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zif-1 translational repression defines a second, mutually exclusive OMA function in germline transcriptional repression

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SUMMARY

Specification of primordial germ cells requires global repression of transcription. In *C. elegans*, primordial germ cells are generated through four rounds of asymmetric divisions, starting from the zygote P0, each producing a transcriptionally repressed germline blastomere (P1-P4). Repression in P2-P4 requires PIE-1, which is provided maternally in oocytes and segregated to all germline blastomeres. We have shown previously that OMA-1 and OMA-2 repress global transcription in P0 and P1 by sequestering TAF-4, an essential component of TFIID. Soon after the first mitotic cycle, OMA proteins undergo developmentally regulated degradation. Here, we show that OMA proteins also repress transcription in P2-P4 indirectly, through a completely different mechanism that operates in oocytes. OMA proteins bind to both the 3′ UTR of the *zif-1* transcript and the eIF4E-binding protein, SPN-2, repressing translation of *zif-1* mRNA in oocytes. *zif-1* encodes the substrate-binding subunit of the E3 ligase for PIE-1 degradation. Inhibition of *zif-1* translation in oocytes ensures high PIE-1 levels in oocytes and germline blastomeres. The two OMA protein functions are strictly regulated in both space and time by MBK-2, a kinase activated following fertilization. Phosphorylation by MBK-2 facilitates the binding of OMA proteins to TAF-4 and simultaneously inactivates their function in repressing *zif-1* translation. Phosphorylation of OMA proteins displaces SPN-2 from the *zif-1* 3′ UTR, releasing translational repression. We propose that MBK-2 phosphorylation serves as a developmental switch, converting OMA proteins from specific translational repressors in oocytes to global transcriptional repressors in embryos, together effectively repressing transcription in all germline blastomeres.

KEY WORDS: OMA proteins, *zif-1*, Translational repression, *C. elegans*, Germline, Oocyte-to-embryo transition, MBK-2

INTRODUCTION

Primordial germ cells, the source of gametes in the adult animal and the genetic link between two generations, are specified early during embryogenesis in most animals. Recent studies in worms, flies and mice show that specification of primordial germ cells requires global repression of transcription, usually through inhibition of initiation or elongation, and chromatin remodeling (Extavour and Akam, 2003; Nakamura and Seydoux, 2008). Defective transcriptional repression in primordial germ cells results in sterility.

In *C. elegans*, the single founder blastomere for the entire germline, P4, is specified through a series of four asymmetric divisions, beginning with the zygote, P0 (Strome, 2005). Each of these divisions results in a smaller germline precursor (P1 through P4, termed the P lineage) and a larger somatic sister cell (Fig. 1). P4 divides symmetrically to generate Z2 and Z3, which generate the entire germline postembryonically. All germline blastomeres are transcriptionally repressed, whereas their somatic sisters undergo rapid transcriptional activation and lineage-specific differentiation, requiring ready reversibility for any repressive mechanism operating in the P lineage (Seydoux and Dunn, 1997; Seydoux et al., 1996). Epigenetic marks characteristic of transcriptionally competent chromatin are found in *C. elegans* germline blastomeres (Schaner et al., 2003), consistent with them being transcriptionally competent but being actively restrained from differentiation-promoting transcription.

Transcriptional repression in the P lineage in *C. elegans* requires at least two groups of maternally supplied proteins. In P0 and P1, two closely related and functionally redundant cytoplasmic proteins, OMA-1 and OMA-2, globally repress transcription initiation by binding to TAF-4, a crucial component of the RNA polymerase II pre-initiation complex (Guven-Ozkan et al., 2008). In P2-P4, PIE-1 globally represses transcription elongation by inhibiting P-TEFb, the kinase which phosphorylates serine 2 (Ser2) residues within heptapeptide repeats of the RNA polymerase II C-terminal domain (Batchelder et al., 1999; Seydoux and Dunn, 1997; Zhang et al., 2003). Ser2 phosphorylation (Ser2P) is required for transcriptional elongation (Komarnitsky et al., 2000; Shim et al., 2002).

OMA-1, OMA-2 and PIE-1 proteins are all expressed in oocytes from maternally supplied mRNAs. OMA-1 and OMA-2 are degraded soon after the first mitotic division and are not detected in subsequent P-lineage blastomeres (Fig. 1) (Detwiler et al., 2001; Lin, 2003). Degradation requires that OMA proteins be phosphorylated by at least two kinases, one of which, the DYRK2-type kinase MBK-2, is developmentally activated in newly fertilized embryos (Cheng et al., 2009; Nishi and Lin, 2005; Shirayama et al., 2006; Stitzel et al., 2006). PIE-1 is segregated asymmetrically to the germline blastomere at each P-lineage blastomere division. In addition, the minor amount of PIE-1

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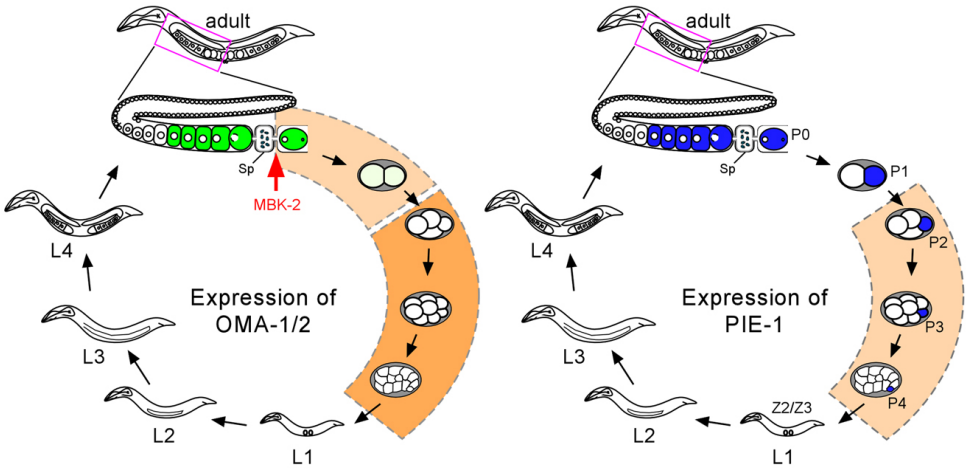

Fig. 1. *C. elegans* germline and the expression of OMA-1/2 and PIE-1 proteins. Spatial and temporal expression of OMA-1/2 (green, left) and PIE-1 (blue, right) proteins in the gonad and embryos during the *C. elegans* lifecycle. The embryonic stages when each protein represses transcription are contained within the dashed lines (light orange, direct repression; orange, indirect repression). Red arrow, MBK-2 activation; P0-P4, germline blastomeres; Sp, spermatheca; Z2/Z3, primordial germ cells in L1.

segregated to the somatic sister is rapidly degraded (Mello et al., 1996; Reese et al., 2000). Repression by both OMA and PIE-1 provide a robust, but readily reversible, way to repress transcription in the P-lineage while maintaining the chromatin primed for transcriptional activation in the somatic sisters.

OMA-1, OMA-2 and PIE-1 have additional functions beyond repressing transcription in germline blastomeres. All three proteins contain tandem CCCH zinc fingers, a domain usually associated with RNA binding (Detwiler et al., 2001; Lai et al., 1999; Mello et al., 1996; Pagano et al., 2007). However, the CCCH zinc fingers are not required for PIE-1 to repress transcription (Tenenhaus et al., 2001) or for the OMA proteins to bind to and sequester TAF-4 (Guven-Ozkan et al., 2008). OMA-1 and OMA-2 activity are required for oocyte maturation, although the molecular basis for this requirement is unknown (Detwiler et al., 2001; Shimada et al., 2002). All three proteins contribute to the restricted expression pattern of a Nanos-related protein, NOS-2, to the P4 germline blastomere. OMA proteins have been shown to bind to the *nos-2* 3′ UTR and repress translation in oocytes, whereas PIE-1 has been shown to maintain the expression level of NOS-2 through an unknown mechanism (Jadhav et al., 2008; Tenenhaus et al., 2001). Recently, OMA proteins have also been implicated in the translational repression of *mei-1* in embryos (Li et al., 2009). One intriguing unanswered question is how the multiple functions of OMA proteins or PIE-1 intersect in vivo. We have shown previously that phosphorylation of OMA-1 by MBK-2, at the same amino acid that triggers its degradation, facilitates OMA-1 binding to TAF-4 (Guven-Ozkan et al., 2008), suggesting coordinated regulation.

Degradation of PIE-1 in somatic cells is carried out by a CUL-2-containing E3 ligase (DeRenzo et al., 2003). The substrate-binding subunit of this E3 ligase, ZIF-1, binds to PIE-1 via its first CCCH zinc finger (DeRenzo et al., 2003). ZIF-1 also binds to and promotes the degradation of tandem CCCH zinc finger proteins MEX-1, POS-1, MEX-5, and MEX-6 in somatic blastomeres (DeRenzo et al., 2003). How the degradation of these ZIF-1 substrates is restricted to somatic blastomeres, and not in germline blastomeres or oocytes, remains unknown. ZIF-1 is not responsible for OMA protein degradation (DeRenzo et al., 2003).

Increasing evidence supports an important role for translational repression during germ cell differentiation in both flies and worms (for a review, see Nakamura and Seydoux, 2008). In *Drosophila*, two RNA-binding proteins, Nanos and Pumilio, repress translation of differentiation-promoting proteins in germline stem cells, maintaining their stem cell character (Parisi and Lin, 2000). In *C. elegans*, spatiotemporal expression of most proteins expressed in the germline is regulated by their 3′ UTR sequences (Merritt et al., 2008). Indeed, many key regulators of germline development are RNA-binding proteins (Kimble and Crittenden, 2005). For example, depletion of two KH-domain RNA-binding proteins expressed in the *C. elegans* gonad, MEX-3 and GLD-1, drives germ cells into precocious somatic differentiation within the gonad (Ciosk et al., 2006).

We show here that OMA proteins have an essential function in maintaining a high level of PIE-1 protein in oocytes and embryos. OMA proteins do so by binding to and repressing translation of *zif-1* mRNA in oocytes, thereby preventing PIE-1 degradation. By preventing PIE-1 degradation, OMA proteins indirectly repress transcription in P2-P4. The two mechanisms by which OMA proteins repress primordial germ cell transcription, namely by direct sequestration of TAF-4 and indirect repression of *zif-1* translation, are distinct. They take place at different developmental stages, require distinct OMA protein domains, can be disrupted independently of each other and are differentially regulated by MBK-2 phosphorylation. We propose that phosphorylation by MBK-2, itself a developmentally regulated kinase, serves as a developmental switch that converts OMA proteins from translational repressors of specific maternal transcripts in the germline to global transcriptional repressors in the early embryo, together ensuring transcriptional repression in germline blastomeres.

MATERIALS AND METHODS

Strains

N2 was used as the wild-type strain. Genetic markers were: LGI, *gld-1(q485)*; LGIII, *unc-119(ed3)*; LGIV, *oma-1(zu405)*, *oma-1(te33)*, *oma-1(te21)*, *mbk-2(ne992)*; LGV, *oma-2(te50);oma-2(te51)*. Plasmids used, strain names and transgenes were as follows:

TX1246 (*teIs113 [$P_{pie\text{-}1}$gfp::h2b::UTR$^{zif\text{-}1,771bp}$]*),
TX1248 (*teIs114 [$P_{pie\text{-}1}$gfp::h2b::UTR$^{zif\text{-}1,771bp}$]*),
TX1240 (*teEx602 [$P_{pie\text{-}1}$gfp::h2b::UTR$^{zif\text{-}1,304bp}$]*),
TX1251 (*teEx604 [$P_{pie\text{-}1}$gfp::h2b::UTR$^{zif\text{-}1,771bp,\ \Delta 4\text{-}63}$]*),
TX1409 (*teEx656 [$P_{pie\text{-}1}$gfp::h2b::UTR$^{zif\text{-}1,771bp,\ \Delta 64\text{-}123}$]*),
TX1272 (*teEx606 [$P_{pie\text{-}1}$gfp::h2b::UTR$^{zif\text{-}1,771bp,\ \Delta 124\text{-}183}$]*),
TX1298 (*teEx607 [$P_{pie\text{-}1}$gfp::h2b::UTR$^{zif\text{-}1,771bp,\ \Delta 184\text{-}243}$]*),
TX1410 (*teEx657 [$P_{pie\text{-}1}$gfp::h2b::UTR$^{zif\text{-}1,771bp,\ \Delta 244\text{-}303}$]*),
TX1311 (*teEx610 [$P_{pie\text{-}1}$gfp::h2b::UTR$^{zif\text{-}1,771bp,\ 64\text{-}183}$]*),
TX1315 (*teEx611 [$P_{pie\text{-}1}$gfp::h2b::UTR$^{zif\text{-}1,771bp,\ \Delta 64\text{-}183}$]*),
TX1375 (*teIs126 [$P_{pie\text{-}1}$gfp::zif-1::UTR$^{zif\text{-}1,771bp}$]*).

JH1436, JH227, AZ212 and TX1162 contain $P_{pie\text{-}1}$*gfp::pie-1 zf1::UTR*$^{pie\text{-}1}$, $P_{pie\text{-}1}$*gfp::pie-1::UTR*$^{pie\text{-}1}$, $P_{pie\text{-}1}$*gfp::H2B::UTR*$^{pie\text{-}1}$ and $P_{oma\text{-}1}$ *oma-1Δ46-80::gfp::UTR*$^{oma\text{-}1}$ transgenes, respectively, as described (Guven-Ozkan et al., 2008; Praitis et al., 2001; Reese et al., 2000).

Plasmid construction

Most plasmids were constructed with the Gateway cloning technology. The *zif-1* 3′ UTR is annotated in Wormbase as being 304 nucleotides long. In order to ensure proper expression in vivo, a longer piece of genomic DNA (771 nucleotides downstream of the stop codon) was used to generate the *zif-1* translational reporter. The 771 nucleotide sequence was cloned downstream of *pie-1* promoter-driven GFP::H2B in the germline expression vector pID3.01B (Reese et al., 2000), which contains the *pie-1* 3′ UTR now downstream of the 771 nucleotide *zif-1* 3′ sequence. All deletion constructs were derived from the 771 nucleotide sequence.

C. elegans transformation

All integrated lines were generated by microparticle bombardment (Praitis et al., 2001), whereas other transgenic lines were generated by complex array injection (Kelly et al., 1997). For each construct, expression was analyzed and found to be consistent in at least two independent lines. Germline expression in some of the non-integrated lines became silenced after 4-6 generations.

RNA interference

Feeding RNAi was performed as described (Timmons and Fire, 1998) using HT115 bacteria seeded on NGM plates containing 1 mM IPTG. L1 larvae were fed for 2 days at 25°C or 3 days at 20°C. *oma-1/2(RNAi);gld-1(q485)* animals were incubated at 25°C.

Immunofluorescence

Immunofluorescence for *C. elegans* gonads and embryos was carried out as described previously for anti-PIE-1 (1/50) (Mello et al., 1996), anti-OMA-1a (1/100) (Shimada et al., 2006), anti-GFP (1/250, rabbit, Invitrogen), anti-MEX-1 (Guedes and Priess, 1997) and anti-Ser2P (1/300, MMS-129R, Covance) (Seydoux and Dunn, 1997). Secondary antibodies used were Alexa488- or Alexa568-conjugated goat anti-rabbit or goat anti-mouse (Invitrogen, 1/250).

Lysate preparation and RNA binding assay

Worm lysates were prepared from 1-day-old gravid adults as described except the following modifications (Lee and Schedl, 2001). Worms were harvested, washed and then disrupted in a Dounce homogenizer by 4-5 strokes with a loose-fitting pestle followed by 15-20 strokes with a tight-fitting pestle. Lysates were spun at 16,000 ***g*** for 20 minutes and the supernatants were used for binding. Protein concentrations averaged 6-8 mg/ml. Synchronized *mbk-2(ne992ts)* (Pang et al., 2004) L1 larvae were cultured at 16°C until L4, then shifted to 25°C for one day before being harvested for lysate.

RNA pulldowns were performed as in Lee and Schedl (Lee and Schedl, 2001). After pulldown and washing, beads were then boiled in SDS sample buffer. Supernatants were loaded on a 10% SDS-PAGE gel and subjected to western blot analysis with the indicated antibodies: anti-OMA-1 (1:50) (Detwiler et al., 2001), anti-SPN-2N (1:2000) (Li et al., 2009) or anti-MBP (1:1000; NEB). More detailed protocols for lysate preparation, RNA synthesis and RNA pulldown are available upon request.

Analysis of embryos, imaging and quantification

Images of immunofluorescence and live embryos were acquired and processed as described (Guven-Ozkan et al., 2008).

RESULTS

OMA-1/2 are required to prevent PIE-1 degradation

Double loss-of-function mutants for *oma-1* and *oma-2* are sterile owing to a defect in oocyte maturation (Detwiler et al., 2001). Embryos with reduced levels of OMA-1 and -2 can be obtained by RNAi [*oma-1(RNAi);oma-2(RNAi)*]. These RNAi embryos have severe defects in cell division (Nishi and Lin, 2005). By analyzing *oma-1(RNAi);oma-2(RNAi)* embryos with two clear pronuclei prior to the first mitotic division, we showed previously that transcription is derepressed in these 1-cell embryos (65%, *n*=20; Fig. 2A) (Guven-Ozkan et al., 2008). We assayed transcription by in situ hybridization of an early zygotic transcript or by immunofluorescence using an antibody recognizing RNA polymerase II phosphorylated at the Ser2 of its C-terminal domain (anti-Ser2P) (Seydoux and Dunn, 1997). With weaker *oma-1;oma-2* RNAi, we could obtain embryos that divided relatively normally beyond the 1-cell stage. Under these RNAi conditions, we detected no transcription in P0 or P1 germline blastomeres but often observed transcription in P2-P4, in addition to somatic blastomeres (79%, *n*=29; Fig. 2A) (Guven-Ozkan et al., 2008). Transcriptional repression in P2, as measured by a lack of Ser2P, requires a high level of PIE-1 protein, and derepression can even be detected in embryos missing one of the two copies of the *pie-1* gene (Tenenhaus et al., 1998). The observed defect in transcriptional repression in P2 in *oma-1/2*-depleted embryos is an indirect effect of a reduced level, or loss, of PIE-1 protein. *oma-1(RNAi);oma-2(RNAi)* embryos have a reduced level of PIE-1 (100%, *n*=9), with the extent of PIE-1 reduction proportional to the severity of transcriptional derepression (the intensity of Ser2P staining) (Guven-Ozkan et al., 2008). In worms homozygous for *oma-1(te33);oma-2(te51)* (both loss-of-function mutations), or worms

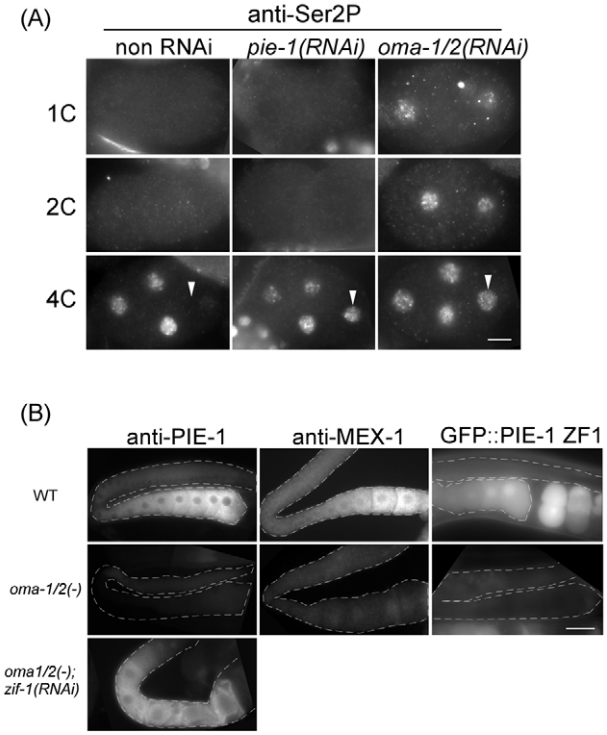

Fig. 2. OMA-1/2 repress transcription in P2 indirectly by preventing PIE-1 degradation. (**A**) Anti-Ser2P staining in 1-cell, 2-cell and 4-cell embryos of wild-type or indicated RNAi animals. Arrowhead indicates the P2 nucleus. (**B**) Immunofluorescence micrographs of anti-PIE-1, anti-MEX-1 and GFP fluorescence micrograph of GFP::PIE-1 ZF1 in the gonads (dashed outline) of wild-type, *oma-1(te33);oma-2(51)* or *oma-1(te33);oma-2(te51);zif-1(RNAi)* animals. Scale bars: 10 μm in A; 30 μm in B.

that have been strongly depleted of *oma-1* and *oma-2* by RNAi, we detected no, or a dramatically reduced level of, PIE-1 in the gonad (94%, *n*=17; Fig. 2B).

The level of many transgenic proteins regulated by the *pie-1* promoter and the *pie-1* 3′ UTR are not affected in *oma-1(RNAi);oma-2(RNAi)* animals (Guven-Ozkan et al., 2008). Therefore, it is unlikely that OMA-1/2 regulate transcription or translation of PIE-1 protein. Three lines of evidence suggest, instead, that OMA-1/2 regulate PIE-1 stability. First, depletion of *zif-1* by RNAi in *oma-1(–)*;*oma-2(–)* animals blocks PIE-1 degradation and suppresses the loss of PIE-1 phenotype (100%, *n*=9; Fig. 2). Second, the first zinc finger of PIE-1 is both necessary and sufficient for its degradation by the ZIF-1-containing E3 ligase (Reese et al., 2000). We show that expression of a reporter GFP protein carrying only the first zinc finger of PIE-1 (GFP::PIE-1 ZF1) (Reese et al., 2000) is dramatically reduced in the oocytes of *oma-1(–)*;*oma-2(–)* animals (100%, *n*=15; Fig. 2). Third, levels of MEX-1, another tandem CCCH zinc-finger protein whose degradation is also mediated by ZIF-1 (DeRenzo et al., 2003), are decreased or abolished in *oma-1(–)*;*oma-2(–)* oocytes (100%, *n*=10; Fig. 2). Together, these results support the model that OMA proteins prevent PIE-1 degradation by inhibiting the ZIF-1-containing E3 ligase activity.

OMA proteins maintain PIE-1 stability and sequester TAF-4 via distinct domains

Sequestration of TAF-4 by OMA-1 requires a 35-amino-acid region (residues 46-80) that resembles the histone-fold domain of TAF-12, the normal binding partner for TAF-4 (Guven-Ozkan et al., 2008). Worms expressing only the mutant OMA-1 Δ46-80, in an otherwise *oma-1(te33);oma-2(te51)* background, do not have an Oma phenotype. Instead, these worms are fertile and their embryos are transcriptionally derepressed in P0 and P1 (89%, *n*=57; Fig. 3A-C) (Guven-Ozkan et al., 2008). PIE-1 protein levels are similarly high, or slightly reduced, in oocytes of worms expressing only OMA-1 Δ46-80, compared with wild-type worms (89%, *n*=9). This is in dramatic contrast to the complete absence of PIE-1 in *oma-1(te33);oma-2(te51)* animals (Fig. 2B).

We now show that maintenance of PIE-1 levels in oocytes requires one or both OMA zinc fingers. *oma-1(te21)* is a missense mutation resulting in the substitution of lysine for a conserved aspartate residue in the first zinc finger of OMA-1 (E141K) (Fig. 3A) (Detwiler et al., 2001). Similarly, *oma-2(te50)* is a missense mutation that substitutes tyrosine for the second cysteine in the second zinc finger of OMA-2 (C162Y) (Detwiler et al., 2001). We showed previously that both OMA-1 (E141K) and OMA-2 (C162Y) were expressed at wild-type levels in *oma-1(te21)* and *oma-2(te50)* animals, respectively (Detwiler et al., 2001). PIE-1 protein levels are greatly reduced or abolished in *oma-1(te21);oma-2(RNAi)* and *oma-1(te21);oma-2(te50)* animals, similar to those observed in animals with no detectable OMA proteins [*oma-1(te33);oma-2(te51)* or *oma-1(RNAi);oma-2(RNAi)*] (Fig. 2B; Fig. 3B; see Fig. S1 in the supplementary material). Although only the first finger of OMA-1 and the second finger of OMA-2 were tested, their high sequence similarity and redundant in vivo function suggest that both zinc fingers are probably required for OMA-1 and OMA-2 to maintain PIE-1 stability.

Neither the first finger of OMA-1 nor the second finger of OMA-2 is required for OMA-dependent transcriptional repression in the P0 or P1 blastomeres. We detected no ectopic Ser2P staining in the majority of 1-cell and 2-cell embryos derived from *oma-1(te21);oma-2(te50)* animals (88%, *n*=25; Fig. 3D). However, 100% of 4-cell embryos (*n*=30) derived from *oma-1(te21);oma-2(te50)* animals demonstrated ectopic Ser2P in the P2 blastomere, supporting our notion above that the defect in transcriptional repression in P2 is not a consequence of a defect in P0, but rather due to the absence of PIE-1 protein.

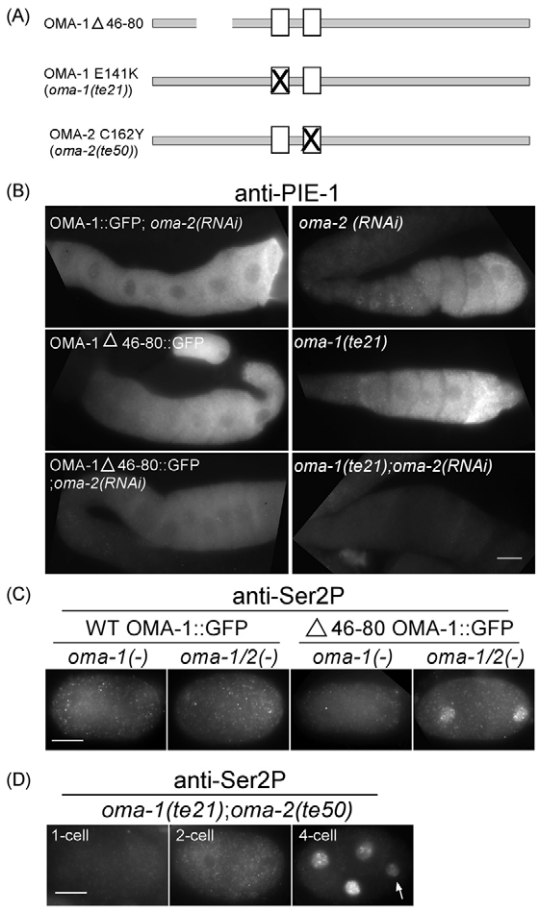

Fig. 3. Translational repression and transcriptional repression require distinct domains of OMA proteins. (**A**) Schematic of OMA-1 Δ46-80, OMA-1 E141K [*oma-1(te21)*] and OMA-2 C162Y [*oma-2(te50)*]. White box, zinc finger. (**B**) Immunofluorescence micrographs of anti-PIE-1 staining in the gonad of *oma-1(te33)* animals expressing either wild-type OMA-1::GFP or OMA-1 Δ46-80::GFP with or without *oma-2* depletion by RNAi (left column), and animals of the indicated genotypes (right column). (**C**) Anti-Ser2P staining of 1-cell embryos from *oma-1(te33)* animals expressing the indicated version of OMA-1::GFP with or without *oma-2(RNAi)*. (**D**) Anti-Ser2P staining in *oma-1(te21);oma-2(te50)* embryos. Note that Ser2P staining is only detected in P2 (arrow) but not P0 or P1. Scale bars: 30 μm in B; 10 μm in C,D.

Taken together, these results suggest that OMA proteins maintain PIE-1 protein levels through a zinc-finger-dependent mechanism that is independent of TAF-4 binding.

OMA proteins prevent PIE-1 degradation by repressing *zif-1* translation

Our results above suggest that OMA proteins prevent PIE-1 degradation by inhibiting the ZIF-1-containing E3 ligase activity. In addition to ZIF-1, this E3 ligase contains the cullin CUL-2, the ubiquitin-like protein elongin B (ELB-1), the adaptor protein

elongin C (ELC-1), and the ring finger protein RBX-1 (DeRenzo et al., 2003; Vasudevan et al., 2007). Depletion of *cul-2* or other components in this E3 ligase complex results in a large variety of phenotypes in the germline and early embryos that were not observed in *zif-1(RNAi)* animals (Liu et al., 2004; Sonneville and Gonczy, 2004). Genetic data are consistent with another E3 ligase containing CUL-2, ELB-1, ELC-1 and RBX-1 being active in wild-type gonads and newly fertilized embryos, where it regulates degradation of other proteins in a ZIF-1-independent manner (Vasudevan et al., 2007). Therefore, it is unlikely that OMA-1 and OMA-2 inhibit the ZIF-1-containing E3 ligase in the germline by inhibiting any of the common components of these complexes. The best candidate whose expression or activity might be repressed by OMA-1 and OMA-2 was ZIF-1 (DeRenzo et al., 2003).

zif-1 mRNA is maternally supplied and is detected at a high level throughout the gonad and early embryo (*C. elegans* expression database), although the in vivo spatiotemporal localization of ZIF-1 protein has not been determined. We raised an antibody against the ZIF-1 protein; however, this ZIF-1 antibody either did not work in our immunofluorescence analyses or failed to detect ZIF-1 protein owing to low abundance. Recent studies have shown that expression of the majority of maternally supplied proteins in *C. elegans* is regulated by the corresponding 3′ UTR sequence (Merritt et al., 2008). We therefore generated a reporter construct that would express nuclear GFP under the control of the *zif-1* 3′ UTR ($P_{pie\text{-}1}$-*gfp::h2b-UTR*^{zif}) as a means to indirectly monitor *zif-1* translation. For the purpose of this paper, we will refer to GFP::H2B expressed from this transgene as GFP::H2B$^{zif\text{-}1}$.

GFP::H2B$^{zif\text{-}1}$ recapitulates the temporal and spatial localization of the known ZIF-1 activity in the gonad and embryo (Fig. 4). That is, ZIF-1 activity and GFP::H2B$^{zif\text{-}1}$ expression are repressed in oocytes and germline blastomeres and both are present in somatic blastomeres. We observed nuclear GFP::H2B$^{zif\text{-}1}$ signal in embryos starting from the 4-cell stage and then only in somatic blastomeres (Fig. 4A). In germline blastomeres, where PIE-1 levels were high, no GFP::H2B$^{zif\text{-}1}$ was detected. GFP::H2B$^{zif\text{-}1}$ expression was also absent in 1-cell and 2-cell embryos and oocytes, where PIE-1 levels are high (Fig. 4). A similar expression pattern was also observed for a reporter in which the sequence-encoding histone H2B was replaced with the full-length *zif-1* coding sequence (GFP::ZIF-1$^{zif\text{-}1}$) (see Fig. S2A in the supplementary material). This is in clear contrast to GFP::H2B expressed under the control of the *pie-1* 3′ UTR (GFP::H2B$^{pie\text{-}1}$) (Praitis et al., 2001), which is ubiquitous, being detected in all nuclei in the gonad and early embryos (Fig. 4B).

Repression of $P_{pie\text{-}1}$-*gfp::h2b-UTR*^{zif} in oocytes is dependent on OMA proteins and, more precisely, on the first finger of OMA-1 and second finger of OMA-2 (Fig. 4B). We observed a high level of GFP::H2B$^{zif\text{-}1}$ in animals homozygous for *oma-1(te33);oma-2(te51)* or depleted of *oma-1* and *oma-2* by RNAi (100%, *n*=19 and 20, respectively). Similarly, GFP::H2B$^{zif\text{-}1}$ was detected in oocytes of *oma-1(te21);oma-2(RNAi)* and *oma-1(RNAi);oma-2(te50)* animals (100%, *n*=10 and 15, respectively). Repression of $P_{pie\text{-}1}$-*gfp::h2b-UTR*^{zif} in the meiotic gonad distal to oocytes is dependent on another RNA-binding protein, GLD-1. GLD-1 has been shown to repress the translation of several oocyte proteins in the distal meiotic gonads, including OMA-1 and OMA-2 (Lee and Schedl, 2001). We found that animals depleted of *oma-1*, *oma-2* and *gld-1* exhibit GFP::H2B$^{zif\text{-}1}$ expression throughout the gonad (67%, *n*=33; see Fig. S2B in the supplementary material). For the remainder of this paper, we will characterize repression of $P_{pie\text{-}1}$-*gfp::h2b-UTR*^{zif} only in oocytes where OMA proteins are normally expressed.

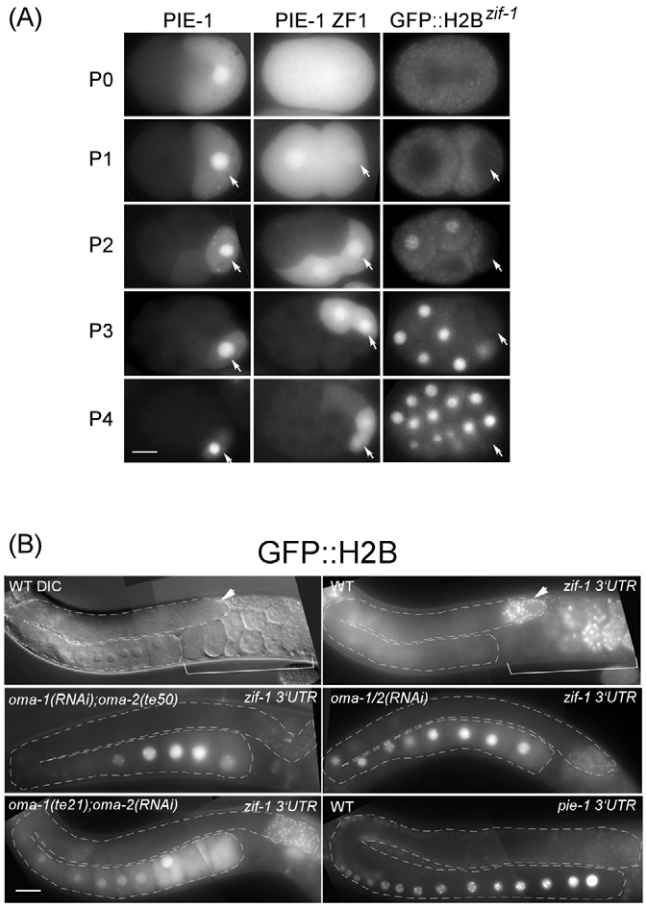

Fig. 4. GFP::H2B$^{zif\text{-}1}$ recapitulates the temporal and spatial localization of ZIF-1 activity. (**A**) Fluorescence micrographs of staged embryos expressing indicated GFP reporters. Embryo stage is indicated to the left by the name of its germline blastomere (arrow). (**B**) GFP::H2B expression from reporters containing indicated 3′ UTR (upper right hand corner) in gonads of different genetic backgrounds (upper left hand corner). A wild-type gonad exhibiting GFP::H2B$^{zif\text{-}1}$ in mitotic germline stem cells (arrowheads), but repressed in meiotic germ cells, is shown with both DIC (top left) and fluorescence micrographs (top right). GFP::H2B expressed from another reporter construct differing only in the 3′ UTR is expressed in oocytes (bottom right). Additional data demonstrating specificity of GFP::H2B$^{zif\text{-}1}$ is shown in Fig. S3 in the supplementary material. Scale bars: 10 μm in A; 30 μm in B.

OMA-1 and OMA-2 are not global translational repressors in oocytes. We found no evidence that they repress the translation of another reporter construct containing the *glp-1* 3′ UTR (Merritt et al., 2008). GLP-1 expression resembles that of GFP::H2B$^{zif\text{-}1}$ and has been shown to be regulated through its 3′ UTR (Evans et al., 1994; Marin and Evans, 2003; Ogura et al., 2003). However, a GFP::H2B reporter containing the *glp-1* 3′ UTR remains repressed in *oma-1(RNAi);oma-2(RNAi)* animals (100%, *n*=15; see Fig. S2C in the supplementary material).

OMA proteins repress *zif-1* mRNA translation by direct binding to its 3′ UTR

The fact that *zif-1* translational repression requires the CCCH zinc-finger domains supports the notion that the OMA proteins bind directly to the *zif-1* 3′ UTR. To test whether OMA proteins are

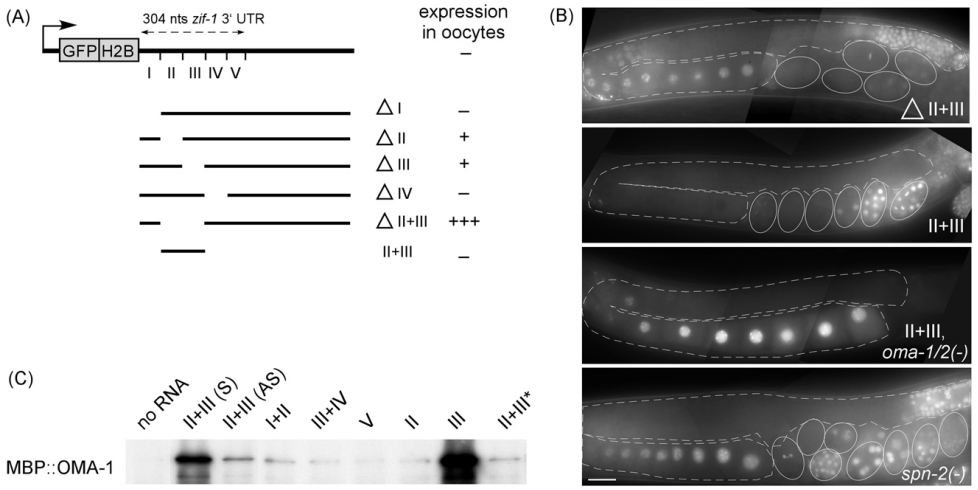

Fig. 5. Biochemical and functional analyses of the *zif-1* 3′ UTR. (**A**) Schematic of the *zif-1* 3′ UTR subregions or deletions assayed in transgenic worms and the degree of *zif-1* reporter derepression in oocytes (+++, strong; +, weak; –, none). (**B**) Fluorescence micrographs of these transgenic worms (also see Fig. S3 in the supplementary material). Repression by regions II and III is sensitive to *oma-1/2* depletion (third panel). Expression of GFP::H2B$^{zif\text{-}1}$ in oocytes was observed in *spn-2(RNAi)* animals (bottom panel). (**C**) In vitro RNA pulldowns using MBP::OMA-1 purified from bacteria and the indicated variant *zif-1* 3′ UTR RNAs. AS, antisense RNA; S, sense RNA; II+III*, middle 60 nucleotides of regions II and III. Scale bar: 30 μm.

capable of specifically binding to the *zif-1* 3′ UTR, we performed a series of in vitro RNA-binding assays (Lee and Schedl, 2001; Mootz et al., 2004). The assay combined protein, either purified from bacteria or endogenous protein in a worm extract, with in-vitro-synthesized, single-stranded biotinylated RNAs corresponding to the *zif-1* 3′ UTR. Following pulldown of the RNA using streptavidin-coated magnetic beads, proteins also pulled down were assayed by SDS-PAGE and western blotting analyses using specific antibodies. We observed that both MBP-tagged OMA-1 protein generated in bacteria and OMA-1 from wild-type worm extracts bound to the *zif-1* 3′ UTR in a strand- and sequence-specific manner (Fig. 5C).

We divided the 300 nucleotide *zif-1* 3′ UTR into five regions (I-V) and observed that region III is both necessary and sufficient for binding by bacterially expressed OMA-1 (Fig. 5A,C). We also assayed various deletions within the 3′ UTR sequence for their ability to repress the expression of GFP::H2B in vivo (Fig. 5B). Consistent with the in vitro binding result that OMA proteins bind to region III, deletion of region III resulted in the derepression, albeit weak, of GFP::H2B in oocytes, as well as some meiotic nuclei preceding cellularization. A stronger derepression was observed when both regions II and III were deleted. Deleting regions I, IV or V individually did not result in derepression in the gonad (see Fig. S3 in the supplementary material). Regions II and III are not only necessary, but also sufficient, for repression of $P_{pie\text{-}1}$-*gfp::h2b-UTR*^{zif} in oocytes. This result suggests that repression of *zif-1* translation in oocytes requires OMA proteins binding to region III. However, it also suggests that one or more additional RNA-binding proteins that bind to region II function in *zif-1* translational repression.

Repression of the *zif-1* reporter requires the eIF4E-binding protein, SPN-2

We showed previously that OMA-1 binds to SPN-2, an eIF4E-binding protein (4E-BP), and that together these two proteins might repress translation of *mei-1*, the meiotic katanin, in the 1-cell embryo (Li et al., 2009; Srayko et al., 2000). 4E-BP binding to eIF4E, the 5′ CAP binding protein, prevents binding of eIF4G to eIF4E, which is required for 40S ribosomal subunit recruitment (Gingras et al., 1999), thereby inhibiting translation initiation. Although some 4E-BPs repress translation of all mRNAs, others, like Maskin and Cup, are targeted to a small number of mRNAs through interactions with specific RNA-binding proteins (Richter and Sonenberg, 2005). The phenotype of *spn-2* mutant embryos suggests that SPN-2 has a limited number of RNA targets (Li et al., 2009). SPN-2 does not contain an RNA-binding motif and is believed to bind to RNA indirectly through its association with eIF4E or other RNA-binding proteins (Li et al., 2009). Therefore, the specificity of SPN-2 targets is likely to be determined by the sequence-specific RNA-binding proteins with which SPN-2 interacts. Because OMA-1 binds to SPN-2, we asked whether SPN-2 is required for translational repression of *zif-1* in oocytes. Indeed, we observed a robust derepression of GFP::H2B$^{zif\text{-}1}$ in oocytes of *spn-2(RNAi)* animals (Fig. 5B). This result supports a model in which the OMA proteins repress translation of *zif-1* by preventing translational initiation via its interaction with SPN-2 and, indirectly, the 5′ CAP binding protein, eIF4E.

Ectopic OMA-1 protein in embryos can repress *zif-1* translation

We showed previously that the *oma-1(zu405)* mutation (P240L) abolishes or greatly reduces MBK-2-mediated phosphorylation and prevents the timely degradation of OMA-1 after the first mitotic cycle (Fig. 6) (Lin, 2003; Nishi and Lin, 2005). In *oma-1(zu405)* embryos, translation of GFP::H2B$^{zif\text{-}1}$ in somatic blastomeres is repressed and degradation of PIE-1 ZF1 is dramatically delayed [94%, *n*=33 and 75%, *n*=76, respectively; Fig. 6] (Lin, 2003). We observed a similar defect in translation of GFP::H2B$^{zif\text{-}1}$ and delayed degradation of PIE-1 ZF1 in embryos depleted of *mbk-2* (88%, *n*=18 and 92%, *n*=13, respectively), as well as *cul-2* (100%, *n*=12 and 88%, *n*=18, respectively; Fig. 6), which is required for MBK-2 activity (Stitzel et al., 2006). OMA-1 degradation is also defective in embryos depleted of *cul-1*, the likely E3 ligase responsible for OMA protein degradation (Shirayama et al., 2006). However, in dramatic contrast to *oma-1(zu405)*, *mbk-2(RNAi)* and *cul-2(RNAi)* embryos, degradation of PIE-1 ZF1 and repression of

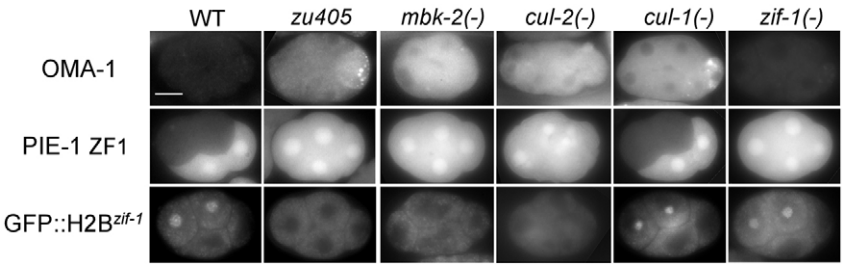

Fig. 6. Ectopic unphosphorylated OMA-1 protein in embryos can repress *zif-1* translation. Immunofluorescence micrographs of 8- to 12-cell embryos stained with anti-OMA-1 (top row) and fluorescence micrographs of 4-cell embryos expressing GFP::PIE-1 ZF1 (middle row) or GFP::H2B^{zif-1} (bottom row) in wild-type, *oma-1(zu405)*, *mbk-2(RNAi)*, *cul-2(RNAi)*, *cul-1(RNAi)* and *zif-1(RNAi)* backgrounds. Scale bar:10 μm.

GFP::H2B^{zif-1} both appear wild-type in *cul-1(RNAi)* embryos, despite a high level of persisting OMA proteins (100%, *n*=16; Fig. 6; data not shown). Whereas MBK-2-dependent OMA-1 phosphorylation is compromised in *cul-2(RNAi)*, *mbk-2(RNAi)* or *oma-1(zu405)* embryos, it is not affected in *cul-1(RNAi)* animals (DeRenzo et al., 2003; Guven-Ozkan et al., 2008). Indeed, we showed previously that ectopic OMA-1 in *cul-1(RNAi)* embryos exhibits MBK-2-dependent TAF-4 sequestration (Guven-Ozkan et al., 2008). Therefore, ectopic OMA protein in embryos is sufficient to repress translation of the *zif-1* reporter and to inhibit PIE-1 degradation in somatic cells. These results also suggest that phosphorylation of OMA-1/2 by MBK-2 inhibits repression of the *zif-1* reporter mediated by ectopic OMA proteins.

Phosphorylation of OMA-1 by DYRK2 displaces SPN-2 from the *zif-1* 3′ UTR RNA

To elucidate the mechanism by which MBK-2 phosphorylation interferes with the translational repression of *zif-1* mRNA by OMA proteins, we performed the following biochemical analyses using the commercially available human DYRK2 (hDYRK2) kinase. First, we asked whether phosphorylation by hDYRK2 prevented OMA-1 association with, or promoted OMA-1 dissociation from, the *zif-1* 3′ UTR. As shown in Fig. 7A-C, pre-phosphorylation of MBP::OMA-1 by hDYRK2 did not interfere with OMA-1 binding to the *zif-1* RNA, nor did phosphorylation of OMA-1 that was already bound to the *zif-1* 3′ UTR promote its dissociation from the RNA. Second, we tested whether MBK-2

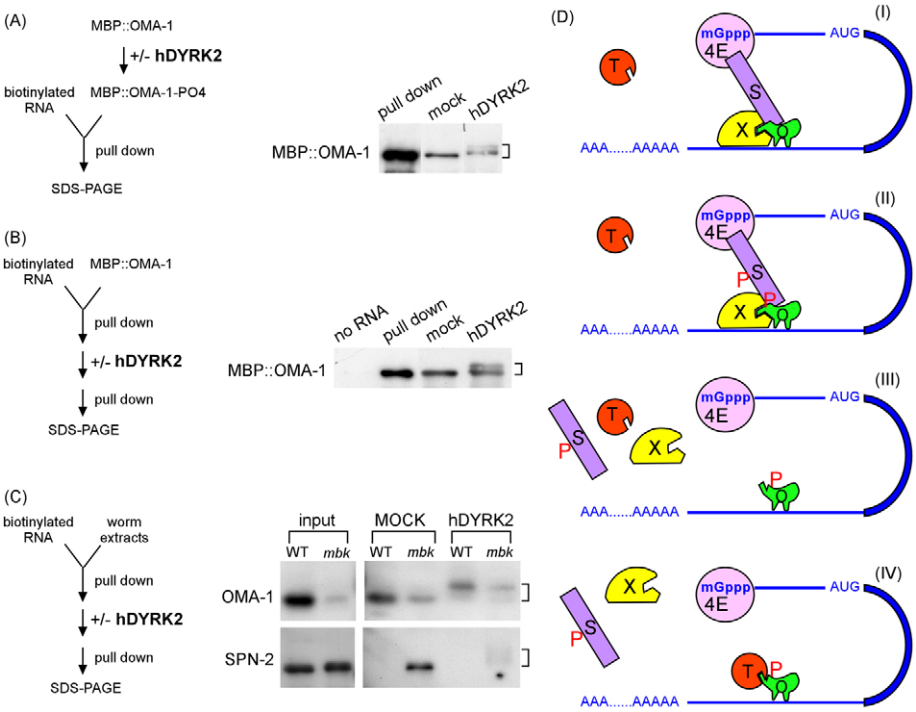

Fig. 7. MBK-2 phosphorylation releases SPN-2 but not OMA-1 from the *zif-1* 3′ UTR. (**A-C**) In vitro RNA pulldown experiments using *zif-1* 3′ UTR regions II+III. Schematic of the pulldown experiments is shown to the left and representative western blot results to the right. Mock reactions were processed identically to kinase reactions except that no hDYRK2 was present. (A,B) MBP::OMA-1 was phosphorylated by hDYRK2 either prior to RNA binding (A) or following RNA binding and pulldown (B). The primary antibody was anti-MBP. (C) Proteins from worm extracts were bound to *zif-1* 3′ RNA and then phosphorylated by hDYRK2. The primary antibodies were anti-OMA-1 and anti-SPN-2. Input: 1.3% of lysate used in the pulldown. Note that SPN-2 binding to the *zif-1* 3′ UTR is only detectable using the *mbk-2* extract (MOCK) and is greatly reduced following hDYRK2 incubation. The small amount of SPN-2 that remains bound to the RNA exhibits retarded gel migration, suggesting it could be phosphorylated. *mbk*, *mbk(ne992)* extract; WT, wild-type extract. (**D**) Model: (I) OMA (O), SPN-2 (S), eIF4E (4E) and other RNA-binding protein(s) (X) form a complex linking the *zif-1* 3′ UTR (blue line adjacent to the poly A tail) and 5′ CAP (mGppp), which inhibits translation. (II) Phosphorylation of OMA-1 and other factors dissociates the complex, (III) releasing SPN-2 and allowing translation. (IV) MBK-2 phosphorylation is likely to promote OMA binding to TAF-4 by inducing a conformational change.

phosphorylation affected the association of SPN-2 with the *zif-1* 3′ UTR. We noticed that the abundance of SPN-2 in wild-type extracts varied dramatically from one sample to another, whereas SPN-2 levels remained similarly abundant in extracts prepared from *mbk-2(ne992)* animals. Interestingly, using extracts with comparable amounts of SPN-2, we were able to pull down SPN-2 with the *zif-1* 3′ UTR only from *mbk-2(ne992)* and not wild-type extracts (Fig. 7C). This result suggests that MBK-2 activity, either directly or indirectly, affects SPN-2 protein levels as well as SPN-2 binding to the *zif-1* 3′ UTR.

Strikingly, upon hDYRK2 phosphorylation, the majority of SPN-2 pulled down from the *mbk-2(ne992)* extracts was displaced from the *zif-1* 3′UTR, whereas bound OMA-1 levels remained unchanged (Fig. 7C). The small amount of SPN-2 that remained bound to the *zif-1* 3′ UTR in the hDYRK2-treated sample showed retarded mobility, suggesting that SPN-2 was phosphorylated by hDYRK2 in vitro. Sequence analysis identified one potential MBK-2 phosphorylation site (T433) in SPN-2. These results demonstrate that phosphorylation of OMA-1/2 (and possibly SPN-2 or other not-yet-identified proteins bound to the *zif-1* 3′ UTR) by hDYRK2 weakens the interaction between SPN-2 and the *zif-1* 3′ UTR-protein complex, releasing SPN-2 from the *zif-1* 3′ UTR (Fig. 7D). This provides a molecular mechanism by which MBK-2 phosphorylation inhibits translational repression of the ZIF-1 reporter in vivo (Fig. 6).

DISCUSSION

We have shown previously that OMA proteins directly repress global transcription in early germline blastomeres in *C. elegans* by sequestering from the nucleus TAF-4, a TATA binding-protein-associated factor and a key component of TFIID. Here, we show that OMA proteins also repress transcription in germline blastomeres indirectly, through a completely different mechanism that operates in oocytes. OMA proteins bind to both the 3′ UTR of the *zif-1* transcript as well as the eIF4E-binding protein, SPN-2, repressing translation of *zif-1* mRNA in oocytes. Although PIE-1 asymmetry in the early embryo does not depend on degradation, but rather on diffusion properties of PIE-1 (Daniels et al., 2009), inhibition of *zif-1* translation in oocytes is crucial to ensure maintenance of PIE-1 levels in oocytes and, as a consequence, PIE-1 levels in germline blastomeres. In this way, the OMA proteins, which are degraded at the end of the first zygotic cell cycle and are absent from the later germline blastomeres, indirectly promote transcriptional repression in these cells. We also show that the two OMA protein functions are strictly regulated in both space and time by MBK-2-dependent phosphorylation, as the MBK-2 kinase itself is only activated following fertilization. Phosphorylation of OMA (and possibly other) proteins in P0 displaces SPN-2 from the *zif-1* 3′ UTR, releasing translational repression. This ensures correct developmental timing for activation of the E3 ligase that will function to degrade the small amount of PIE-1 protein that remains in somatic blastomeres following the early embryonic cell divisions. We propose that MBK-2 phosphorylation serves as the developmental switch that converts OMA proteins from repressors of specific protein translation in the gonad to global transcriptional repressors in the earliest germline precursors. Both OMA protein functions, one acting directly in the earliest germline blastomeres and the other acting earlier and indirectly in the oocytes, together help guarantee transcriptional repression in all germline blastomeres.

Our results demonstrate that repression of *zif-1* translation requires the sequence-specific RNA-binding proteins, OMA-1/2, and the 4E-BP, SPN-2. 4E-BPs compete with eIF4G for binding to eIF4F, an essential subunit for binding of 40S ribosomes to mRNA. Through its interaction with OMA proteins at the 3′ UTR and eIF4E at the 5′ CAP of the *zif-1* mRNA, SPN-2 enables the formation of an inhibitory closed loop that precludes the binding of the translational initiation complex (Fig. 7D) (Jackson et al., 2010). It has been speculated that all *C. elegans* tandem CCCH zinc-finger proteins bind RNA with less stringent RNA sequence requirements compared with the vertebrate TIS11 proteins (Pagano et al., 2007). Sequence specificity toward target mRNAs for *C. elegans* CCCH zinc-finger proteins probably comes from cooperative binding of multiple RNA-binding proteins. Our in vivo reporter assay suggested that an additional factor(s) binds to region II of the *zif-1* 3′ UTR and contributes to its translational repression. We believe that this factor(s) probably contributes to the stability and/or specificity of *zif-1* mRNA binding by the OMA proteins.

OMA proteins are dual functional proteins with distinct domains performing distinct functions. One intriguing aspect of their dual functionality is how the RNA-binding-dependent functions intersect with their RNA-binding-independent functions as global transcriptional repressors. Multifunctional proteins are well-known in many different systems. The majority of these proteins display multifunctionality within the same biochemical process. For example, plant MFP possesses four enzymatic activities involved in fatty acid β-oxidation (Preisig-Muller et al., 1994). Other multifunctional proteins are involved in completely different processes. For example, in addition to its function in protein synthesis, tryptophanyl tRNA synthetase can bind to VE-cadherin and inhibit angiogenesis (Zhou et al., 2010). Examples of multifunctional proteins where the mechanism of the switch in function is known are few. Proteolytic cleavage of tryptophanyl tRNA synthetase produces a smaller peptide that can bind VE-cadherin (Zhou et al., 2010). Phosphorylation induces a conformational change that converts phosphofructokinase 2, the enzyme that regulates the rates of glycolysis versus gluconeogenesis, from exhibiting a kinase activity to exhibiting a phosphatase activity (Kurland et al., 1992).

We show here that MBK-2 phosphorylation not only facilitates OMA-1 and TAF-4 binding in mammalian tissue culture cells (Guven-Ozkan et al., 2008), but also simultaneously inactivates the RNA-binding-dependent OMA-1 function required in oocytes. Unlike the allosteric mode of regulation for phosphofructokinase 2 or the irreversible cleavage of tryptophanyl tRNA synthetase, the dual functions of OMA proteins regulate completely different biochemical processes and are switched by a reversible modification. The mutual exclusivity of the two OMA functions is very different from a modification that simply adds a second function. It suggests that both functions must not overlap within the organism, and this is supported by the available genetic evidence. There might be a developmental requirement that the first function be completed before the second function initiates or that the second function must initiate immediately upon termination of the first function. This very stringent functional switch is best achieved via a single dual-function protein whose modification, which is also stringently timed, shuts down the first function while simultaneously activating the second function. On top of that, phosphorylation of the OMA proteins by MBK-2 results not only in their switch in function, but also marks the proteins for proteasomal degradation, delimiting the second OMA protein function, sequestration of TAF-4, to the 1-cell embryo. The OMA proteins, along with MBK-2, play an important role, perhaps the key role, in orchestrating the oocyte-to-embryo transition.

We present a molecular mechanism by which the two functions of OMA proteins can be switched by MBK-2 phosphorylation. We propose that OMA proteins, regardless of their phosphorylation state, can bind to the *zif-1* mRNA 3′ UTR. The phosphorylation status of the OMA proteins, however, dictates the association of other proteins with the *zif-1* mRNA. Phosphorylation of OMA-1 at T239 by MBK-2 results in the dissociation of SPN-2, releasing the translational repression. We feel it is unlikely that phosphorylation of OMA-1 at T239 directly interferes with SPN-2 binding, as such a mechanism would predict that phosphorylation by MBK-2 would affect all targets regulated by both OMA and SPN-2 proteins. Currently, there is no indication that translational repression of *mei-1*, which also requires OMA-1 and SPN-2, is regulated by MBK-2 phosphorylation (Li et al., 2009). Therefore, we believe it more likely that phosphorylation of OMA proteins interferes with the binding of OMA protein with another protein that is specific to the *zif-1* mRNA/RNP complex, indirectly affecting the binding of SPN-2. Our in vitro data suggest that SPN-2 might also be a substrate of MBK-2 in vivo. It has been shown that phosphorylation of 4E-BPs in response to numerous stimuli weakens their interaction with eIF4E, thereby initiating the translation of target mRNAs (Gingras et al., 1999; Jackson et al., 2010; Van Der Kelen et al., 2009). As the in-vitro-synthesized, biotinylated *zif-1* 3′ UTR RNA used in our pulldown assays does not possess a 5′ CAP, the hDYRK2-induced dissociation of SPN-2 can not be explained by a dissociation from eIF4E. It is, however, possible that in vivo phosphorylation of SPN-2 induces its dissociation from eIF4E, contributing to the derepression of *zif-1* translation during the egg-to-embryo transition. TAF-4 binding could be the result of a conformational change of the OMA proteins due to MBK-2 phosphorylation, or a consequence of newly available binding sites caused by the phosphorylation-promoted dissociation of other factors. Our previous studies showed that MBK-2 phosphorylation facilitates OMA-1 and TAF-4 binding in mammalian tissue culture cells, supporting the first possibility.

The identification of *zif-1* as a target mRNA for OMA proteins is very significant because translational derepression of *zif-1* in oocytes is detrimental to transcriptional repression in germline blastomeres. The two independent functions of OMA proteins have the same goal: to guarantee the totipotency of germline blastomeres by keeping them transcriptionally repressed. The multiple levels of regulation utilized to achieve this aim, including translational repression of *zif-1* in oocytes and transcriptional repression in P0 and P1 by the two functionally redundant OMA proteins, as well as transcriptional repression in P2-P4 by PIE-1, highlight just how important it must be for the animal to ensure transcriptional repression in germline blastomeres. Our results suggest that PIE-1 function is not sufficient to repress transcription in P0 or P1 because animals expressing only Δ46-80 OMA-1 are transcriptionally derepressed in P0 and P1, despite a high level of PIE-1. In light of our findings that OMA proteins exhibit dual, mutually exclusive functions, we suggest that PIE-1 might have a function in oocytes or 1-cell embryos that is distinct from its later function as a transcriptional repressor.

Not all phenotypes associated with the *oma-1(zu405)* mutation can be explained by the persistence of known ZIF-1 target proteins (PIE-1, MEX-1, POS-1, MEX-5 and MEX-6), making it probable that OMA proteins repress translation of additional proteins as well (Lin, 2003). Indeed, at least two other target RNAs of OMA proteins, *nos-2* and *mei-1*, have recently been identified (Jadhav et al., 2008; Li et al., 2009). However, unlike what we present here for ZIF-1 translational repression, repression of MEI-1 by SPN-2 and OMA-1 appears to take place in the embryo and not in oocytes (Li et al., 2009). Although the precise timing for OMA- and SPN-2-mediated *mei-1* translational repression in embryos has not been determined, it appears that the repression is not subject to MBK-2 regulation. We propose that SPN-2 is an obligate component of many OMA-mediated translational repressions and that OMA proteins regulate different mRNA targets by cooperating with different accessory RNA-binding proteins, allowing the repression to be subject to different modes of regulation.

Finally, the *C. elegans* germline lineage bears some resemblance to a stem cell lineage, and repression of expression of genes associated with lineage specific differentiation is a common feature of various types of stem cells. Our studies on OMA-1 and -2, both on TAF-4 sequestration and translational repression leading to the stabilization of PIE-1, might shed light on an additional mechanism(s) by which transcription can be repressed in vertebrate stem cells.

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Competing interests statement

The authors declare no competing financial interests.

Supplementary material

Supplementary material for this article is available at
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