# Supplemental Data



# The *C. elegans* DYRK Kinase MBK-2 Marks Oocyte Proteins for Degradation in Response to Meiotic Maturation

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### Supplemental References


S1. Pellettieri, J., Reinke, V., Kim, S.K., and Seydoux, G. (2003). Coordinate activation of maternal protein degradation during the egg-to-embryo transition in C. elegans. Dev. Cell *5*, 451–462.

S2. Pintard, L., Kurz, T., Glaser, S., Willis, J.H., Peter, M., and Bowerman, B. (2003). Neddylation and deneddylation of CUL-3 is required to target MEI-1/Katanin for degradation at the meiosis-to-mitosis transition in C. elegans. Curr. Biol. *13*, 911–921.

S3. Lin, R. (2003). A gain-of-function mutation in oma-1, a C. elegans gene required for oocyte maturation, results in delayed degradation of maternal proteins and embryonic lethality. Dev. Biol. *258*, 226–239.

S4. L'Hernault, S.W., Shakes, D.C., and Ward, S. (1988). Developmental genetics of chromosome I spermatogenesis-defective mutants in the nematode Caenorhabditis elegans. Genetics *120*, 435–452.

S5. Wallenfang, M.R., and Seydoux, G. (2000). Polarization of the anterior-posterior axis of C. elegans is a microtubule-directed process. Nature *408*, 89–92.


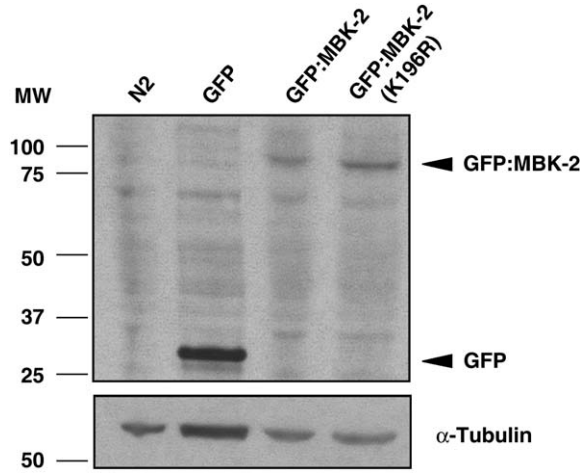


Figure S1. GFP:MBK-2 and GFP:MBK-2(K196R) Expression Levels in *mbk-2(pk1427)* Hermaphrodites

One hundred and twenty-five hermaphrodites for each indicated genotype were processed for an anti-GFP western blot (see Experimental Procedures). The membrane was reprobed with anti-tubulin for loading control. GFP and GFP:MBK-2(K196R) were expressed 2.8-fold and 1.9-fold higher, respectively, than GFP:MBK-2. MW denotes molecular weight and is expressed in kDa.

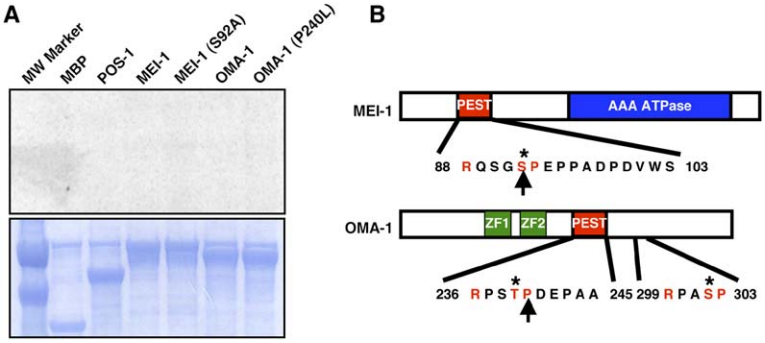


Figure S2. MBP:MBK-2(K196R) Does Not Phosphorylate MBP Substrates

(A) A kinase assay was performed as in Figure 1C except that MBP:MBK-2(K196R) was used in this experiment.

(B) Schematic diagram of MEI-1 and OMA-1. PEST domain residues are shown below with DYRK consensus residues ($RX_{1-3}S/TP$) highlighted in red. Asterisk denotes the predicted MBK-2 phosphorylation site. Arrows indicate residues mutated in MEI-1 (S92A) and OMA-1 (P240L). ZF denotes CCCH-type zinc finger.

Table S1. Detection of P-MEI-1 in Wild-Type and Mutant Embryos

| Developmental Stage | Wild-Type | *mei-1(ct101)* | *mbk-2 (pk1427)* |
|---|---|---|---|
| Metaphase I | − (10/10) | − (3/3) | − (10/10) |
| Anaphase I/Telophase I | + (12/14) | − (6/6) | − (2/2) |
| Metaphase II | + (11/11) | − (5/5) | − (10/10) |
| Anaphase II/Telophase II | ++ (8/10) | ND | − (3/3) |
| Pronuclear Formation / Migration | +++ (16/19) | − (13/13) | − (19/20) |
| Pronuclear Meeting (Prophase) | ++ (17/19) | − (5/6) | − (24/24) |
| Mitosis | + (10/11) | − (4/4) | − (7/7) |
| 2-cell | + (15/16) | − (4/4) | N/A* |

ND denotes not determined; N/A denotes not applicable. Scoring of staining intensity correlates with Figure 2C as follows: − = no staining above background; + = weak staining (0%–33% of maximum); ++ = moderate staining (34%–66% of maximum); +++ = strong staining (67%–100% of maximum); and * indicates that *mbk-2(pk1427)* embryos fail to complete cytokinesis.

Table S2. P-MEI-1 Subcellular Distribution in Wild-Type Embryos

| Developmental Stage | # of Embryos with P-MEI-1 on Chromosomes | # of Embryos with P-MEI-1 on Polar Bodies |
|---|---|---|
| Metaphase I | 0/10 | N/A |
| Anaphase I | 6/11* | N/A |
| Telophase I | 4/5* | 4/5 |
| Metaphase II | 2/11* | 10/11 |
| Anaphase II | 5/9* | 6/9 |
| Telophase II | 1/3* | 3/3 |
| Pronuclear Formation / Migration | 0/21 | 20/21 |
| Pronuclear Meeting (Prophase) | 0/19 | 17/19 |
| Mitosis | 0/13 | 13/13 |
| 2-cell | 0/16 | 16/16 |

* indicates that P-MEI-1 was detected only on maternal chromatin.

Table S3. *C. elegans* Strains Used in This Study

| Name | Description | Genotype | References |
|---|---|---|---|
| JH 1576 | $P_{pie-1}$:GFP:MBK-2 | *unc-119(ed3);axIs1140[pJP1.02]* | [S1] |
| JH 1714 | $P_{pie-1}$:GFP:MBK-2(K196R) | *unc-119(ed3);axIs1227[pJP1.08]* | This study |
| JH 1886 | *mbk-2(null)*;$P_{pie-1}$:GFP:MBK-2 | *unc-24(e1172) mbk-2(pk1427)/nT1;unc-119(ed3);axIs1140[pJP1.02]* | [S1] |
| JH 1867 | *mbk-2(null)*;$P_{pie-1}$:GFP:MBK-2(K196R) | *unc-24(e1172) mbk-2(pk1427)/nT1;unc-119(ed3);axIs1227[pJP1.08]* | This study |
| JH 1580 | *mbk-2(null)* | *unc-24(e1172) mbk-2(pk1427)/nT1* | [S1] |
| EU 1065 | $P_{pie-1}$:GFP:MEI-1 | *unc-119(ed3);orIs1[$P_{pie-1}$:GFP:MEI-1]* | [S2] |
| TX 189 | $P_{oma-1}$:OMA-1:GFP | *unc-119(ed3);teIs1[pRL475 + pPDMM016]* | [S3] |
| | *mei-1 (null)* | *unc-13 mei-1(ct101)/hT2* | Gift from Paul Mains |
| BA 708 | *spe-9 (ts)* | *spe-9 (hc52)* | [S4] |
| JH1360 | *mat-1 (ts)* | *mat-1(ax227)* | [S5] |
| JH1903 | $P_{pie-1}$:GFP | *unc-119(ed3);[$p_{pie-1}$:GFP]* | This study |
| JH1924 | $P_{pie-1}$:GFP:MEI-1(M1L, R36C) | *unc-119(ed3);[pMS4.06]* | This study |
| JH1925 | $P_{pie-1}$:GFP:MEI-1(M1L, R36C, S92A) | *unc-119(ed3);[pMS4.07]* | This study |
| JH1977 | *mat-1(ts)*;$P_{pie-1}$:GFP:MBK-2 | *mat-1(ax227);unc-119(ed3);axIs1140[pJP1.02]* | This study |
| JH1978 | *spe-9(ts)*;$P_{pie-1}$:GFP:MBK-2 | *spe-9(hc52);unc-119(ed3);axIs1140[pJP1.02]* | This study |
| JH1979 | *spe-9(ts)*;$P_{pie-1}$:GFP:MEI-1 | *spe-9(hc52);unc-119(ed3);orIs1[$P_{pie-1}$:GFP:MEI-1]* | This study |