmetric distribution identical to that previously described for a group of oocyte polarity mutants (reviewed in Anderson, 1995).

Mutations in mael also disrupt the microtubule-dependent migration of the oocyte nucleus from the posterior of the cell to the future dorsal anterior margin during stage 7 of oogenesis. The nucleus remained at the oocyte posterior in 17% (13/79) of stage 8 mael egg chambers (see Fig. 6G). Hence mael mutants disrupt the localization of the earliest morphological marker for the A/P axis in the oocyte.

**mael and Egfr pathway mutants have similar posterior follicle cell defects**

Defects in N, Egfr or Pka signalling all cause A/P axis defects similar to those observed in mael mutants (see Introduction), but differ in their specification of posterior follicle cell fates. Only mutations in genes of the N and Egfr pathways cause posterior follicle cell fate transformations (reviewed in Rongo and Lehmann, 1996). Mutants defective for N signalling have too many posterior polar cells, while mutants defective for Egfr signalling express anterior markers in posterior follicle cells. Mutations in mael do not alter the number of posterior polar cells (data not shown), suggesting that the N signalling pathway is not affected in these mutants. To test for alterations in posterior follicle cell fate comparable to those observed in mael mutants (see Introduction), we used three enhancer trap insertions that mark anterior follicle cells: BB127, 6B4 and 5A7. BB127 and 6B4 mark centripetal cells, while 5A7 identifies border cells (Fig. 2A.D.G). None of these markers are normally expressed in posterior follicle cells but, in mael mutants, BB127, 6B4 and 5A7 were expressed at the posterior in 16%, 18% and 87% of the stage 10 egg chambers, respectively (Fig. 2B.E.H; Table 1). Therefore, in mael mutants, posterior follicle cells adopt anterior fates. The patterns of marker expression are qualitatively similar to those in grkDC29/grk2B6 heterozygotes, which are defective for Egfr pathway signalling (Fig. 2C.F.I; Table 1; Roth et al., 1995).

Mutations in mael also alter follicle cell fates along the D/V axis, as inferred from eggshell phenotypes. Whereas wild-type eggs have two distinct anterodorsal respiratory appendages, 90% (337/374) of eggs from mael females have a single fused appendage of variable length at the dorsal midline (Fig. 3). The dorsal follicle cell prints on eggs with severely reduced 4663
appendages are rounded instead of elongate, suggesting that overlying follicle cells have adopted lateral or ventral fates. These D/V phenotypes are similar to those observed in weak grk mutants.

Taken together, the follicle cell fate transformations described above suggest that mael interferes directly or indirectly with Egfr signalling. Consistent with this interpretation, through a screen for enhancers of the mael mutant phenotype, we observed an interaction between Star, a component of the Egfr pathway, and a deficiency for mael (Df1) (Ž. B., N. J. C. and H. R.-B., unpublished data).

**mael acts in the germline**

To determine whether mael is required in the germline, the dominant female-sterile technique was used to create a homozygous mael germline surrounded by wild-type follicle cells (Chou et al., 1993). Seven females with a homozygous mael germline displayed the same egg shell and oocyte polarity defects as detected in mael 


The phenotypes of mael mutants are similar to those of mutants defective for Egfr signalling. Since mael acts in the germline, we asked whether mael mutants affect Grk, the germline component of the Egfr signalling pathway.

In stage 3-6 wild-type egg chambers, grk mRNA is localized and protein accumulates at the extreme posterior of the oocyte, between the nucleus and the plasma membrane (Figs 4A, 5A,C; Neuman-Silberberg and Schüpbach, 1993). Remarkably, in 86% (154/179) of stage 3-6 mael mutant egg chambers, grk

<table>
<thead>
<tr>
<th>Genotype</th>
<th>P/A transformation</th>
<th>Av. number transformed cells</th>
<th>Range</th>
</tr>
</thead>
</table>
| BB127/+; mael 


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**Fig. 2.** Follicle cell fate transformations in mael 


<table>
<thead>
<tr>
<th>Genotype</th>
<th>P/A transformation</th>
<th>Av. number transformed cells</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB127/+; mael</td>
<td>16% (15/97)</td>
<td>4</td>
<td>1-10</td>
</tr>
</tbody>
</table>
| BB127/+; grk 


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**Table 1.** Posterior follicle cells adopt anterior fates in mael and grk mutants
mRNA is mislocalized either at the anterior or along the lateral margins of the oocyte (see Fig. 4B). Therefore, mael is required for early mRNA localization within the oocyte. The mislocalization of grk mRNA in mael mutants correlates with greatly reduced levels of Grk protein (an 8-fold reduction; Fig. 5B,D). Since posterior follicle cell fate and oocyte A/P axis specification requires Grk, A/P axis defects in mael could be explained by the loss of grk function.

In later stages of wild-type oogenesis, grk mRNA and protein both form a perinuclear cap facing the overlying follicle cells (Fig. 5E; Neuman-Silberberg and Schüpbach, 1993; Roth et al., 1995). In stage 8-9 mael mutant oocytes, grk RNA is found along the anterior margin of the oocyte and the level of Grk staining is reduced (Fig. 5F). This reduction may account for mael eggshell defects, since dorsal follicle cells are probably specified in these stages (reviewed in Ray and Schüpbach, 1996). Later, in stage 10 mael mutant oocytes, some grk mRNA and protein are detected adjacent to the nucleus, suggesting that, even though mael affects earlier stages, mael does not drastically affect Grk at this stage.

mael mutants mislocalize several mRNAs in early oocytes

To determine whether mael mutations disrupt mRNAs other than grk, we extended our analysis to osk and bcd transcripts. In young wild-type egg chambers, these mRNAs are transported into the oocyte where they accumulate at the extreme posterior of the cell (see Micklem, 1995; Pokrywka and Stephenson, 1995). osk mRNA accumulates in oocytes throughout stages 1-6, while bcd mRNA accumulates in stage 5 (Fig. 4C,E; Ephrussi et al., 1991; Kim-Ha et al., 1991; Berleth et al., 1988; St. Johnston et al., 1989). In stage 3-6 mael mutant oocytes, osk and bcd mRNAs are mislocalized 89% (106/119) and 29% (10/38) of the time, respectively (Fig. 4D,F). These data demonstrate that mael is required for an mRNA localization process, early in oogenesis, that does not exclusively affect grk mRNA.

Although mael mutants affect the distribution of at least three mRNA species that are normally sequestered at the oocyte posterior (i.e. grk, osk, bcd), they do not alter the uniform distribution of transcripts that normally disperse throughout the egg chamber (data not shown). Nor do they disrupt general translation or intracellular targeting of proteins. In wild-type stage 1-6 oocytes, the 65F mRNA (encoding a

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**Table 2. mael is required in the germline**

<table>
<thead>
<tr>
<th>Allele</th>
<th>Females screened</th>
<th>Mosaics*</th>
<th>mael/+ mosaics†</th>
<th>Eggshell defect</th>
<th>A/P Axis defect‡</th>
<th>mael/mael mosaics†</th>
<th>Eggshell defect</th>
<th>A/P Axis defect†‡</th>
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<tbody>
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<tr>
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<td>29</td>
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<td>NO</td>
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<tr>
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<td>24§</td>
<td>12</td>
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<td>NO</td>
<td>3</td>
<td>YES</td>
<td>YES</td>
</tr>
</tbody>
</table>

*Females that laid eggs were mosaic and had some germline stem cells that were either mael+ or mael/mael. Two genotypes were possible because P🚀[Povo D1]1X48 is centromere-distal to mael.
†Assayed by testing the fertility of the offspring. The potential germline mosaics were mated with mael/TM3 males.
‡Assayed by the position of Kin:B-Gal in the oocyte of stage 9 egg chambers
§Nine mosaics died before progeny testing.
protein of unknown function) is translated and the protein is transported into the nucleus. In mael mutant oocytes, the protein is still translated and accumulates normally in the nucleus (data not shown). Taken together, these data suggest that the primary defect in stage 3-6 mael mutant egg chambers is in subcellular mRNA localization within the oocyte. Messenger RNA transport from the nurse cells to the oocyte is unaffected even in strong mael mutants such as maelM391. Hemizygous maelM391/Df1 females disrupt RNA localization at stage 3-6 egg chambers only within the oocyte.

mael affects the distribution and dynamics of oocyte microtubules

The mislocalization of RNAs other than grk in early oocytes suggested that the mael mutants might have additional late phenotypes unrelated to the Egfr pathway. A bias towards Egfr-like defects might simply reflect the sensitivity and importance of the Egfr pathway during early oogenesis. Since all oocyte polarity mutants investigated to date have a defective microtubule cytoskeleton (Lane and Kalderon, 1994; Micklem et al., 1997; Newmark et al., 1997), we compared the microtubule cytoskeleton of stage 7-9 mael and grk egg chambers to look for subtle phenotypic differences.

In stage 1-6 egg chambers, microtubules extend from a microtubule organizing centre (MTOC) at the posterior of the oocyte, through the ring canals and into the nurse cells (Theurkauf et al., 1992; Fig. 6A). During stages 7 and early 8, the posterior MTOC degenerates and microtubules re-organize at the anterior of the oocyte, creating a broad anterior-to-posterior concentration gradient of anti-tubulin staining throughout stages 8-10a (Fig. 6B,C; Theurkauf et al., 1992). At stage 10b, the microtubule cytoskeleton re-arranges again into an elaborate pattern of subcortical microtubule bundles that mediate endoplasmic streaming (Fig. 6D; Theurkauf et al., 1992).

As expected, grk and mael mutants have a defective microtubule cytoskeleton similar to that previously described for the oocyte polarity mutants PKA and mago nashi; however, the grk and mael cytoskeletons are not identical. Both mutants have a
high concentration of microtubules at the posterior of the oocyte in stages 8 and 9 (Fig. 6F-H,J) when microtubules are normally concentrated at the oocyte anterior (Fig. 6C). But, in stage 7, mael microtubules are tightly bundled around the cortex (Fig. 6E), while grk mutants have a more diffuse network (Fig. 6I). The latter configuration is associated with rapid microtubule-dependent cytoplasmic streaming, a phenomenon that is probably required to evenly disperse unanchored components throughout the oocyte. We therefore asked whether mael mutant ooplasm prematurely streams.

Initially we used time-lapse videomicroscopy to detect abnormal, premature cytoplasmic motion in stage 8-9 oocytes. In wild-type oocytes, only minor back-and-forth motion is detected during these stages (Gutzeit and Koppa, 1982) but, in 87% of mael mutant oocytes, rapid cytoplasmic streaming was observed throughout the oocyte. The ‘swirling’ phenomenon was further documented using time-lapse laser scanning confocal microscopy (Fig. 7). In mael oocytes, cytoplasmic streaming is evident when yolk granules first accumulate in early stage 8 (Fig. 7B). Earlier stages could not be assayed because there are no autofluorescent particles in the oocyte.

Next we compared the microtubule cytoskeleton of mael mutants to another mutation that causes premature cytoplasmic streaming: capu G7 (Theurkauf, 1994b; Emmons et al., 1995). In stages 8-10a, both capu G7/capu G7 and mael mutant oocytes have microtubules all around the periphery of the oocyte (Fig. 6K,F-H); however, the capu mutant lacks bundles of microtubules that penetrate the centre of mael mutant oocytes (Fig. 6H). Thus mael and capu microtubule cytoskeletons are somewhat different, although they both cause premature swirling.

Defective follicle-cell-to-oocyte signalling, which in turn causes a defective microtubule organization, could lead to abnormal cytoplasmic motion within the oocyte (Larkin et al., 1996). Alternatively, mael mutants may mislocalize mRNAs in the early oocyte that are required later in development for normal regulation of the oocyte cytoskeleton.

**Cloning mael**

Fragments of genomic DNA adjacent to the P[w + lacZ]11A4 (mael 11A4) enhancer trap insertion site were cloned and hybridized to wild-type egg chambers. A single 1800 nt transcript is present in wild-type (wt) but absent in the mael mutant. An actin genomic clone was used as a control for RNA loading. (C) Western blot of a bacterially expressed Mael protein, Mael in wild-type (wt) and mael r20 /Df1 egg chambers. No Mael protein is evident in the mael mutant; equivalent amounts of total protein were loaded.
Therefore, the transcript we have described is the described in mixing were rescued 50% (95/100) and 38% (10/26) of the females, 33% (468/1435) of the eggs hatched; with two copies of mael mutants can be rescued by a transgene. Germline transformation with a 4.5 kb genomic fragment resulted in two transformant lines with independently derived transgenes (XP3 and XP5) segregating on the second chromosome. Both transgenes are able to rescue the mael mutant phenotypes. No hatching is detected in hemizygous mael mutants, but when one copy of the rescue construct XP3 was introduced into mael1/Df1 females, 33% (468/1435) of the eggs hatched; with two copies of XP3, 86% (70/80) hatched. A single copy of XP3 fully rescued the fused dorsal appendage and cell fate defects, whereas Kin-β-Gal mislocalization and premature cytoplasmic mixing were rescued 50% (95/190) and 38% (10/26) of the time, respectively. In conclusion, all of the phenotypes described in mael mutants can be rescued by a transgene. Therefore, the transcript we have described is the mael gene.

Mael protein distribution in egg chambers

Immunohistochemical staining was used to determine the distribution of Mael in wild-type and mael mutant ovaries. In wild-type germaria and egg chambers, punctate anti-Mael staining is detected in the germline. In the germarium, Mael is present in all regions, including region I where the germ cells are dividing (Fig. 10A). In early egg chambers, Mael is uniformly distributed throughout the nurse cells and oocyte (Fig. 10B) but, by stage 5, it is most concentrated around the outer margins of the cells, closest to the periphery of the egg chamber (Fig. 10C,D). Mael staining decreases in stages 5 and 6, but most noticeably in the oocyte, where little Mael remains (Fig. 10D). There is no detectable Mael protein from stage 8 onward. In mael egg chambers, no Mael staining is detected.

DISCUSSION

The mael gene plays an essential role in establishing polarity in the developing egg chamber. Mutations in mael disturb the normal localization of osk, bcd and grk mRNAs to the posterior pole of the oocyte in stage 3-6 egg chambers (Fig. 11B). grk mRNA mislocalization correlates with reduced levels of Grk protein. Later (stage 7-10) maelstrom mutant phenotypes, which include oocytes with two anterior poles and posterior follicle cells with anterior fates, are probably a consequence of the early lack of grk function. Consistent with an early role in mRNA localization, Mael protein is detected throughout the
mael affects early oocyte polarity

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mael affects early oocyte polarity prior to stage 6, but disappears from the oocyte in later stages. The ability of mael mutations to disturb the earliest subcellular distribution of mRNAs in the oocyte without affecting long-range transport of RNAs from the nurse cells is thus far unique.

Is the posterior localization of grk mRNA essential for the establishment of A/P polarity in the oocyte?

St. Johnston and colleagues have suggested that the posterior localization of grk mRNA directs Grk signalling towards the adjacent terminal follicle cells to establish posterior follicle cell fates and therefore the oocyte A/P axis (González-Reyes et al., 1995). Interestingly, in mael mutants, grk mRNA is not localized to the posterior of the cell, the levels of Grk protein are reduced and terminal follicle cells fail to adopt posterior fates (summarized in Fig. 11). These data suggest that posterior grk RNA localization in the oocyte is required for Grk translation or stability. Translational regulation at the level of mRNA localization has been demonstrated for both oskar and nanos (Curtis et al., 1995; Kim-Ha et al., 1995; Gavis and Lehmann, 1994). Although our data indicate a correlation between posterior localization of grk mRNA and Grk protein translation or stability, we cannot rule out the possibility that mael mutants independently affect both of these processes during stages 3-6 of oogenesis. A direct test of the requirement for grk mRNA localization in Egfr signalling would be to specifically mislocalize grk mRNA in stage 3-6 oocytes. To reach this goal, further studies of grk mRNA are necessary to delineate the elements within the transcript that mediate posterior localization in stages 3-6.

Intracellular and subcellular mRNA transport in the germline

Messenger RNA transport has been studied from two different perspectives, transport from nurse cells to the oocyte and localization within the oocyte. Kim-Ha et al. (1993) proposed that these two events are functionally separable. While the majority of studies focus on mRNA localization in stages 7-9, some mutants affect mRNA localization in earlier stages.

Mutants have been identified that affect mRNA localization during stages 1-6, when an MTOC is located at the posterior of the oocyte. These mutants disturb transport of mRNA from the nurse cells to the oocyte (Ephrussi et al., 1991, Suter and Steward, 1991; Kim-Ha et al., 1993; Ran et al., 1994; Hawkins et al., 1996). Mutations in Bicaudal-D, egalitarian and encore (which appear to be required for oocyte determination), as well as 3’ UTR mutations in the osk gene, all cause osk mRNA to disperse throughout the early egg chamber (Ephrussi et al., 1991; Suter and Steward, 1991; Kim-Ha et al., 1993; Ran et al., 1994; Hawkins et al., 1996). Most of these mutations probably interfere directly or indirectly with the establishment and/or maintenance of the microtubule network that is used for mRNA transport from nurse cells to the oocyte (see Theurkauf, 1994a). mael is unique in this group, in that it only affects RNA localization within the oocyte, not nurse-cell-to-oocyte transport (Fig. 11B). Even a strong allele (mael$^{M391}$, unpublished) accumulates mRNA in stage 1-6 oocytes.

In later developmental stages (stages 7-9), when the MTOC...
is located at the anterior of the oocyte, few mutants affect mRNA transport from the nurse cells to the oocyte (Gillespie and Berg, 1995) but several affect mRNA localization within the oocyte (Micklem, 1995).

**mael is required for early posterior mRNA localization**

*mael* is required for subcellular mRNA localization within the stage 3-6 oocyte. This is the earliest stage that oocyte-specific mRNA mislocalization has been observed. Mutations in *Bicaudal-D*, *egalitarian* and *encore* may also affect posterior mRNA localization within stage 3-6 oocytes, but their earlier phenotypes make this process hard to study. This distinction makes *mael* mutants particularly useful tools for analyzing how mRNA is localized in the early oocyte.

What is the normal function of the *mael* gene? Two testable possibilities are (1) that *mael* is required for the proper placement and anchoring of the MTOC within the early (stage 3-6) oocyte or (2) that *mael* acts in a short-range transport mechanism required to bring mRNAs to the posterior pole. Punctate cytoplasmic distribution of the Mael protein at stages 1-6 can be reconciled with either model. Studies to distinguish between these possibilities are in progress.

In conclusion, *mael* mutants reveal and will facilitate further study of a mechanism for subcellular localization of mRNA within the oocyte prior to stage 6 in oogenesis. This mechanism is essential for the early establishment of polarity.

We thank Drs E. Ginger, P. Webster and D. Baker, and the anonymous reviewers for helpful comments on the manuscript. We also thank the Jan laboratory, especially Dr B. Hay for helping to establish the P[w* lacZ]mael11A4 stock, Dr S. Younger-Shiperd who recombined a second P-element off the *mael*11A4 chromosome, and Dr E. Grell who determined the Df1 breakpoints. Dr T. Schüpbach kindly provided Grk antibody and a grk cDNA. We thank Dr C. VanBuskirk for advice on staining with anti-Grk. This work was supported by a fellowship from the Cytotherapeutics Corporation. L. S. was supported by an NSF Pre-doctoral Fellowship.

**REFERENCES**


(Accepted 2 September 1997)