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# 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

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**Abstract**

In vertebrates, oocytes undergo maturation, arrest in metaphase II, and can then be fertilized by sperm. Fertilization initiates molecular events that lead to the activation of early embryonic development. In *Caenorhabditis elegans*, where no delay between oocyte maturation and fertilization is apparent, oocyte maturation and fertilization must be tightly coordinated. It is not clear what coordinates the transition from an oocyte to an embryo in *C. elegans*, but regulated turnover of oocyte-specific proteins contributes to the process. We describe here a gain-of-function mutation (*zu405*) in a gene that is essential for oocyte maturation, *oma-1*. In wild type animals, OMA-1 protein is expressed at a high level exclusively in oocytes and newly fertilized embryos and is degraded rapidly after the first mitotic division. The *zu405* mutation results in improper degradation of the OMA-1 protein in embryos. In *oma-1(zu405)* embryos, the C blastomere is transformed to the EMS blastomere fate, resulting in embryonic lethality. We show that degradation of several maternally supplied cell fate determinants, including SKN-1, PIE-1, MEX-3, and MEX-5, is delayed in *oma-1(zu405)* mutant embryos. In wild type embryos, SKN-1 functions in EMS for EMS blastomere fate specification. A decreased level of maternal SKN-1 protein in the C blastomere relative to EMS is believed to be responsible for this cell expressing the C, instead of the EMS, fate. Delayed degradation of maternal SKN-1 protein in *oma-1(zu405)* embryos and resultant elevated levels in C blastomere is likely responsible for the observed C-to-EMS blastomere fate transformation. These observations suggest that *oma-1*, in addition to its role in oocyte maturation, contributes to early embryonic development by regulating the temporal degradation of maternal proteins in early *C. elegans* embryos.


*Keywords:* *C. elegans*; *oma-1*; Embryo; Fate transformation; Protein degradation; SKN-1; Gain-of-function

## Introduction

In *Caenorhabditis elegans*, blastomeres become committed to distinct developmental fates very early during embryogenesis. Following the first embryonic division, for example, the posterior and anterior blastomeres have already committed to different lineage patterns and tissue types. The posterior blastomere, P1, is the germline precursor and divides in a stem cell-like pattern, giving rise to a germline precursor and a somatic sister at each division (see Fig. 1). The anterior blastomere, AB, divides symmetrically and produces tissues with distinct fates. After a sequence of five asymmetric divisions in early embryos, six embryonic founder cells are born that have distinct lineage patterns and tissue types.

Factors required for proper development of early *C. elegans* embryos are provided maternally and prepackaged in oocytes. Many maternal proteins important for cell fate determination have distinct spatial and temporal localization patterns (Bowerman et al., 1993; Mello et al., 1996). The combinatorial expression of these maternal factors in early blastomeres plays a key role in the commitment of blastomeres to distinct patterns of differentiation during the rapid cell divisions characteristic of early embryogenesis (see reviews by Bowerman, 2000; Rose and Kemphues, 1998). The inappropriate localization of these maternal proteins results in cell fate transformations of particular early

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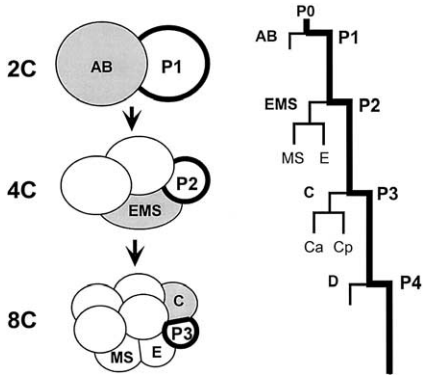


Fig. 1. Schematic diagrams of early embryos and cleavages of *C. elegans* embryos. Horizontal lines on the lineage diagram represent cell divisions. The branch of the lineage that produces germline precursors is indicated by thick black line. Names of blastomeres mentioned in the text are listed. In the embryo diagram, germline blastomeres are outline in thick black lines and their corresponding somatic sisters are shown shaded. Anterior is to the left and dorsal is up.

blastomeres (Bowerman et al., 1993; Guedes and Priess, 1997; Hunter and Kenyon, 1996; Schubert et al., 2000).

Mechanisms regulating asymmetric distribution of key regulators have been well characterized in several model systems. For example, in *Drosophila* neuroblasts, the homeodomain protein Prospero is targeted to a crescent in the basal cortex of the dividing neuroblast and is therefore asymmetrically inherited by the basal daughter (Hirata et al., 1995; Knoblich et al., 1995). In contrast, the asymmetric localization of the budding yeast Ash1p protein is a result of asymmetric localization of its mRNA to the daughter cell (Bertrand et al., 1998; Long et al., 1997; Takizawa et al., 1997). A recent study has shown that, in mollusc embryos, several mRNAs are asymmetrically segregated to daughter cells by localizing to particular centrosomes prior to division (Lambert and Nagy, 2002). In *C. elegans,* although most maternal regulatory proteins are asymmetrically localized in early embryos, the majority of these proteins are supplied as mRNA, and most of these mRNAs are evenly distributed throughout all early embryonic cells (Seydoux and Fire, 1994). RNA localization is therefore unlikely to be the major mechanism for the regulation of asymmetric protein localization in early *C. elegans* embryos. At least two maternal proteins, GLP-1 and PAL-1, are regulated by asymmetric translation of mRNA in the daughter cells following a division (Evans et al., 1994; Hunter and Kenyon, 1996). Protein degradation can also regulate asymmetric distribution of maternal regulators and asymmetric cell fates in *C. elegans.* Reese et al. (2000) showed that the PIE-1 protein is asymmetrically localized to the germline lineage through two distinct mechanisms: asymmetric enrichment of the PIE-1 protein to that side of the cell destined to become a germline precursor after cell division, and selective degradation of the PIE-1 protein in the somatic sister cells. These two mechanisms underlying asymmetric PIE-1 localization are mediated by distinct domains within the PIE-1 protein. PIE-1 belongs to a family of proteins that contain two TIS11-like (CCCH) zinc fingers (Mello et al., 1996). The first zinc finger is necessary and sufficient to direct PIE-1 protein degradation in the somatic blastomeres (Reese et al., 2000).

In addition to PIE-1, several other TIS11 zinc finger-containing proteins have been shown genetically to function in the specification of early blastomere fate (Guedes and Priess, 1997; Schubert et al., 2000; Tabara et al., 1999). MEX-1 and POS-1, like PIE-1, are also germline-specific proteins. Like PIE-1, the germline-specific distribution pattern of these two proteins also relies on first zinc finger-based selective degradation in somatic sister cells (Reese et al., 2000). Two other TIS11 zinc finger proteins, MEX-5 and MEX-6, are nearly identical proteins with redundant functions during embryogenesis (Schubert et al., 2000). The spatial distribution pattern of MEX-5 is complementary to that of PIE-1, MEX-1, and POS-1, being detected only in somatic sisters and not germline precursors (Schubert et al., 2000). The mechanism that regulates the distribution pattern of MEX-5 has not been determined.

OMA-1 and OMA-2 are also closely related TIS11 finger proteins with redundant biological function during *C. elegans* development (Detwiler et al., 2001). They are unique compared with other TIS11 finger proteins characterized so far in that they play an essential role in the development of the *C. elegans* germline, specifically in oocyte maturation (Detwiler et al., 2001). We have shown previously that both OMA-1 and OMA-2 express in developing oocytes, peaking in the maturing oocyte. Both OMA-1 and OMA-2 proteins were also detected in newly fertilized embryos, but it was not known whether their functions are required in embryo development as *oma-1; oma-2* mutant animals are sterile (Detwiler et al., 2001).

We describe here the very unique temporal distribution of OMA-1 protein in early *C. elegans* embryos and an embryonic lethal phenotype resulting from inappropriate degradation of the OMA-1 protein. Using a GFP fusion, we show that the level of OMA- 1 protein remains high in wild-type fertilized zygotes but is rapidly degraded immediately after the first mitotic division. This degradation is important for embryonic development as a gain-of-function mutation *zu405* that results in a defect in the proper degradation of OMA-1 protein results in embryonic lethality. In *zu405* mutant embryos, the C blastomere adopts the fate of the EMS blastomere. We show further that the C-to-EMS fate transformation is likely a result of delayed degradation of the maternal transcription factor, SKN-1. The degradation of several other maternal proteins, including PIE-1, POS-1, MEX-1, MEX-3, and MEX-5, is also delayed in *zu405* embryos. These results suggest that correct temporal regulation of OMA-1 protein degradation is critical for proper embryo development. Furthermore, our data suggest an intriguing model whereby the OMA-1 protein functions, in addition to in oocyte maturation, in early embryos to

regulate the correct temporal degradation of a subset of maternal factors.

## Materials and methods

### *Strains*

The N2 Bristol strain was used as the wild-type strain. The genetic markers used were: LGIV: *eDf18, fem-3(e1950), unc-129(ev554), oma-1(zu405).* All strains were cultured as described by Brenner (1974). Transgenic strains containing $P_{med\text{-}1}gfp$ (JR1186) or $P_{pie\text{-}1}gfp$ were provided by Joel Rothman (Maduro et al., 2001) and Geraldine Seydoux (Reese et al., 2000), respectively.

### *Genetics and rescue of oma-1(zu405)*

Homozygous *oma-1(zu405)* worms grown at 15°C have a low percentage (1–2%) of animals exhibiting defects in the posterior portion of the body. In these worms, the tail fails to undergo proper morphogenesis and often form a nob-like structure. We mapped the *zu405* mutation to chromosome IV and showed that the *zu405* locus is deleted in the deficiency *eDf18.* Hermaphrodites heterozygous for the deficiency *eDf18* produce approximately 25% of dead embryos, suggesting that the amount of dead embryos observed with *zu405*/+ hermaphrodites were not due to haploid insufficiency. Three-factor mapping places *zu405* approximately 0.04 map units to the left of the cloned gene *fem-3* and very close to *unc-129.* We have assayed cosmids surrounding the genomic region where the *unc-129* gene resides for their ability to rescue the embryonic lethality associated with *zu405.* Injection was performed by using the complex array method as described in Kelly et al (1997). Injected worms were cultured at 15°C until $F_2$. For each transgenic line, $F_2$ transgenic animals were shifted to 25°C at L4, and the viability of $F_3$ progeny was scored. While all other cosmids assayed produced no viable $F_3$ progeny, $F_2$ animals containing the cosmid C27B7 or C09G9 gave approximately 3–5 viable larvae, suggesting weak rescue. The rescuing activity was further narrowed down by injecting PCR amplified fragments covering the genomic region of overlap between these two cosmids. When a 6-kb region that contains only two transcription units, *C27B7.1* and *C09G9.6,* was injected, we obtained a higher number of viable $F_3$ larvae, up to approximately 30 progeny from 1 transgenic line. Depleting either of these two genes in wild type embryos by RNAi failed to reveal a phenotype. However, depleting *C09G9.6,* but not *C27B7.1,* in *zu405* mutant worms rescued the embryonic lethality at 25°C.

### *Generation of OMA-1-GFP-expressing strain*

The $P_{oma\text{-}1}gfp$ plasmid was constructed as follows: the 6-kb rescuing genomic sequence containing the entire OMA-1 coding sequence, 2.1 kb upstream, and 2.7 kb downstream coding sequences was cloned into the Bluescript pSK. An *Sfi*I site was engineered by site directed mutagenesis at the last nucleotide of *oma-1* coding region (pRL472). The *gfp* sequence was PCR amplified from pPD104.53 with primers each containing an *Sfi*I site and subcloned into the *Sfi*I site of pRL472 resulting in pRL475. Transgenic animals expressing OMA-1::GFP (TX189) were generated by microparticle bombardment according to the protocol by Praitis et al. (2001). TX189 segregates 100% transgenic animals, consistent with it being an integrated line (teIs1).

### *Analysis of embryos and imaging*

Light microscopy was performed with a Zeiss Axioskop II or an Axioplan II microscope equipped with epifluorescence, polarizing, and differential interference contrast (DIC) optics. Digital images were acquired and processed either with a C5985 chilled CCD camera (Hamamatsu Photonics) or with a MicroMax-512EBFT CCD camera (Princeton Instruments). Embryos were processed for light microscopy following the procedure of Sulston et al. (1983) and for immunofluorescence as described in Lin et al. (1998). Intestinal cells were identified by their birefringent gut-specific granules and by staining with the monoclonal antibody MabICB4 (Kemphues et al., 1988). Pharyngeal cells were assayed by staining with Mab3NB12, a monoclonal antibody recognizing a subset of pharyngeal muscles (Priess and Thomson, 1987). Laser ablations were performed at 25°C by using a VSL337 laser power (Laser Science) attached to a Zeiss Axioskop microscope as described in Avery and Horvitz (1989). After laser operation, embryos were incubated at 25°C for 12 h and examined under the light microscope and by immunofluorescence microscopy following antibody staining.

In situations where the GFP expression was monitored in live embryos, GFP and DIC images were collected with the MicroMax-512EBFT CCD camera and processed by using a custom program EditView4D as previously described (Rogers et al., 2002). Consecutive time points are 30 s apart. The raw pixel values were within the linear range of the CCD camera (0-4095). The 90% reduction of OMA-1::GFP after the first embryonic division was an estimation based on the fluorescence intensity on selected focal planes.

### *Antibody staining*

Both wild type and *oma-1(zu405)* mutant embryos were prepared for immunofluorescence as follows: worms were synchronized and cultured at 15°C until L4, shifted to 25°C for 16 h, and fixed for immunofluorescence at 25°C. Fixation protocols for OMA-1 (Detwiler et al., 2001), SKN-1 (Lin et al., 1998), MEX-1 (Guedes and Priess, 1997), MEX-3 (Draper et al., 1996), MEX-5 (Schubert et al., 2000), and POS- 1 (Tabara et al., 1999) were as described. Antibody dilutions used were: OMA-1 affinity purified, 1:2;

SKN-1 monoclonal F2A+F4C, no dilution (Bowerman et al., 1993); MEX-1 affinity purified, 1:5; MEX-3 monoclonal, no dilution; MEX-5 monoclonal, 1:5; POS-1, polyclonal 1:200; goat anti-mouse or goat anti-rabbit Alexa488 secondary antibody (Molecular Probes), 1:500.

*RNA interference*

Double-stranded RNA corresponding to *wrm-1* and *skn-1* were derived from clones yk283g8 and pRL803, respectively, and were injected at concentrations of 1–3 mg/ml (Hsu et al., 2000). All injections were performed into young adults raised at 15°C, then shifted to 25°C after injection (Hsu et al., 2000). Embryos collected 16 h after injection were scored for phenotype.

## Results

*Isolation of the temperature-sensitive, maternal-effect lethal mutation, zu405*

The *zu405* mutation was isolated in a screen for temperature-sensitive, maternal- effect, lethal mutations that result in excess intestinal tissues. Embryos from *zu405* homozygous mothers (hereafter *zu405* mutant embryos) produce 100% dead embryos at a nonpermissive temperature (25°C, $n > 1000$) and 30−80% dead embryos at a permissive temperature (15°C). *zu405/+* embryos derived from homozygous *zu405* hermaphrodites mated with wild type males at 25°C do not hatch, indicating that the mutant phenotype shows strict maternal effect. The temperature-sensitive lethality of *zu405* is semidominant, as *zu405/+* hermaphrodites generate approximately 80% dead embryos at 25°C ($n = 500$). At a nonpermissive temperature, embryos from mothers *trans*-heterozygote for *zu405* and the deficiency *eDf18* that removes the *zu405* locus appear indistinguishable from embryos produced by *zu405* homozygous mothers (see Materials and methods).

*The zu405 mutation is in the gene oma-1*

We positioned the *zu405* mutation by classic genetic mapping and cloned the gene mutated by transformation rescue (see Materials and methods). The *zu405* mutation is in the gene *oma-1,* which we have shown to function in *C. elegans* oocyte maturation (Detwiler et al., 2002). The *oma-1* gene encodes a protein containing two zinc fingers of a class first identified in the mouse TIS11 protein and found in more than 20 proteins in *C. elegans* (Detwiler et al., 2001; Guedes and Priess, 1997; Mello et al., 1996; Schubert et al., 2000; Tabara et al., 1999; Varnum et al., 1989). TIS11 zinc fingers are found in a small subset of the zinc finger protein superfamily and consist of C-$X_{8-10}$-C-$X_5$-C-$X_3$-H, where X refers to any amino acid (Bai and Tolias, 1996; DuBois et al., 1990; Varnum et al., 1989). We have shown that null mutations in *oma-1* have no abnormal phenotype but double loss-of-function mutations in *oma-1* and its most closely related *C. elegans* gene, *oma-2,* are sterile with a defect in oocyte maturation (Detwiler et al., 2002). The *zu405* mutation contains a single nucleotide change (C→T) in the coding region of *oma-1,* which converts proline 240 to leucine. Proline 240 is located within the C-terminal domain of the OMA-1 protein downstream of both TIS-11 zinc fingers. Depleting *oma-1* by *RNAi* in *oma-1(zu405)* mutant worms rescued the embryonic lethality at 25°C, indicating that *zu405* is a gain-of-function mutation (Detwiler et al., 2002).

*OMA-1 protein is detected at high levels in the one-cell embryo but is then rapidly and asymmetrically degraded*

OMA-1 and OMA-2 have redundant functions in oocyte maturation and have very similar expression patterns. Using antibodies specific to either OMA-1 or OMA-2, these two proteins are expressed in the cytoplasm with increasing levels in growing oocytes, peaking in the oocyte undergoing maturation (Detwiler et al., 2001). The levels of OMA-1 and OMA-2 proteins remain very high following fertilization as well as throughout the first mitotic cycle. The antibody staining often included a slight punctate pattern of fluorescence. Immediately after the first mitotic division, the levels of OMA-1 and OMA-2 proteins rapidly decreased and became difficult to detect with antibody staining (Fig. 2A, and data not shown).

The rapid decrease in the level of OMA-1 and OMA-2 after the first mitotic division was intriguing as it distinguished these two proteins from all other *C. elegans* maternal proteins characterized to date. Like mRNAs for most other maternally supplied regulatory proteins, the mRNA for *oma-2* is detected at a high level in all early blastomeres up to at least the four-cell stage (The Nematode Expression Pattern DataBase, http://nematode.lab.nig.ac.jp/db/index.html) Therefore, the rapid decrease in OMA protein levels is likely the result of rapid protein degradation. To investigate the dynamic regulation of the OMA-1 protein, we created transgenic animals expressing OMA-1::GFP under the control of its own promoter (see Materials and methods). The OMA-1::GFP fluorescence in the developing oocytes and one-cell embryos recapitulated the wild-type spatial and temporal patterns of OMA-1 antibody staining (Fig. 2B, and data not shown). The punctate staining appeared more pronounced and resembled the characteristic pattern of germline P granules. Starting with the onset of the first mitotic division, the intensity of OMA-1-GFP fluorescence rapidly decreased, and by the time the division was complete, only approximately 10% remained (Fig. 2B). Interestingly, that remaining 10% of the GFP signal in the two-cell embryo was predominantly found in the germline precursor, P1, associated with what appeared to be P granules (Fig. 2C). The GFP signal continued to decrease in two-cell embryos and again was asymmetric after the next

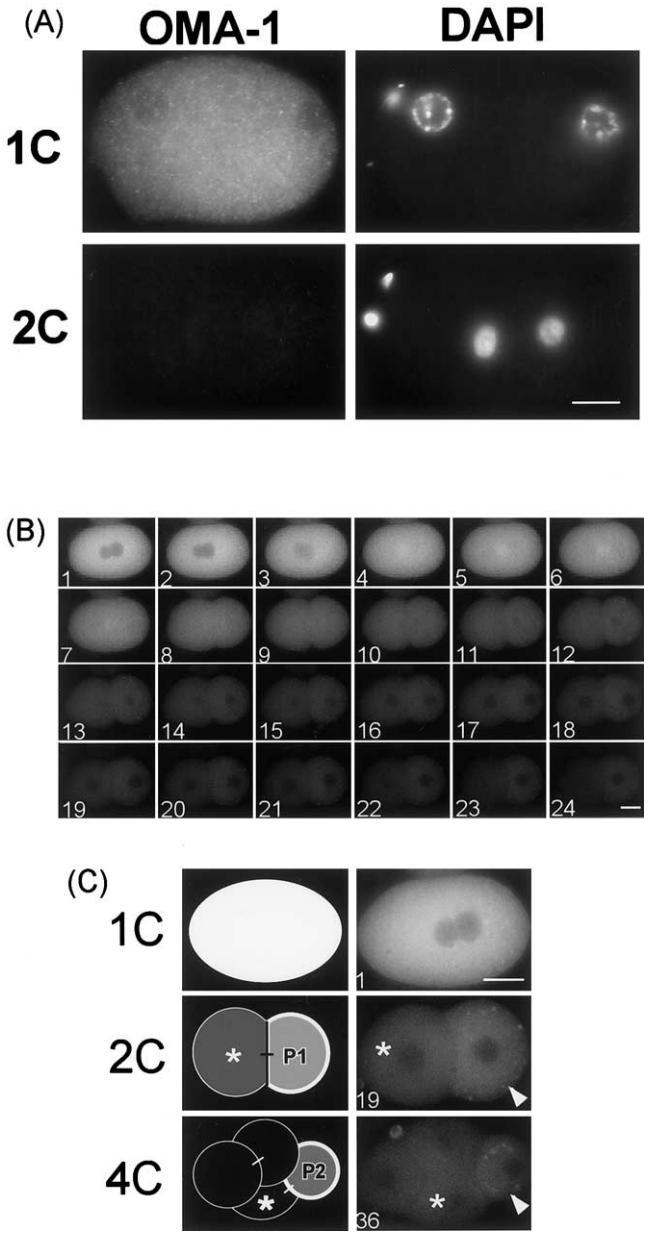


Fig. 2. Expression of the OMA-1 protein in embryos. (A) Fluorescence micrographs of 1-cell (top panels) and 2-cell (bottom panel) wild type embryos stained with affinity-purified OMA-1 antibody (left) and DAPI (right). A similar pattern was obtained with OMA-2 antibody (data not shown). (B) A montage of selected images from a movie recording of OMA-1::GFP from one-cell to two-cell stage. Adjacent frames are recorded approximately 1 min apart. Frame numbers are indicated at lower left corners. (C) OMA-1::GFP in one-cell, two-cell, and four-cell embryos. Schematic representation of the OMA-1::GFP is shown on the left-hand column, whereas the highly enhanced GFP images are shown on the right-hand column. Shades of gray represent different levels of GFP, with white being the brightest. The GFP signal shown in two-cell and four-cell embryos is highly enhanced (compare frames 19 in B and C). With the same scaling shown in (B), the signal in four-cell is not easily detected. Arrowheads, germline precursors; asterisks, corresponding somatic sisters. Short bars between cells indicate sister blastomeres. Bar, 10 $\mu$m.

division, with most of the remaining fluorescence segregated to P2, where it was also predominantly associated with granules (Fig. 2C). The OMA-1-GFP signal became too weak to detect in the embryo after the four-cell stage. The asymmetric segregation of OMA-1::GFP to the germline precursors is characteristic of three other TIS11 germline proteins: PIE-1, MEX-1, and POS-1 (Guedes and Priess, 1997; Mello et al., 1996; Tabara et al., 1999). Our ability to detect OMA-1 better in two-cell and four-cell embryos by GFP versus antibody staining probably reflects an increased sensitivity of detection with this method.

### *OMA-1 protein is not properly degraded in zu405 mutant embryos*

To understand the nature of the *zu405* gain-of-function mutation, we examined the expression of the OMA-1 and OMA-2 proteins in *oma-1(zu405)* mutant embryos. By antibody staining, we observed that, while OMA-2 protein levels in *oma-1(zu405)* mutant embryos appeared normal (data not shown), OMA-1 protein levels were elevated in embryos past the 1-cell stage compared with wild type embryos (Fig. 3). OMA-1 protein could be detected in more than 80% of the *zu405* embryos up to the 16-cell stage, in contrast to wild-type embryos where OMA-1 antibody staining was not detected in any 4- cell or older embryos. Although the temporal regulation of OMA-1 protein is defective in *zu405* embryos, its spatial localization appeared to be properly regulated. That is, the OMA-1 protein was still detected primarily in the germline precursors in *zu405* mutant embryos. OMA-1 protein in *zu405* mutant embryos is both cytoplasmic and P-granule- associated. The P-granule association of OMA-1 was confirmed by costaining with various antibodies known to recognize P-granules, such as OIC4, MEX-1 antibody, and POS-1 antibody (data not shown). The persistence of OMA-1 protein in germline precursors in *oma-1(zu405)* mutant embryos suggests that sequences surrounding $P_{240}$ are important for its rapid degradation. $P_{240}$ resides within a probable PEST domain, with PESTfind (Rechsteiner and Rogers, 1996) giving a score of $+9.7$. PESTfind rates sequences from $-50$ to $+50$ with a score of $+5$ or more signifying a probable PEST domain. When $P_{240}$ is mutated to leucine as in the *oma-1(zu405)* mutant, the PESTfind score for the sequence immediately surrounding this residue drops from $+9.7$ to $-0.2$.

### *oma-1(zu405) mutant embryos produce excess pharyngeal and intestinal cells*

To investigate why the *oma-1(zu405)* mutation results in embryonic lethality, we first characterized its embryonic lethal phenotype. *oma-1(zu405)* mutant embryos do not undergo proper morphogenesis and arrest before hatching with differentiated tissues, including intestinal, pharyngeal, neuronal, skin, and muscle cells (Fig. 4, and data not shown). The majority (75%) of *oma-1(zu405)* mutant embryos have excess pharyngeal and intestinal tissues as detected either by Nomarski optics or by staining with antibodies specific to either tissue (Fig. 4). Many mutant

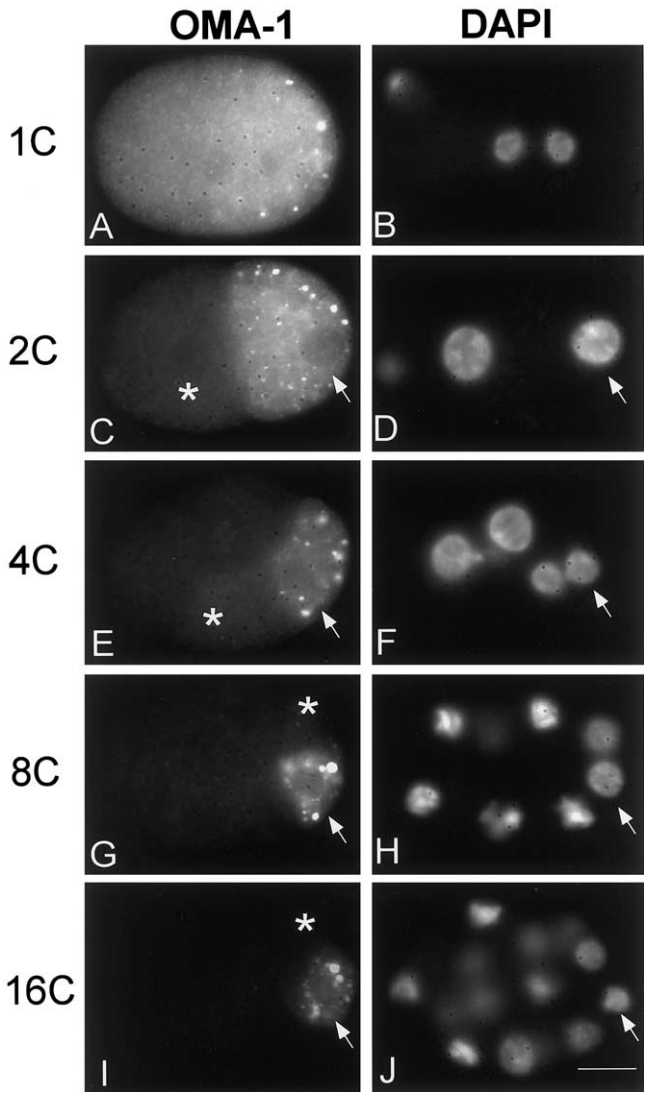


Fig. 3. The OMA-1 protein fails to degrade properly in *oma-1(zu405)* mutant embryos. Immunofluorescence micrographs of various staged *oma-1 (zu405)* mutant embryos stained with the affinity-purified antibody to OMA-1 (left-hand column) and DAPI (right-hand column). Arrowheads, germline precursors; asterisks, corresponding somatic sisters. Bar, 10 $\mu$m.

embryos have up to 40 intestinal cells, twice the number found in wild-type embryos. In most embryos observed, the excess intestinal cells are well organized and are located at the posterior–dorsal portion of the embryo parallel to the endogenous intestine.

During wild-type embryogenesis, only one blastomere in a 4-cell embryo, EMS, produces pharyngeal and intestinal cells. When EMS is killed, no pharyngeal or intestinal cells are produced (Priess and Thomson, 1987). We used a laser microbeam to ablate EMS in 10 *oma-1(zu405)* embryos. Eight of the operated embryos produced pharyngeal and intestinal cells, suggesting that, in *oma-1(zu405)* embryos, an additional blastomere or blastomeres adopts an EMS-like fate. Because of the posterior location of the extra pharyngeal and intestinal tissues in *oma-1(zu405)* mutant embryos, we asked whether these extra tissues are derived from the posterior blastomere, P2, in 4-cell embryos. The fate of individual blastomeres can be assayed by killing all other blastomeres and allowing the partial embryo to develop in isolation. In such experiments, wild-type EMS produces pharyngeal muscles, body-wall muscles, and intestinal cells, whereas wild-type P2 produces skin, body-wall muscles, and 2 germ cells, corresponding to the fates of EMS and P2 in intact embryos, respectively (Mello et al., 1992). After laser ablation of blastomeres in *oma-1(zu405)* embryos, an isolated P2 blastomere produces pharyngeal muscles, body-wall muscles, and intestinal cells, tissue types similar to those normally produced by a wild-type EMS blastomere (Table 1, and data not shown). This defect is reminiscent of the phenotype observed for a previously described mutant, *pie-1,* in which P2 adopts the fate of EMS and produces EMS-derived tissues (Mello et al., 1992). However, unlike *pie-1* mutant embryos in which no germ cells are produced, all *oma-1(zu405)* mutant embryos examined produced the wild-type number of 2 germ cells ($n = 30$; data not shown).

In wild-type embryos, germ cells are derived from one daughter of P2, termed P3, and not the somatic sister blastomere, C. The C blastomere normally produces skin and muscle cells that are located at the posterior–dorsal portion of the embryos. Because *oma-1(zu405)* mutant embryos produced germ cells and often lacked skin and muscle cells at the posterior–dorsal portion, we reasoned that the extra-

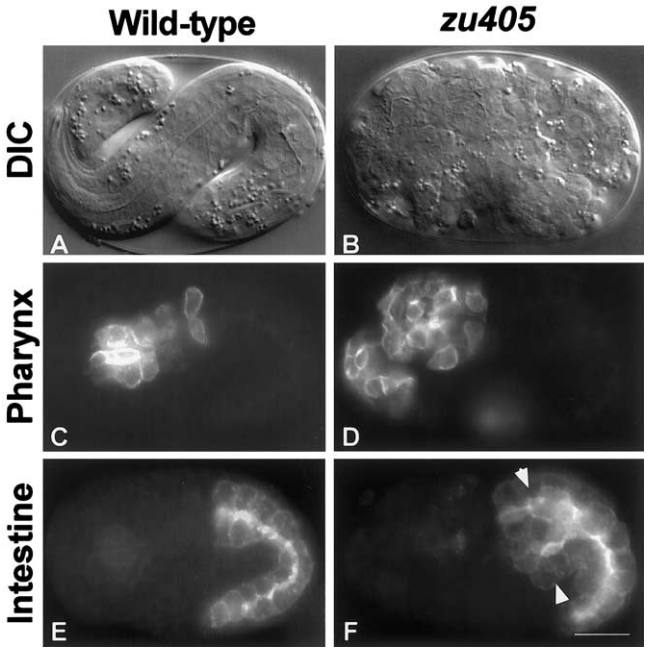


Fig. 4. *oma-1(zu405)* mutation results in excess pharyngeal and intestinal muscle. Tissue differentiation in wild type (A, C, E) and *oma-1(zu405)* mutant embryos (B, D, F) as shown by DIC images (A, B), and immunofluorescence images with pharynx-specific antibody Mab3NB12 (C, D) or intestine-specific antibody MabICB4 (E, F). Arrowheads in (F) point to a focal plane where two intestinal lumens can be clearly recognized. Anterior is to the left and dorsal is up. Bar, 10 $\mu$m.

Table 1
Tissue types produced by wild type and *oma-1(zu405)* partial embryos

| Blastomeres ablated | Wild type embryos | | | *oma-1(zu405)* embryos | | |
|---|---|---|---|---|---|---|
| | Pharynx and gut | Pharynx, no gut | No pharynx, no gut | Pharynx and gut | Pharynx, no gut | No pharynx, no gut |
| EMS | 0% ($n = 15$) | 0% ($n = 15$) | 100% ($n = 15$) | 80% ($n = 10$) | 20% ($n = 10$) | 0% ($n = 10$) |
| All but P2 | 0% ($n = 13$) | 0% ($n = 13$) | 100%[a] ($n = 13$) | 100% ($n = 7$) | 0% ($n = 7$) | 0% ($n = 7$) |
| All but C | 0% ($n = 10$) | 0% ($n = 10$) | 100%[a] ($n = 10$) | 43.4% ($n = 22$) | 10.8% ($n = 22$) | 45.8%[a] ($n = 22$) |
| All but EMS | 100% ($n = 15$) | 0% ($n = 15$) | 0% ($n = 15$) | 73.9% ($n = 23$) | 26.1% ($n = 23$) | 0% ($n = 23$) |

[a] Partial embryos produce skin and muscles in these experiments.

pharyngeal and intestinal tissues in *oma-1(zu405)* embryos could be derived from a transformation of the C blastomere to an EMS-like fate. We investigated this possibility by ablating with a laser microbeam all blastomeres except the C blastomere in *zu405* embryos. In contrast to wild-type C blastomeres, which produce skin and muscle in these experiments, over half (56%, $n = 22$) of C blastomeres from *oma-1(zu405)* embryos produced either pharyngeal cells or pharyngeal and intestinal cells (Table 1). Conversely, when P3 was isolated in *oma-1(zu405)* embryos, we never observed pharyngeal or intestinal tissues (data not shown). These results suggest that, in *oma-1(zu405)* embryos, the C blastomere adopts an EMS-like fate, resulting in extrapharyngeal and intestinal tissues.

### *The C blastomere in the oma-1(zu405) mutant expresses high levels of EMS transcription factor MED-1 and SKN-1*

In wild-type embryos, production of the EMS-derived pharyngeal and intestinal tissues requires four factors: SKN-1, POS-1, MED-1, and MED-2 (Bowerman et al., 1992; Maduro et al., 2001; Tabara et al., 1999). In the absence of these factors, EMS adopts the fate of the C blastomere and produces skin and muscle. SKN-1, MED-1, and MED-2 are all transcription factors whose activities are restricted to the EMS lineage (Bowerman et al., 1992, 1993; Maduro et al., 2001). POS-1 is not expressed in EMS, and its function in EMS fate remains unclear (Tabara et al., 1999). We examined whether the C blastomere in *oma-1(zu405)* embryos expresses any of these EMS factors and whether this expression could account for the transformation to an EMS-like fate.

In wild-type embryos, SKN-1 protein is detected at high levels in the nuclei of EMS and P2 but at low levels in the four daughters of EMS and P2, the MS, E, C, and P3 blastomeres (Fig. 5B–D; Bowerman et al., 1993). In contrast, in *oma-1(zu405)* mutant embryos, the level of SKN-1 protein remains high in the daughters of EMS and P2 (18 of 19 embryos examined; Fig. 5F–H). In fact, the level of SKN-1 protein in the C blastomere of *oma-1(zu405)* mutant embryos is comparable with that in a wild-type EMS blastomere. The level of SKN-1 protein decreases in the 12-cell-stage *oma-1(zu405)* embryos and is not detectable after the 12-cell stage. Generation of the extrapharyngeal and intestinal tissues in *zu405* mutant embryos requires SKN-1, as RNAi of *skn-1* in *zu405* embryos results in embryos without any pharyngeal tissues (data not shown). Approximately 30% ($n > 200$) of the RNAi embryos still produce intestinal tissues, consistent with the percentage of embryos producing intestine in *skn-1* null mutant embryos (Bowerman et al., 1992).

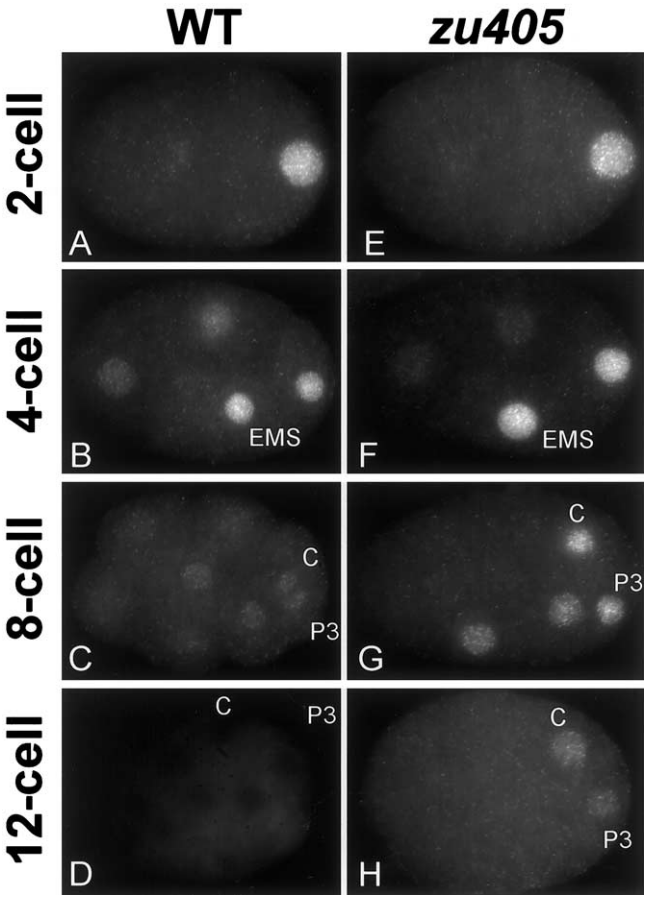


Fig. 5. Delayed degradation of maternal SKN-1 protein in *oma-1(zu405)* embryos. Fluorescence micrographs of wild-type (A–D) and *oma-1(zu405)* mutant embryos (E–H) shown stained with SKN-1 antibodies. Embryonic stages are indicated to the left. EMS, C, and P3 nuclei are indicated by blastomere names. MS and E nuclei are at metaphase in (H) and have no apparent nuclear SKN-1 staining. Note the high level of SKN-1 in the C blastomere in (G). Bar, 10 $\mu$m.

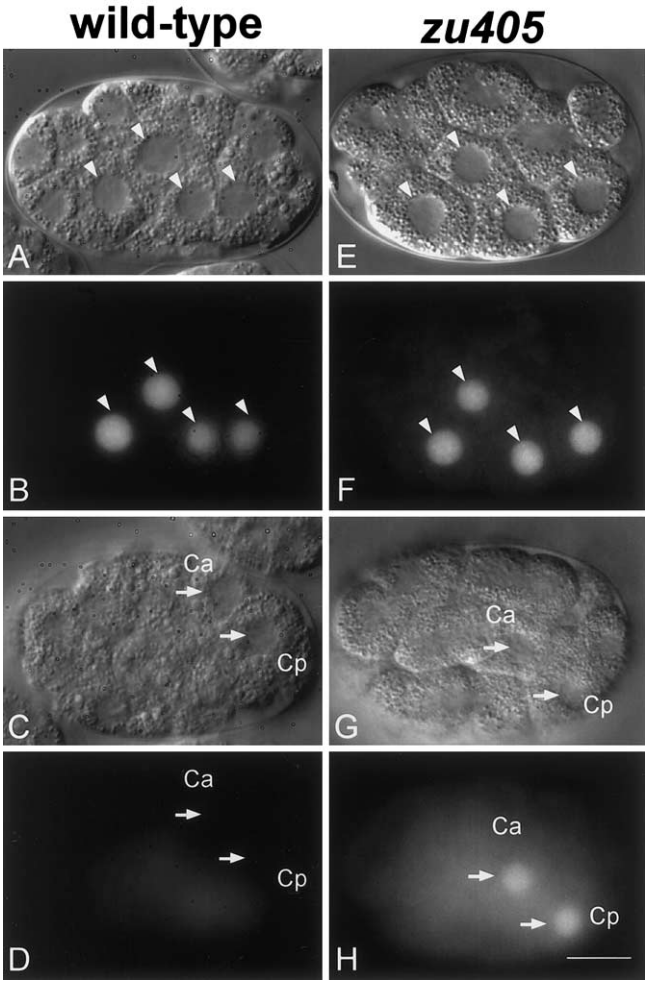


Fig. 6. The C blastomere in *oma-1(zu405)* expresses an EMS-specific transcription factor MED-1. Fluorescence micrographs depicting MED-1::GFP expression in 24-cell-stage wild-type (A–D) and *oma-1(zu405)* mutant (E–H) embryos. Arrowheads in (A, B, E, F) point to 4 EMS descendents, whereas arrows in (C, D, G, H) indicate C descendents, Ca and Cp, on a different focal plane. Bar, 10 $\mu$m.

MED-1 and MED-2 are functionally redundant, zygotically expressed GATA-like transcription factors. The expression of both proteins is dependent on SKN-1 and is restricted to EMS and its descendants in wild-type embryos (Maduro et al., 2001). It has been shown that ectopic expression of MED-1 is sufficient to induce EMS-derived cell types from a non-EMS lineage (Maduro et al., 2001). A $P_{med\text{-}1}gfp$ reporter construct expresses GFP exclusively in MS and E, the daughters of EMS, and their descendants (Fig. 6A–D; Maduro et al., 2001). When we introduced the $P_{med\text{-}1}gfp$ reporter into *oma- 1(zu405)* mutant worms, we consistently detected GFP in the descendants of the C blastomere, Ca and Cp, in addition to EMS descendents (100%, $n = 39$; Fig. 6E–H). The intensity of MED-1::GFP in Ca and Cp is never as bright as that in MS and E. These results suggest that a high level of SKN-1 in the *oma-1(zu405)* C blastomere induces the synthesis of MED-1 and an EMS-like developmental fate. The persistence of maternal SKN-1 protein therefore would appear to be key to the C-to-EMS fate transformation observed in *oma-1(zu405)* embryos.

### *C blastomere-derived intestine in oma-1(zu405), like wild-type EMS blastomere-derived intestine, requires the Wnt signaling component, WRM-1*

In wild type *C. elegans* embryos, development of intestine requires a Wnt-like signal at the four-cell stage from the P2 blastomere to the EMS blastomere (Goldstein, 1992, 1993, 1995; Rocheleau et al., 1997; Thorpe et al., 1997). In the absence of the P2 signal, the E blastomere develops like its sister MS and produces pharyngeal and body-wall muscles. In order to test whether the intestinal cells derived from the C blastomere in *oma-1(zu405)* embryos also required a Wnt-like signal, we removed one essential Wnt signaling component, a $\beta$-catenin homolog (WRM-1), via RNAi. Intestinal cells are easily identified by their morphology under differential interference contrast (DIC) optics as well as by intestine-specific markers, the gut granules, which appear birefringent under polarized light (Fig. 7A and B). When WRM-1 was depleted by RNAi in wild-type animals, the resulting embryos produced no intestinal cells, as observed by the DIC image and by the lack of birefringent granules, and produced excess pharyngeal tissues (Fig.7C and D; Rocheleau et al., 1997). When *wrm- 1(RNAi)* was carried out in *oma-1(zu405)* animals, intestinal tissues were also completely eliminated (0%, $n = 50$; Fig. 7E and F) and the RNAi embryos instead also produced excess pharyngeal tissues (Fig. 7E, and data not shown). These data suggest that, like the E- derived intestinal tissue in wild-type embryo, the ectopic, C-derived intestinal tissue in *oma-1(zu405)* embryos required a Wnt signaling pathway.

### *P2-EMS signaling is defective in oma-1(zu405) mutant embryos*

Although the C blastomere is transformed to an EMS-like fate in *oma-1(zu405)* embryos, this transformation is not fully penetrant despite an almost complete defect in the degradation of the SKN-1 and MED-1 proteins. Specifically, only 43% of the isolated C blastomeres from *oma-1(zu405)* embryos generated gut and pharynx (Table 1). Forty-six percent of the isolated C blastomeres from *oma-1(zu405)* embryos generated skin and muscle similar to a wild-type C blastomere. The remaining 11% of isolated C blastomeres from *oma-1(zu405)* embryos generated only pharyngeal tissue but no intestine, suggesting an EMS-like transformation with defective induction of intestinal tissue from the EMS-like blastomere.

To determine whether the partial penetrance of the fate transformation of intestinal cells in *zu405* embryos reflected a general defect in Wnt-signaling, we examined whether P2-to-EMS signaling is defective in *zu405* mutant embryos. In wild type embryos, P2-to-EMS signaling is responsible for three easily scored properties of the wild type embryo:

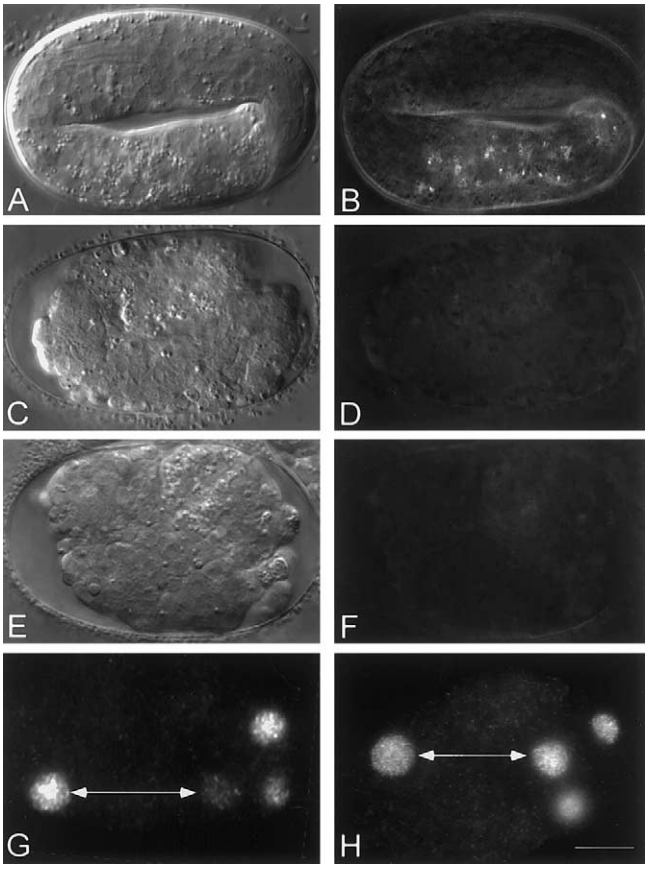


Fig. 7. Wnt signaling and endoderm formation in wild type and *oma-1(zu405)* mutant embryos. (A–F) Endoderm differentiation in wild type (A, B), *wrm-1(RNAi)* (C, D), and *oma- 1(zu405);wrm-1(RNAi)* (E, F) embryos. Endoderm differentiation is shown as DIC images (A, C, E) and viewed with polarized microscopy as birefringent granules (B, D, F). (G, H) Immunofluorescence micrographs of eight-cell-staged wild type (G) and *oma- 1(zu405)* (H) embryos stained with mabRL2, a monoclonal antibody to POP-1. Each doubleheaded arrow points to an MS nucleus (left) and an E nucleus (right). Bar, 10 $\mu$m.

First, endoderm is formed from the posterior daughter of EMS, E (Goldstein, 1992, 1993, 1995; Rocheleau et al., 1997; Thorpe et al., 1997). Second, nuclear levels of the transcription factor POP-1 are lower in E than in MS (Fig. 7G; Lin et al., 1998; Rocheleau et al., 1997; Thorpe et al., 1997). Third, P2 to EMS signaling determines the division axis of EMS parallel to the anterior–posterior axis of the wild-type embryo. In a subset of mutants that are defective in P2–EMS signaling, this EMS cleavage axis is abnormal, often skewed to the left–right axis of the embryo (Bei et al., 2002; Schlesinger et al., 1999).

We assessed these three aspects of embryonic development in *oma-1(zu405)* mutant embryos. We found that, in contrast to wild-type embryos, 26% of EMS blastomeres of *oma-1(zu405)* embryos isolated by laser ablation did not produce intestinal cells but instead produced excess pharyngeal and body-wall muscles (Table 1). Second, in *oma-1(zu405)* mutant embryos, 30% ($n = 20$) of the embryos had symmetric levels of POP-1 protein in the daughters of EMS as assessed by staining with a monoclonal antibody (mabRL1) that detects POP-1 protein (Fig. 7H). Third, 50% of *oma-1(zu405)* embryos examined ($n = 38$) had a cleavage axis rotated at least 60° relative to the a-p axis. These data suggest that the EMS blastomeres of *oma- 1(zu405)* embryos have defects consistent with abnormalities in the Wnt signaling pathways that normally determine EMS fate.

### *Regulated degradation of several maternal proteins is defective in oma-1(zu405) mutant embryos*

Although the production of ectopic intestinal and pharyngeal tissues in *oma- 1(zu405)* mutant embryos can be attributed to the delayed degradation of the SKN-1 transcription factor (see above), it is not clear why SKN-1 degradation should be delayed or why P2-to-EMS signaling is partially defective in *oma-1(zu405)* mutant embryos. By examining the expression patterns of several other maternal factors, we have observed a more general effect of the *oma-1(zu405)* mutation on the degradation of these proteins. In particular, protein degradation in the somatic blastomeres appears delayed.

In wild-type embryos, the PIE-1 protein is segregated to the germline precursor (Fig. 8A and B; Mello et al., 1996). The asymmetric localization of PIE-1 protein is regulated by two distinct mechanisms: asymmetric enrichment toward the future germline precursor cell prior to cell division and selective degradation of PIE-1 in the somatic sister cells (Reese et al., 2000). In *oma-1(zu405)* mutant embryos carrying a $P_{pie\text{-}1}$*gfp* reporter construct, PIE-1::GFP is preferentially segregated to the germline precursors, as in wild-type embryos, but, in contrast to wild type embryos, higher than normal levels of GFP are observed in the somatic sisters (Fig. 8C and D). The asymmetric enrichment of PIE-1::GFP toward the future germline precursor cell prior to cell division was clearly observed in *oma-1(zu405)* mutants (data not shown), suggesting that degradation of PIE- 1::GFP in the somatic sisters is abnormal in these embryos. To test whether the persistence of PIE-1::GFP in the somatic sister cells might reflect a more general defect in the degradation of germline proteins, we examined segregation of the MEX-1 and POS-1 proteins in *zu405* mutant embryos. We observed a similar but less severe persistence of MEX-1 and POS-1 proteins in somatic cells in *oma-1(zu405)* embryos by antibody staining (Fig. 8E–L).

The delay in degradation of maternal proteins in *oma-1(zu405)* mutant embryos was not a germline protein-specific phenomenon because it was also observed for two other maternal proteins not specifically localized to the germline precursors, MEX-5 and MEX-3. MEX-5 is a TIS11 zinc finger protein expressed in a pattern opposite to germline proteins: it is expressed for approximately one cell cycle in the somatic daughter after each division of the germline precursors (Fig. 8M and N; Schubert et al., 2000). For example, MEX-5 is detected in wild-type four-cell

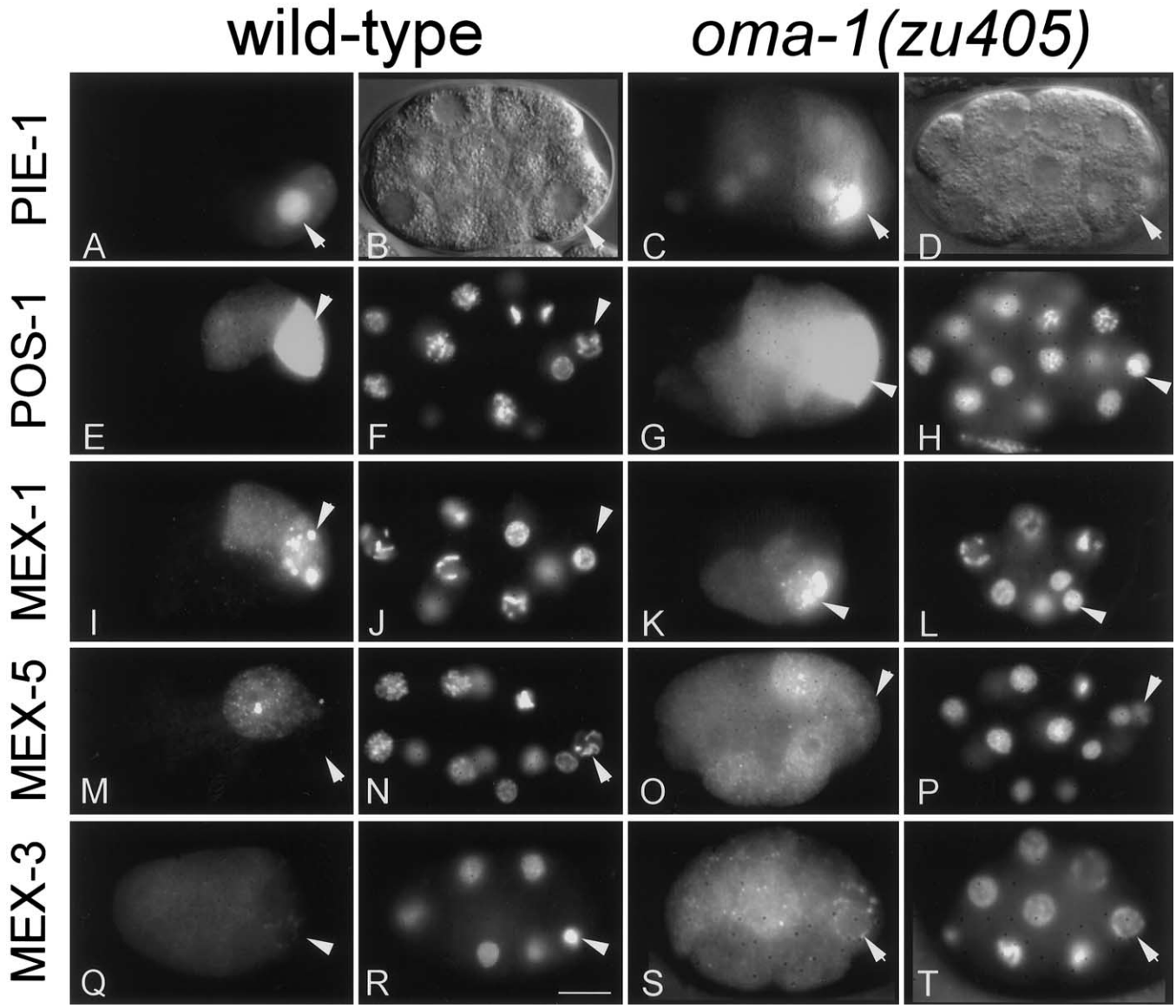


Fig. 8. Delayed degradation of several maternal proteins in *oma-1(zu405)* mutant embryos. Expression of PIE-1 (A–D), POS-1 (E–H), MEX-1 (I–L), MEX-5 (M–P), and MEX-3 (Q–T) in wild-type (A–B, E–F, I–J, M–N) and *oma-1(zu405)* mutant (C–D, G–H, K–L, O–P) embryos. The expression of PIE-1 protein is assayed using PIE-1::GFP (A, C), and the stage of embryos is shown with DIC images (B, D). Expression of all other proteins is shown by immunofluorescence using specific antibodies. (E, G, I, K, M, O, Q, S) Immunofluorescence micrographs; (F, H, J, L, N, P, R, T) DAPI staining. For each protein, the embryos shown for wild type and the *oma-1(zu405)* mutant are at a comparable stage. Germline blastomeres are indicated with arrowheads. The germline nucleus in the embryo shown in (O) and (P) is not visible on this focal plane. The exposure is the same for wild-type and *oma-1(zu405)* embryos with each antibody. Note that the delayed degradation of MEX-1 and POS-1 is not as dramatic as seen with the other proteins and the photographs have been purposely enhanced to demonstrate this point. Bar, 10 $\mu$m.

embryos in EMS, the somatic sister of the germline precursor cell P2, and in eight-cell embryos in C, the somatic sister of P3. As for wild type embryos, antibody staining of *oma-1(zu405)* embryos detects MEX- 5 in EMS and C, the somatic sister cells of the P2 and P3 germline precursors, respectively. However, in contrast to wild type embryos, the MEX-5 protein could be detected in the progeny generated by EMS and C for at least one further cell cycle compared with wild type. As a result, we consistently detected MEX-5 protein in multiple somatic cells (Fig. 8O and P).

We obtained similar results for the MEX-3 protein. In wild type 2-cell embryos, the protein MEX-3 has been shown to localize to the cytoplasm of the anterior cell, AB, and to be associated with P-granules in P1 (Draper et al., 1996). In 4-cell embryos, MEX-3 localizes to the cytoplasm of the daughters of AB and is associated with P- granules in P2. By the 8-cell stage, MEX-3 protein is no longer detected (Fig. 8Q and R). In *oma-1(zu405)* embryos, we consistently detected a significant level of cytoplasmic MEX-3 protein in the descendants of AB blastomeres and P-granule-associated MEX-3 in the germline precursor in 8-cell-and 12-cell-stage embryos (Fig.8S and T). For both MEX-5 and

MEX-3, it appears that their synthesis, both temporally and spatially, is properly regulated but their degradation is delayed. These observations suggest that the early degradation of the OMA-1 protein in the embryo somehow contributes to the later degradation of several other maternal regulators in the fate specification of early blastomeres (see Discussion).

## Discussion

We describe here a novel embryonic lethal phenotype resulting from misregulated degradation of OMA-1 protein in the gain-of-function *oma-1(zu405)* mutant. Wild-type OMA-1 protein is expressed at a very high level in the newly fertilized embryo. After the first mitotic division, OMA-1 is degraded rapidly and the remaining protein is preferentially segregated into the germline precursor cell. Degradation of OMA-1 and several other maternally supplied proteins, including SKN-1, PIE-1, MEX-1, POS-1, MEX-3, and MEX-5, is delayed in *oma-1(zu405)* mutants, suggesting that this allele of *oma-1* interferes with degradation of these early embryonic cell fates determinants. *oma-1(zu405)* mutant embryos have extra pharynx and intestinal cells, and we show that these defects are a result of abnormally high levels of the SKN-1 protein in the C blastomere and transformation of the C blastomere to an EMS-like fate.

### *Defect in the degradation of maternally supplied proteins in oma-1(zu405) mutant embryos*

We show that several maternally supplied proteins, SKN-1, PIE-1, MEX-5, MEX-3, and likely POS-1 and MEX-1 as well, are all detectable at later stages in *oma-1(zu405)* embryos than they are normally detected in wild type embryos. These observations suggest that somehow the prolonged elevation of OMA-1 protein in *zu405* embryos allows the persistence of these maternal proteins. Because the CCCH TIS11 zinc finger protein has been implicated in mRNA binding (Bai and Tolias, 1996; Barabino et al., 1997; Lai et al., 1999), it is possible that OMA-1 protein in *zu405* embryos directly interferes with the degradation of mRNAs for these maternal proteins. These transcripts are Class II maternal mRNAs characterized by distribution throughout the early blastomeres but degraded progressively in the somatic cells after the four-cell stage (Draper et al., 1996; Guedes and Priess, 1997; Mello et al., 1996; Seydoux and Fire, 1994; Tabara et al., 1999). Interference with the degradation of Class II maternal mRNAs could result in the observed persistence of corresponding maternal proteins in somatic cells after the four-cell stage. However, it has been shown that the amino acid, and not nucleic acid, sequence is responsible for the degradation of PIE-1, MEX-1, and POS-1 proteins in somatic sisters (Reese et al., 2000). This result makes the above scenario unlikely, and instead suggests that OMA-1 protein in *zu405* embryos may result in the persistence of maternal proteins by interfering with protein degradation.

How, then, would the persistence of OMA-1 protein in germline precursors interfere with the degradation of maternal proteins in somatic cells? It is especially puzzling for a protein such as MEX-5 that is expressed exclusively in nongermline cells. One possible explanation is that, in wild-type embryos, the degradation of proteins in somatic cells requires an activity from the germline precursor (see next section for more detail). In each division of the germline precursor, some of the activity is segregated to the somatic sister. The overabundance of OMA-1 protein in germline precursors in *zu405* embryos somehow interferes with this segregation process.

### *The potential function of OMA-1 and OMA-2 in embryo development*

The identical localization pattern of OMA-1 and OMA-2 proteins and their redundant function in oocyte maturation suggest a redundant function for these two proteins in early embryo development. Unfortunately, *oma-1;oma-2* mutant animals are sterile (Detwiler et al., 2001), which makes it technically difficult to address their function in embryos. It is conceivable that neither OMA-1 nor OMA-2 has any function in wild- type embryos. The observed phenotype in *zu405* embryos could be due to a neomorphic OMA-1 protein with a novel activity. Alternatively, the overabundance of OMA-1 protein in the germline blastomere could saturate the degradation machinery that normally degrades maternal proteins. We do not believe this to be likely because wild- type embryos overexpressing germline proteins, such as PIE-1, MEX-1, and POS-1, undergo normal embryogenesis (Reese et al., 2000).

Assuming that the *zu405* phenotype is the result of an overabundance of OMA-1 protein performing its normal function, we suggest the following function for OMA-1 and OMA-2 in wild-type embryos. We propose that OMA-1 and OMA-2 are present at high levels in newly fertilized embryos to prevent premature activation of a protease activity (or activities) responsible for the degradation of many maternal proteins. As some TIS11 proteins have been implicated in RNA binding, this interference could be via binding to the mRNA encoding a protease component, thereby preventing its synthesis. After the first embryonic division, the majority of OMA-1 and OMA-2 is degraded, releasing the mRNA and allowing the initiation of protease synthesis. The protease activity is predominantly in the somatic cell, AB, as the protease mRNA segregated to the P1 blastomere remains bound and inactivated by the residual OMA-1 and OMA-2. When P1 divides, the further degradation of OMA-1 and OMA-2 and the asymmetric segregation of the remaining OMA proteins to the P2 blastomere allow the synthesis of more protease(s) in the somatic sister of P2, EMS. The same pattern reiterates in each division of a

germline blastomere, allowing the sequential synthesis of a protease(s) in somatic sisters.

In contrast to wild-type embryos, in *oma-1(zu405)* mutant embryos where the level of OMA-1 protein remains high in P1 after the first division, a higher portion of mRNA remains bound to the residual OMA-1 protein in P1 and a corresponding smaller amount of protease mRNA is released and translated in the somatic sister AB. In each of the following germline blastomere division, compared with wild-type embryos, a smaller amount of protease mRNA is released to the somatic sister. Therefore, in *zu405* embryos, the protease activity is lower in somatic cells, leading to the observed delayed degradation of maternal proteins.

*Rescue the gain-of-function mutation*

Assuming the *zu405* phenotype is the result of an overabundance of OMA-1 protein performing its normal function, it is somewhat counterintuitive as to why the embryonic lethality of *oma-1(zu405)* could be weakly rescued by a wild-type *oma-1* gene (see Materials and methods). This rescue result can be explained by a process known as cosuppression. Cosuppression was first described in plants and referred to the silencing of the endogenous gene triggered by the introduction of transgenic DNA (Matzke and Matzke, 1995). Because *oma-1* null mutant worms are viable and produce live progeny (Detwiler et al., 2001), silencing of the endogenous, mutant *oma-1* gene should therefore suppress the embryonic lethality of *zu405*. Cosuppression of the endogenous, mutant *oma-1* gene by *oma-1* transgenes has recently also been observed by Rosa Alcazar and Andrew Fire (personal communication).

*C-to-EMS fate transformation*

The C blastomere in *oma-1(zu405)* mutant embryos not only produces EMS- derived tissue types, but also exhibits other EMS-specific properties, including expression of the MED-1 transcription factor and dependence on the *wrm-1* activity to produce intestine.

In wild-type embryos, the level of SKN-1 protein is high in both the EMS and P2 blastomeres but remains inactive in P2 due to the presence of the germline-specific repressor protein PIE-1 (Bowerman et al., 1993; Mello et al., 1992). When P2 divides, the C blastomere inherits SKN-1 and, although not inheriting PIE-1, does not produce SKN-1-dependent tissues. This lack of SKN-1-dependent tissues from the wild-type C blastomere has been attributed to insufficient levels of SKN-1 protein in C at the eight- cell stage (Hunter and Kenyon, 1996). We observed that the C blastomere in *oma- 1(zu405)* mutant embryos contained a higher than wild-type level of SKN-1 protein and produced EMS-derived tissues. These data strongly support the model that the difference in fate specification between wild-type EMS and C is primarily a result of SKN-1 protein level differences between these two blastomeres.

Although we have shown that a P2–EMS Wnt-signaling component is required for intestine formation from the C blastomere in *oma-1(zu405)* embryos, it is currently not clear whether a signaling cell is required. It has been shown previously that an isolated P2 blastomere retains its capability to polarize EMS even after it divides into P3 and C (Goldstein, 1995). However, it has not been determined whether one, or both, of the P2 daughters inherit this signaling ability. If a signaling cell is required for *oma-1(zu405)* C blastomere to produce intestine, a highly likely candidate is the germline precursor P3.

*Defect in P2-to-EMS signaling*

In two other *C. elegans* mutants, *gsk-3* and *src-1,* intestinal cells are generated from the C blastomere (Bei et al., 2002; Maduro et al., 2001; Schlesinger et al., 1999). Although the *oma-1(zu405)* phenotype bears some similarity to these mutants, *oma- 1(zu405)* also has characteristics that distinguish it from *gsk-3* and *src-1*. *gsk-3* and *src-1* are similar to *zu405* in that: (1) mutant embryos exhibit partially penetrant defects in P2- to-EMS signaling; (2) some percentage of C blastomeres in these mutants generate intestine rather than skin and muscle; and (3) the EMS division axis is rotated with respect to the embryonic A-P axis. However, unlike *oma-1(zu405),* the C blastomeres from *gsk-3* or *src-1* mutant embryos do not produce MS-derived tissues, and it is this characteristic that uniquely defines the *oma-1(zu405)* phenotype.

It is currently not clear why *oma-1(zu405)* mutant embryos exhibit defects in P2- to-EMS signaling. Given our observations that *oma-1(zu405)* mutant embryos have defects in the degradation of several proteins, one possible explanation is that proper protein degradation plays a role in *C. elegans* P2-to-EMS signaling. Alternatively, it is possible that the *oma-1(zu405)* phenotype is due to an indirect result of inactivation of GSK-3 or SRC-1 proteins.

*Oocyte maturation and early embryonic events*

In vertebrates, oocytes undergo maturation before arresting in metaphase II, at which point they can be fertilized by sperm. Activation of protein and DNA synthesis occurs within several minutes of fertilization and is brought about by an increase in the intracellular $Ca^{2+}$ concentration and pH (see review by Ben-Yosef and Shalgi, 1998). A high level of $Ca^{2+}$ mediates the degradation of cyclin B and consequent inactivation of MPF (maturation promoting factor) and CSF (cytostatic factor) upon fertilization of *Xenopus* embryos, allowing entry into the first mitotic cycle (Lorca et al., 1993). In an animal like *C. elegans,* where all factors required for early embryonic development come packaged in the egg and no delay between oocyte maturation and fertilization is apparent, oocyte maturation, fertilization, and initiation of embryogenesis must be tightly coordinated. Such coordination might include turnover or

inactivation of oocyte-specific proteins and the synthesis or activation of zygote-specific proteins important for early embryonic development. One attractive model for the coordinated regulation of these two processes would be for the activation of zygote-specific proteins to be contingent on the degradation of a key regulator of oocyte maturation. The interesting expression pattern of OMA proteins and the *oma-1(zu405)* gain-of-function phenotype suggest the intriguing model that OMA-1 and OMA-2 coordinate oocyte maturation with early embryonic events in *C. elegans.* Further efforts are underway to investigate this model.

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