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Maternal control of a zygotic patterning gene in *Caenorhabditis elegans*

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SUMMARY

The transition from maternal to zygotic gene control is a key process in embryogenesis. Although many maternal effect genes have been studied in the *C. elegans* embryo, how their activities lead to the positional expression of zygotic patterning genes has not yet been established. Evidence is presented showing that expression of the zygotic patterning gene *vab-7* does not depend on cell position or cell contacts, but rather on the production of a C blastomere. Furthermore, *pal-1*, a *caudal* homologue with maternal product necessary for the proper development of the C blastomere, is both necessary and sufficient for *vab-7* expression. This provides a link between maternal gene activity and zygotic patterning gene expression in *C. elegans*. The results suggest that zygotic patterning genes might be generally controlled at the level of blastomere fate and not by position.

Key words: *Caenorhabditis elegans*, pattern formation, *vab-7*, *glp-1*, *apx-1*, *skn-1*, *pie-1*, *mex-3*, *pal-1*, heat shock

INTRODUCTION

The study of numerous maternal effect genes in the *C. elegans* embryo has led to an increasingly detailed view of early patterning events (Bowerman, 1995; McGhee, 1995). Maternal gene activities become localised to blastomeres during early cell divisions and their activities help direct blastomere fates, which are defined by their characteristic cell lineages and by the types of tissues they produce (Sulston et al., 1983). In most animals, a major function of maternal genes is to set the positional expression of zygotic patterning genes, but little is known about the mechanism of this transition in *C. elegans*. For example, it is not yet clear whether localised maternal activities control zygotic patterning genes within blastomeres and their descendants, or whether a larger scale patterning system might provide positional information to direct zygotic patterning gene expression. Expression of the *C. elegans* HOX gene *mab-5* was recently reported to be dependent on cell lineage (which is linked with blastomere fate) but not position, giving support to the former model (Cowing and Kenyon, 1996). However, a different interpretation of the data suggested that position could still be important for expression (Schnabel and Schnabel, 1997). The genes that regulate *mab-5* in the embryo have not yet been identified, so this issue is still unresolved.

The zygotic patterning gene *vab-7* is a member of the *even-skipped* family of homeodomain-containing proteins, and is required for patterning posterior muscle and epidermal cells (Ahringer, 1996). Early *vab-7* expression is confined to some of the descendants of the C blastomere, which is positioned at the posterior of the embryo (Fig. 1A,B). What cues guide *vab-7* expression in these posterior cells? Is their descent from the C blastomere important or does their position at the posterior determine their fate? To explore how early zygotic patterning genes come to be positionally expressed in *C. elegans*, I have examined the expression of *vab-7* in a number of maternal effect embryonic lethal mutants that have changes in early blastomere fates. If a posterior position is important for *vab-7* expression, then anteriorly located C blastomeres should not express the gene and *vab-7* should be expressed at the posterior whether or not a C blastomere is made. However, if blastomere fate but not position is important, *vab-7* should be expressed in C blastomeres no matter what position they are in. To assay *vab-7* expression, I used a *vab-7::lacZ* reporter gene (Ahringer, 1996) that has the same early expression pattern as the endogenous *vab-7* protein (Ahringer and Neades, unpublished). The results show that *vab-7::lacZ* expression depends on the production of a C blastomere regardless of position and that *pal-1* gene activity, which is necessary for the development of the C blastomere, is responsible for the expression of *vab-7* in its descendants. This supports a model where the position of zygotic patterning gene expression is regulated at the level of blastomere fate, and not by cell position.

MATERIALS AND METHODS

Strains

The following strains were used in this study: CB4078: *glp-1(e2144ts)*, EU91: *apx-1(or3)/dnT1*, EU001: *skn-1(zu67)/dnT1*, JJ532: *pie-1(zu154) unc-25(e156)/qC1*, JJ518: *mex-3(zu155)* dpy-*5(e61)/hT1*, CF918: *dpy-17(e164) pal-1(ct224) ncl-1(e1865) unc-36(e251) ; sDp3 ; lin-2(e1309)*.

Standard genetic methods were used to make the above strains homozygous for *eIs24* (Ahringer, 1996), an integrated *vab-7::lacZ* reporter gene.

X-gal staining

Hermaphrodites homozygous for *eIs24* and the maternal effect mutant

were picked to a plate and allow to lay eggs overnight. These embryos plus those still inside the mothers were collected and stained with X-Gal. Embryos were stuck to polylysine-coated slides in water, slightly squashed under a coverslip by wicking liquid away, then frozen on dry ice. The coverslip was flicked off and slides immersed in –20°C acetone for 4 minutes, then taken through an acetone series at room temperature to 10%. Staining was overnight at 37°C as described (Fire et al., 1990).

22/22 *glp-1* and 20/20 *apx-1* mutant embryos examined had normal *vab-7::lacZ* expression. 38/41 *pie-1* mutant embryos examined had no *vab-7::lacZ* expression; in 3/41 embryos, 3-6 cells stained. These were late stage embryos and the stained cells were probably the few AB descendants that express *vab-7::lacZ* late. Ectopic expression of *vab-7::lacZ* in *skn-1* mutants was variable, consistent with the variable phenotype of this mutant (Bowerman et al., 1992). The strongest existing allele was used (*skn-1(zu67)*), but this is known not to be null; in some *skn-1(zu67)* mutant embryos, the EMS blastomere produces gut and pharynx as it does in wild-type instead of being transformed to producing two blastomeres with C-like fates (Bowerman et al., 1992). In *skn-1(zu67)* mutants, 15/18 embryos examined had ectopic *vab-7::lacZ* expression; up to three times the normal number of cells expressed *vab-7::lacZ*. The ectopically expressing cells were in the position of the EMS descendants. 47/47 *mex-3* mutant embryos examined expressed *vab-7::lacZ* ectopically. An embryo of genotype *mex-3(zu155) dpy-5(e61); eIs24*. was lineaged and then fixed and stained to identify ectopic *lacZ* expressing cells; cells derived from both ABa and ABp ectopically expressed *vab-7::lacZ*.

vab-7::lacZ expression in homozygous *pal-1* mutants laid by mothers contributing maternal product (progeny of the genotype above that did not inherit a copy of the duplication) was indistinguishable from their siblings that inherited a wild-type copy of *pal-1*. During morphogenesis, *pal-1* homozygotes continued to express *vab-7::lacZ* and could be identified by their abnormal development; expression was sometimes reduced in late stage embryos.

pal-1 germline mosaics were identified in the *pal-1* strain as described (Hunter and Kenyon, 1996). Broods of 5 *pal-1* germline mosaics were examined; no *vab-7::lacZ* was detected in premorphogenesis embryos. 62/132 embryos examined had no staining and the remaining 70/132 expressed *vab-7::lacZ* in 1-5 cells. These were all late stage embryos and the staining probably represents the cells from the AB lineage that express *vab-7::lacZ* during morphogenesis (Ahringer, 1996). In wild-type late stage embryos, about 30 cells stain.

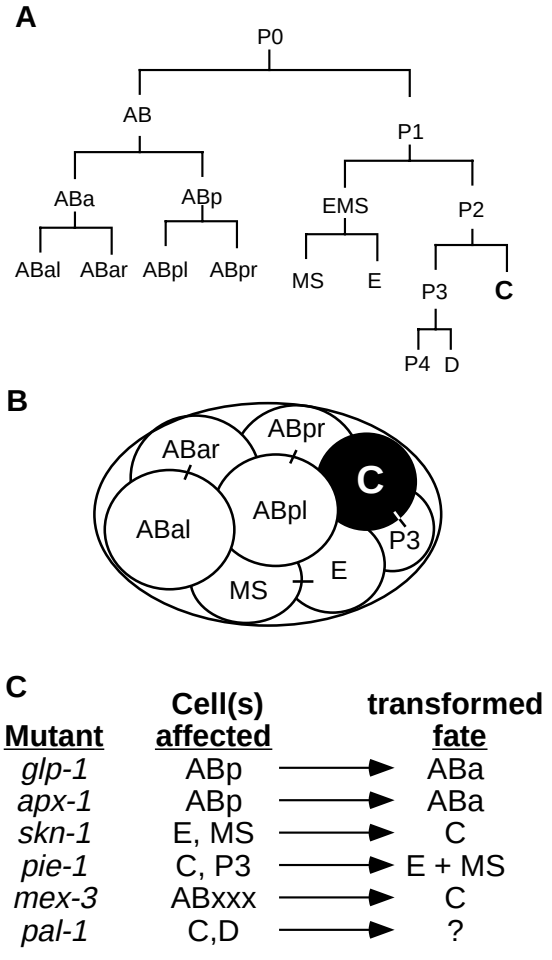
Heat-shock *pal-1* experiments

The HS-PAL-1 construct was made by PCR amplifying the 830 bp *pal-1* coding region from embryonic cDNA using primers PAL1F (5′GGGGTACCCCAATGTCGGTCGATGTCAAGTCG3′) and PAL1R (5′CATGCCATGGCATGGTACTTATAGCCGAATCTTCTG3′) containing *Kpn*I and *Nco*I restriction sites, respectively. This fragment was digested with *Kpn*I and *Nco*I and cloned in to *Kpn*I/*Nco*I cut pPD49.83 (containing the heat shock promoter *hsp16-41* (Stringham et al., 1992), obtained from A. Fire) to create plasmid pJA21. A mixture of pJA21 (40 μg/ml), pJA15 (*vab-7::lacZ*; 40 μg/ml) + pRF4 (Mello et al., 1991) (*rol-6(d)*; 100 μg/ml) was transformed into wild-type hermaphrodites as in (Mello et al., 1991) to create strains *weEx1*, *weEx2* and *weEx3*. The *weEx1* extrachromosomal array was integrated using 3500 rads X-rays to create strains *weIs1*, *weIs2* and *weIs3*. Without heat shock, *weEx1-3* and *weIs1-3* all have a pattern of expression identical to *eIs24*, containing pJA15. Plates of *weIs1-3* were heat shocked at 33°C for 45 minutes, allowed to recover for 15 minutes at 20°C, then embryos harvested by hypochlorite treatment. Embryos were fixed and stained for β-galactosidase (using a mouse anti-β-galactosidase antibody; Promega) and PAL-1 (using a rabbit anti-PAL-1 antibody provided by C. Hunter; (Hunter and Kenyon, 1996)) at 1 hour intervals after heat shock. PAL-1 was strongly expressed in all cells at 1 hour after heat shock. Ectopic β-galactosidase could be weakly detected by 1 hour post-heat shock and was strong by 3 hours. No ectopic *vab-7::lacZ* expression occurred after heat shock of *eIs24*. Nearly all pre-morphogenesis embryos contained ectopic β-galactosidase after heat shock, but heat-shocked embryos that had begun morphogenesis had a normal *vab-7::lacZ* expression pattern.

RESULTS

The ABp fate is not necessary for *vab-7* expression in the C blastomere

Fig. 1B shows a schematic diagram of an 8-cell *C. elegans* embryo. The zygotic patterning gene *vab-7* is primarily expressed in descendants of the C blastomere (Ahringer, 1996), which is located at the posterior of the embryo. Some of the cells that contact C and its descendants are derived from the ABp blastomere. The genes *glp-1* and *apx-1* are required for normal ABp development (Hutter and Schnabel, 1994; Mango et al., 1994; Mello et al., 1994; Moskowitz et al., 1994). In the absence of either gene product, ABp adopts the fate of its sister, ABa, and there is no ABp fate in the embryo (Fig. 1C). To determine whether ABp produces cells that induce the expression of *vab-7* in C descendants, I examined the



C

Mutant	Cell(s) affected		transformed fate
glp-1	ABp	→	ABa
apx-1	ABp	→	ABa
skn-1	E, MS	→	C
pie-1	C, P3	→	E + MS
mex-3	ABxxx	→	C
pal-1	C,D	→	?

Fig. 1. Blastomere origins, positions and fates in mutants. (A) Partial early cell lineage; horizontal bars indicate cell divisions, vertical bars time. The C blastomere is the daughter of the P2 cell. (B) Cartoon of an 8-cell embryo. The C blastomere is positioned at the posterior of the embryo. (C) Phenotypes of the maternal effect mutants used in this study. References are given in the text.

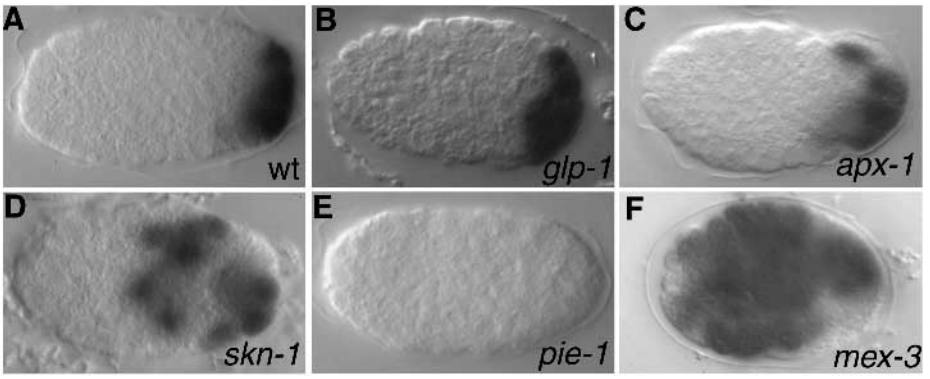


Fig. 2. Expression of *vab-7::lacZ* in different mutant backgrounds. (A) Wild-type, (B) *glp-1(e2144ts)* at restrictive temperature, (C) *apx-1(or3)*, (D) *skn-1(zu67)*, (E) *pie-1(zu154)*, (F) *mex-3((zu155)*. Complete genotypes are given in Materials and Methods

expression of *vab-7::lacZ* in *glp-1* and *apx-1* mutant embryos. The reporter gene is expressed normally in both mutant backgrounds (Fig. 2B,C), arguing that ABp fate cells are not required to induce *vab-7* expression.

vab-7 expression depends on the production of a C blastomere

I next asked whether *vab-7::lacZ* expression is affected in two mutants with an altered number of C-like blastomeres. First, embryos mutant for the maternal effect gene *skn-1* frequently have two additional C-like blastomeres in place of the EMS daughters (Bowerman et al., 1992). These transformed blastomeres are anterior to the normal C blastomere and have different cell contacts. In *skn-1* mutant embryos, there is ectopic expression of *vab-7::lacZ* in these additional C-like cells (Fig. 2D). This shows that the expression of *vab-7* does not depend on a posterior position in the embryo or on particular cell contacts.

skn-1 encodes a bZIP transcription factor (called SKN-1) required for the EMS fate. SKN-1 is localised to the P2 and EMS blastomeres, but is only active in EMS (Bowerman et al., 1992, 1993). Maternal *pie-1* activity normally inhibits *skn-1* in P2, the mother of the C blastomere (Mello et al., 1992). In *pie-1* mutant embryos, SKN-1 is active in both P2 and EMS, and no blastomere with a C fate is made (Mello et al., 1992). In these embryos, *vab-7::lacZ* is not expressed (Fig. 2E). The finding that there is ectopic *vab-7::lacZ* expression in *skn-1* mutants (where there are extra C blastomeres), and no *vab-7::lacZ* expression in *pie-1* mutants (where there is no C blastomere) argues that *vab-7* expression depends on the production of a C blastomere. Therefore, a maternal factor found in the C blastomere might control *vab-7* expression. The ectopic expression of *vab-7::lacZ* in descendants of the 'EMS' cell lacking the SKN-1 transcription factor suggests that EMS also has an inherent ability to express *vab-7*, but that SKN-1 normally inhibits its expression there.

pal-1 activity is necessary for *vab-7* expression

Recently, it was reported that maternal *pal-1* product is located in the EMS and P2 blastomeres (Hunter and Kenyon, 1996), cells predicted from the above experiments to contain a positive regulator of *vab-7*. The *pal-1* gene encodes a homologue of *Drosophila caudal* (Waring and Kenyon, 1991) and is required for the fates of the posterior blastomeres C and D (Hunter and Kenyon, 1996). Its activity also appears to be inhibited by SKN-1 (Hunter and Kenyon, 1996). This raises the possibility that *pal-1* might be a regulator of *vab-7*.

The *pal-1* gene has essential maternal and zygotic functions (Yandell et al., 1994; Hunter and Kenyon, 1996). In *pal-1* homozygotes that have inherited wild-type maternal *pal-1* product, *vab-7::lacZ* is expressed in the normal pattern (Fig. 3A; see Materials and Methods). To ask whether *pal-1* activity is necessary for *vab-7* expression, I generated mosaic animals in which the soma has a wild-type copy of *pal-1*, but the germline is homozygous mutant. Most progeny of these mosaic animals (which lack both maternal and zygotic *pal-1* activity) do not express *vab-7::lacZ* (Fig. 3B), showing that *pal-1* activity is necessary for early *vab-7* expression. Late stage embryos sometimes had a few cells that stained (see Materials and Methods); these are probably the few AB descendants that express *vab-7::lacZ* late in embryogenesis (Ahringer, 1996). Inhibition of *pal-1* activity by injection of antisense RNA was also shown to reduce the expression of *vab-7::lacZ* (Hunter and Kenyon, 1996). These results support the idea that PAL-1 positively regulates *vab-7*.

PAL-1 is sufficient for *vab-7* expression

Maternal *pal-1* RNA is under negative translational control such that it is only translated in posterior blastomeres (Hunter

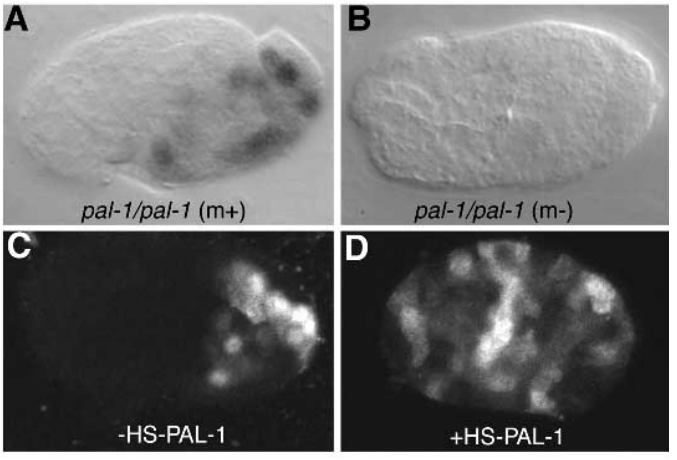


Fig. 3.*pal-1* is both necessary and sufficient for *vab-7* expression. *vab-7::lacZ* expression in (A) *pal-1*(ct224)/*pal-1*(ct224) (m+), inherited wild-type maternal *pal-1* activity; (B) *pal-1*(ct224)/*pal-1*(ct224) (m–), inherited no maternal *pal-1* activity; (C) *weIs2* (HS-PAL-1 + *vab-7::lacZ*) not heat shocked; expression is normal; (D) *weIs2* (HS-PAL-1 + *vab-7::lacZ*) 4 hours post heat shock; abundant ectopic expression. Full genotypes are given in Materials and Methods.

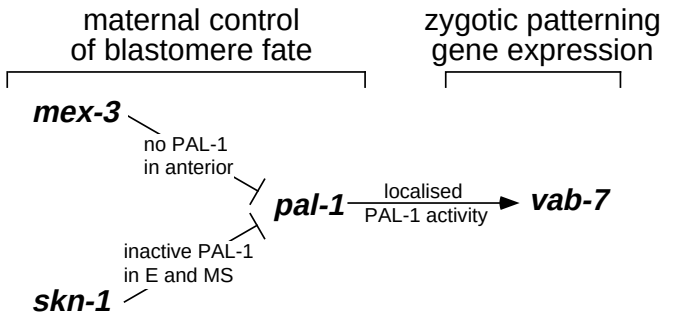


Fig. 4. Model: maternal PAL-1 helps to determine the C blastomere fate by regulating zygotic genes such as *vab-7*. Active *pal-1* protein (PAL-1) in the C blastomere positively regulates the zygotic patterning gene *vab-7*, either directly or indirectly. The products of at least two maternal genes, *mex-3* and *skn-1*, contribute to the localisation of active maternal PAL-1 (Draper et al., 1996; Hunter and Kenyon, 1996). *mex-3* encodes a KH domain RNA-binding protein proposed to negatively regulate translation of *pal-1* RNA in anterior cells. *skn-1* protein (SKN-1) inhibits PAL-1 activity in the blastomere EMS by an unknown mechanism; PAL-1 is unable to activate *vab-7* in wild-type EMS blastomeres, where SKN-1 is active, or in C blastomere of *pie-1* mutants, where SKN-1 is thought to be ectopically active (Bowerman et al., 1992).

and Kenyon, 1996). This control requires maternal MEX-3, which is a KH domain RNA-binding protein (Draper et al., 1996). In *mex-3* mutants, *pal-1* is ectopically expressed in anterior cells. If PAL-1 is a regulator of *vab-7*, then this ectopic *pal-1* protein would be expected to drive *vab-7* expression in the anterior, where it is not normally found. Fig. 2F shows that there is abundant anterior expression of *vab-7::lacZ* in *mex-3* mutants, consistent with this proposal.

To test directly whether *pal-1* is sufficient for *vab-7* expression, I constructed a transgene to express *pal-1* ectopically. The *pal-1* coding sequence was fused to a *C. elegans* heat-shock promoter (Stringham et al., 1992) and transformed together with the *vab-7::lacZ* construct into wild-type animals. Embryos from this strain were then tested for *vab-7::lacZ* expression in the absence or presence of heat shock. Without heat shock, *vab-7::lacZ* expression is normal (Fig. 3C). However, after heat shock, *vab-7::lacZ* expression is induced in many cells (Fig. 3D). Interestingly, PAL-1 only induced ectopic *vab-7::lacZ* expression when the heat shock occurred before differentiation began. These results show that *pal-1* activity is both necessary and sufficient for *vab-7* expression.

DISCUSSION

These experiments establish a link between the specification of the C blastomere fate by maternal components and the zygotic expression of *vab-7* (Fig. 4). Blastomere fates are specified during early cell divisions by cell interactions, translational control and the segregation of regulatory molecules (Bowerman, 1995; McGhee, 1995). These fates appear to direct particular lineage programs, though how this is done is not known. In the case of the C blastomere, PAL-1 activity (which is necessary for C blastomere development) is localised through translational control of maternal RNA and through negative regulation of its activity (Hunter and Kenyon, 1996). Active PAL-1 could then help to direct a C blastomere program of development by regulating genes such as *vab-7*. It is possible that this interaction is direct, since PAL-1 is found in cells expected for a *vab-7* regulator. However, *pal-1* is also expressed in and required for the development of the D blastomere (Hunter and Kenyon, 1996), where *vab-7* is not expressed, so there are likely to be additional factors that contribute to the *vab-7* expression pattern.

This work identifies a possible direct transcriptional connection between maternal and zygotic genes in *C. elegans*. In previous work, other examples of the maternal influence of zygotic gene expression have been identified. Cells in which maternal GLP-1 (an LNG transmembrane receptor family member) has been activated induce the expression of zygotic *lin-12* and repress zygotic *lag-1* (Moscowitz and Rothman, 1996). Another link between maternal gene activity and a zygotic gene's expression comes from work with regulatory elements of the *ges-1* gene, which encodes an esterase that is a component of the differentiated gut. The expression of different *ges-1* reporter genes was correlated with particular blastomere fates in wild-type and maternal effect mutant embryos (Fukushige et al., 1996). However, in none of these examples have putative maternal transcriptional regulators been identified.

More generally, these results with *vab-7* suggest that the initial expression of zygotic patterning genes in *C. elegans* is controlled at the level of specification of blastomere fate. Expression of the *C. elegans* zygotic patterning gene *mab-5*, a member of the HOX cluster, was reported to depend on cell lineage but not cell position (Cowing and Kenyon, 1996), but an alternative interpretation of this data left open the possibility that position could affect *mab-5* expression (Schnabel and Schnabel, 1997). The experiments presented here support a model where blastomere fate and not global positional information controls zygotic patterning gene expression. Though early positional information appears not to be prelocalised as in *Drosophila* (St. Johnston and Nüsslein-Volhard, 1992), the localisation of maternal regulatory factors that occurs during early *C. elegans* cell divisions can be thought of as an analogous process, determining early blastomere fates that control zygotic patterning.

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