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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, 1994). 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 (Egfr) 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, Egfr 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, *maelstrom* (*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 Egfr 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 Lindsley and Zimm (1992). grk^{2B6}, grk^{DC29} and grk^{HK36} are described in Schüpbach (1987) and Neuman-Silberberg and Schüpbach (1993); $capu^{G7}$ is described in Manseau and Schüpbach (1989). Enhancer trap insertion lines *P[w⁺ lacZ]5A7* and *P[w⁺ lacZ]BB127* are described in Roth et al. (1995). *P[w⁺ lacZ]6B4* and *P[w⁺ lacZ]11A4* 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. *Kinesin 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 Δ2-3 transposase (Robertson et al., 1988) into a $mael^{11A4}$ background. Heterozygous $mael^{11A4}$/Δ*2-3, Sb* males were crossed to *w/w, 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 $mael^{11A4}$ ($mael^{r15}$ and $mael^{r20}$) were selected for this study. In the assays described here, $mael^{r15}$/$mael^{r20}$ and *mael/Df(3L)79E-F* females have the same phenotypes. The two alleles differ in the amount of P-element that remains inserted in the *mael* gene (data not shown). A strong *mael* allele, $mael^{M391}$, was isolated in a similar screen. The ovaries of $mael^{M391}$ hemizygotes have two independent defects: (1) germline cells are rapidly depleted after eclosion, and (2) egg chambers have A/P and D/V axis defects identical to those observed in $mael^{r15}$ and $mael^{r20}$ mutants. Both $mael^{M391}$ phenotypes can be rescued using a *mael* transgene (see below).

Df(3L)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 lacto-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; $mael^{x}$/TM3, Sb* × *yw; P[ovo^{D1}]^{2X48}/TM3* were gamma-irradiated at a constant dose of 1000 rads (^{x} indicates either $mael^{r15}$ or $mael^{r20}$). Females were crossed to $mael^{x}$*/TM3, Sb* males, and those with a mosaic germline were identified by their ability to lay eggs. Because *P[ovo^{D1}]^{2X48}* 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 *mael* and the centromere produces a homozygous germline, but could result in two phenotypic outcomes. If *mael* were required in the germline, mothers would be sterile, Kin:β-Gal would be mislocalized in stage 9 oocytes and eggs would have fused appendages. Alternatively, if *mael* were not required in the germline, all F_1 progeny would be fertile. A cross-over between ovo^{D1} and *mael* (which yields no information about the site of *mael* action) would result in a 1:1 ratio of sterile and fertile F_1 progeny. Germline mosaic analysis was performed three times. Control crosses replacing $mael^{x}$ 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 *Egfr* 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 $Star^{S5671}$/+ females had fused appendages. In contrast, 62% (550/884) of the eggs laid by $Star^{5671}$*/+; Df1/+ trans*-heterozygotes had fused appendages. Similar phenotypic enhancement was observed in flies *trans*heterozygous for *Df1* and *Df(2L)ast2*, 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* cDNA#29 (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 interferance contrast, or a Zeiss Axiophot 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 PBSS (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 PBSS 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). Samples were taken from four separate experiments, representing a total of 40-50 stage 8 and 15-20 stage 6 egg chambers.

For staining with rat anti-Grk antibodies (Roth et al., 1995), ovaries were fixed while shaking vigorously in 4% paraformaldehyde-heptane including 0.5% NP40 for 20 minutes, rinsed in NP40 wash (50 mM Tris [pH 7.5], 150 mM NaCl, 0.5% NP40, 1 mg/ml BSA, 0.02% azide), and incubated in NP40 + 2% normal goat serum + 0.1% BSA for 2 hours. Grk antibody (1:3000; preabsorbed with fixed grk^{HK36}/grk^{HK36} ovaries) was added and the ovaries were incubated overnight at 4°C. After 4× 20 minutes in NP40 wash, samples were incubated with Texas Red-conjugated anti-rat antibody overnight. Samples were washed 4× 20 minutes in NP40 wash, then mounted in glycerol. Staining was quantitated using NIH Image software.

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 D504985 (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 *Xba*I/*Pst*I fragment into the w^+ P-element vector pCaSpeR4 (Pirrotta, 1988). P-element-mediated germline transformation into w^{1118} 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 (GGGGATCCAACTCAGGCGGCAAGG; see Fig. 10, bp 419-435) and a primer in the pBluescript (Stratagene) polylinker. After digestion with *Bam*HI and *Kpn*I restriction endonucleases, the DNA fragment was ligated into the pQE series of vectors. Transformed bacteria were screened for in-frame expression of the protein.

Wild-type and mutant ovaries of equivalent developmental stage were homogenized in 2× SDS loading buffer (2% β-mercaptoethanol, 5% SDS, 20% glycerol, 130 mM Tris pH 6.8, 0.25% bromophenol blue) supplemented with protease inhibitors (5 μg/ml leupeptin, 5 μg/ml pepstatin, 0.5 mM PMSF, 1 mM DTT). Samples were heated at 95°C for 5 minutes, frozen, thawed and spun at 13000 ***g*** in a microfuge for 2 minutes. Supernatant was loaded on an 8% SDS-polyacrylamide gel. Equivalent protein loading was verified using Ponceau S red staining. Densitometry (IP Lab Software) of multiple randomly chosen proteins revealed a ratio of 1.06 for staining in wild-type versus *maelstrom* lanes. After electrophoresis, protein was transferred to 0.45 μm nitrocellulose (BioRad) and blocked with TBST (25 mM Tris pH 7.5, 150 mM NaCl, 0.05% Tween 20) supplemented with 5% nonfat powdered milk. Blots were incubated with affinity-purified polyclonal anti-Mael antibodies (1:2000; see below) overnight at 4°C, washed in block and incubated with alkaline phosphatase-conjugated goat anti-rabbit secondary (1:1000) (Southern Biotech Associates). Bands were visualized using a colorimetric reaction (NBT/BCIP; Boehringer Mannheim). Maelstrom protein was undetectable in mutant ovaries. Based on quantitation, the detection limit of the western blot is one twelfth the intensity of the wild-type Maelstrom band. We conclude that Maelstrom is at least 12-fold lower in the mutant than in wild type.

Polyclonal *mael* antisera were purified against blotted, recombinant Mael. Affinity-purified antibodies were eluted from western blots with strip buffer (0.2 M glycine [pH 2.3], 0.5% Tween) and then neutralized with 1 M Tris (pH 10.5).

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,E; 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 mutants defective for Egfr signalling, 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 grk^{DC29}/grk^{2B6} 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

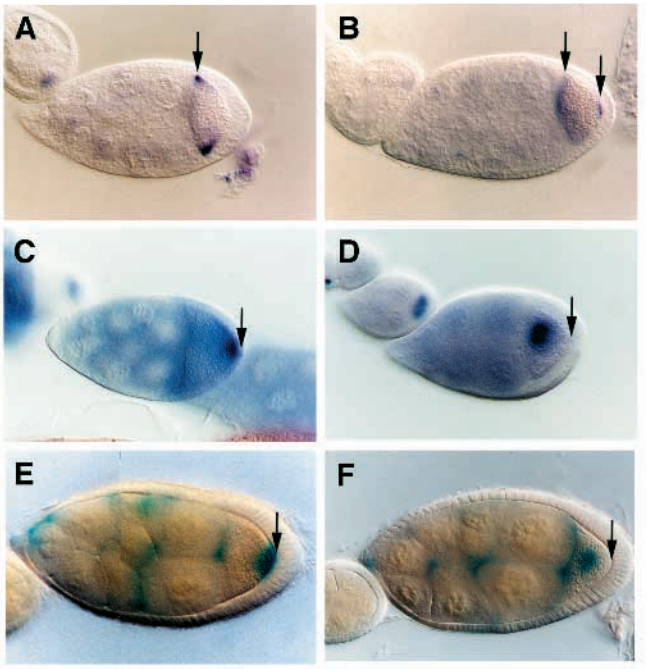

Fig. 1. *mael* mutants have two anterior organizing centres. (A,B) *bcd* cDNA was hybridized to wild type and *mael*^{r20}*/Df1* stage 8 egg chambers. (C,D) *osk* cDNA and (E,F) Kin:β-Gal activity were assayed in wild type and *mael*^{r15}*/mael*^{r20} egg chambers. (A) In wild-type oocytes, *bcd* mRNA is localized to the anterior of the oocyte (arrow). (B) In *mael* mutants, *bcd* mRNA has a bipolar distribution (36/45) (arrows). The posterior *bcd* RNA signal disappears in stage 9. (C) *osk* mRNA is located at the posterior of the oocyte in wild-type egg chambers (arrow) but (D) in the center of mutant oocytes (36/36) (posterior marked with an arrow). (E) Kin:β-Gal has a posterior distribution in wild-type oocytes (arrow) and (F) a central location in *mael* mutants (109/109) (posterior marked with an arrow).

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*) (Z. 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*^{r20}*/Df1* females (Table 2). Therefore, *mael* gene function is required in the germline for correct specification of the A/P axis.

mael mutants mislocalize *grk* mRNA and reduce the level of Grk protein within the oocyte

The phenotypes of *mael* mutants are similar to those of mutants

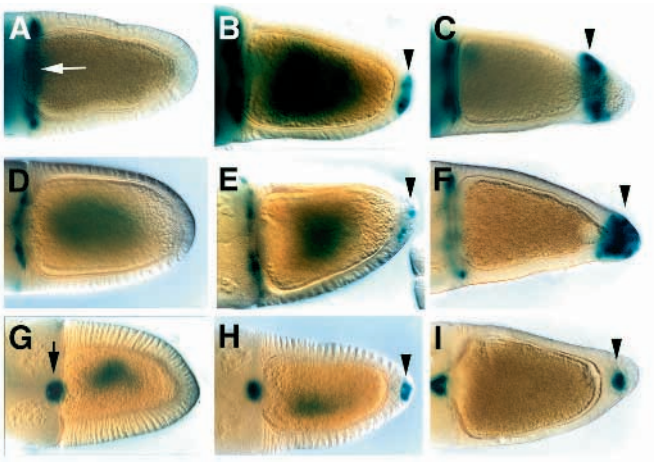

Fig. 2. Follicle cell fate transformations in *mael*^{r20}*/Df1* and *grk*^{2B6}*/grk*^{DC29} stage 10 egg chambers are similar. (A) β-Gal expression from the *BB127* enhancer trap line in control egg chambers. Staining is evident in nurse cells and centripetal cells at the anterior margin of the oocyte (arrow). (B) *BB127* is expressed at the posterior in 16% of *mael* egg chambers. Both the anterior centripetal cells and a few (1-10; Table 1) follicle cells at the oocyte posterior are stained (arrowhead). (C) *BB127* expression in *grk* egg chambers. In a strong *grk* mutant, many (10-36; Table 1) posterior cells stain in addition to the anterior population. In this specimen, the stained cells appear to have been migrating, pinching off part of the oocyte (arrowhead). (D) β-Gal expression from the *6B4* enhancer trap line labels centripetal cells in control egg chambers. (E) *6B4* expression in 20% of *mael* egg chambers. A small population (1-7; Table 1) of unclustered cells exists in the oocyte posterior (arrowhead). (F) *6B4* expression in 100% of *grk* egg chambers. Numerous (31-75; Table 1) posterior cells are stained (arrowhead). The nucleus is ectopically positioned at the oocyte posterior. (G) A control egg chamber demonstrating β-Gal expression in the *5A7* border cell enhancer trap line (arrow). (H) *5A7* expression in 87% of *mael* egg chambers (arrowhead). The same number of cells stain at each pole. (I) *5A7* expression in 96% of *grk* egg chambers (arrowhead). For the *5A7* marker, the penetrance of the phenotype and the number of posterior-to-anterior transformed cells is similar in *grk* and *mael* egg chambers (3-8;1-11, respectively; Table 1).

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 1. Posterior follicle cells adopt anterior fates in mael and grk mutants

Genotype	P/A transformation	Av. number transformed cells	Range
BB127/+; mael^{r20}*/Df1*	16% (15/97)	4	1-10
BB127/+; grk^{2B6}*/grk*^{DC29}	100% (27/27)	16	10-36
mael^{r20}*/6B4 Df1*	20% (20/98)	2	1-7
grk^{2B6}*/grk*^{DC29}*; 6B4/+*	100% (27/27)	53	31-75
mael^{r20}*/5A7 Df1*	87% (146/169)	4	1-11
grk^{2B6}*/grk*^{DC29}*; 5A7 Df1/+*	96% (42/44)	5	3-8

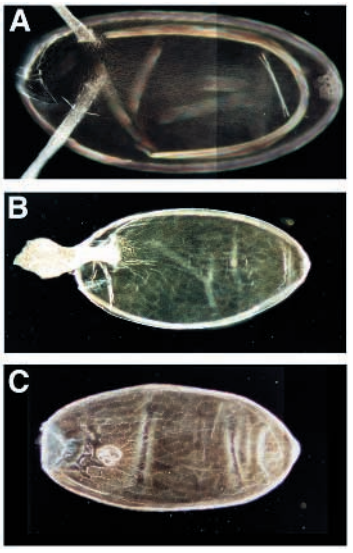

Fig. 3. Eggshell phenotypes of $mael^{r20}/Df1$. (A) A wild-type egg with two dorsal appendages separated by a space along the dorsal midline. The picture was cropped, reducing the length of the appendages. (B,C) The dorsal appendages of $mael^{r20}/Df1$ mutants are fused or lost (337/374), and the follicle cell imprints on the dorsal side of the egg are more rounded than in wild-type (compare A and C), suggesting that the overlying follicle cells have changed fate. Mutant eggs are slightly smaller than wild-type eggs.

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.

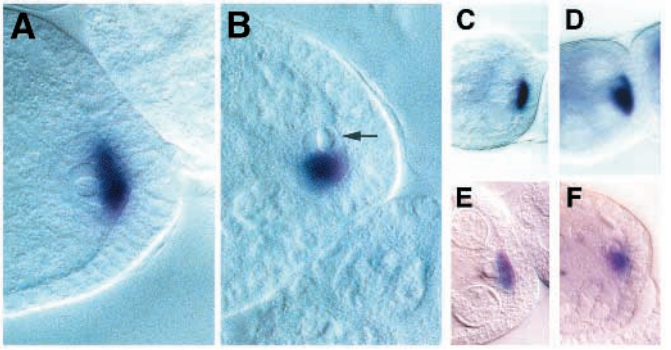

Fig. 4. Mislocalization of mRNAs in $mael^{r20}/Df1$ stage 3-6 oocytes. (A) *grk* mRNA in a wild-type stage 5 egg chamber is tightly localized at the posterior of the oocyte, between the nucleus and the plasma membrane, but is found mislocalized along the lateral or anterior margin in *mael* oocytes (posterior marked with an arrow) (B). This mislocalization is detected in 86% (154/179) of stage 3-6 mutant oocytes. (C) *osk* mRNA is tightly localized at the posterior of a stage 4 wild-type oocyte, but is mislocalized in a *mael* mutant oocyte (D). *osk* mRNA is mislocalized in 89% (106/119) of stage 3-6 *mael* mutant oocytes. (E) In stage 5, *bcd* mRNA accumulates at the posterior of wild-type oocytes but is mislocalized in 29% (10/38) of stage 5 *mael* oocytes (F).

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 RNA 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

Table 2. *mael* is required in the germline

Allele	Females screened	Mosaics*	*mael*/+ mosaics†	Eggshell defect	A/P Axis defect‡	*mael*/*mael* mosaics†	Eggshell defect	A/P Axis defect‡
$mael^{r15}$	1160	15	14	NO	NO	1	YES	YES
$mael^{r20}$	3490	32	29	NO	NO	3	YES	YES
$mael^{r20}$	1030	24§	12	NO	NO	3	YES	YES

*Females that laid eggs were mosaic and had some germline stem cells that were either *mael*/+ or *mael*/*mael*. Two genotypes were possible because $P[ovo^{D1}]^{2X48}$ 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:β-Gal in the oocyte of stage 9 egg chambers

§Nine mosaics died before progeny testing.

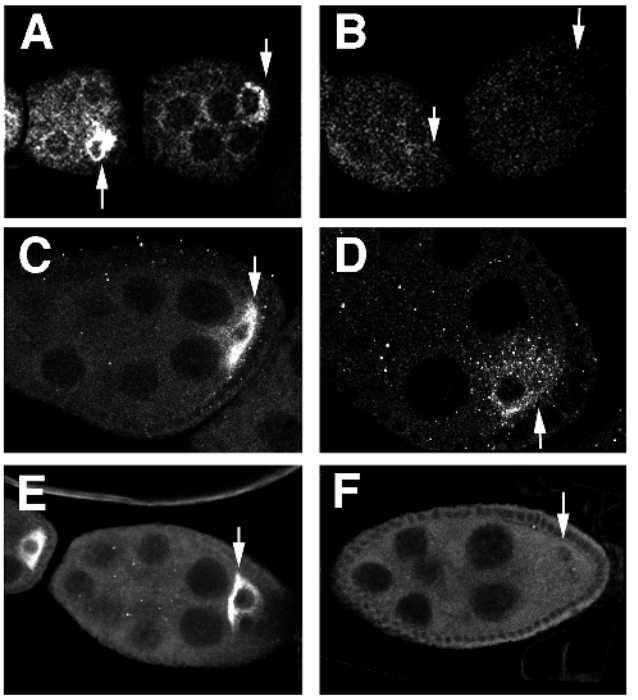

Fig. 5. Grk protein level is reduced in *mael*^{r20}*/Df1* egg chambers. Confocal images of wild-type (A,C,E) or *mael*^{r20}*/Df1* (B,D,F) egg chambers reacted with a polyclonal Grk antibody. Compared to wild-type, Grk staining is reduced in 79% (79/100) of stage 3-6 *mael* mutant oocytes. (A) In stage 3-4 wild-type egg chambers, Grk protein concentrates in the oocyte (arrows). (B) In contrast, very little Grk protein is detected in stage 3-4 *mael* mutant oocytes (arrows; an 8-fold reduction relative to wild-type). (C) In stage 6 wild-type egg chambers, Grk accumulation is detected at the posterior of the oocyte between the nucleus and the plasma membrane (arrow). (D) In stage 6 *mael* oocytes, Grk staining is highly reduced and uniform (arrow). (E) Grk is localized adjacent to the nucleus at the anterior of the early stage 8 wild-type oocyte (arrow). In the upper left-hand corner of the picture is a stage 6-7 oocyte. (F) Grk staining is severely reduced in the late stage 8 *mael* oocyte (arrow). The following paired images have the same magnification: A and B, C and D, E and F.

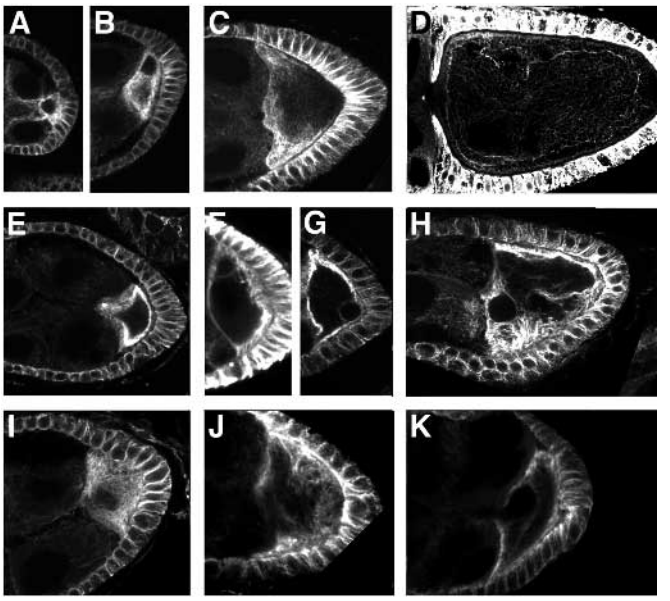

Fig. 6. Microtubule distribution in wild-type and mutant oocytes. (A-D) Wild-type oocytes at stages 5, 7, 8 and 10b. Note the intensely stained posterior region of the stage 5 oocyte (A), a transitional stage with uniform staining at stage 7 (B), and an intensely stained anterior region at stage 8 (C). At stage 10b (D; 0.5× magnification) another transition results in subcortical localization of elongated bundles of microtubules. This morphology facilitates endoplasmic streaming and the distribution of unanchored macromolecules throughout the oocyte. (E-H) Microtubules in *mael*^{r15}*/mael*^{r20} oocytes. (E) In stage 7, intensely stained bundles are evident at the posterior and anterior margins of the oocyte. At stages 8 (F,G) through 10b, these bundled microtubules remain, generally, in a subcortical location, although some course through the oocyte interior (H). (I,J) In *grk*^{2B6}*/grk*^{DC29} amorphs, microtubules stain uniformly throughout the oocyte in late stage 7 (I). Intensely stained bundles are evident in stage 8 and 9 oocytes (J). (K) Microtubule staining in a stage 8 *capu*^{G7}*/capu*^{G7} oocyte. Like *mael* mutants (F,G), premature bundling of microtubules is apparent. Except for F, each image is a single optical section of approximately 1 μm; F has a greater depth of field.

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 *mael*^{M391}. Hemizygous *mael*^{M391}*/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

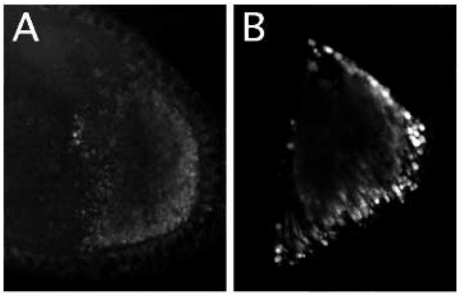

Fig. 7. Endoplasmic streaming occurs prematurely in *mael* oocytes. Confocal images of autofluorescent yolk granules show no granule movement in wild-type, stage 8 oocytes (A). (B) In $mael^{r15}/mael^{r20}$ mutant stage 8 oocytes, vigourous unidirectional movement is detected (27/31). Note that image A's intensity has been digitally enhanced; the yolk autofluorescence is much greater in *mael* mutants than in wild-type at this early stage.

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). This bundling is similar to the continuous subcortical array of microtubules in wild-type stage 10b oocytes (Fig. 6D). 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. An egg chamber-specific pattern was found associated with sequences on one side of the P element (data not shown). Northern analysis using the same fragments and a cloned cDNA identified a 1.8 kb mRNA that is present in wild-type flies and $mael^{11A4}$ revertants but is absent in *mael* mutants (Fig. 8). A comparison of cloned genomic and cDNA sequences positioned the mutagenic transposon in the 5′ untranslated region of *mael*. Conceptual translation of the cDNA creates a protein of approximately 50 kDa

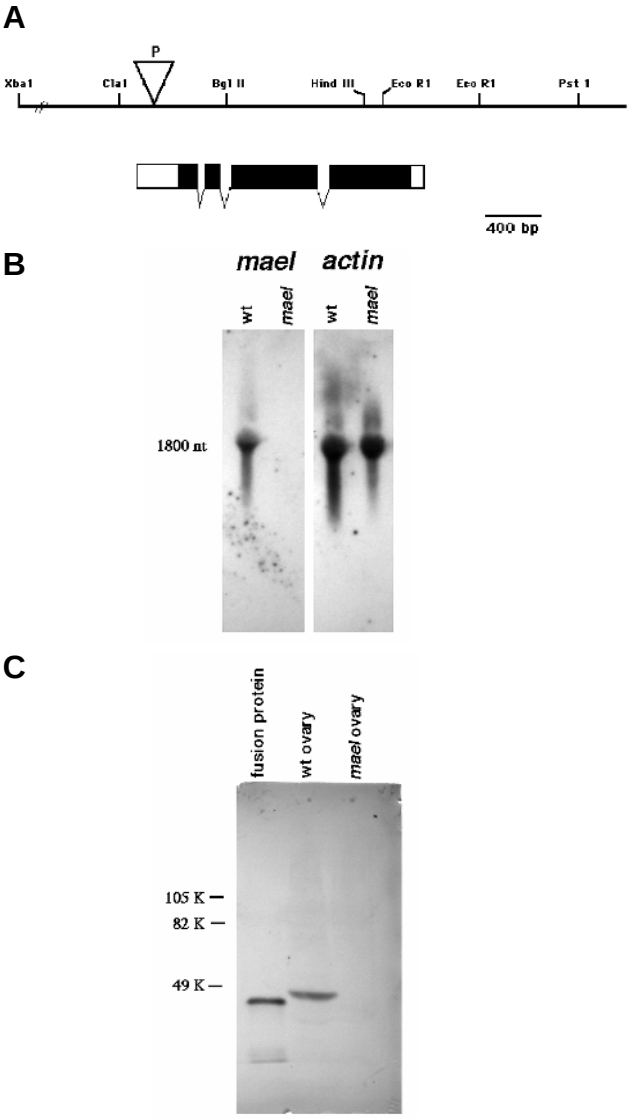

Fig. 8. The *mael* transcription unit and Mael protein. (A) Molecular map of the *mael* locus. In $mael^{11A4}$ a P-element (P) is inserted in the 5′ untranslated region of the gene. The translated region of the gene is indicated in black. (B) Northern blot of RNA from wild-type and $mael^{r20}/Df1$ mutant ovaries hybridized with *mael* cDNA (cDNA29). 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.

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   1    ACGGCATTAGAATTGTGTGATATATTCCACAGACCAGATCATTGGAAGAGAAGTCAATCGTGCGTGAGAGAAGCCAGAAGAGAAGCCACCCGCGACATTCCGAAGTAACCATTTCTGTA

 120    GGGTCAGACGTACATACAAACAACATTCATATAAATTGCTCGTGCTGTTCATTGGTTACCGCCTTTAGTCGCTACCTCGCTCGCACACGCGCTCAACACCTACCCTTACCCGTCAAAAAT

   1                              M  A  P  K  K  H  S  G  F  M  M  F  V  N  E  W  R  N  R  N  A  E  G  R  R  M  T  L  A  Q
 240    GCCGACCTTCTTCTTGCCACGCCCGTAAAGATGGCTCCTAAGAAGCATAGTGGGTTTATGATGTTCGTAAACGAGTGGAGAAACCGGAACGCCGAGGGCCGACGCATGACGCTGGCGCAG

  31    A  V  S  H  C  G  T  I  W  E  K  M  N  T  Q  Q  R  G  P  Y  N  S  G  G  K  D  A  N  V  A  Q  R  A  K  R  E  S  S  N  G
 360    GCTGTTTCCCACTGCGGCACCATATGGGAGAAAATGAACACCCAGCAGCGTGGTCCGTACAACTCAGGCGGCAAGGACGCAAATGTGGCGCAACGCGCCAAAAGGGAAAGTTCCAACGGC

  71    H  G  Q  V  D  K  A  Q  R  E  A  T  E  S  L  M  D  M  K  R  T  I  E  R  L  V  L  N  A  K  M  S  H  D  L  E  N  A  K  F
 480    CACGGCCAGGTGGACAAGGCCCAAAGGGAGGCCACCGAGAGCTTGATGGACATGAAACGTACCATCGAGCGCCTCGTGCTAAACGCCAAGATGTCCCATGATCTGGAGAATGCCAAGTTT

 111    V  F  V  A  F  N  Y  F  T  K  A  L  T  T  D  V  Y  V  P  A  E  F  A  A  C  E  Y  S  L  K  E  G  I  R  S  I  Y  S  T  M
 600    GTCTTCGTTGCCTTCAACTACTTCACTAAAGCCTTGACCACCGACGTCTATGTGCCCGCCGAGTTTGCGGCATGCGAATATTCGTTGAAGGAGGGAATCCGATCCATCTACAGCACCATG

 151    I  D  P  G  Q  I  I  F  G  Q  G  S  D  A  L  L  H  S  S  T  T  H  D  L  P  L  P  P  N  A  L  G  E  K  N  M  T  K  L  Y
 720    ATTGACCCCGGTCAGATCATTTTTGGGCAAGGTAGCGATGCCCTGCTTCACTCTTCAACCACTCACGACCTGCCGCTGCCGCCCAACGCGCTGGGGGAGAAGAACATGACCAAGCTGTAC

 191    R  N  I  V  D  Y  L  S  K  C  Q  G  K  G  K  T  L  V  V  F  T  P  A  E  N  I  T  M  V  K  S  C  F  R  Y  L  E  C  D  D
 840    CGCAACATTGTTGACTACTTATCCAAATGCCAGGGAAAAGGCAAGACCCTAGTAGTCTTTACACCCGCCGAAAATATTACCATGGTGAAGTCATGCTTCCGCTATTTGGAGTGCGATGAT

 231    D  F  R  D  G  A  E  K  I  Q  V  F  D  I  Q  Y  F  L  F  I  L  K  K  E  V  M  N  V  A  D  L  N  D  E  K  I  N  K  F  A
 960    GACTTCAGGGACGGGGGCGAAAAAATCCAGGTGTTTGATATCCAGTACTTCCTATTCATATTGAAGAAGGAGGTCATGAATGTCGCTGATTTGAATGACGAGAAAATCAACAAATTCGCG

 271    T  D  A  F  F  K  K  D  F  F  E  F  T  A  G  I  A  C  Q  Y  H  E  D  N  D  R  T  K  Y  C  T  Q  S  M  V  T  R  W  A  Y
1080    ACGGATGCTTTTTTTAAGAAAGACTTTTTCGAGTTTACCGCAGGGATTGCGTGTCAGTACCACGAGGATAATGATCGAACCAAGTACTGTACCCAGAGTATGGTGACTAGATGGGCGTAT

 311    T  F  T  D  F  M  C  G  D  L  A  I  T  V  Q  P  G  K  H  I  P  A  Q  T  K  P  N  Y  L  I  I  S  S  Y  A  S  S  L  D  H
1200    ACCTTCACTGATTTTATGTGCGGAGACCTGGCCATAACCGTCCAACCCGGAAAGCACATACCTGCCCAAACAAAACCCAACTACCTTATCATAAGCTCGTACGCCTCCTCACTCGACCAC

 371    E  S  S  F  D  S  F  Y  S  L  P  G  S  G  V  K  K  E  S  Q  P  E  A  C  S  L  S  S  S  R  L  S  V  A  S  S  S  Y  K  P
1320    GAGTCCTCCTTCGACTCTTTTTATTCGCTTCCCGGATCTGGGGTCAAGAAGGAAAGCCAACCCGAAGCTTGTTCGCTTTCTAGCAGTCGACTTTCCGTCGCATCAAGCTCCTACAAGCCC

 411    I  D  H  T  S  F  A  A  N  L  N  E  V  S  E  F  P  S  L  G  M  R  N  S  S  K  H  H  G  I  A  A  S  A  Q  R  E  W  N  A
1440    ATAGACCACACTTCCTTTGCCGCAAATTTAAACGAAGTTTCTGAATTCCCCAGCCTCGGCATGCGTAATTCTAGTAAGCATCACGGTATCGCCGCTAGTGCTCAGCGGGAATGGAACGCT

 451    R  N  L  P  T  H  S  R  L  I  R  K  V  S  D  N  D  F  S  V  N  G  A  D  G  K  L  K  K  *
1560    AGGAACTTACCGACGCACTCGCGTTTAATTCGGAAGGTTTCAGACAACGACTTCTCCGTAAACGGCGCTGATGGGAAACTTAAAAAATAAGCCGCTTTGGTCTATCAATTGGACTAAGAA

1680    ACAATGATTCCTTATAGATAATTTTCTTATCGCATTAAACTGTACAAATACTATTACTTGTATTTAAAAATAAATTCGTTCAAACGG
```

Fig. 9. DNA and protein sequence of the *mael* gene. Multiple potential start sites are present at the 5′ end of the gene. No previously reported sequence motifs are evident in the conceptually translated protein. Polyadenylation begins after the last nucleotide. Accession number, GenBank AF025953.

(Fig. 9); however, there are multiple possible start codons. The protein has no similarity to other known proteins in the GenBank database. An antibody raised against recombinant *mael* protein recognizes a single 48 kDa protein in wild-type oocytes (Fig. 8). The amount of *mael* protein is severely reduced in *mael* mutants, at least 12 fold (Fig. 8).

Germline transformation and phenotypic rescue

P-element-mediated germline transformation confirmed that the disrupted gene is *mael*. Germline transformation with a 4.5 kb *Xba*I/*Pst*I 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 $mael^{r15}/Df1$ 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% (95/100) 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

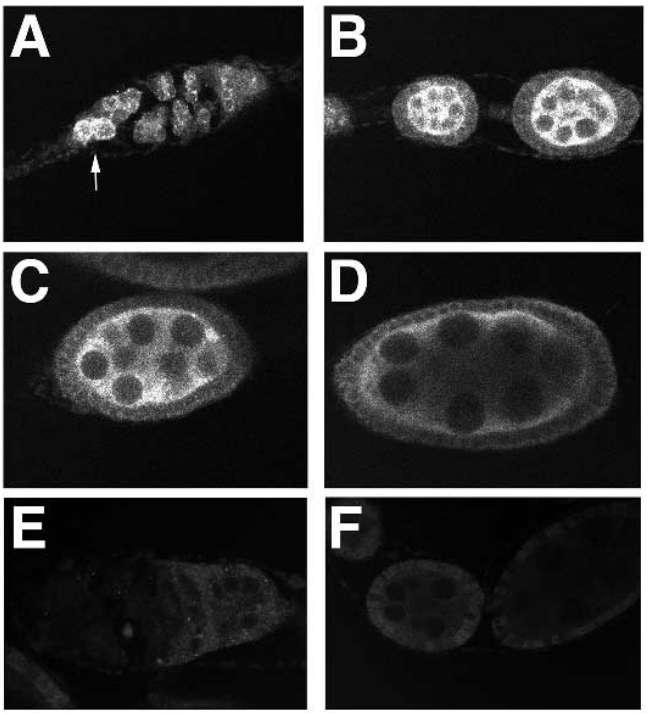

Fig. 10. Mael protein is expressed in the early germline. Confocal micrographs showing Mael localization during oocyte maturation. (A) Punctate Mael staining in the germarium is restricted to the germline. Staining is observed in the germ cells in region I (arrow). (B) Mael is distributed throughout the germline in early egg chambers (stages 2 and 3 shown). (C) At stage 5, Mael staining is greatest in the region of nurse cell cytoplasm adjacent to the follicle cells. (D) Mael staining is weaker in the oocyte of more mature (stage 7) egg chambers and cannot be detected in stage 8 or later egg chambers. (E,F) Absence of Mael staining in $mael^{r20}/Df1$ egg chambers.

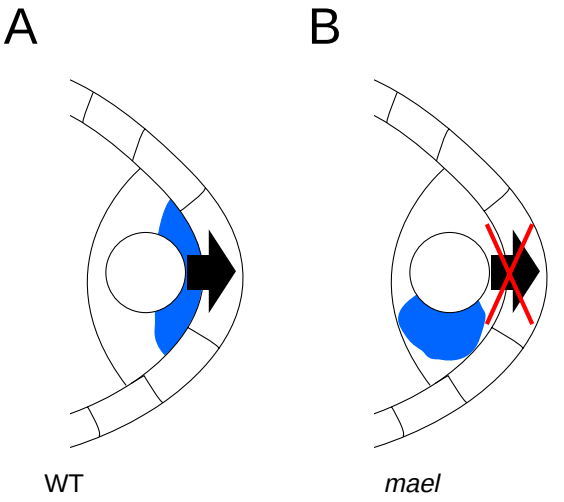

Fig. 11. Summary. (A) In wild-type stage 1-6 egg chambers, *grk* mRNA accumulates at the extreme posterior of the oocyte, between the nucleus and the posterior membrane. González-Reyes et al. (1995) proposed that the posterior localization of *grk* mRNA is required for proper Egfr signalling. (B) In *mael* mutants, *grk* mRNA is mislocalized in the early oocyte. This mislocalization correlates with defects in Egfr signalling in *mael* egg chambers.

germline 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.

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