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The Drosophila MOS Ortholog Is Not Essential for Meiosis

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Summary

In metazoan oocytes, a metaphase arrest coordinates the completion of meiosis with fertilization. Vertebrate *mos* maintains the metaphase II arrest of mature oocytes and prevents DNA replication between the meiotic divisions. We identified a *Drosophila* homolog of *mos* and showed it to be the *mos* ortholog by two additional criteria. The *dmos* transcripts are present in *Drosophila* oocytes but not embryos, and injection of *dmos* into *Xenopus* embryos blocks mitosis and elevates active MAPK levels. In *Drosophila*, MAPK is activated in oocytes, consistent with a role in meiosis. We generated deletions of *dmos* and found that, as in vertebrates, *dmos* is responsible for the majority of MAPK activation. Unexpectedly, the oocytes that do mature complete meiosis normally and produce fertilized embryos that develop, although there is a reduction in female fertility and loss of some oocytes by apoptosis. Therefore, *Drosophila* contains a *mos* ortholog that activates a MAPK cascade during oogenesis and is nonessential for meiosis. This could be because there are redundant pathways regulating meiosis, because residual, low levels of active MAPK are sufficient, or because active MAPK is dispensable for meiosis in *Drosophila*. These results highlight the complexity of meiotic regulation that evolved to ensure accurate control over the reproductive process.

Results and Discussion

Identification of a *Drosophila* Gene Homologous to *mos*

The MOS kinase is crucial for establishment of the metaphase II arrest in vertebrates. MOS is a component of cytostatic factor (CSF) [1], an activity present in oocytes prior to activation but absent from mitotically dividing embryos. We searched for a *Drosophila mos* homolog in order to evaluate the role of *mos* and MAPK signaling in meiotic progression and maintenance of the metaphase I arrest in the mature oocyte. Homology searches with the vertebrate MOS protein against the *Drosophila* genome identified the CG8767 open reading frame as its closest homolog. *Xenopus* MOS and CG8767 are 30% identical and 45% similar across the entire coding sequence (Figure 1A). CG8767 was the only serine/threonine protein kinase in the *Drosophila* genome that was identified in the homology search when the BLASTP server was used and *Xenopus* MOS as a query, and the kinase domain of CG8767 is more similar to *Xenopus* MOS than to any other protein kinase. Therefore, CG8767 is a good candidate for the *Drosophila* ortholog of MOS and will be referred to as DMOS.

The vertebrate MOS protein is characterized by a spatially and temporally regulated expression pattern present in oocytes, but rapidly degrades at fertilization [2–7]. We found that the *dmos* transcript is clearly present throughout egg chambers from stage 7, becomes abundant at stage 9, and remains present to stage 14 of development (Figures 1B–1C). In contrast, *dmos* transcript was absent from early embryos that had undergone activation (Figures 1D–1E). Lack of signal with the control, sense-strand probe showed that the staining was specific (Figures 1F–1I). Therefore, *dmos* displayed an expression pattern analogous to that of vertebrate *mos* and suggestive of a role in meiosis.

DMOS Has Cytostatic Activity

The CSF activity that arrests mitosis at metaphase when vertebrate MOS is injected into *Xenopus* embryos is the most rigorous criterion for MOS function [1]. To determine whether DMOS has CSF activity, we injected *dmos* mRNA into one blastomere of a two-cell *Xenopus* embryo. We found that the injected blastomere divided one or two times and ceased dividing, whereas the uninjected blastomere in the same embryo divided numerous times during the same period (Figure 2C). In uninjected embryos (Figure 2A) or with a kinase-dead mutant of *dmos* (Figure 2B), both blastomeres divided an equal number of times in the same period. We injected unrelated mRNAs (β-catenin and GSK3) as additional controls and did not observe a mitotic arrest (data not shown). Between 25 and 50 embryos were injected with each mRNA, and the results were uniform. We conclude that DMOS is the *Drosophila* ortholog of MOS based on three independent criteria: (1) sequence homology, (2) expression pattern, and (3) mitotic cell cycle arrest activity in *Xenopus* embryos.

The CSF activity of *Xenopus* MOS is mediated by activation of the MAPK cascade [8–10]. The phosphorylation levels of the MAPK protein are a reliable readout for activation of the cascade and can be monitored by an antibody specific to the dually phosphorylated MAPK. To determine whether DMOS activated the MAPK cascade when injected into *Xenopus* embryos, we analyzed the extent of MAPK phosphorylation in *Xenopus* embryo extracts. We compared the level of dually phosphorylated MAPK in extracts from embryos injected with DMOS with that in β-catenin-injected control extracts

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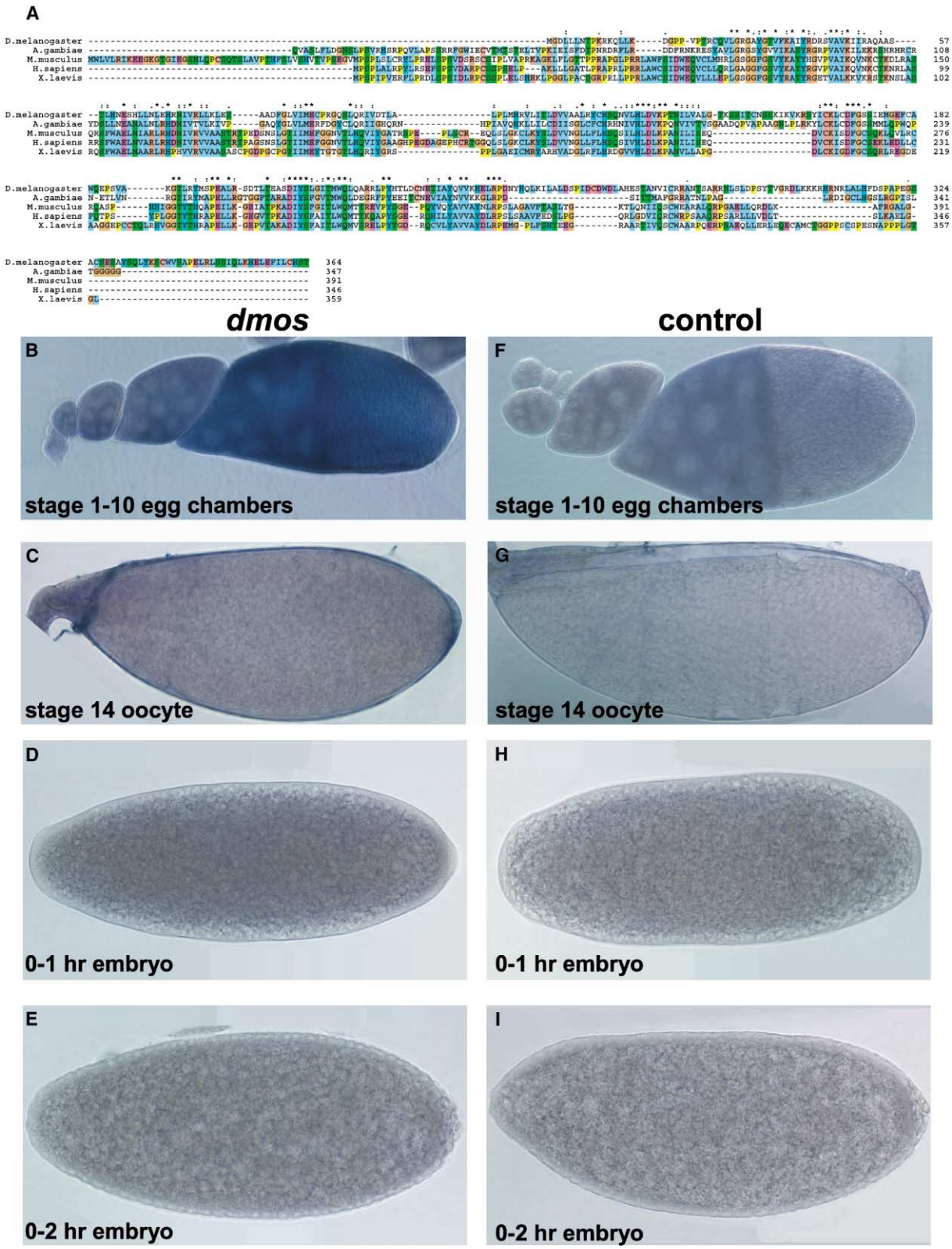

Figure 1. DMOS Is the *Drosophila* Ortholog of MOS

(A) The DMOS protein sequence was aligned against *A. gambia*, *M. musculus*, *H. sapiens*, *X. laevis*, and *A. thaliana* sequences. Identical or similar residues between the sequences are indicated with an asterisk or a dot, respectively. Color shading indicates residues that share similar properties. (B–I) In situ hybridization of *Drosophila* ovaries and embryos with antisense (B–E) or sense (F–I) probe. The *dmos* transcript was present in stage 9 (B) through stage 14 (C) egg chambers, but it was absent from early (D) 0–1 hr or (E) 1–2 hr embryos.

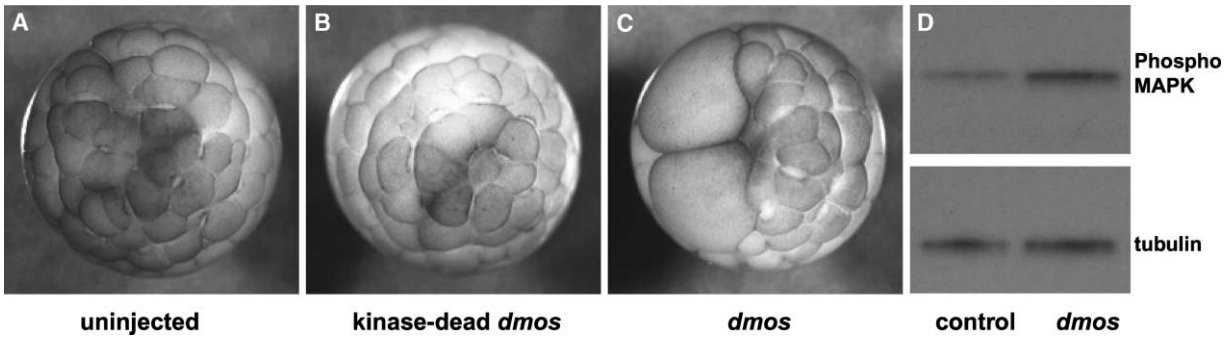

Figure 2. DMOS Has Cytostatic Activity

One cell of two-cell stage *Xenopus* embryos was either uninjected (A) or injected with kinase-dead HA-*dmos* mutant (B) or wild-type HA-*dmos* (C) mRNA. Mitotic division ceased in the *dmos*-injected cell but continued in the uninjected cell in the same embryo. As controls, both cells of the uninjected embryos or the kinase-dead *dmos* mutant-injected cell proceeded to divide. (D) Injection of *dmos* mRNA into *Xenopus* embryos led to an increase in the phosphorylation level of MAPK (top panel). In the bottom panel, the same Western blot was stripped and reprobed with an anti-tubulin antibody to provide a measure for the amount of protein loaded in each lane.

and detected a 2-fold increase (Figure 2D). Therefore, injection of DMOS into *Xenopus* embryos led to activation of MAPK and to an arrest of the embryonic mitotic cycles. These results indicate that DMOS has a CSF activity mediated by the MAPK cascade and that it is the bona fide *Drosophila* ortholog of MOS.

MAPK Is Downstream of *dmos* in *Drosophila* Ovaries

The elevation of MAPK phosphorylation after injection of *dmos* in *Xenopus* embryos raised the possibility that the MAPK cascade is also active in *Drosophila* oocytes and that it may be downstream of DMOS. To test these hypotheses, we first generated a deletion of *dmos* (see the Supplemental Data available with this article online and Figure 3A), and we then analyzed the level of MAPK and MEK1/2 (MAPK kinases) phosphorylation in wild-type and *dmos* mutant flies. We found that MAPK and MEK1/2 are phosphorylated in wild-type *Drosophila* ovaries, during prophase I (stages 1–13) and metaphase I (stage 14) (Figure 3B). Strikingly, the phosphorylation level of MAPK in the *dmos* mutant ovaries was reduced 15-fold in metaphase I-arrested oocytes and 4-fold in prophase I-arrested oocytes (Figure 3B). The levels of MEK1/2 phosphorylation were also greatly reduced. In contrast, MAPK and MEK1/2 phosphorylation levels were unaffected in mutant female carcasses from which the ovaries had been completely removed and in mutant males (Figure 3B), suggesting that DMOS does not affect the MAPK cascade outside the ovaries. These results indicate that the MAPK cascade is active in *Drosophila* ovaries and that DMOS is required for activation of MAPK specifically in the ovaries. A residual level of MAPK phosphorylation in the mutant ovaries suggests that MAPK can be phosphorylated by a redundant pathway.

Mutations in *dmos* Compromise Female Fertility

Having demonstrated that *dmos* activates the MAPK cascade in *Drosophila* oocytes, we investigated the phenotypic consequences of deleting the gene. Flies mutant for *dmos* developed into adults at the expected Mendelian ratios, showing that *dmos* does not affect viability. Males mutant for *dmos* were fully fertile. In contrast, female flies mutant for *dmos* had fewer progeny than heterozygous sibling controls. This reduction ranged from 72% of normal progeny in young females (0–3 days old) to 45% of normal progeny in old females (12–15 days old), showing that the defect is exacerbated with age of the flies (Table 1). Therefore, deletions of *dmos* lead to a female-specific fertility defect.

The reduction in progeny number of the *dmos* mutant females could be due to reduction in the number of viable eggs produced or deposited (oogenesis defect), a reduction in the number of embryos that develop into viable adults (developmental defect), or a combination of both. We found that the majority of embryos deposited by *dmos* mutant females developed normally past the postblastoderm stage (data not shown), and thus embryogenesis defects are not the primary cause of the reduction in progeny number. In addition, during the course of these experiments, the number of eggs deposited by *dmos* mutant females was not noticeably different from that of the heterozygous sibling controls, suggesting that egg deposition is not defective in the mutant.

To examine whether elimination of *dmos* leads to meiotic defects, we dissected the ovaries of *dmos* mutant females and stained them with DAPI. We did not observe any significant differences in the ovaries from the *dmos* mutant as compared to *dmos/CyO* in females aged up to 9 days (data not shown). The metaphase I arrest of mature oocytes appeared to be normal, indicating that *dmos* is not essential for meiosis. The ovaries of *dmos* mutant females aged for 9–15 days displayed more apoptotic egg chambers at stage 7–8 (Figures 3C and 3D) than did those of *dmos/CyO* (36.0% in *dmos* mutant versus 11.2% in *dmos/CyO*). In *Drosophila* ovaries, apoptosis is induced at stage 7–8 in abnormally developing follicles predominantly in old females and can be triggered in younger females by mutations affecting oocyte development or meiotic progression [11]. Our results suggest that deletion of *dmos* leads to defects that trigger apoptosis at a younger age and to a greater extent than in wild-type ovaries, consistent with *dmos* functioning in meiosis. The extent of apoptosis observed does not completely account for the reduction in progeny

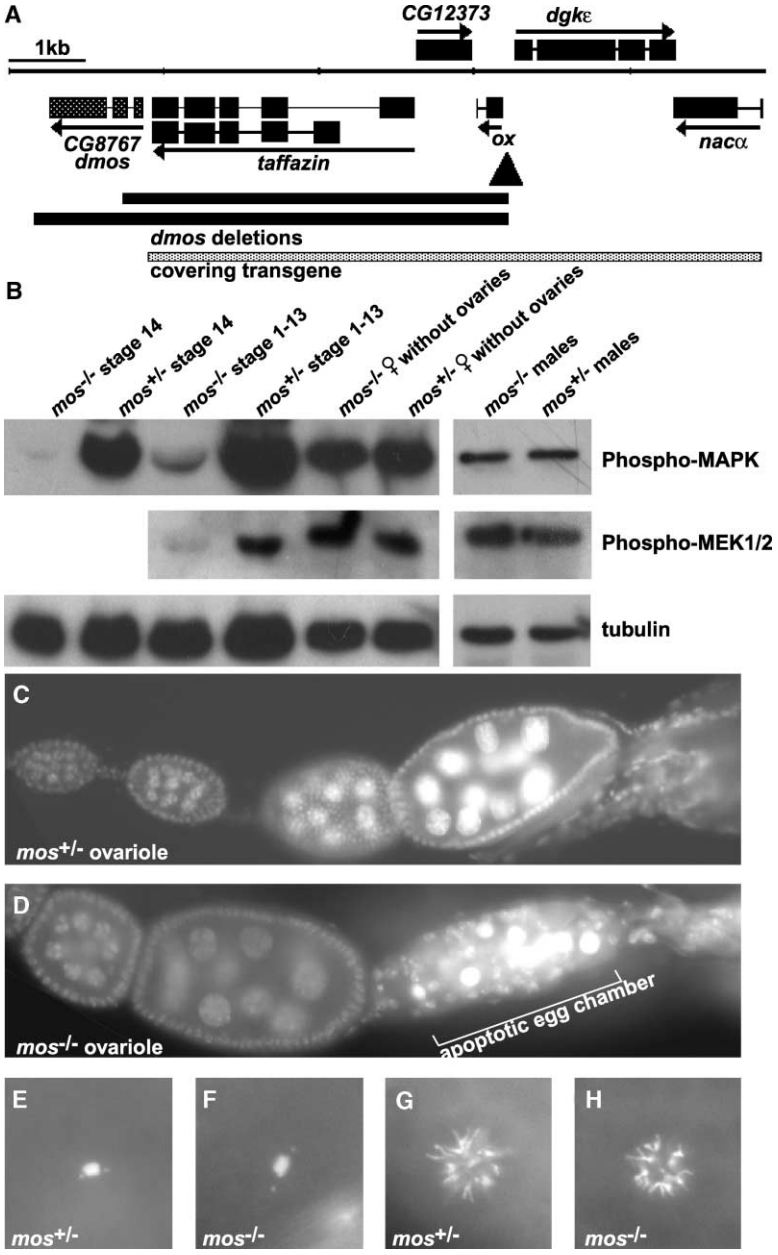

Figure 3. DMOS Is Upstream of MAPK in *Drosophila* Ovaries

(A) The genomic region surrounding *dmos*. The *dmos* open reading frame is represented by dotted rectangles, and the neighboring genes are represented by solid rectangles. The original position of the P element is represented by a triangle, and chromosomal regions deleted in the partial and full deletions of *dmos* are represented by black bars at the bottom of panel A. The hatched bar represents the covering transgene that was introduced into the *dmos*-deleted lines to complement the neighboring genes that were also deleted. (B) MAPK (top panels) and MEK1/2 (middle panels) phosphorylation levels are greatly reduced in ovaries of *dmos* mutant females, whereas they are unaffected by the mutation in the rest of the female body or in males. In the bottom panel, the same Western blots were stripped and reprobed with anti-tubulin antibody to provide a measure for the amount of protein loaded in each lane. (C–D) In contrast to their heterozygous siblings (C), *dmos* mutant females (D) showed an increase in apoptosis at stage 8 of egg chamber development. In *Drosophila*, a normal metaphase I spindle is characterized by the small, nonexchange fourth chromosomes migrating toward the spindle poles [35]. This chromosomal configuration showing a normal metaphase I arrest is present in both the control and the *dmos* mutant stage 14 oocytes (E and F). In addition, the rosette structures (polar bodies) (G and H) were indistinguishable between the mutants and the controls.

Table 1. *dmos* Mutant Females Produce Fewer Progeny

	Progeny Number			Percent mutant/heterozygotes
	Wild-Type	*dmos/CyO*	*dmos/dmos*	
0–3 days	1161	1317	954	72
3–6 days	1432	1385	724	52
6–9 days	1226	1326	626	47
9–12 days	1246	989	546	55
12–15 days	882	749	340	45

Females from the three genotypes indicated in the top row were aged for the time indicated in the left column. For each genotype, five vials with five virgin females in each vial and 5 wild-type (*y, w*) males were mated for 3 days. After 3 days, the flies were transferred to a fresh vial supplemented with dry yeast. The number of adult progeny that emerged from the last vials in each aging period was counted and the number given in the "Progeny #" columns represent total of the five vials for each genotype. The percent mutants/heterozygotes represents the ratio of progeny by *dmos/dmos* divided by the progeny of *dmos/CyO*. At the end of each aging period, the females were recovered and their ovaries were dissected and stained with DAPI.

number. The apoptotic egg chambers were frequently followed by stage 14 oocytes with normal metaphase I spindles (Figure 3F); thus, absence of DMOS function could be overcome so that some egg chambers did not undergo apoptosis and developed.

To determine whether *dmos* is required for progression through meiosis beyond the metaphase I arrest, we analyzed unfertilized embryos laid by *dmos* mutant females. The post-meiotic chromosomes in the unfertilized embryos condense into a characteristic rosette structure (Figure 3G). We found that the rosettes in the unfertilized eggs laid by *dmos* mutant females appeared to be normal (Figure 3H). These results show that *dmos* is not essential for progression through meiosis I and II or for blocking DNA replication between the two meiotic divisions. In summary, mutations in *dmos* compromise fertility in females as a result of a combination of apoptosis at stage 7–8 and a secondary defect. In those egg chambers that escape apoptosis, however, the meiotic divisions are completed normally, and viable embryos result. Thus, it appears that despite its requirement for activating the MAPK cascade in the *Drosophila* ovaries, *dmos* is nonessential for meiosis in *Drosophila*, in contrast to starfish, in which *mos* is absolutely required [12]. Our results are analogous to the Mos mouse knockout, for which fertility is greatly impaired but not completely eliminated [13, 14]. In *Xenopus*, Mos is not essential for meiosis I [15] and does not lead to a metaphase I arrest despite full Mos-dependent activation of MAPK [16], consistent with our findings that *dmos* is not essential for meiosis I in *Drosophila*. Our results suggest that DMOS and the MAPK cascade play a role in meiotic progression either by regulating a nonessential process or by regulating an essential process in conjunction with a redundant pathway.

DMOS-Redundant Pathways

Given the essential function of *mos* in vertebrates and the requirement for DMOS in activation of the MAPK cascade in *Drosophila* ovaries, we explored the existence of *dmos*-redundant pathways in three ways. First, another MAPKKK may activate MAPK in the *dmos* mutant ovaries, providing the residual 7% of activity and sufficient function to mask any phenotypic consequences of deleting *dmos*. The RAF-1 protein, a conserved homolog of the *v-raf* oncogene [17], activates the MAPK cascade in *Drosophila* somatic tissues (for a recent review see [18]) and is therefore a good candidate for redundancy with DMOS. To test this hypothesis, we introduced one copy of a null *raf-1* mutation in the *dmos* mutant background and thereby reduced the levels of RAF-1 by half. The *raf-1* mutation did not dominantly enhance the *dmos* phenotype (data not shown), and thus either RAF-1 is not redundant with DMOS or the levels of RAF-1 are not limiting in the ovaries.

The MAPK cascade activates the Cyclin B/CDK1 complex, so a second possibility is that Cyclin B/CDK1 is activated by a pathway redundant with MOS. In vertebrates, both *mos* and *cyclin B* are translationally activated by the CPEB protein [19]. The *Drosophila* ortholog of CPEB is the oo18 RNA binding (ORB) protein [20, 21]. Analogously to CPEB, ORB may also function to translationally regulate *mos* and *cyclin B*. We explored a role for ORB in establishment of the metaphase I arrest and found that orb^{mel} mutant ovaries had normal metaphase I spindles in stage 14 oocytes (data not shown). If *cyclin B* and *mos* are both targets of ORB, and if they function redundantly in establishing the metaphase I arrest, then reducing the amount of *mos* in a weak orb^{mel} mutant may unmask a role for ORB in metaphase I arrest. However, we found that reducing the levels of *mos* in the orb^{mel} mutant background did not enhance the orb^{mel} mutant phenotype (data not shown), suggesting that ORB does not regulate a pathway parallel to MOS.

Third, the PAN GU (PNG) protein kinase complex is required for sustaining high levels of Cyclin B by activating *cyclin B* posttranscriptionally [22]. PNG and its two activating subunits, PLU and GNU, promote mitosis specifically in the early embryonic divisions, but all of the proteins are present during oogenesis [23–25]. Thus, DMOS and PNG could act redundantly to control Cyclin B and active CDK1/Cyclin B (or Cyclin B3) during meiosis. We tested this hypothesis by examining the phenotypes of double mutants, but we did not observe any genetic interactions between *png* and *dmos*. The stage 14 oocytes in the ovaries of *png;dmos* females had normal metaphase I spindles, and the embryos had the same phenotype as the *png* single mutant (data not shown). Therefore, the PNG pathway is not redundant with DMOS.

Additional mechanisms independent of MAPK may control female meiosis in parallel with *dmos*, as supported by recent observations of pathways acting in parallel to *mos* in vertebrates [26]. For example, the APC inhibitor, Emi1, and the CyclinE/CDK2 complex have been shown to have CSF activity [27–29]. The existing alleles of *CyclinE* and *rca1*, the *Drosophila emi1* homolog, are lethal or disrupt the early stages of meiosis; thus, it has not been possible to test their role specifically in the metaphase I arrest [30–32].

Our analysis of *dmos* illustrates the divergence of meiotic regulatory mechanisms and supports the emerging paradigm that meiosis is subjected to parallel, compensatory controls to ensure the proper completion of this developmental process that is critical for reproductive success. It will be important to evaluate the role of MAPK in *Drosophila* female meiosis as well as to test the requirements for CDK1/Cyclin activity, APC-mediated proteolysis, and the spindle checkpoint. The mechanism by which the metaphase I arrest is maintained and released is particularly intriguing. It has been demonstrated that chiasmata are essential for signaling the arrest [33, 34], but the role of CDK1/Cyclin remains unknown. To date, a genetic evaluation of these components in oocytes has been hampered by the lack of alleles that could distinguish between roles during earlier mitotic divisions in the germline and later meiosis in the oocyte. Alternative approaches to producing conditional phenotypes are being developed, and these should permit such analyses in the future.

Supplemental Data

Supplemental Experimental Procedures and a supplemental figure are available online at http:www.current-biology.com/cgi/content/full/14/1/75/DC1/.

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