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Report

Conserved Functions for Mos in Eumetazoan Oocyte Maturation Revealed by Studies in a Cnidarian

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Summary

The kinase Mos, which activates intracellularly the MAP kinase pathway, is a key regulator of animal oocyte meiotic maturation. In vertebrate and echinoderm models, Mos RNA translation upon oocyte hormonal stimulation mediates "cytostatic" arrest of the egg after meiosis, as well as diverse earlier events [1–5]. Our phylogenetic survey has revealed that MOS genes are conserved in cnidarians and ctenophores, but not found outside the metazoa or in sponges. We demonstrated MAP kinase-mediated cytostatic activity for Mos orthologs from *Pleurobrachia* (ctenophore) and *Clytia* (cnidarian) by RNA injection into *Xenopus* blastomeres. Analyses of endogenous Mos in *Clytia* with morpholino antisense oligonucleotides and pharmacological inhibition demonstrated that Mos/MAP kinase function in postmeiotic arrest is conserved. They also revealed additional roles in spindle formation and positioning, strongly reminiscent of observations in starfish, mouse, and *Xenopus*. Unusually, cnidarians were found to possess multiple Mos paralogs. In *Clytia*, one of two maternally expressed paralogs accounted for the majority MAP kinase activation during maturation, whereas the other may be subject to differential translational regulation and have additional roles. Our findings indicate that Mos appeared early during animal evolution as an oocyte-expressed kinase and functioned ancestrally in regulating core specializations of female meiosis.

Results and Discussion

Mos Is a Highly Conserved Kinase Specific to the Eumetazoa

We recovered candidate MOS sequences from complete genomes of a range of metazoans, choanoflagellates, and fungi, as well as from *Clytia hemisphaerica* (cnidarian) and *Pleurobrachia pileus* (ctenophore) EST collections, and verified their orthology by phylogenetic analysis (Figure 1; data set details and sequence alignments in Table S1 and Figures S1 and S2 available online). All genomes examined from eumetazoan (bilaterian + ctenophore + cnidarian) species, except the nematode *Caenorhabditis elegans*, contained MOS gene orthologs showing well-conserved kinase domains (Figure S3). MOS genes were also identified unambiguously in Placozoa, but not in available sponge, choanoflagellate, or fungi genomes. The absence of MOS in *Amphimedon* (sponge) suggests that this gene may have originated in a common eumetazoan ancestor or was secondarily lost in the sponges.

Unexpectedly, we identified four distinct MOS sequences in the genome of the anthozoan-class cnidarian *Nematostella vectensis* and two in our EST collection from the hydrozoan *Clytia*. The retention in cnidarians of more than one MOS gene after ancestral duplication(s) (Figure 1) contrasts markedly with the situation in bilaterians, in which only single intact genes were found despite well-documented whole-gene duplications [6, 7]. We can speculate that diversification of Mos kinase function in cnidarians accompanied a complex evolutionary history involving changing characteristics of gametogenesis and/or reproduction.

We confirmed that MOS genes from basally diverging eumetazoans encode kinases with conserved activity using the classical assay of injection into of *Xenopus* embryo blastomeres [8]. RNAs for the *Clytia* paralogs CheMos1 and CheMos2 and for *Pleurobrachia* PpiMos all provoked cleavage arrest in *Xenopus* embryos within one or two cell cycles (Figure 2A), with elevated levels of the diphosphorylated (activated) form of p42 MAP kinase (ERK1/2) (Figure 2B). Activation of MAP kinase was consistently less pronounced after injection of CheMos2 than of CheMos1 RNA. Consistently, injection of CheMos1 RNA was as effective as mouse Mos RNA in inducing *Xenopus* oocyte maturation (100% oocytes injected with 0.5 μg/μl RNA), whereas CheMos2 was ineffective at this concentration but caused 50% maturation at 1 μg/μl (groups of 10–15 oocytes injected in parallel). The lower activity of CheMos2 RNA could reflect reduced kinase activity, protein stability, RNA translation, and/or RNA stability.

In oocytes of hydrozoans such as *Clytia*, cytostatic arrest occurs in the G1"pronuclear" stage after completion of both meiotic divisions [9, 10]. CheMos1 and CheMos2 RNAs were thus injected into blastomeres of *Clytia* two-cell-stage embryos to test their ability to mediate this interphase arrest. At 0.5 μg/μl, both RNAs caused rapid cell-cycle arrest with one or two interphase nuclei per cell (Figure 2C) and elevated levels of diphospho MAP kinase (Figure 2D), whereas the non-injected or dextran-injected cells continued to divide normally. Lower concentrations of Mos RNA caused cleavage arrest with multiple nuclei or spindles within a common cytoplasm (not shown), probably reflecting effects of partially elevated MAP kinase activation on microtubule dynamics [11].

Cnidarian Mos Translation Controls Oocyte Cytostatic Arrest and Polar Body Emission

In vertebrates (*Xenopus* and mouse) and starfish, translation of maternally stored Mos RNA during oocyte maturation activates the MAP kinase cascade, and has been shown contribute both to cytostatic arrest (in MII or post-meiotic G1 respectively) and to a variety of preceding meiotic events [4, 5, 12]. In

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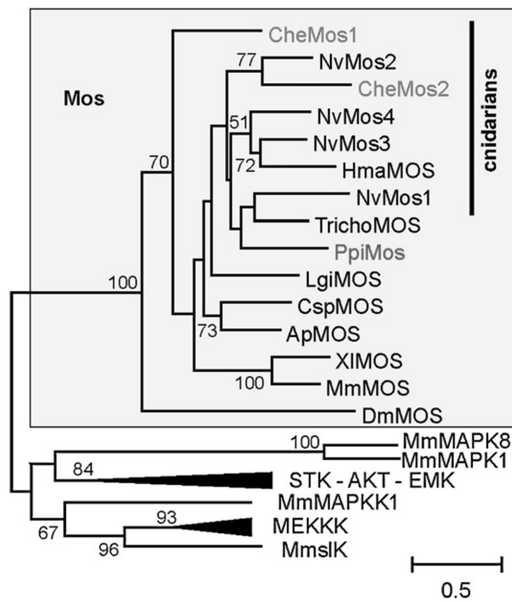

Figure 1. MOS Genes in Basally Diverging Eumetazoans

Phylogenetic analysis of Mos sequences. Bootstrap values over 50% are shown. Abbreviations are as follows: Ap, *Asterina pectinifera*; Csp, *Capitella* sp.; Che, *Clytia hemisphaerica*; Dm, *Drosophila melanogaster*; Hma, *Hydra magnipapillata*; Mm, *Mus musculus*; Nv, *Nematostella vectensis*; Ppi, *Pleurobrachia pileus*; Spu, *Strongylocentrotus purpuratus*; Tricho, *Trichoplax adherens*; and Xl, *Xenopus laevis.* The *Lottia gigentea* Mos sequence strongly perturbed the relationships of the cnidarian Mos sequences and so was excluded. The dispersion of *Nematostella* and *Clytia* MOS sequences indicates at least one ancestral duplication in the cnidarian lineage, with subsequent gene losses accounting for single MOS in *H. magnipapillata.*

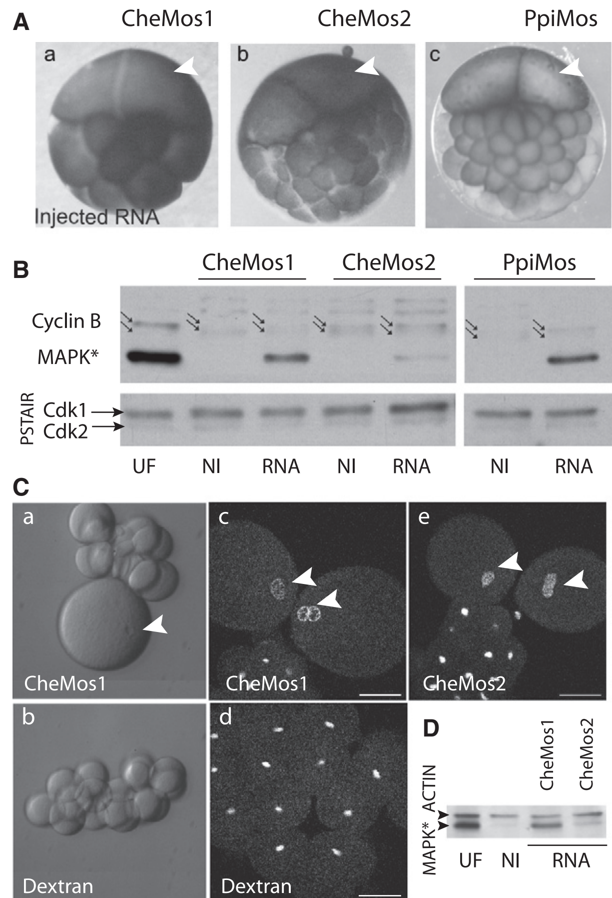

Figure 2. Cytostatic Activity of *Clytia* and *Pleurobrachia* Mos Kinases

(A) *Xenopus* embryos showing blastomere cleavage arrest (indicated by arrowheads) following injection of Mos RNAs as indicated into single blastomeres at the two-cell stage.

(B) Western blot of activated diphospho-MAP kinase (MAPK*) in unfertilised *Xenopus* eggs (UF), noninjected 16- to 32-cell embryos (NI) and cleavage-arrested embryos injected with Mos RNAs after fertilization. Consistent with an arrest in metaphase, Mos-injected embryos showed higher levels of the slower-migrating, mitotically phosphorylated isoform of cyclin B2 than the uninjected controls (indicated by arrows). Cdk1 and Cdk2 kinases (anti-PSTAIR) serve as loading controls.

(C_a and C_b) Living *Clytia* embryos injected with Mos RNA or dextran into one blastomere at the two-cell stage (DIC images).

(C_c–C_e) Hoechst staining of fixed 16-cell-stage embryos injected at the two-cell stage with *CheMos1* (0.25 μg/μl) or *CheMos2* (0.5 μg/μl) RNA or dextran. Mos-RNA-injected blastomeres rapidly stopped dividing, with one or two interphase nuclei (indicated by arrowheads). Scale bars represent 10 μm.

(D) Anti-phospho-MAP kinase (MAPK*) western blot of unfertilized eggs (UF) and 16-cell-stage embryos uninjected (NI) or preinjected with Mos RNAs as described above. Parallel detection with an actin antibody serves as a loading control.

hydrozoans, MAP Kinase activity is known to be involved in this G1 arrest, but the role of endogenous Mos has not been addressed [10]. *Clytia* is well suited for experimental analysis of oogenesis and oocyte maturation. The transparent gonads can undergo autonomous daily cycles of growth and spawning in culture, providing access to oocytes during the growth phase [13]. Fully-grown oocytes can be isolated manually and stimulated to undergo maturation by treatment with the cell-permeable cAMP analogue Br-cAMP [13, 14]. This mimics the physiological cAMP rise [15] normally triggered by a factor (peptide) released by the gonad [16] following light stimulus after a dark period (or a light /dark transition in some hydrozoans).

We tested the requirement for Mos translation during oocyte maturation in *Clytia* using morpholino antisense oligonucleotides targeted against the translation initiation codon of CheMos1 (Mos1-MO) and/or CheMos2 (Mos2-MO) RNAs. We first showed that expression of both Mos paralogs was restricted to gonads (Figure S4)—to growing oocytes of all stages in females, and to the peripheral zone of final spermatid differentiation [17] in males. After Br-cAMP stimulation, oocytes preinjected with Mos2-MO underwent all the events of maturation in parallel with controls, but ones injected with Mos1-MO showed marked perturbations (Figure 3). First, although nuclear envelope breakdown (GVBD) occurred on time, most Mos1-MO-injected oocytes showed a complete failure to emit both first and second polar bodies. Nevertheless, "M-phase" cytoplasmic movements were consistently observed in parallel at the time of meiosis I and II, followed by disorganized cortical contractions in parallel with a second polar body emission in controls (Figures 3A and 3B; Movie S1). The second major phenotype was the absence of a cytostatic arrest, as manifest by a series of cortical contractions after the end of the normal maturation period. Coinjection of Mos2-MO with Mos1-MO consistently increased the severity of the phenotypes, with complete cell divisions rather than abortive "pseudocleavages" observed more frequently (Figures 3A and 3B; Movie S1 and Table S2).

Cytostatic arrest could be restored in Mos1-MO-injected oocytes by introduction of CheMos1 ORF RNA (lacking the MO target site) after the onset of maturation, demonstrating

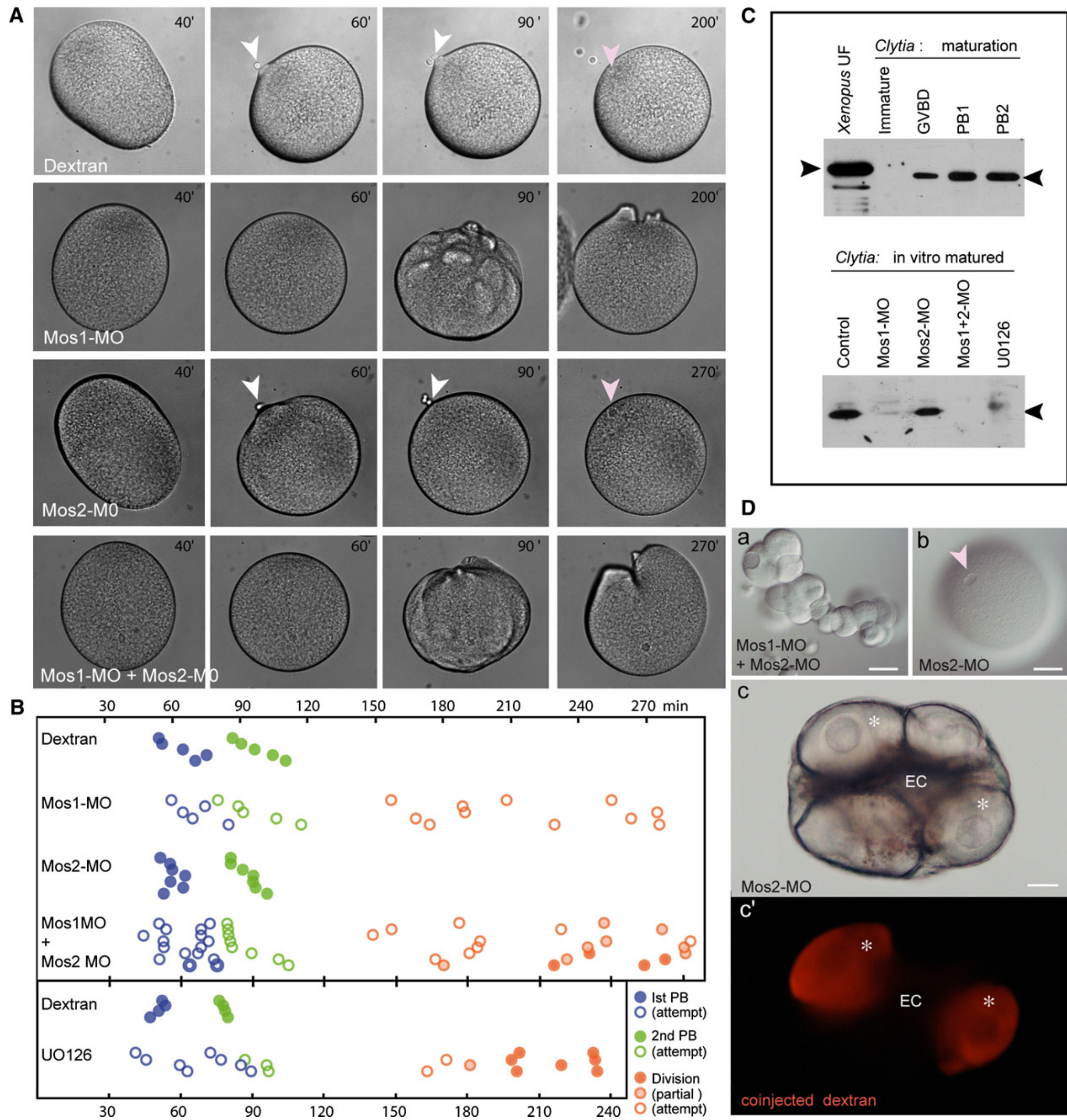

Figure 3. CheMos1 Synthesis Is Required for Postmeiotic Arrest

(A) Images from time-lapse recordings of *Clytia* oocytes injected with anti-Mos morpholinos prior to cAMP induced maturation (see Movie S1). Times post-Br-cAMP are indicated. Mos1-MO oocytes show visible contractions in parallel with polar body formation in dextran- and Mos2-MO-injected oocytes (indicated by white arrowheads), and cytostatic arrest failure manifest as repeated furrowing after meiosis completion. Pink arrowheads indicate arrested nuclei.
(B) Timing of maturation events after MO injection as in (A) (top panel; Movie S1) or U0126 treatment to prevent MAPK activation (bottom panel; Movie S2). Each row of circles represents an individual, filmed oocyte. Filled circles indicate the onset of first polar body (PB) emission, second PB emission, or cell division as indicated by their colors (see inset). Open circles indicate abortive contraction movements. Mos1-MO + Mos2-MO- and U0126-treated oocytes showed two indistinct movements (blue circles) during first meiosis. Division attempts started at the predicted time of first division in an egg fertilized at ovulation (120 min maturation + 50 min mitotic cycle). Pale orange represents regressed cleavage furrows; dark orange represents complete divisions.
(C) Anti-diphospho-MAPK western blot of groups of ten *Clytia* oocytes harvested at successive maturation stages (top), or 2 hr after Br-cAMP treatment, following injection of 1 mM Mos1-MO, Mos 2-MO, or both morpholinos or treated with 10 μM U0126 (bottom). Side arrows indicate activated MAP kinase in *Clytia* (right) and in *Xenopus* unfertilized eggs (left).
(D) Phenotypes resulting from injection of morpholinos into growing oocytes within isolated gonads 3–5 hr after the previous spawning and cultured through a dark period to induce ovulation and maturation (quantification in Table S3). (D_a) shows a Mos1-MO + Mos2-MO-injected oocyte, which underwent multiple divisions after ovulation. (D_b) shows an ovulated oocyte that was injected with Mos2-MO and that showed a typical bipartate female pronucleus (indicated by a pink arrowhead). (D_c) shows a Mos2-MO-injected oocyte (*), identified within an isolated gonad by coinjection of rhodamine dextran ($D_{c'}$), which grew to full size but failed to ovulate. EC, endodermal cavity. Scale bars represent 50 μm.

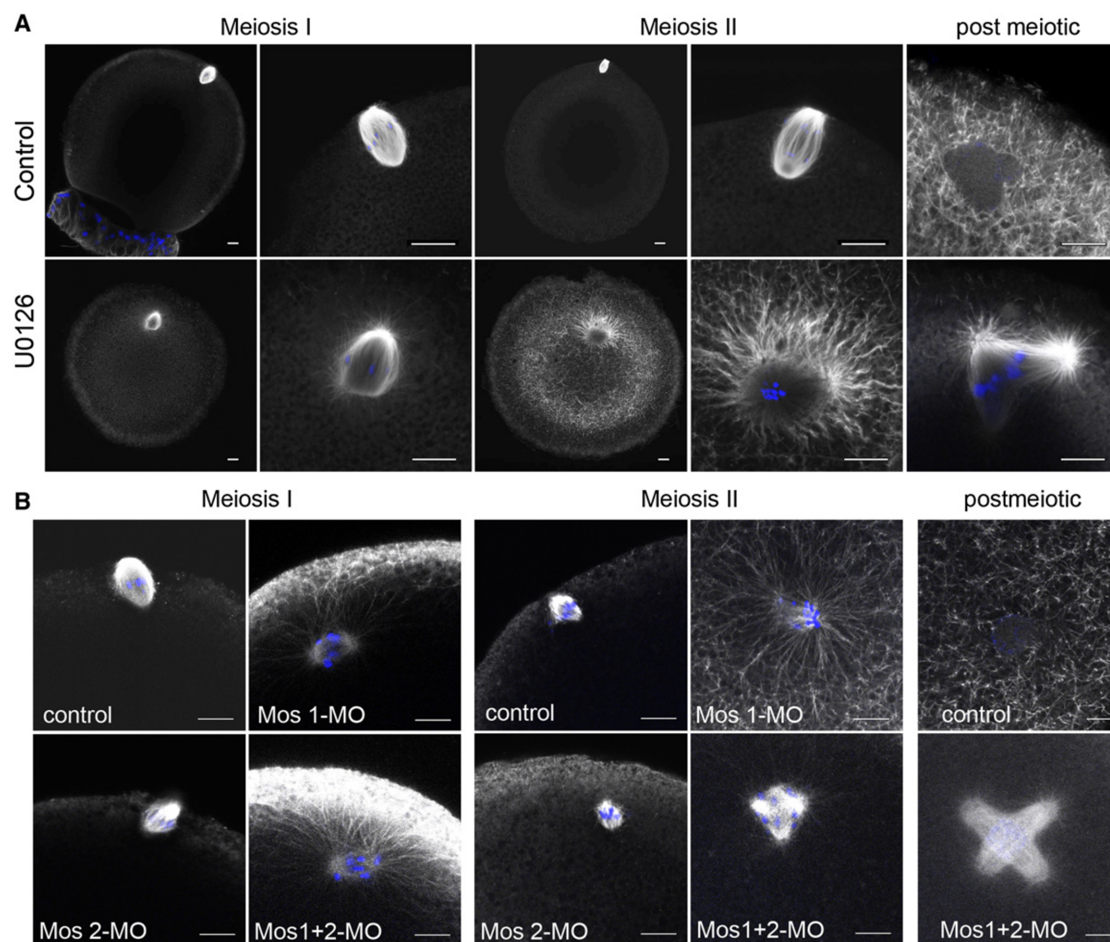

Figure 4. Mos/MAPK Regulated Spindle Formation and Positioning

Confocal images showing antitubulin immunofluorescence of isolated oocytes fixed during Br-cAMP-induced maturation at the times of meiosis I and meiosis II and of first mitosis after spontaneous activation.

(A) An experiment comparing untreated (top) and U0126-treated oocytes (bottom).

(B) An experiment comparing oocytes injected prior to maturation with morpholinos as indicated. Hoechst-stained chromatin overlaid in blue. Scale bars represent 10 μm.

the specificity of the morpholino effect (Movie S4). Furthermore, a transient polar-body-like protrusion was observed in some oocytes, coinciding with second polar body formation in uninjected controls. The specificity of the Mos-MO phenotypes was further confirmed with U0126, a highly specific pharmacological inhibitor of the ERK1/2-activating kinase MEK [18], which produced effects strikingly similar to the double morpholino injection (Figures 3B and 3C; Movie S2). Furthermore, MAP kinase activation during GVBD, which normally attains high levels by the time of first and second polar body emission (Figure 3C, top panel), was completely abolished by either coinjection of oocytes of Mos2-MO + Mos1-MO or by U0126 treatment. Mos1-MO injection alone caused an extensive but incomplete inhibition of MAP kinase activation (Figure 3C, bottom panel). These analyses provide good evidence that the injected Mos2-MO effectively prevents Mos2 translation, despite the absence of an effect on maturation when injected alone, and indicates that the combined translation of CheMos1 and CheMos2 RNA accounts fully for MAP kinase activation during oocyte maturation in *Clytia.* This activation, mainly due to CheMos1, is required for both polar body emission and postmeiotic cytostatic arrest.

Mos/MAP Kinase Mediates Microtubule Organization in Cnidarian Oocytes

We examined microtubule organization in oocytes treated with U0126 or preinjected with Mos morpholinos and fixed during maturation (Figure 4). In U0126-treated oocytes, spindle structures were detected at the time of first meiosis in controls, however they were not associated closely with the cortex, and showed longer and more abundant astral and cytoplasmic microtubules (Figure 4A). At the time of meiosis II, U0126-treated embryos contained extremely abnormal spindle structures, with long microtubules focused at multiple sites radiating around the condensed chromosomes. Oocytes injected with Mos1-MO alone or together with Mos2-MO showed very similar phenotypes, while those injected with Mos2-MO alone were indistinguishable from controls (Figure 4B).

The presence of microtubular structures focused around condensed chromosomes in U0126 and Mos1-MO injected oocytes at the time of both meiosis I and meiosis II, together with the unaffected GVBD and the cortical contractions observed in parallel with polar body formation (Figure 3B), indicate that in *Clytia* as in mouse Mos/MAPK does not regulate the initiation or duration of the meiotic cycles. In line with

this conclusion, injection of CheMos1, CheMos2, or mouse Mos RNAs into immature *Clytia* oocytes did not provoke GVBD, although it did cause aberrant morphological distortion followed by oocyte degeneration (data not shown). In starfish oocytes, meiosis II appears to be absent after Mos inhibition [12]. This may reflect difficulty in detecting the transitory M2 event of starfish oocytes in the absence of polar body emission or may reflect a real distinction in the role of Mos in this echinoderm.

Our data clearly indicate that Mos/MAP kinase activity in *Clytia* oocytes is required for the correct formation and peripheral positioning of the meiotic spindles (Figure 4), as has been described in mouse [5, 19], *Xenopus* [20], and starfish [12] oocytes. *Clytia* oocytes resemble starfish oocytes in that the proximity of the GV to the cortex prior to maturation reduces the distance required for meiotic spindle migration. In starfish, spindle positioning involves a combination of actin and microtubule-mediated processes following GVBD [21], and similar cytoskeletal reorganisations have been detected in *Clytia* [13]. Modulation of actin and microtubule dynamics to assure meiotic spindle formation and positioning may represent an ancient and conserved role of Mos-activated MAP kinase. Another conserved role for Mos/MAP kinase, and its downstream target p90rsk, may be in the reduction of maternal centrosomal material [22], important for preventing cleavage after accidental parthenogenetic egg activation [23]. Thus in *Clytia*, as in starfish [12], Mos/MAP kinase invalidation can lead to parthenogenetic development with complete cleavages and multiple asters and spindle poles during mitotic cycling (Figures 3A, 3B, and 4). In mouse, however, maternal centrosome inactivation does not appear to be Mos dependent, given that monopolar spindles form after spontaneous activation in oocytes from mice lacking Mos [5]. Another proposed conserved function for MAP kinase during oocyte maturation is the suppression of microtubule aster growth on the sperm-derived centrosome during the completion of meiosis [24]. Intriguingly, this function is detectable also in species in which maturation and MAP kinase activation is triggered by fertilization without prior (Mos) translation [24].

Evidence for Additional Periods of CheMos2 Translation

Mos RNA translational activation during maturation in deuterostome models has been well characterized to be precisely regulated by cytoplasmic polyadenylation and other sequence elements in the 3′ UTR [25, 26]. Unusually for a Mos, we detected at the extreme 5′ end of the CheMos2 cDNA a characteristic 5′ TOP (*t*erminal *o*ligo*p*yrimidine) sequence, found in many RNAs translated under control of the growth-associated kinase TOR (*T*arget *o*f *R*apamycin) [27]. In immature *Xenopus* oocytes, TOR-dependent translation of 5′ TOP RNAs appears to contribute to suppressing translation of other RNAs including Mos RNA until hormonal stimulation of maturation [28]. We obtained preliminary evidence for selective TOR-dependent translation of CheMos2 RNA during maturation in *Clytia* by treating maturing oocytes with rapamycin (Movie S3). Rapamycin treatment alone had no effect on polar body formation, but Mos1-MO-injected oocytes matured in rapamycin closely resembled those coinjected with Mos2-MO, by frequently exhibiting complete mitotic cleavages.

To check for an earlier role for CheMos2, we coinjected morpholinos with a fluorescent dextran tracer into growing oocytes through the ectodermal cell layer of isolated gonads [13] (Figure 4D). The efficiency of morpholinos injected was lower than in isolated fully grown oocytes, as attested by the reduced (15%–20%) proportion of spontaneous activation after ovulation in oocytes injected with Mos1-MO or Mos1-MO + Mos2-MO (Figures 3D_a and 3D_b; Table S3), perhaps because of morpholino degradation and/or dilution. Mos2-MO injected into growing oocytes did not cause spontaneous activation (Figure 3D) but did have an inhibitory effect on GVBD and ovulation, with 27% (12/44) of Mos2-MO and 20% (9/46) of double-morpholino-injected oocytes remaining blocked in the gonad with their nuclei intact (Figures 4D_c and $D_{c'}$). This ovulation failure did not seem to result from interference with oocyte growth; 8/8 Mos1-MO-injected oocytes and 8/10 Mos2-MO-injected oocytes monitored reached full size by the time of ovulation, compared with 13/14 in oocytes injected with dextran alone. Mos1-MO did not inhibit maturation, but did appear to affect oocyte release (Table S3), perhaps as a consequence of polar body inhibition and/or the accompanying oocyte polarization [13].

These results suggest that Mos2 translation during the growth period of oogenesis, perhaps under the control of the TOR pathway, may contribute to the subsequent ability of oocytes to mature, for instance contributing to the acquisition of maturation competence. A more effective means of preventing CheMos2 translation during the 15 hr oocyte growth phase, as well as specific tools to monitor levels and activation of the two *Clytia* kinases, will be required to address this possibility.

Mos Evolution in the Eumetazoa

We have provided evidence that MOS genes are highly conserved and specific to the Eumetazoa. Mos kinases should thus be seen not as core regulators of meiosis, which is a much older process, but of a particularity of meiosis in (eu)metazoans. Furthermore, we have shown that in a cnidarian, as in the main bilaterian experimental models, Mos kinases are indispensable for the key oocyte-specific events of asymmetric meiotic spindle positioning and polar body emission, as well as cytostatic arrest, and propose that these functions of Mos are ancestral for Eumetazoa. Mos may also have had an ancestral role in male gametogenesis, given that Mos expression is detectable in developing spermatids in mouse [29] as well as in *Clytia* (Figure S4); however, MOS-deficient male mice show normal fertility with no defects in meiosis or MAPK activation during spermatogenesis [30]. It is particularly interesting that the cytostatic arrest at the end of meiosis in eumetazoan oocytes under the control of the Mos protein appears to be homologous in Eumetazoa despite species-specific differences in the cell-cycle stage and of the Mos targets implicated in this arrest [1]. In vertebrates Mos cooperates with the cyclosome inhibitor Emi2 to prevent MII exit [31, 32], whereas G1 cytostatic arrest in starfish like *Xenopus* but not mouse requires the MAP kinase substrate p90rsk [22]. It would be of interest to test whether p90rsk is implicated in *Clytia* cytostatic arrest.

There is not enough information currently available to decide which innovations of oocyte (or wider germ cell) function during evolution relate to MOS gene evolution. One possibility is anisogamy evolution in the metazoa, involving egg size maximization through production of tiny polar bodies. Another is release of female gametes (ovulation/spawning) for delayed external fertilization, which imposes a variable delay between meiotic completion and fertilization and thus favors cytostatic arrest. Adoption of internal fertilization in the *Caenorhabditis* and *Drosophila* lineages may explain the respective loss or relaxation [33] of the cytostatic role of Mos. In the sawfly,

a maternally expressed Mos has been characterized and probably acts upstream of MAP-kinase-mediated MI arrest [34].

Our study has raised the attractive possibility that Mos may have acquired new roles during evolution after sequence changes in the UTRs affecting translational timing [25], although clearly our evidence is preliminary. Acquisition of a 5′ TOP sequence in one *Clytia* Mos paralog may have resulted in a shift in translational timing, leading to functions distinct during maturation and possibly oocyte growth. The extensively studied but apparently atypical participation of *Xenopus* Mos in initiating oocyte maturation [3, 4] may similarly have arisen after temporal advancement in Mos translation as a result of 3′ UTR changes in this species.

Experimental Procedures

Mos Sequence Retrieval and Phylogenetic Analysis

Gene searches were performed by tBLASTn on publicly available genomic data from metazoans (see Table S1). *Clytia hemisphaerica* and *Pleurobrachia pileus* Mos sequences were retrieved from EST collections, sequenced by the Genoscope (Evry, France). We aligned amino acid sequences with Mos sequences from *X. laevis*, *M. musculus*, and *D. melanogaster* and an out-group comprising other *M. musculus* serine-threonine kinases (e.g., MAPKs and MEKs) with CLUSTALW in BioEdit and then corrected the alignments by eye. Positions with more than 50% gaps were excluded. Maximum likelihood (ML) analyses were performed on the kinase domain with PhyML, with a WAG + Γ (10 categories) + I model of amino acid substitution and 500 bootstrap replicates.

Clytia Oocyte Manipulation

Adult *Clytia* (*Phialidium*) *hemisphaerica* were obtained from permanent laboratory colonies, and in situ hybridization performed as described previously [35]. Isolated gonads (shown in Figure 4D), which function autonomously and undergo daily cycles of oocyte growth and ovulation, were dissected and cultured as described [13]. Fully grown oocytes, isolated manually were induced to mature by 5–10 min incubation in 2–4 mM in Br-cAMP. An Eppendorf femtojet apparatus was used to inject spawned eggs, fertilized embryos, isolated oocytes, and oocytes within transparent isolated gonads. Injection volumes were estimated as approximately 3% of cell volume. The MEK inhibitor U0126 (Cell Signaling) was used at 10 μM, diluted from a 10 mM stock in DMSO immediately before use. The mTOR inhibitor rapamycin was used at 10 μM, from a 5 mM stock (Calbiochem). Antisense morpholino oligonucleotides (GeneTools) targeted to straddle the putative AUG initiation codons of CheMos1 and CheMos2 RNAs were injected at concentrations of 0.8–1.6 mM: Mos1-MO: 5′-AUAUCAACUUUUAUCGUCAUGUU CG-3′; Mos2-MO: 5′-CAUUCUGUGCCCAGCGGUUAUUUG-3′.

mRNA for microinjection was transcribed from linearized plasmids with mMesssage machine (Ambion) and resuspended in $H_2$0. Mouse Mos RNA was transcribed from a pRN3 vector [36]. For CheMos1 and CheMos2, equivalent results were obtained with full-length RNAs synthesized from original cDNA clones in Express1 vector containing native UTR sequences or ORFs cloned into TOPO plasmid with the p/ENTR/D TOPO cloning kit (Invitrogen) and then into pSPE3 destination vectors [37] modified to introduce a C-terminal RFP or M-Cherry tag. We were unable to exploit the fluorescent tags for localization studies, given that both *Clytia* Mos kinases were found to be active at levels well below those required for their detection and were toxic at detectable levels.

Cytostatic Activity Assay, *Xenopus* Oocyte Maturation Assay, and Western Blotting

Isolated *Xenopus laevis* oocytes or spawned fertilized eggs were injected with a Drummond microinjection apparatus. To assay MAP kinase phosphorylation, we harvested groups of three embryos at the 8- to 16-cell stage and processed them for western blotting using anti-dpERK1/2 (Cell Signaling Technology), an anti *Xenopus* cyclin B2 serum (gift of E. Shibuya), monoclonal anti-PSTAIR (Santa Cruz), and rabbit anti-actin (Sigma, #2066).

Microscopy

For anti-tubulin (DM1A: Sigma) immunofluorescence, specimens were fixed in 0.1 M HEPES (pH 6.9)/50 mM EGTA/10 mM $MgSO_4$/0.5 M Maltose/4% paraformaldehyde [31]. Nuclei were stained with Hoechst dye 33258. Time-lapse recordings were made on a Zeiss Axiovert microscope with a motorized stage and camera driven by Metamorph software.

Accession Numbers

The Genbank accession numbers for Mos sequences reported in this paper are as follows: PpiMos,: FJ026394; CheMos1, FJ026395; and CheMos2, FJ026396.

Supplemental Data

Supplemental Data include three tables, four figures, and four movies and can be found with this article online at http://www.current-biology.com/supplemental/S0960-9822(09)00633-2.

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References

1. Sagata, N. (1998). Introduction: Meiotic maturation and arrest in animal oocytes. Semin. Cell Dev. Biol. *9*, 535–537.
2. Kishimoto, T. (2003). Cell-cycle control during meiotic maturation. Curr. Opin. Cell Biol. *15*, 654–663.
3. Abrieu, A., Doree, M., and Fisher, D. (2001). The interplay between cyclin-B-Cdc2 kinase (MPF) and MAP kinase during maturation of oocytes. J. Cell Sci. *114*, 257–267.
4. Haccard, O., and Jessus, C. (2006). Oocyte maturation, Mos and cyclins–a matter of synthesis: Two functionally redundant ways to induce meiotic maturation. Cell Cycle *5*, 1152–1159.
5. Verlhac, M.H., Kubiak, J.Z., Weber, M., Geraud, G., Colledge, W.H., Evans, M.J., and Maro, B. (1996). Mos is required for MAP kinase activation and is involved in microtubule organization during meiotic maturation in the mouse. Development *122*, 815–822.
6. Dehal, P., and Boore, J.L. (2005). Two rounds of whole genome duplication in the ancestral vertebrate. PLoS Biol. *3*, e314.
7. Pavlicev, M., and Mayer, W. (2006). Multiple copies of coding as well as pseudogene c-mos sequence exist in three lacertid species. J. Exp. Zoolog. B Mol. Dev. Evol. *306*, 539–550.
8. Masui, Y. (2001). From oocyte maturation to the in vitro cell cycle: The history of discoveries of Maturation-Promoting Factor (MPF) and Cytostatic Factor (CSF). Differentiation *69*, 1–17.
9. Freeman, G., and Ridgway, E.B. (1993). The role of intracellular calcium and pH during fertilization and egg activation in the hydrozoan Phialidium. Dev. Biol. *156*, 176–190.
10. Kondoh, E., Tachibana, K., and Deguchi, R. (2006). Intracellular Ca2+ increase induces post-fertilization events via MAP kinase dephosphorylation in eggs of the hydrozoan jellyfish Cladonema pacificum. Dev. Biol. *293*, 228–241.
11. Hoshi, M., Ohta, K., Gotoh, Y., Mori, A., Murofushi, H., Sakai, H., and Nishida, E. (1992). Mitogen-activated-protein-kinase-catalyzed phosphorylation of microtubule-associated proteins, microtubule-associated protein 2 and microtubule-associated protein 4, induces an alteration in their function. Eur. J. Biochem. *203*, 43–52.
12. Tachibana, K., Tanaka, D., Isobe, T., and Kishimoto, T. (2000). c-Mos forces the mitotic cell cycle to undergo meiosis II to produce haploid gametes. Proc. Natl. Acad. Sci. USA *97*, 14301–14306.
13. Amiel, A., and Houliston, E. (2009). Three distinct RNA localization mechanisms contribute to oocyte polarity establishment in the cnidarian *Clytia hemisphærica*. Dev. Biol. *327*, 191–203.

14. Freeman, G., and Ridgway, E.B. (1988). The role of cAMP in oocyte maturation and the role of the germinal vesicle contents in mediating maturation and subsequent developmental events in hydrozoans. Rouxs Arch. Dev. Biol. *197*, 197–211.
15. Takeda, N., Kyozuka, K., and Deguchi, R. (2006). Increase in intracellular cAMP is a prerequisite signal for initiation of physiological oocyte meiotic maturation in the hydrozoan Cytaeis uchidae. Dev. Biol. *298*, 248–258.
16. Ikegami, S., Honji, N., and Yoshida, M. (1978). Light-controlled production of spawning-inducing substance in jellyfish ovary. Nature *272*, 611–612.
17. Roosen-Runge, E.C., and Szollosi, D. (1965). On biology and structure of the testis of Philidium Leuckhart (Leptomedusae). Z. Zellforsch. Mikrosk. Anat. *68*, 597–610.
18. Favata, M.F., Horiuchi, K.Y., Manos, E.J., Daulerio, A.J., Stradley, D.A., Feeser, W.S., Van Dyk, D.E., Pitts, W.J., Earl, R.A., Hobbs, F., et al. (1998). Identification of a novel inhibitor of mitogen-activated protein kinase kinase. J. Biol. Chem. *273*, 18623–18632.
19. Verlhac, M.H., Lefebvre, C., Guillaud, P., Rassinier, P., and Maro, B. (2000). Asymmetric division in mouse oocytes: With or without Mos. Curr. Biol. *10*, 1303–1306.
20. Bodart, J.F., Baert, F.Y., Sellier, C., Duesbery, N.S., Flament, S., and Vilain, J.P. (2005). Differential roles of p39Mos-Xp42Mpk1 cascade proteins on Raf1 phosphorylation and spindle morphogenesis in Xenopus oocytes. Dev. Biol. *283*, 373–383.
21. Lenart, P., Bacher, C.P., Daigle, N., Hand, A.R., Eils, R., Terasaki, M., and Ellenberg, J. (2005). A contractile nuclear actin network drives chromosome congression in oocytes. Nature *436*, 812–818.
22. Mori, M., Hara, M., Tachibana, K., and Kishimoto, T. (2006). p90Rsk is required for G1 phase arrest in unfertilized starfish eggs. Development *133*, 1823–1830.
23. Manandhar, G., Schatten, H., and Sutovsky, P. (2005). Centrosome reduction during gametogenesis and its significance. Biol. Reprod. *72*, 2–13.
24. Gould, M.C., and Stephano, J.L. (1999). MAP kinase, meiosis, and sperm centrosome suppression in Urechis caupo. Dev. Biol. *216*, 348–358.
25. Belloc, E., Pique, M., and Mendez, R. (2008). Sequential waves of polyadenylation and deadenylation define a translation circuit that drives meiotic progression. Biochem. Soc. Trans. *36*, 665–670.
26. Lapasset, L., Pradet-Balade, B., Verge, V., Lozano, J.C., Oulhen, N., Cormier, P., and Peaucellier, G. (2008). Cyclin B synthesis and rapamycin-sensitive regulation of protein synthesis during starfish oocyte meiotic divisions. Mol. Reprod. Dev. *75*, 1617–1626.
27. Hamilton, T.L., Stoneley, M., Spriggs, K.A., and Bushell, M. (2006). TOPs and their regulation. Biochem. Soc. Trans. *34*, 12–16.
28. Schwab, M.S., Kim, S.H., Terada, N., Edfjall, C., Kozma, S.C., Thomas, G., and Maller, J.L. (1999). p70(S6K) controls selective mRNA translation during oocyte maturation and early embryogenesis in Xenopus laevis. Mol. Cell. Biol. *19*, 2485–2494.
29. Goldman, D.S., Kiessling, A.A., Millette, C.F., and Cooper, G.M. (1987). Expression of c-mos RNA in germ cells of male and female mice. Proc. Natl. Acad. Sci. USA *84*, 4509–4513.
30. Inselman, A., and Handel, M.A. (2004). Mitogen-activated protein kinase dynamics during the meiotic G2/MI transition of mouse spermatocytes. Biol. Reprod. *71*, 570–578.
31. Madgwick, S., and Jones, K.T. (2007). How eggs arrest at metaphase II: MPF stabilisation plus APC/C inhibition equals Cytostatic Factor. Cell Div *2*, 4.
32. Inoue, D., Ohe, M., Kanemori, Y., Nobui, T., and Sagata, N. (2007). A direct link of the Mos-MAPK pathway to Erp1/Emi2 in meiotic arrest of Xenopus laevis eggs. Nature *446*, 1100–1104.
33. Ivanovska, I., Lee, E., Kwan, K.M., Fenger, D.D., and Orr-Weaver, T.L. (2004). The Drosophila MOS ortholog is not essential for meiosis. Curr. Biol. *14*, 75–80.
34. Yamamoto, D.S., Tachibana, K., Sumitani, M., Lee, J.M., and Hatakeyama, M. (2008). Involvement of Mos-MEK-MAPK pathway in cytostatic factor (CSF) arrest in eggs of the parthenogenetic insect, Athalia rosae. Mech. Dev. *125*, 996–1008.
35. Chevalier, S., Martin, A., Leclère, L., Amiel, A., and Houliston, E. (2006). Polarised expression of FoxB and FoxQ2 genes during development of the hydrozoan Clytia hemisphaerica. Dev. Genes Evol. *216*, 709–720.
36. Verlhac, M.H., Lefebvre, C., Kubiak, J.Z., Umbhauer, M., Rassinier, P., Colledge, W., and Maro, B. (2000). Mos activates MAP kinase in mouse oocytes through two opposite pathways. EMBO J. *19*, 6065–6074.
37. Roure, A., Rothbacher, U., Robin, F., Kalmar, E., Ferone, G., Lamy, C., Missero, C., Mueller, F., and Lemaire, P. (2007). A multicassette Gateway vector set for high throughput and comparative analyses in ciona and vertebrate embryos. PLoS ONE *2*, e916.