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Two Zinc Finger Proteins, OMA-1 and OMA-2, Are Redundantly Required for Oocyte Maturation in *C. elegans*

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Summary

Oocytes are released from meiotic prophase I arrest through a process termed oocyte maturation. We present here a genetic characterization of oocyte maturation, using *C. elegans* as a model system. We show that two TIS11 zinc finger-containing proteins, OMA-1 and OMA-2, express specifically in maturing oocytes and function redundantly in oocyte maturation. Oocytes in *oma-1;oma-2* mutants initiate but do not complete maturation and arrest at a defined point in prophase I. Two maturation signal-induced molecular events, including the maintenance of activated MAP kinase, do not occur in Oma oocytes. The Oma prophase arrest is released by inactivation of a MYT-1-like kinase, suggesting that OMA-1 and OMA-2 function upstream of MYT-1 as positive regulators of prophase progression during meiotic maturation.

Introduction

In addition to its maternal genome, the oocyte provides a wealth of mRNAs and proteins responsible for early embryonic patterning as well as for oocyte development itself. In almost all animal species studied, oocytes arrest at prophase of the first meiotic division (Masui and Clarke, 1979). Oocyte maturation releases this prophase arrest, allowing the resumption of meiosis and the development of the oocyte to a stage when fertilization can occur (Masui, 1967). This process is dependent upon reception of an appropriate stimulatory signal. Maturation encompasses two developmental programs, one nuclear and the other cytoplasmic (see review in Eppig, 1996). Nuclear maturation refers to the nuclear events associated with the resumption of meiotic cell cycle progression. Cytoplasmic maturation encompasses at least three events: the reception of the maturation signal at the cell surface, the transduction of this signal to the nucleus, and any subsequent cytoplasmic and/or membrane changes that allow fertilization by sperm.

Although the basic outline of oocyte maturation is highly conserved in vertebrates and invertebrates, some variation exists between species. For example, the duration and precise point of meiotic arrest can vary considerably. Whereas meiotic arrest can occur for several decades in humans and for several years in amphibians, this arrest is not apparent in certain nematode species where the stimulus for oocyte maturation is constitutively present (see below). In addition, the stimulus that triggers oocyte maturation varies between species. Maturation is induced by progesterone in *Xenopus* and mammals (Masui, 1967), 1-methyladenine in starfish (Kanatani, 1969), and serotonin in oysters (Krantic et al., 1991). The stimulus for oocyte maturation in *C. elegans* has recently been shown to be a sperm-specific cytoskeletal protein, MSP (Miller et al., 2001). Despite these variations, many key components of the maturation pathway appear to be ubiquitous. These include components of the maturation-promoting factor (MPF), as well as the mitogen-activated protein kinase pathway (MAPK; Kosako et al., 1994). MPF was initially defined as an activity present in mature *Xenopus* oocytes that is capable of inducing the maturation of immature oocytes in the absence of a progesterone signal (Masui and Markert, 1971). MPF is a heterodimer of a cyclin-dependent protein kinase (CDC2 in most animals) and cyclin B (Lohka et al., 1988; Maller et al., 1989). Activated MPF is believed to phosphorylate target proteins involved in chromosome condensation, spindle formation, and nuclear envelope breakdown.

Although the biochemical events leading to the activation of MPF have been extensively studied, the initial events of oocyte maturation that occur immediately following stimulation are not as well understood. For example, many maternal mRNAs synthesized during oocyte growth are dormant in immature oocytes (see review in Richter, 1999). Progesterone activation selectively relieves this dormancy, making particular mRNAs available for translation. Although the biochemical pathways linking progesterone stimulation and unmasking of dormant mRNAs, as well as the mechanism of mRNA unmasking itself, are not fully understood, one mechanism likely to play a role in the activation of masked maternal mRNA is the addition of a poly A tail. Indeed, it has been demonstrated that the translation of maternal cyclin B mRNA in response to progesterone activation requires the addition of a long poly A tail (Stebbins-Boaz et al., 1996).

The nematode *Caenorhabditis elegans* should provide an excellent model for genetic analysis of the regulation of oocyte maturation. *C. elegans* has a female soma and a hermaphroditic germline, which first generates sperm and then switches completely to the generation of oocytes (Hirsh et al., 1976, Schedl, 1997). Oocytes grow and undergo maturation in a single-file-assembly line fashion in a tube-like gonad, with the most developed oocyte most proximal to the spermatheca. The fully mature oocyte is ovulated into the lumen of the spermatheca, where fertilization takes place. Because the stimulus for oocyte maturation, MSP, is present at all times in young adult hermaphrodites due to the constitutive presence of sperm, oocyte maturation can be defined as the period from the time an oocyte assumes the most proximal position to the time fertilization occurs. The maturation process, despite the constitutive presence of the stimulus, is regulated; it occurs one oocyte at a time, once every 23 min, and only in the most proximal position (McCarter et al., 1999). Therefore, oocyte maturation in *C. elegans* would appear to be dependent on

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the ability of an oocyte to respond to or to process the MSP signal. Because *C. elegans* is transparent, oocyte maturation, ovulation, and fertilization can be visualized in live adult animals and has been well characterized morphologically (McCarter et al., 1999). No mutations have been identified that are defective specifically in the oocyte maturation process, although many mutants defective in various aspects of early female germline development (Austin and Kimble, 1987; Church et al., 1995; Francis et al., 1995), as well as in ovulation and fertilization, have been isolated (Hodgkin, 1986; Iwasaki et al., 1996; Rose et al., 1997). The lack of genetic mutants defective in oocyte maturation strongly suggests that genes involved in this process are functionally redundant or are essential for developmental events prior to adulthood.

We report here the identification by mutation of two closely related TIS11 zinc finger-containing proteins, OMA-1 and OMA-2, and demonstrate their redundant function in oocyte maturation in *C. elegans*. We show that while loss-of-function mutations in either *oma-1* or *oma-2* alone do not have a phenotype, *oma-1;oma-2* double mutant animals are sterile, containing oocytes arrested prior to the completion of maturation. The maturation defect is specific to oocytes, as we detect no sperm defect in double mutant animals. OMA-1 and OMA-2 proteins are both expressed specifically in growing oocytes, with expression for both peaking in maturing oocytes. Suppression of Oma prophase arrest by removal of a meiotic MYT-1-like kinase suggests that OMA-1 and -2 function upstream of MPF activation. The identification of *oma-1* and *oma-2* mutations should facilitate further genetic and molecular analyses of oocyte maturation, a process that has been difficult to characterize genetically due to a lack of genetic mutants.

Results

Identification of the *oma-1* and *oma-2* Genes

We have isolated and characterized a mutation in *C. elegans*, *zu405*, based on its early embryonic phenotype. Through transformation rescue, we showed that the *zu405* mutation was in the gene *C09G9.6* (see Experimental Procedures). Somewhat surprisingly, inactivation of *C09G9.6* in wild-type worms through RNAi revealed no observable phenotype. This result suggested either that *C09G9.6* does not have an essential function or that its function is redundant with another gene. Coinjection of wild-type worms with *C09G9.6* dsRNA and that derived from its closest sequence homolog, *ZC513.6* (also no phenotype on its own), resulted in the sterility of the injected animals. This result strongly suggests that these two genes have redundant functions in the adult germline. We show here that this sterile phenotype is due to a specific defect in oocyte maturation, and will be referred to as Oma (for *o*ocyte *ma*turation defective). *C09G9.6* and *ZC513.6* will hereafter be referred to as *oma-1* and *oma-2*, respectively. The original mutation, *zu405*, is a gain-of-function (gof) allele of *oma-1*, and the characterization of its embryonic phenotype is the subject of a separate paper.

oma-1 and *oma-2* Encode TIS11, CCCH Zinc Finger-Containing Proteins

oma-1 and *oma-2* are predicted to encode 407 and 393 amino acid proteins, respectively, that contain two TIS11-like zinc fingers spaced 22 amino acids apart (Figure 1A). The TIS11 zinc finger is found in a small subset of zinc finger family proteins and consists of C-$X_{8–10}$-C-X_5-C-X_3-H, where X refers to any amino acid (Figure 1B; Varnum et al., 1989; DuBois et al., 1990; Bai and Tolias, 1996). The TIS11 zinc finger was first described in the mouse TIS11 protein and is shared by five *C. elegans* proteins, MEX-1, PIE-1, POS-1, MEX-5, and MEX-6, that have been shown to function in early blastomere fate determination (Varnum et al., 1989; Mello et al., 1996; Guedes and Priess, 1997; Tabara et al., 1999; Schubert et al., 2000). OMA-1 and OMA-2 are nearly identical within the two zinc fingers and share 64% identity throughout the entire protein, consistent with them being functional homologs.

Isolation of Loss-of-Function Mutations in the *oma-1* and *oma-2* Genes

To better characterize the Oma sterile phenotype, we conducted a two-step genetic screen to isolate loss-of-function (lof) mutations in both the *oma-1* and *oma-2* genes. Our RNAi result suggested that lof mutations in the *oma-1* gene would have no phenotype. However, *oma-1* RNAi in the *zu405* gof mutant rescued the embryonic lethality (see Experimental Procedures). Therefore, lof mutations in *oma-1* should be isolated as dominant suppressors of *zu405*. In the first step of the screen, we isolated 12 dominant suppressors of the *zu405* mutation, of which ten are lof or reduced function mutations in *oma-1*. In the second step of the screen, we isolated two mutations that resulted in the synthetic Oma phenotype in the *oma-1(te33)* background. The nature of these *oma-1* and *oma-2* mutations are summarized in Figure 1C. *oma-1(te33)* is a nonsense mutation upstream of both zinc fingers resulting in no detectable OMA-1 protein (Figure 3A), and represents a null mutation. *oma-2(te51)* is a splice site mutation that results in no detectable OMA-2 protein (Figure 3A). The *oma-1(te33); oma-2(te51)* mutant phenotype is similar to that of *oma-1(RNAi);oma-2(RNAi)* animals. All data presented below, unless stated otherwise, were derived using the double mutant *oma-1(te33);oma-2(te51)* strain TX183. The same results were seen with the *oma-1(RNAi);oma-2(RNAi)* animals.

oma-1 and *oma-2* Function Redundantly in Fertility

The reproductive system of wild-type *C. elegans* hermaphrodites consists of two distal gonad arms (ovaries) that are joined proximally to a shared uterus (Figures 2A and 2B). Each gonad arm is a syncitium filled with germ nuclei that proliferate mitotically before entering meiotic prophase I and initiating oogenesis. Following cellularization and late oogenesis, oocytes grow tremendously both in nuclear and cytoplasmic volumes. Fully grown oocytes remain in diakinesis of prophase I. In the presence of sperm, oocytes sequentially undergo maturation and are ovulated into the spermatheca where

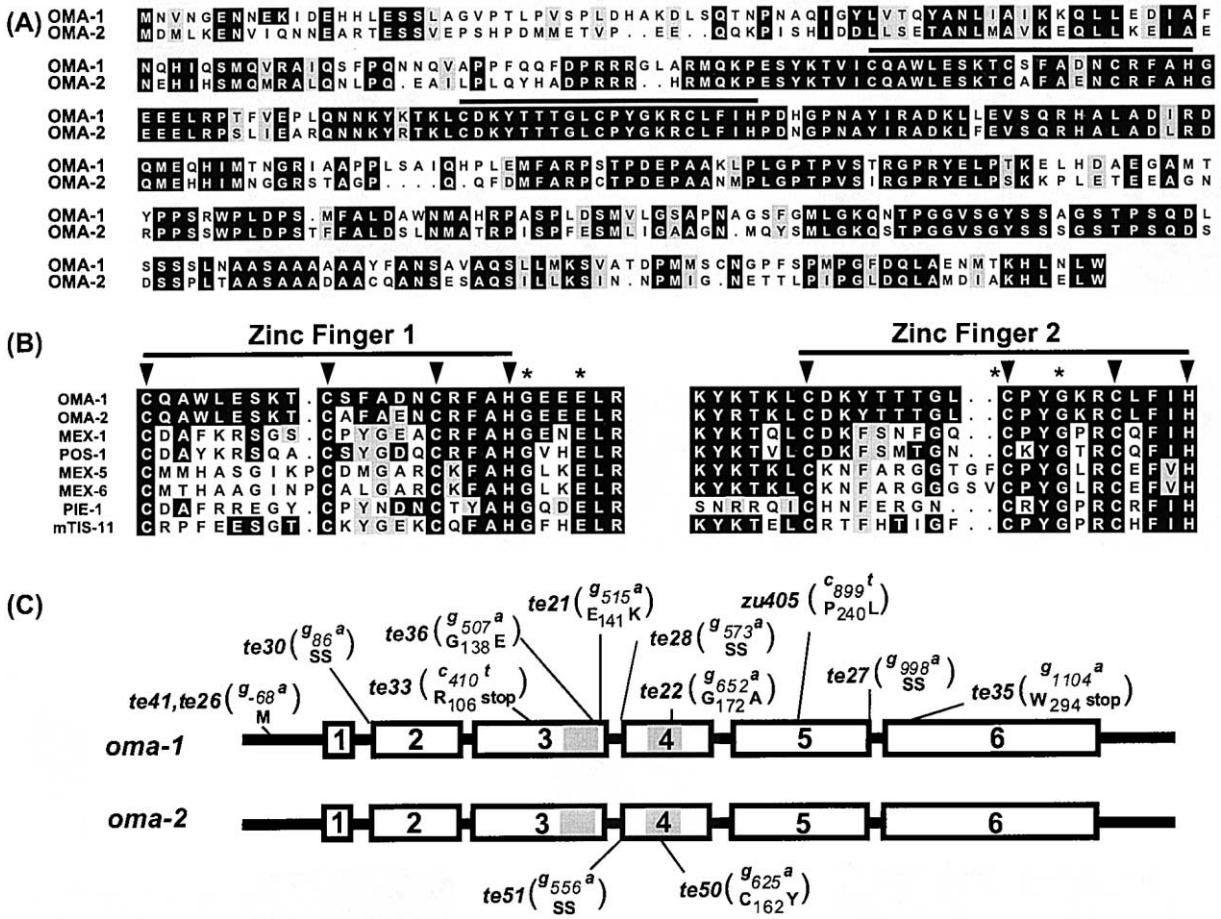

Figure 1. Sequence Analysis of *oma-1* and *oma-2* Genes, Mutant Alleles, and Related Sequences

(A) Alignment of OMA-1 and OMA-2 protein sequences with TIS11 fingers indicated by lines above.

(B) Alignment of the two zinc fingers of OMA-1, OMA-2, five other *C. elegans* proteins, and mouse TIS11 (mTIS11). Black and gray backgrounds indicate residues identical and similar, respectively, to those in OMA-1. Arrows indicate the conserved C-C-C-H residues. Asterisks mark amino acids changed in four missense mutations (*te36*, *te21*, *te22*, and *te50*).

(C) Schematic diagrams of *oma-1* and *oma-2* genomic sequences and positions of each mutation. Mutations are indicated by allele numbers, with nucleotide changes in small letters and amino acid changes in caps. Boxes are exons and lines are introns. Position 1 of the nucleotide sequence refers to the "a" of the initiating atg. M, ectopic initiator methionine; ss, splice site mutation; stop, nonsense mutation. Gray indicates zinc fingers.

fertilization occurs, and are then passed into the uterus. Immediately after fertilization, the oocyte nucleus in the 1-cell embryo completes two rounds of meiotic division to generate the oocyte pronucleus. After ovulation of the most proximal oocyte (position relative to spermatheca, hereafter the minus one [−1] oocyte), the next most proximal oocyte (the −2 oocyte), assumes the most proximal position and the process of maturation repeats.

Although Oma animals produce both sperm and oocytes, they do not produce embryos and have an empty uterus (n = 300; Figure 2D). Gonad arms in Oma animals fill with an abnormally high number of oocytes (an average of 15 oocytes per gonad arm versus 7 for wild-type). Oma oocytes are unusually large; each is approximately three to five times the volume of a fully grown, wild-type oocyte (Figures 2C and 2D). Due to their size and number, oocytes occupied approximately 70% of the gonad volume in Oma animals, and extended much more distally than in wild-type gonads. By examining individual live Oma animals as they transitioned from L4 to adulthood, we observed oocytes accumulating first proximally and then more distally over time. This result suggests a block in proximal oocyte development underlying the Oma sterile phenotype.

oma-1;oma-2 Mutants Are Defective Specifically in the Female, but Not the Male, Germline

To determine the cause of Oma sterility, we examined whether sperm and oocytes derived from Oma mutant animals were functional. *C. elegans* hermaphrodites typically fertilize their oocytes from an endogenous sperm supply and produce self-progeny. However, exogenous wild-type sperm can be introduced via mating with a male and are used preferentially to produce cross-progeny. We tested whether sperm from Oma mutants could fertilize non-Oma oocytes by mating *oma-1(te33);oma-2(te51)* males to young, uncoordinated (*unc-24*) hermaphrodites. Single *oma-1*;*oma-2* males mated with

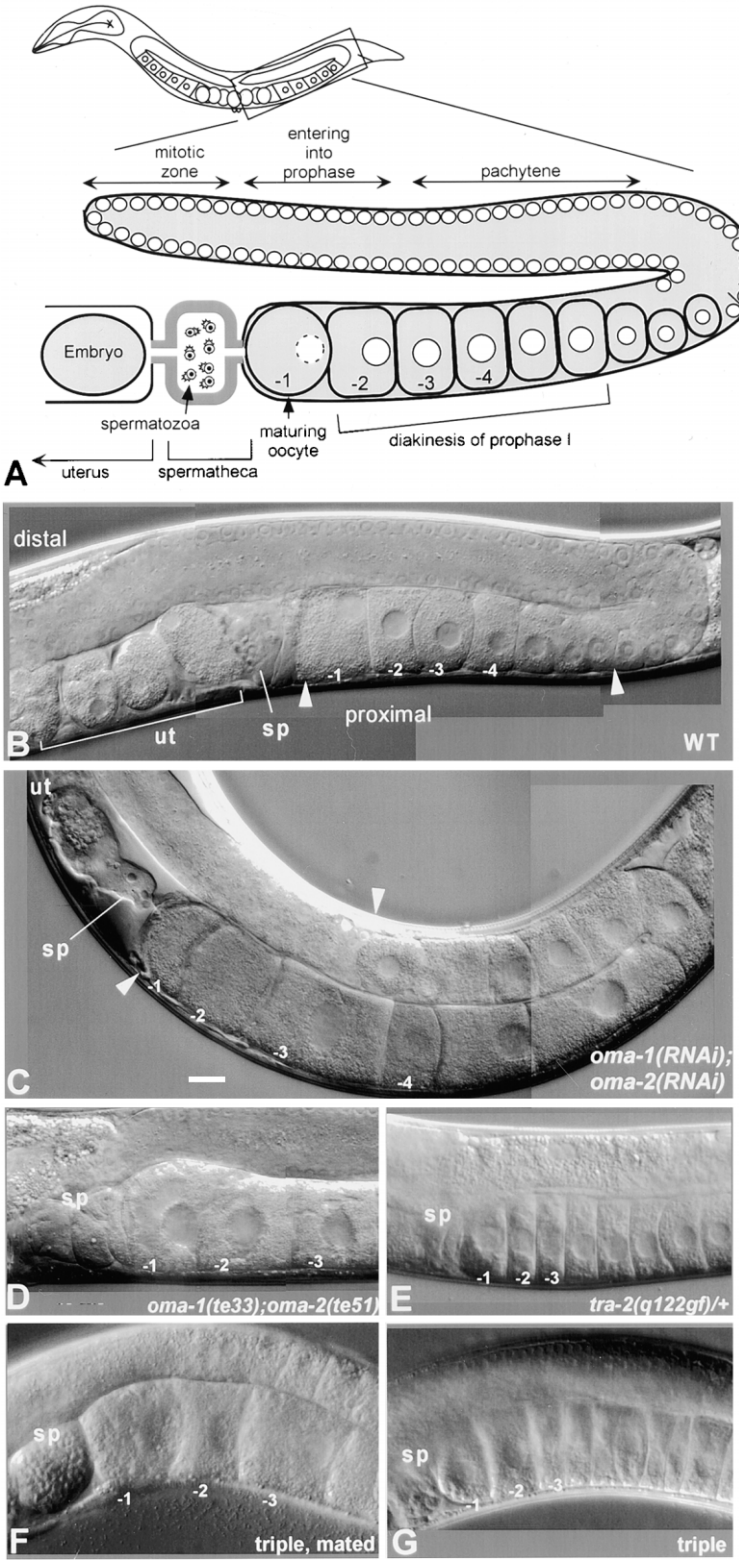

Figure 2. Comparison of Gonad Morphology for Wild-Type, Oma, and Female Animals

(A) Schematic diagram of a wild-type *C. elegans* adult hermaphrodite gonad arm. Germ cells proliferate mitotically in the distal region and then enter prophase of meiosis I. Distal germ nuclei (open circles) are in a syncitial cytoplasm, whereas proximal germ cells are enclosed by membranes. The −1 oocyte is the most proximal and most developed oocyte.

(B–G) Light micrographs, all at the same magnification, of wild-type (B), *oma-1(RNAi); oma-2(RNAi)* (C), *oma-1(te33);oma-2(te51)* (D), and *tra-2(q122gf)/+* (E), *tra-2(q122gf)/+; oma-1(te33);oma-2(te51)*, mated with wild-type N2 males (F), *tra-2(q122gf)/+;oma-1(te33); oma-2(te51)*, unmated (G) gonads. The portion of each gonad arm containing oocytes is indicated between the two arrowheads in (B) and (C). ut, uterus; sp, spermatheca. The scale bar represents 10 μm.

single *unc-24* hermaphrodites yielded an average of 146 non-Unc cross-progeny per mating (n = 24 matings), similar to the number of cross-progeny obtained (140) when a wild-type N2 male was mated with an *unc-24* hermaphrodite (n = 8). This result suggests that *oma-1;oma-2* sperm are fully functional and points toward a defect in oocytes as the cause of the sterility. No cross-progeny were ever observed when *oma-1(te33);oma-*

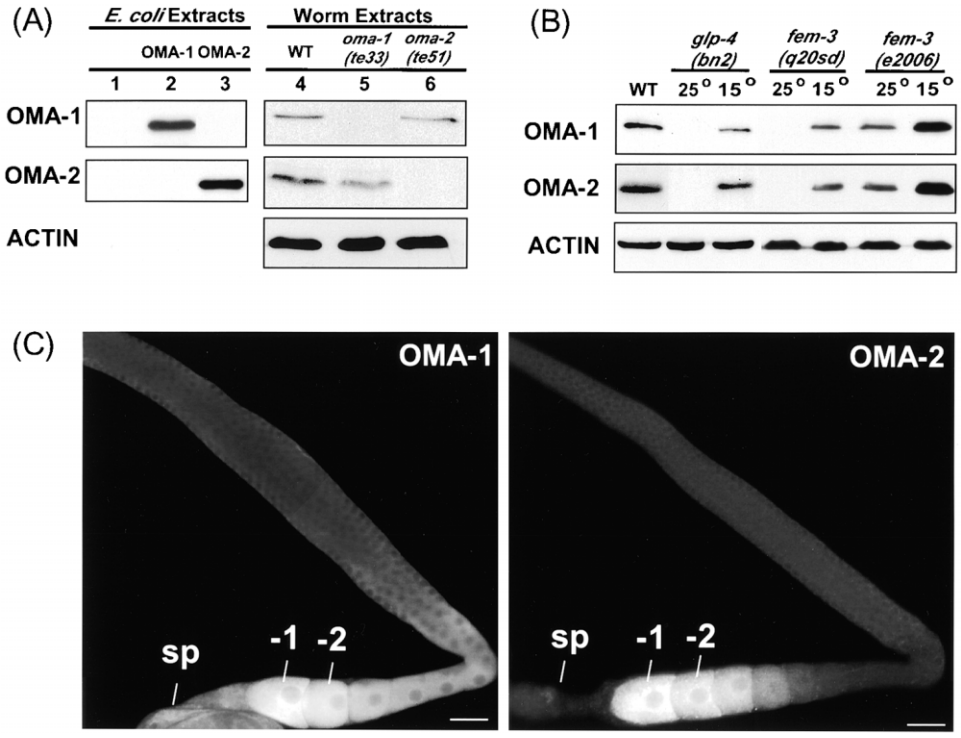

Figure 3. Expression of OMA-1 and OMA-2 Proteins

(A) Extracts of bacteria expressing no fusion protein (lane 1), GST-OMA-1 (lane 2), or GST-OMA-2 (lane 3), and whole-worm extracts from wild-type N2 (lane 4), *oma-1(te33)* (lane 5), and *oma-2(te51)* (lane 6) were probed with antibodies to OMA-1, OMA-2, or actin.

(B) Worm lysates from wild-type N2, *glp-4(bn2)*, *fem-3(q20sd)*, or *fem-3(e2006)* grown at either 15°C or 25°C probed with antibodies to OMA-1, OMA-2, or actin.

(C) Dissected wild-type gonads stained with affinity-purified antibodies to either OMA-1 or OMA-2. The scale bar represents 10 μm.

2(te50), *oma-1(te33)*;*oma-2(te51)*, or *oma-1*(RNAi);*oma-2*(RNAi) hermaphrodites were mated with wild-type N2 males, confirming that both OMA-1 and OMA-2 function are required in oocytes but not in sperm.

OMA-1 and OMA-2 Expression Is Restricted to the Female Germline and Peaks in the −1 Oocyte

To characterize the spatiotemporal distribution of OMA-1 and OMA-2 proteins, we raised antibodies to OMA-1 and OMA-2. Figure 3A shows the specificity of antibodies 7704 to OMA-1 and 7703 to OMA-2, respectively. We also showed, by Western blotting, that the level of OMA-1 protein is not affected in *oma-2* mutant extracts and vice versa, consistent with their functional redundancy. Two mutant alleles, *oma-1(te21)* and *oma-2(te50)*, have missense mutations in conserved amino acid residues in or near the zinc finger domain (Figure 1C). These two mutations do not affect protein levels but do clearly affect protein function, suggesting that the zinc finger domains of OMA-1 and OMA-2 are critical for their biological function. The absolute specificity of the two antisera enabled us to distinguish between the two identically sized proteins (see below).

A developmental Western blot using synchronized populations indicates that both OMA-1 and OMA-2 are expressed only in the adult with very little, if any, expression detectable in L1 through L4 larval stages (data not shown). The predominant developmental difference between an L4 animal and an adult is the differentiation of the germline. To examine the expression of OMA-1 and OMA-2 in the adult germline, we prepared extracts from three temperature-sensitive mutant strains that are defective in different aspects of germline development (Figure 3B). At the permissive temperature (15°C) in all three strains, both OMA-1 and OMA-2 are expressed. At the nonpermissive temperature (25°C), *glp-4(bn2)* mutant worms do not produce a germline (Beanan and Strome, 1992) and do not express either OMA-1 or OMA-2. *fem-3(e2006)* mutant worms produce only oocytes and no sperm at 25°C (Hodgkin, 1986) and express both OMA-1 and OMA-2. Semidominant mutants *fem-3(q20sd)* at 25°C produce only sperm and no oocytes (Barton et al., 1987) and do not express either OMA-1 or OMA-2. We conclude that OMA-1 and OMA-2 proteins are expressed primarily in adult oocytes but not in sperm, consistent with the functional assay.

We also carried out immunofluorescence to analyze expression of OMA-1 and OMA-2 in the adult gonad (Figure 3C). OMA-1 expression is detectable prior to oocyte cellularization, whereas OMA-2 is expressed only in fully cellularized oocytes. In both cases, expression is cytoplasmic and increases as the oocytes develop, peaking in the −1 maturing oocyte. Neither OMA-1 nor OMA-2 expression is detected in sperm or

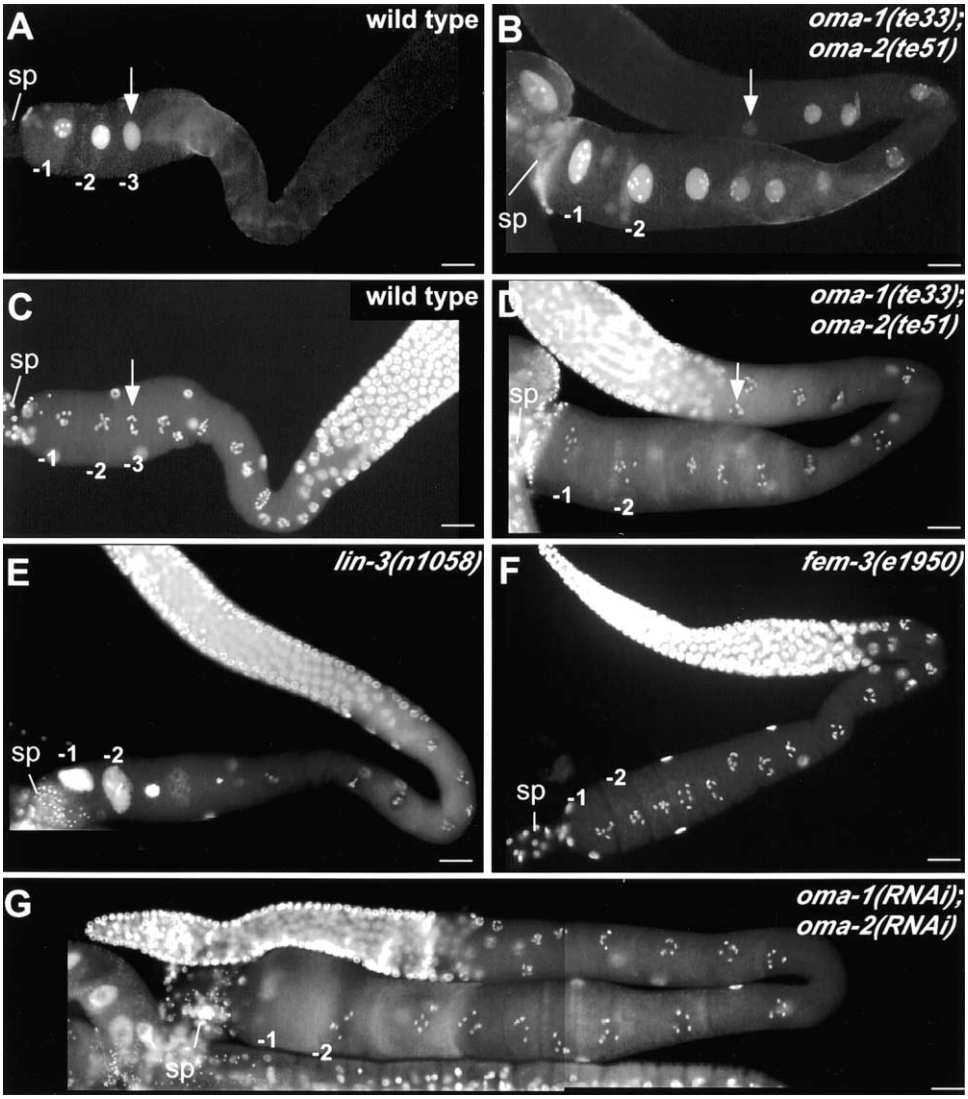

Figure 4. Oocytes Are Arrested at Diakinesis in Oma Gonads

Dissected gonads from wild-type (C), *oma-1(te33);oma-2(te51)* (D), *lin-3(n1058)* (E), *fem-3(e1950)* (F), and *oma-1(RNAi);oma-2(RNAi)* (G) were stained with DAPI. Wild-type (A) and Oma (B) gonads were also stained with the H3P antibody. Arrows in (A)–(D) point to the most distal oocytes with detectable H3P staining. sp, spermatheca. The scale bar represents 10 μm.

the spermatheca, nor are they detected in *oma-1(te33);oma-2(te51)* mutant oocytes (data not shown). The expression of both OMA-1 and OMA-2 continues in the 1-cell embryo, but neither is detected in 2-cell or older embryos (data not shown). The restricted and overlapping expression profiles of OMA-1 and OMA-2 in the proximal oocytes strongly support the suggestion that OMA-1 and OMA-2 function redundantly in oocytes and do not play a role in sperm development.

Oma Oocytes Initiate but Fail to Complete Maturation

Several aspects of late oogenesis, including increased nuclear and cellular volume, the disappearance of nucleoli, and nuclear migration (McCarter et al., 1999), appear normal in Oma animals (Figures 2C and 2D and data not shown). To determine the developmental stage at which oocytes in Oma gonads arrest, we used an antibody to phosphorylated histone H3 (H3P). In a wild-type gonad, only the most proximal three to four oocytes stain with the H3P antibody (Figure 4A; Hsu et al., 2000). In Oma gonads, all proximal to distal oocyte nuclei have detectable H3P staining (Figure 4B), suggesting that all Oma oocytes are at a developmental stage between the −4 oocyte and a fully mature oocyte.

C. elegans oocyte maturation has not been previously characterized genetically, but has been examined in detail morphologically in live animals (McCarter et al., 1999). Nuclear envelope breakdown (NEBD) and cortical rearrangement, two visible, sperm-dependent events, occur in the last 6 min of the 23-min oocyte maturation cycle. NEBD initiates in the −1 oocyte 6 min prior to

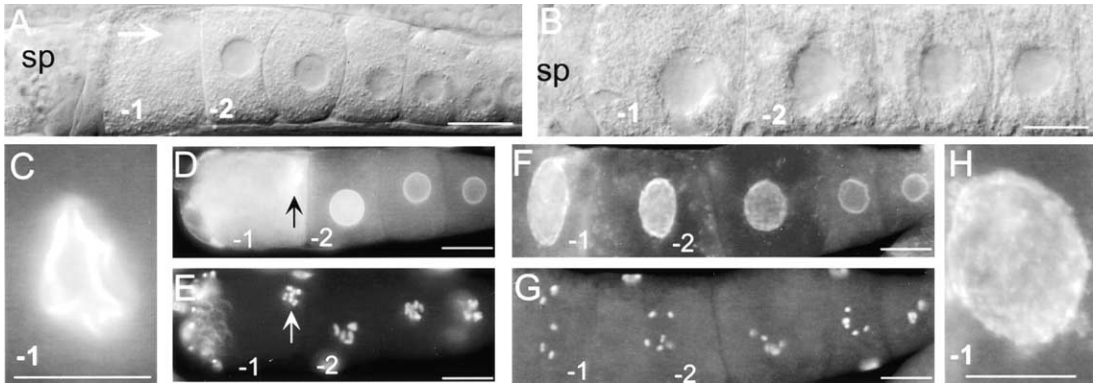

Figure 5. Comparison of Nuclear Envelope in Wild-Type and Oma Oocytes

(A and B) Close up micrographs of live wild-type (A) and *oma-1(te33);oma-2(te51)* (B) proximal gonads.

The arrows in (A), (D), and (E) point to nuclei in the −1 oocyte. Note that the −1 oocyte in (A) and (D) has initiated NEBD and that chromosomes in (E) have compacted further to form a tight cluster, whereas chromosomes in Oma oocytes remain scattered (F and G).

(C and H) High magnification of wild-type (C) and Oma (H) −1 oocyte nuclear lamin staining.

In wild-type, the lamin staining becomes wrinkled (C) then cytoplasmic (D) upon oocyte maturation. sp, spermatheca. The scale bar represents 10 μm.

fertilization, and is characterized by a decrease in the definition of the nuclear boundary under Nomarski optics (Figure 5A, arrow). Cortical rearrangement, occurring 3 min after the initiation of NEBD, results in a shape change of the oocyte from cylindrical to spherical (Figures 2A and 2B). Well-developed wild-type oocytes are in diakinesis of prophase I and contain six bivalents scattered in the nucleus (Figure 4C). During late-stage meiotic maturation, chromosomal bivalents undergo further compaction and congression (clustering; Figure 5E; Albertson and Thomson, 1993).

The morphology of Oma oocytes suggests that some events associated with oocyte maturation have been initiated. First, some Oma oocytes are spherical, suggesting that they have begun cortical rearrangement (Figures 2C and 2D). Second, the nuclear envelope of Oma oocytes appeared poorly defined under Nomarski optics, reminiscent of nuclei in wild-type −1 oocytes that have initiated NEBD. No molecular markers specific for cortical rearrangement are as yet available, and therefore we were unable to examine this change molecularly. We did, however, examine the nuclear envelope morphology using antibodies to three *C. elegans* nuclear envelope components, B-type lamin, nuclear envelop complex, and Ce-emerin (Lee et al., 2000; Liu et al., 2000). B-type lamin staining revealed the most pronounced change and is shown here, although all three antibodies yielded similar results. In wild-type gonads, lamin staining of the nuclear envelope was uniformly detected in all germ nuclei except when the −1 oocyte initiated NEBD (Figure 5D). At that time, the nuclear staining took on a wrinkled appearance, coincident with further compaction of the chromosomes and an increase in cytoplasmic staining (Figures 5C and 5E). Nuclear lamin staining of the −1 oocyte was not detected in some animals, presumably due to the completion of NEBD. By comparison, lamin staining was irregular and punctate in most Oma oocytes, although uniform staining was still observed in distal germ nuclei in Oma gonads (Figures 5F and 5H). We never observed stained Oma nuclei that had a wrinkled appearance, nor did we observe Oma oocytes without nuclear lamin staining. We conclude that the nuclear envelope composition is altered in Oma oocytes, and favor the notion that NEBD initiates but does not complete. However, we can not exclude the possibility that the observed phenotype is due to a change in the nuclear membrane unrelated to NEBD.

Normally, no DNA rereplication occurs in wild-type oocytes. However, if oocytes mature but remain in the gonad due to a defect in ovulation, they can replicate their DNA (endoduplication) and become polyploid. This phenomenon has been demonstrated in ovulation-defective mutants such as *emo-1(oz1)* and *lin-3(n1058)* (Figure 4E and data not shown; Ferguson and Horvitz, 1985; Iwasaki et al., 1996; Clandinin et al., 1998). Oocytes in *oma-1(te33);oma-2(te51)* animals were not observed to undergo endoduplication (n = 200 gonads), and contained six bivalents (Figure 4D) similar to wild-type oocytes, consistent with the conclusion above that they fail to complete the maturation process. However, in old Oma animals (3–4 day adults), we often observed oocytes with congressed chromosomes and occasionally observed rereplicated DNA. It is possible that *te51* is not a true null, and therefore some old oocytes do escape arrest. Alternatively, it is possible that *C. elegans* oocytes in general have difficulty maintaining an arrest indefinitely.

Taken together, our results suggest that Oma oocytes initiate oocyte maturation but fail to complete the maturation process. The observed failure of Oma oocytes to undergo ovulation is likely a consequence of this defect in oocyte maturation. This demonstrates that ovulation in *C. elegans* is dependent on oocyte maturation.

Oma Phenotype Is Dependent on Sperm

Oocytes in female (e.g., *fem-3* or *tra-2*) animals are also arrested at diakinesis of prophase I (Figure 4F), but are distinct from Oma oocytes in the following ways. First, oocytes in females appear thin, cylindrical, and tightly

packed (Figure 2E). Oma oocytes, on the other hand, are abnormally large and do not appear tightly packed (Figures 2C and 2D). Second, oocytes in female worms undergo stochastic, sperm-independent maturation at a rate that is approximately 1/40 that of the wild-type oocyte maturation rate. This stochastic maturation event releases mature but unfertilized oocytes to the uterus (McCarter et al., 1999). Oma oocytes have not been observed to undergo stochastic maturation or ovulation (n = 300). Third, and most important, female oocytes can be fertilized to produce normal viable embryos in the presence of exogenous sperm, whereas Oma oocytes cannot, suggesting an inherent defect in Oma oocytes that prevents them from being fertilized. We will refer to the developmental block observed in Oma oocytes as Oma prophase arrest to distinguish it from female prophase arrest.

We examined whether the phenotypic difference between Oma and female oocytes is a result of the presence or absence of sperm by comparing oocytes in *oma-1(te33);oma-2(te51)*, *oma-1(te33);oma-2(te51);tra-2(q122gf)/+*, and *tra-2(q122gf)/+* animals. *tra-2(q122gf)* is a dominant gain-of-function mutation that feminizes hermaphrodites to develop into spermless females. We showed that oocytes in Oma Tra triple mutant females (Figure 2G) resemble those in *tra-2/+* female animals (Figure 2E) in that they are tightly packed, cylindrical, and have a well-defined nuclear morphology and a smooth lamin staining (data not shown). Unlike *tra-2* oocytes, the oocytes in the triple mutant could not be fertilized when exogenous sperm was introduced via mating. However, in the presence of sperm, oocytes in triple mutants continued to develop and became indistinguishable from oocytes in *oma-1(te33);oma-2(te51)* double mutants (Figures 2D and 2F and data not shown for lamin staining). This result demonstrates that the Oma phenotype is dependent on sperm, consistent with the conclusion above that Oma oocytes have initiated maturation.

In triple mutants, we never observed rereplication of oocyte DNA but we did observe a high number of nuclei with congressed chromosomes, and the number increased as the worms aged, suggesting that in *oma-1;oma-2* mutants, sperm is not required for chromosome congression. We also observed that the triple mutant oocytes are consistently bigger than female oocytes, suggesting that OMA-1 and OMA-2 have an additional function in repressing oocyte growth independently of sperm (see Discussion).

Two Sperm-Dependent Events Do Not Occur in the Oma Gonad

In wild-type *C. elegans*, the MAP kinase, MPK-1, is activated in germ nuclei arrested in pachytene and in proximal oocytes (Figure 6A), as detected by an antibody directed against activated MAP kinase (MAPK-YT antibody; Church et al., 1995; Yung et al., 1997; Miller et al., 2001). MAPK-YT staining peaks in the −1 oocyte, and this high level of staining is dependent on the presence of sperm. In *fem-3* mutants, MAPK-YT staining is not detected in proximal oocytes but is often detected at a low level in one or two distal oocytes (Figure 6C, arrowhead). *oma-1;oma-2* mutant worms have a similar MAPK-YT staining pattern (Figure 6B). However, this absence of MAPK-YT staining in Oma mutants is age dependent. In young adults producing their first few oocytes, MAPK-YT staining is clearly detectable in these oocytes (data not shown). In both Fem and Oma gonads, MAPK-YT staining in the pachytene zone is unaffected. From these results, we conclude that the maintenance of activated MPK-1 in proximal oocytes, a sperm-dependent event, requires either wild-type *oma-1* or *oma-2* activity. We also demonstrate, however, that the initial sperm signal for MPK-1 activation has been received by Oma oocytes.

A second molecular event known to occur during oocyte maturation is the association with chromatin of an aurora-like kinase, AIR-2, specifically in the −1 oocyte (Figure 6G; Schumacher et al., 1998). AIR-2 chromatin association coincides with the congression of chromosomes and only occurs in the last 4–6 min of the 23-min maturation cycle. In *fem-3* mutants, AIR-2 remains cytoplasmic and does not bind to chromatin (Figure 6I; Schumacher et al., 1998). The AIR-2 homolog in *Xenopus*, Eg2, has been shown to play an important role in oocyte maturation (Mendez et al., 2000). However, despite the sequence similarity with Eg2 and the interesting localization pattern coincident with oocyte maturation, there is no evidence that *C. elegans* AIR-2 plays a role in oocyte maturation because *air-2(RNAi)* embryos do mature, ovulate, and get fertilized. We examined AIR-2 localization in Oma gonads and detected only faint cytoplasmic AIR-2 staining in all oocytes, indicating that *oma-1* and *oma-2* are redundantly required for the association of AIR-2 with chromatin in the −1 oocyte (Figure 6H).

Because two sperm-dependent events do not occur in Oma double mutant animals, we conclude that wild-type *oma-1* or *oma-2* activity is required for the oocyte to execute certain downstream events upon sperm signaling.

Oma Prophase Arrest Can Be Suppressed by *wee-1.3(RNAi)*

In *Xenopus* and other animals, meiotic prophase arrest is thought to require a WEE-1-like kinase, MYT-1. When MYT-1 activity is removed in *Xenopus* oocytes, maturation is induced, presumably by subsequent activation of CDC2/MPF activity (Nakajo et al., 2000). In *C. elegans*, it is not clear whether a CDC2-like kinase function is required for oocyte maturation (Boxem et al., 1999; see Discussion). It has been shown, however, that a *C. elegans* MYT-1-like kinase, WEE-1.3, might play a role in oocyte maturation, as germline development is affected when *wee-1.3* is removed by RNAi (M. Kosinski and A. Golden, personal communication; see below). We tested whether Oma prophase arrest could be suppressed if the *C. elegans* MYT1-like protein was removed.

When wild-type worms were fed *wee-1.3(RNAi)* bacteria, NEBD and ovulation (without fertilization) occurred more often and with increased irregularity (see Experimental Procedures) compared to non-RNAi animals. NEBD was observed to occur sometimes in more distal oocytes, in addition to the −1 oocyte, and was accompanied by the further compaction of the six bivalents,

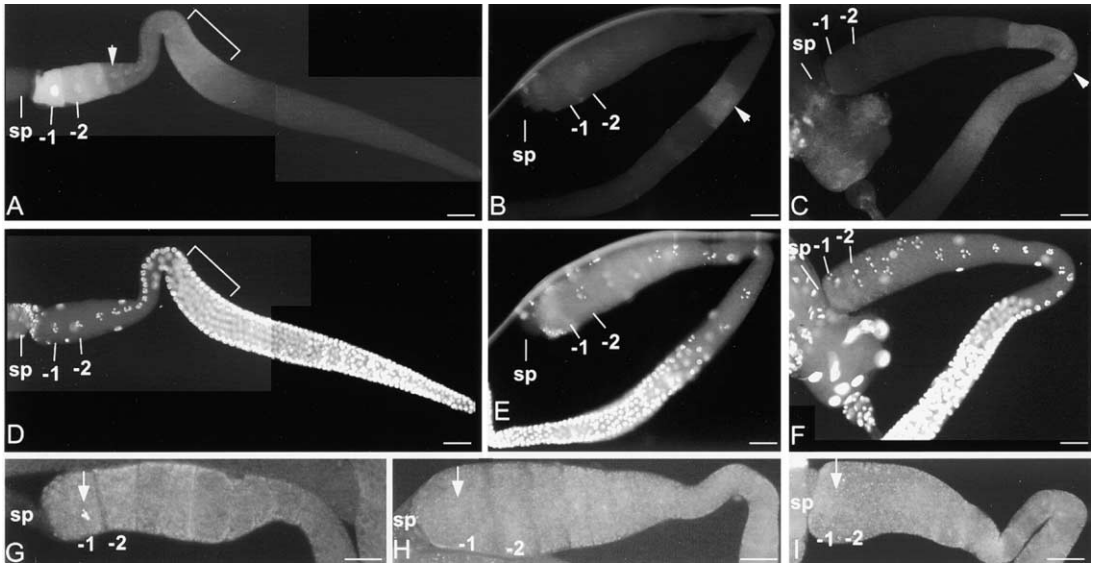

Figure 6. Two Sperm-Dependent Events Do Not Occur in Oma Gonads

Gonads dissected from wild-type (A, D, and G), *oma-1(te33);oma-2(te51)* (B, E, and H), or *fem-3(e1950)* (C, F, and I) are stained with MAPK-YT antibody (A–C), DAPI (D–F), or AIR-2 antibody (G–I). Brackets in (A) and (D) indicate the pachytene zone where MAP kinase is also activated to a low degree. Arrowheads in (A)–(C) point to weak MAPK-YT staining in distal oocytes. Arrows in (G)–(I) point to the position of −1 oocyte nuclei. Neither Oma nor Fem-3 gonads show chromosomal association of AIR-2. sp, spermatheca. The scale bar represents 10 μm.

as monitored by H2B-GFP. Ovulated oocytes accumulated in the uterus, where they were eventually compressed and severely damaged (Figure 7A). By 28 hr after RNAi initiation, ovulation ceased in most animals and the uterus was completely filled with a mass of damaged oocytes, presumably preventing new oocytes from being ovulated. Newly developed oocytes continued to undergo NEBD but remained in the gonad and became polyploid (Figure 7C). These results suggest that *C. elegans wee-1.3* plays a role in negative regulation of NEBD, and likely prophase arrest.

Recall that *oma-1;oma-2* mutant worms do not ovulate nor do they have polyploid oocytes (n = 300). Twenty-one hr after being fed *wee-1.3* RNAi bacteria, ovulated Oma oocytes were observed in the uterus (Figure 7B). These ovulated oocytes were not fertilized, and were eventually compressed and damaged in the uterus, similar to what was observed for wild-type *wee-1.3(RNAi)* worms. By 28 hr, the uterus became full, ovulation stopped, and some proximal oocytes became polyploid. Because Oma prophase arrest in *oma-1;oma-2* animals can be released by removing *wee-1.3* activity, this result supports the conclusion with wild-type worms that *C. elegans wee-1.3* functions as a negative regulator of prophase progression. In addition, this result suggests that OMA-1 and OMA-2 either function upstream of WEE-1.3 or in parallel with WEE-1.3 as positive regulators of prophase progression.

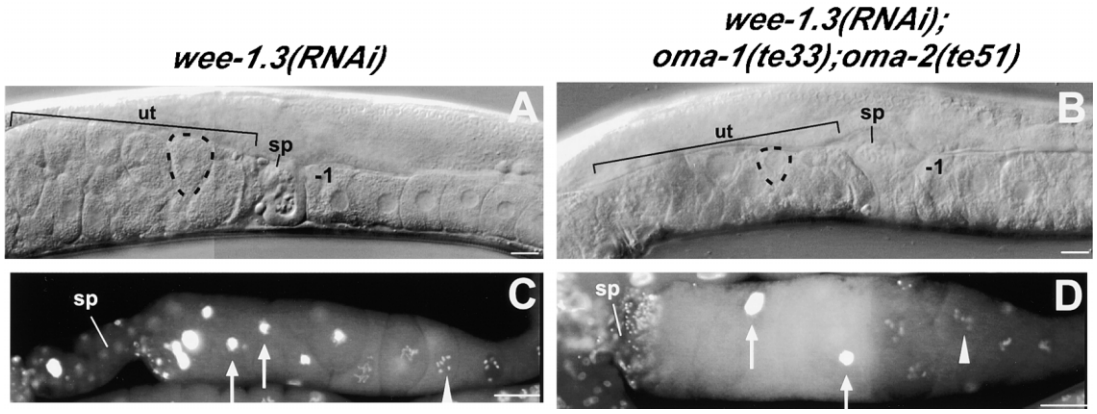

Figure 7. *wee-1.3(RNAi)* Suppresses Oma Prophase Arrest

Live micrographs (A and B) or DAPI staining (C and D) of wild-type (A and C) or *oma-1(te33);oma-2(te51)* (B and D) animals fed *wee-1.3* dsRNA-producing bacteria. Brackets point to the uterus. One oocyte from each uterus is outlined with a dashed line. Arrows point to polyploid nuclei in the gonad arm, and arrowheads indicate nuclei remaining in diakinesis with six chromosomal bivalents. ut, uterus; sp, spermatheca. The scale bar represents 10 μm.

Discussion

We report here the identification of two TIS11 finger proteins, OMA-1 and OMA-2, that function redundantly during oocyte maturation in *C. elegans* using genetic mutants specific to this process. We conclude from our characterization that Oma worms initiate oocyte maturation but are defective in its completion for the following four reasons. (1) Events that normally occur during late oogenesis appear to occur properly in Oma oocytes. (2) Chromosomes in Oma oocytes remain diakinetic, but two events associated with oocyte maturation, NEBD and cortical rearrangement, appear to initiate in Oma oocytes. (3) The Oma phenotype is dependent on the presence of sperm. In the absence of sperm, oocytes in *oma-1;oma-2* animals resemble those in female animals. (4) Maintenance of MPK-1 kinase activation and the association of AIR-2 with chromosomes, two sperm-dependent events, do not occur in Oma oocytes, and yet Oma sperm are functional. We conclude that OMA-1 and OMA-2 are not required for the initial reception of the sperm signal, but instead are required for correct processing of that signal for oocyte maturation.

TIS11 Finger-Containing Proteins

Five TIS11 finger proteins, MEX-1, PIE-1, POS-1, MEX-5, and MEX-6, have been characterized genetically in *C. elegans* for their roles in early blastomere fate determination (Mello et al., 1996; Guedes and Priess, 1997; Tabara et al., 1999; Schubert et al., 2000). With the exception of PIE-1, these TIS11 finger proteins are all cytoplasmic. PIE-1 is nuclear as well as cytoplasmic. Consistent with their cytoplasmic localization, it has been suggested that they regulate cell fates via RNA targets (Bai and Tolias, 1996; Barabino et al., 1997; Lai et al., 1999). In fact, it is likely that most TIS11 zinc finger proteins function by regulating RNA targets. Mouse TIS11 has been shown to bind directly to an AU-rich element in the TNFα 3′ UTR, leading to depolyadenylation and destabilization of the message (Lai et al., 1999). In *Drosophila*, the TIS11 finger protein Clipper functions as a double-stranded RNase that mediates the degradation of RNA hairpin structures (Bai and Tolias, 1996). Therefore, given their cytoplasmic localization, it is reasonable that OMA-1 and OMA-2 function by regulating target RNA(s). An alternative, but not exclusive, possibility is that OMA-1 and OMA-2 function via interaction with each other or another protein(s). This notion is supported by our observation that the gain-of-function mutation *zu405* can be rescued by introducing wild-type *oma-1* genes, consistent with a protein-protein interaction.

Possible Roles for OMA-1 and OMA-2 in Oocyte Maturation

Our data suggest that OMA-1 and OMA-2 function as redundant positive regulators of a key step downstream of sperm signal in oocyte cytoplasmic maturation required for prophase progression. In the absence of OMA-1 and OMA-2 function, meiotic progression is blocked. Our result with *wee-1.3* suppression suggests that OMA-1 and OMA-2 could function upstream of the conserved kinase WEE-1.3 and, likely, MPF. Alternatively, it is also possible that OMA-1 and OMA-2 regulate oocyte maturation through a yet unknown pathway parallel to the WEE-1.3/MPF function.

The functional redundancy of OMA-1 and OMA-2 suggests either that they regulate the same target, or that they each function in two redundant pathways leading to nuclear maturation. As yet, we are unable to distinguish between these two possibilities. OMA-1 and OMA-2, by analogy to other TIS11 finger proteins, are likely to function by binding to and regulating an RNA target. Two models present themselves: in the first, an RNA that is a negative regulator of oocyte maturation is in turn negatively regulated by OMA-1 and OMA-2 binding. In the absence of OMA-1 and OMA-2, the negative regulator is abundant and oocyte maturation is inhibited. As the oocyte develops and OMA-1 and OMA-2 levels continue to increase, the level of this negative regulator decreases, triggering maturation in the −1 oocyte. One possible candidate target for OMA-1 and OMA-2 proteins in this scenario would be the *wee-1.3* mRNA. This possibility is currently under investigation. In the second model, OMA-1 and OMA-2 positively regulate an RNA target essential for oocyte maturation. No obvious candidate target RNAs for this model have presented themselves as yet.

As is the case for many vertebrates, most maternally supplied mRNAs in *C. elegans* are dormant in the gonads, but are made available for translation later during embryogenesis (Evans et al., 1994; Hunter and Kenyon, 1996). Inappropriate temporal translation of some of these dormant mRNAs in immature oocytes has been shown to disrupt normal embryogenesis (Hunter and Kenyon, 1996). Activation of masked maternal mRNAs in several systems involves regulation at the level of transcript 3′ polyadenylation (see review in Richter, 1999). The addition of a 3′ poly A tail is regulated by the cytoplasmic polyadenylation element binding (CPEB) protein. Four CPEB homologs have been identified in *C. elegans* from the genome sequence and all four have been shown by RNAi, either alone or in all combinations, to be dispensable for oogenesis and oocyte maturation (Luitjens et al., 2000). Therefore, either the CPEB RNAi does not completely inactivate the function of these genes or an alternative mechanism, possibly involving OMA-1 and OMA-2, is used in *C. elegans* for the activation of maternally supplied masked mRNA. Indeed, several maternal mRNAs, such as *mex-1*, *mex-5*, and *skn-1*, are first translated in the 1-cell embryo (Bowerman et al., 1993; Guedes and Priess, 1997; Schubert et al., 2000), coincident with the degradation of OMA-1 and OMA-2 proteins. This raises the possibility that removal of OMA-1 and OMA-2 could lead to the inappropriate translational activation of these maternal messages. We examined by immunofluorescence whether any of these three proteins were inappropriately translated in double mutant Oma oocytes and saw no indication of ectopic translation (R.L., unpublished data). We believe, therefore, that OMA-1 and OMA-2 do not function as global translational repressors for maternal messages in the gonad. Instead, we believe that OMA-1 and OMA-2 regulate specific RNA or protein target(s) in the gonad that function in oocyte maturation. The nature of these potential targets is currently under investigation.

Size Control of Oocytes in *C. elegans*

Prophase-arrested oocytes in female (e.g., *fem-3*) worms do not grow beyond the size of a wild-type −1 oocyte, suggesting a size control mechanism for *C. elegans* oocytes in the absence of sperm. Oma oocytes, on the contrary, despite the arrest, continue to grow, sometimes becoming up to ten times as large in volume as wild-type −1 oocytes. The striking lack of growth control in Oma oocytes suggests the following possibilities. First, OMA-1 and OMA-2 function, directly or indirectly, in meiotic cell size control, in addition to oocyte maturation. This is consistent with our observation that in the absence of sperm, Oma oocytes are still noticeably bigger than oocytes in female worms. This possibility is intriguing because a TIS11 finger-containing protein has been implicated in cell size control. A *Drosophila* TIS11-like gene was isolated as a high copy number suppressor for the metaphase arrest phenotype of *cdr1-34;cdc25-22* double mutant fission yeast (Warbrick and Glover, 1994). The *S. pombe* gene *cdr1* is believed to function upstream of *wee1* to relieve mitotic inhibition, and mutations in *cdr1* affect mitotic size control (Feilotter et al., 1991). An alternative possibility is that oocyte cellular growth, although requiring sperm, is independent of meiotic maturation in *C. elegans*. In this model, oocyte maturation requires the oocyte to grow to a certain size, but arresting at maturation does not block normal growth. Consistent with this notion, elegant studies in yeast have shown that blocking mitotic cell cycle progression does not prevent cellular growth, but growth impediment results in cell cycle arrest (Nurse, 1975).

MPF and Oocyte Maturation in *C. elegans*

Despite differences in how MPF is activated in different animals, it appears to play a key role in oocyte maturation in most organisms examined. However, it is not clear in *C. elegans* what roles, if any, MPF plays in oocyte maturation. Genome sequencing reveals several CDC2-like kinases in *C. elegans*, of which NCC-1 appears to be the only one required for meiotic division (Boxem et al., 1999). Inactivation of the *ncc-1* gene by RNAi prevents the completion of meiotic divisions. However, oocytes in *ncc-1(RNAi)* animals do mature, ovulate, and become fertilized to form defective 1-cell embryos. There are two likely explanations for the lack of a maturation defect in *ncc-1(RNAi)* embryos. First, NCC-1 may have a function in *C. elegans* oocyte maturation similar to that of CDC2 in other animals, but *ncc-1(RNAi)* results in an incomplete inactivation of *ncc-1* in the gonad. Genetic *ncc-1* mutations result in larval lethality and therefore their effect on oocyte maturation can not be assayed (Boxem et al., 1999). Second, it is possible that *C. elegans* NCC-1 has a function in meiotic divisions, but does not play a role during oocyte maturation. Because both meiotic divisions in *C. elegans* are completed after fertilization, this germline uncouples the molecular events of oocyte maturation from those of completion of the meiotic cell divisions. This provides a unique opportunity to dissect the roles of known key players in oocyte maturation as it uncouples the molecular events of oocyte maturation from those of completion of the meiotic cell divisions. We believe our findings on OMA-1 and OMA-2 will greatly facilitate further genetic analysis and will help uncover new players in this developmental process.

Experimental Procedures

Strains, Alleles, and Genetic Analyses

The Bristol strain N2 was used in this study as the standard wild-type strain. The genetic markers used in this paper are listed by chromosome as follows: LGI: *glp-4(bn2ts).* LGII: *tra-2(q122gf).* LGIV: *unc-24(e138)*, *fem-3(e1950, e2006ts, q20sd,ts)*, *lin-3(n1058)*, DnT1, *mes-6(bn66)*, *unc-129(ev554).* LGV: *dpy-11(e224)*, DnT1.

zu405 is a temperature-sensitive, semidominant, maternal effect lethal mutation: *zu405/+* and *zu405/zu405* mothers produce 30%–80% and 100% dead embryos, respectively, at 25°C. The mutation was mapped between *mes-6* and *fem-3*, close to *unc-129* on LGIV. Weak rescue of *zu405* embryonic lethality was obtained with cosmid C09G9. A 6-kb genomic fragment containing C09G9.6 and C27B7.1 resulted in clear rescue with 30–50 viable progeny recovered from each of the four rescued lines. *zu405* worms have a single nucleotide change in C09G9.6 but not in C27B7.1. The semidominancy and rescue by RNAi of the *oma-1* gene demonstrate that *zu405* is a gof mutation.

tra-2(q122gf)/+;oma-1(te33);oma-2(te51) triple mutants were generated by crossing *tra-2(q122gf)* males to *oma-1(te33);oma-2(te51)/dnT1* hermaphrodites. The Unc female cross-progeny were then crossed with *oma-1(te33);oma-2(te51)* males. From the second cross, Unc female cross-progeny were backcrossed with *oma-1(te33);oma-2(te51)* males and non-Unc females were *tra-2(q122gf)/+;oma-1(te33);oma-2(te51).*

Unless specified otherwise, all phenotypic characterizations were performed using 1-day-old adult hermaphrodites.

Isolation of *oma-1* and *oma-2* Mutant Alleles

oma-1 lof alleles were isolated in an F1 screen for dominant suppressors of *zu405* embryonic lethality. L4 *zu405* animals were mutagenized with EMS and 18,000 F1s were shifted to 25°C as L2. Twelve independent dominant suppressors were isolated and sequenced, confirming ten *oma-1* lof alleles: *te21*, *te22*, *te26*, *te27*, *te28*, *te30*, *te33*, *te35*, *te36*, and *te41*. We did not detect any mutations in the coding region of the *oma-1* gene in *te31* and *te34*. Using 7704 antibody, OMA-1 protein was not detected in *te22*, *te27*, *te28*, *te30*, *te31*, *te33*, *te34*, *te35*, and *te41*, detected at a reduced level in *te26* and *te36*, and detected at a wild-type level in *te21*.

oma-2 lof alleles were isolated as follows: L4 TX131 *oma-1(te33)*(IV);*dpy-11*(V) animals were mutagenized and F1 hermaphrodites were mated with *oma-1(te33)* males. Seventeen hundred F2 cross-progeny were cloned, and F3 progeny were scored for the segregation of 25% sterile Dpys (*oma-2* and *dpy-11* are on LGV). All sterile Dpys were examined with Nomarski optics to confirm the Oma phenotype. Two recessive alleles, *te50 and te51*, were isolated and sequencing confirmed that both alleles harbor mutations in the *oma-2* gene. *oma-2(te51)* was unlinked from *dpy-11* by picking recombinants occurring in this interval. The size of Oma oocytes were estimated based on the difference in their cell diameters compared to wild-type −1 oocytes.

RNA Interference

Clones used to generate dsRNA are: *oma-2*, yk89e11; *oma-1*, pRL446 (1.5-kb genomic DNA); *wee-1.3*, pRL502 (cDNA from yk14f9 subcloned into the feeding vector pPD129.36). Double-stranded RNAs were prepared and concentrations were estimated as described in Hsu et al. (2000). Approximately 1 mg/ml dsRNA was used for injection. All injections were performed by injecting dsRNA into both gonad arms of either L4 or young adult hermaphrodites (Fire et al., 1998). Feeding RNAi was performed as described in Timmons and Fire (1998) except that L3 larvae were added to bacterial lawns and scored at various time intervals. The timing of NEBD and ovulation was based on visual examination of nonanesthetized worms for a period of 1–2 hr each. Ovulation was scored as release of oocytes to spermatheca (8–47 min/ovulation; $n = 9$), whereas NEBD was scored as change in nuclear definition and dispersal of H2B-GFP into the cytoplasm (9–32 min/NEBD; $n = 12$). NEBD and

ovulation are well coordinated in wild-type animals (21–27 min/cycle; n = 4).

Generation of OMA-1 and OMA-2 Antibodies

Rabbit polyclonal antibody 7704 was raised against the OMA-1 peptide NGENNEKIDEHHLC, whereas antibody 7703 was raised against the OMA-2 peptide ETVPEEQQKPISHIC. Peptides were synthesized (BioSynthesis, Inc.) and injected into two rabbits each according to Waddle et al. (1994; Charles River Co.). The serum was affinity purified using nitrocellulose-bound GST-fusion OMA-1 and OMA-2 proteins as described in Robinson et al. (1988).

Western Blot and Cell Lysates

For each worm strain, ~10,000 adult worms were fixed in −20°C DMF for 1 hr, washed in PBS, resuspended in NEST (100 mM Tris [pH 6.8], 5% SDS, and 5 mM EDTA), sonicated for 3 min, and boiled for 5 min. Each Western blot lane corresponds to ~120 adult worms. The antibody dilutions used were 1:200 for affinity-purified 7703 and 7704, and 1/10,000 for actin C4 antibody (ICN, #69100).

Immunofluorescence and Imaging

Antibodies and dilutions used were: mouse MAPK-YT (Sigma, #M8159), 1:300; rabbit H3P, 1:5000; rabbit AIR-2, 1:200; rabbit Celamin, 1:400; mouse mab414, 1:500; Ce-emerin, 1:100; 7703, 1:50; 7704, undiluted. Gonads were dissected and stained as described in Hsu et al. (2000). All images were analyzed by light microscopy with a Zeiss Axioskop II equipped with epifluorescence and differential interference contrast (DIC) optics. Digital images were acquired and processed using a C5985 chilled CCD camera (Hamamatsu Photonics).

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