Development 122, 4105-4117 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
DEV3495

lin-12 and *glp-1* are required zygotically for early embryonic cellular interactions and are regulated by maternal GLP-1 signaling in *Caenorhabditis elegans*

Ivan P. G. Moskowitz and Joel H. Rothman*

Department of Biochemistry, 420 Henry Mall, University of Wisconsin, Madison, WI 53706, USA

*Current address: Neuroscience Research Institute and Department of MCD Biology, University of California, Santa Barbara, CA 93106, USA

SUMMARY

Cell-cell interactions mediated by LIN-12 and GLP-1, members of the LNG (LIN-12, Notch, GLP-1) family of receptors, are required to specify numerous cell fates during development of the nematode *Caenorhabditis elegans*. Maternally expressed GLP-1 participates in two of at least four sequential inductive interactions that specify the fates of early embryonic descendants of the AB founder cell. We report that GLP-1 and LIN-12, and apparently their ligand, LAG-2, as well as a downstream component, LAG-1, are required in the latter two inductions. We find that LAG-2 is expressed in the signaling cells and LIN-12 is expressed in cells receiving the inductions, consistent with their proposed roles as ligand and receptor, respectively. Furthermore, we report that maternal GLP-1 activity is required (1) to repress early zygotic *lag-2* expression and (2) to activate zygotic *lin-12* expression in the early embryo. The patterning of both receptor and ligand expression by maternal GLP-1 signaling establishes competence for the zygotic LNG-mediated cellular interactions and localizes these interactions to the appropriate cells. We propose that activation of maternal GLP-1 regulates zygotic *lin-12* and *lag-2* expression by a regulatory mechanism analogous to that described for the post-embryonic gonad.

Key words: cellular interaction, *Caenorhabditis elegans*, *Notch*, *lin-12*, *glp-1*, signal transduction, pattern formation

INTRODUCTION

The evolutionarily conserved LIN-12/Notch/GLP-1 (LNG) transmembrane receptors have been implicated in a diverse set of cell fate decisions that occur during the development of both invertebrates and vertebrates (reviewed by Artavanis-Tsakonas et al., 1995). LNG receptors act pleiotropically, mediating cell fate decisions in multiple germ layers and specifying a variety of cell types (e.g., Greenwald et al., 1983; Austin and Kimble, 1987; Priess et al., 1987; Hartenstein et al., 1992; Coffman et al., 1993). These receptors often act in sequential cellular interactions, in which the outcome of one interaction influences the occurrence and outcome of subsequent interactions (Hartenstein and Posakony, 1990; Hutter and Schnabel, 1994; Moskowitz et al., 1994; Posakony, 1994; Newman et al., 1995; Rulifson and Blair, 1995). The mechanisms that restrict the expression of LNG receptors and their ligands to the appropriate cells and that regulate sequential LNG-mediated cellular interactions are not well understood.

The *lin-12* and *glp-1* genes of the nematode *Caenorhabditis elegans* encode homologous members of the LNG receptor family (Greenwald, 1985; Yochem et al., 1988; Austin and Kimble, 1989; Weston et al., 1989; Yochem and Greenwald, 1989), which are functionally interchangeable (Lambie and Kimble, 1991; Mango et al., 1991; Fitzgerald et al., 1993). Both LIN-12 and GLP-1 are necessary for the reception of intercellular signals and accordingly act autonomously within receiving cells to specify cell fates (Austin and Kimble, 1987; Seydoux and Greenwald, 1989). Whereas LIN-12 mediates both lateral signaling between cells of equivalent developmental potential and inductive interactions between non-equivalent cells (Greenwald et al., 1983; Newman et al., 1995), GLP-1 is only known to be required for inductive interactions (Austin and Kimble, 1987; Priess et al., 1987).

Maternally provided GLP-1 is required for two early embryonic inductions that specify the fate of descendants of AB, the anterior daughter of the zygote. These two inductions promote differences in cell fates along the dorsal-ventral and left-right axes of the developing embryo, respectively (Bowerman et al., 1992; Hutter and Schnabel, 1994; Mango et al., 1994; Mello et al., 1994; Moskowitz et al., 1994). Maternal GLP-1 is present in all AB descendants when these inductions occur, consistent with a role for GLP-1 as a receptor (Evans et al., 1994).

Zygotically expressed LIN-12 and GLP-1 are functionally redundant in the embryo. The zygotic lethal phenotype of *lin-12 glp-1* double mutants is distinct from either the *lin-12* or *glp-1* single mutant phenotypes, implicating LIN-12 and GLP-1 in previously unknown interactions for which either receptor is sufficient (Lambie and Kimble, 1991). *lin-12 glp-1* double mutants have defects in head morphogenesis, lack an excretory cell and rectum, and die as L1-stage larvae; this collection of defects is known as the Lag (for *lin-12* and *glp-1*) phenotype (Lambie and Kimble, 1991). Screens for mutations that result

in a Lag phenotype identified two genes, *lag-1* and *lag-2* (Lambie and Kimble, 1991). The loss-of-function phenotype of *lag-1* and *lag-2* is identical to that of the *lin-12 glp-1* double mutant, suggesting that LAG-1 and LAG-2 represent components of the signal transduction cascade mediated by LIN-12 and GLP-1 (Lambie and Kimble, 1991). Consistent with this suggestion, LAG-1 shows sequence similarity to Suppressor of Hairless [Su(H)], a sequence-specific DNA-binding protein required to transduce Notch-mediated signaling in *Drosophila* (Bailey and Posakony, 1995; Lecourtois and Schweisguth, 1995; Christensen et al., 1996). Moreover, LAG-2 is structurally similar to Delta and Serrate, transmembrane proteins that are putative ligands of the Notch receptor (Henderson et al., 1994; Tax et al., 1994). *lag-2* is expressed postembryonically in the signaling cells of LIN-12- and GLP-1-mediated cell interactions, consistent with the apparent action of LAG-2 as a signaling ligand in this pathway (Henderson et al., 1994; Wilkinson et al., 1994). We will refer to *lin-12, glp-1, lag-1* and *lag-2* collectively as the Lag pathway.

In this report, we demonstrate a zygotic requirement for components of the Lag pathway in two inductive interactions that specify the fates of certain AB descendants in the early embryo. We find that *lag-2* is expressed in the signaling cells and that *lin-12* is expressed in the receiving cells of both interactions, consistent with the prediction that LAG-2 acts as a ligand and LIN-12 acts as a receptor in these inductions. Furthermore, we demonstrate that maternal GLP-1 activity regulates the zygotic expression of *lag-2* and *lin-12* in the early embryo: maternal GLP-1 activity is both necessary and sufficient to repress *lag-2* transcription and is necessary to activate *lin-12* expression. The regulation of *lag-2* and *lin-12* expression by the activity of an LNG receptor, maternal GLP-1, establishes competence for and restricts later LNG-mediated induction events to the appropriate cells in the early embryo.

MATERIALS AND METHODS

Strains and culture

Nematode strains were maintained according to Brenner (1974). The wild-type strain used was *C. elegans* var Bristol (N2). The following alleles were used in this study: *glp-1(q224ts, q231ts, e2142ts), lin-12 (q269), unc-32(e189), dpy-19(e1259ts), lag-1*(*q385, q476*); *deb-1(st385), lag-2*(*q387, q477*), *qIs8* [pRF4, pJK375 (*lag-2::lac-Z*)] (Henderson et al., 1994), and *arIs11*[pRF4, pBGSLE (*lin-12::lac-Z*)] (Wilkinson et al., 1994). Experiments were conducted at 20°C, except where noted.

Reporter constructs

The *lag-2::lacZ* reporter construct used in this work consists of the 5′ portion of *lag-2* fused to the *lacZ* coding region and a nuclear localization signal (NLS) (Henderson et al., 1994). This construct removes all *lag-2* coding regions. The *lag-2::lacZ* construct has been shown to be an accurate reporter of *lag-2* expression: first, strains carrying this construct have been previously shown to expresses β-galactosidase in cells known to require *lag-2* function (Henderson et al., 1994); second, the construct contains all the non-coding sequences required to rescue *lag-2* mutants (Henderson et al., 1994); third, several independent lines of each construct gave identical staining patterns; fourth, a *lag-2*::*lacZ* construct that can rescue a *lag-2* mutant (*lag-2intra::lac-Z*) gives a staining pattern identical to the construct analyzed here.

The *lin-12::lacZ* reporter construct is the insertion of a *lacZ* gene into the *lin-12* coding region at position 6 in frame with the *lin-12* ATG (Wilkinson et al., 1994). The *lin-12*::*lacZ* construct appears to be an accurate reporter of *lin-12* expression: first, strains carrying this construct have previously been shown to express β-galactosidase in cells known to require *lin-12* function (described in Wilkinson et al., 1994); second, the construct contains all non-coding sequences required to rescue *lin-12* mutants; third, several independent lines carrying integrated arrays of the construct gave identical staining patterns (Wilkinson et al., 1994).

Laser ablations

Eggs were cut from gravid adults in M9 buffer (Sulston and Hodgkin, 1988). 4-cell embryos were collected, placed on a 4% agar pad, covered with a coverslip, and sealed with Vaseline according to Sulston et al. (1983). Ablations of embryonic blastomeres were performed according to Sulston and White (1980) and Avery and Horvitz (1989), with a VSL-337 nitrogen laser (Sulston and White, 1980; Avery and Horvitz, 1989). A DLM-110 dye laser module and coumarin 440 dye were all supplied by Laser Science, Inc. Ablated embryos were incubated at 20°C for 60-90 minutes, transferred to poly-L-lysine-coated slides and processed for immunofluorescence.

Blastomere fusion

Fusion of embryonic blastomeres was performed by ablating the membrane between the relevant cells with a laser, as described by Schierenberg (1984). If blastomere fusion was not achieved within 3 minutes of the birth of the relevant cells or if fusion required greater than 10 pulses of the laser, the embryo was discarded. Successful fusions cause the two nuclei within the fused cell to migrate together and contact one another. The division of the fused cell is tetrapolar creating four daughter cells, two from each nucleus. Embryos with fused blastomeres were incubated at 20°C for 60-90 minutes and processed for immunofluorescence.

Immunofluorescence

Fixation of embryos with methanol and acetone and antibody-staining procedures were carried out as described by Albertson (1984) and Sulston and Hodgkin (1988). Slides were treated with a solution of 0.1% poly-L-lysine obtained from Sigma Chemical Co. Embryos were incubated with monoclonal antibody MH27 (Priess and Hirsh, 1986; Waterston, 1988) or anti-β-galactosidase antibody (Sigma Chemical Co. catalogue no. G8021) followed by fluorescein (DTAF)-, or rhodamine (TRITC)-conjugated secondary antibodies from Jackson ImmunoResearch Laboratories, Inc. Embryos were mounted in glycerol mounting medium supplemented with 1,4 diazobicyclo-[2,2]-octane (DABCO) as described (Sulston and Hodgkin, 1988). 0.2 μg/ml 4′6-diamidino-2-phenolindole (DAPI, Sigma Chemical Co.) was added to the mounting medium to allow visualization of nuclei.

Homozygous *lin-12 glp-1, lag-1* or *lag-2* embryos were identified on MH27-stained slides by the absence of a rectum (Lambie and Kimble, 1991). Seam cells can be distinguished morphologically, by their lateral location and a failure to fuse into the epidermal syncytium (Sulston et al., 1983) and, molecularly, by the expression of a construct, called SCM, that is seam-cell specific (Hope, 1991; Gendreau et al., 1994).

Lineage analysis

Lineage analysis (Sulston et al., 1983) was performed by 4-D time-lapse analysis (Schnabel, 1991; Hird and White, 1993) on a Nikon Microphot-SA microscope equipped with Nomarski differential interference contrast optics at 600×. A Hamamatsu Newvicom videocamera was used to record images. The 4-D time-lapse system consists of a computer-controlled Bio-Rad focusing drive motor and a Sony LVR-5000/LVS-5000P optical disk recorder. Eggs were cut from gravid adults in M9 buffer (Sulston and Hodgkin, 1988). Four-cell embryos were collected, placed on a 4% agar pad, covered with a coverslip and sealed with Vaseline according to Sulston et al.

(1983). Intact embryos were recorded for at least 7 hours of development.

Cell divisions were reported as terminal only if the nuclei of the resulting cells could be followed through at least three hours of subsequent development without dividing. The number and pattern of cellular divisions and cellular morphology were used for lineage comparisons. Cells with granular cytoplasm, large nuclei with smooth nucleoplasm, and a large nucleolus were scored as epidermal. Cells with a small cytoplasm-to-nucleus size ratio, and granular nuclei and which migrated to the interior of the embryo were scored as neurons.

RESULTS

Background

The AB founder cell generates the majority of somatic cells of the *C. elegans* embryo. The cell lineage of AB is complex, giving rise to a diverse set of cell types that contribute primarily to three distinct organ types: the epidermis, the nervous system, and the neuromuscular feeding organ, or pharynx (Sulston et al., 1983). Five events that establish cell fate diversity among early embryonic AB descendants have been identified (Gendreau et al., 1994; Hutter and Schnabel, 1994; Mango et al., 1994; Mello et al., 1994; Moskowitz et al., 1994; Hutter and Schnabel, 1995a,b).

The first two of these events promote anterior/posterior differences in the AB lineage (Fig. 1A). The first breaks equivalence between the daughters of AB; ABp is induced to be different from its sister ABa by a neighboring cell, P_2 (Fig. 1A) (Hutter and Schnabel, 1994; Mango et al., 1994; Mello et al., 1994; Moskowitz et al., 1994). The second breaks equivalence between sister cells at the 12-cell (8-AB-cell) stage; each AB granddaughter undergoes a developmentally asymmetric cell division along the A/P axis, producing non-equivalent daughters (Fig. 1A) (Gendreau et al., 1994; Moskowitz et al., 1994; Hutter and Schnabel, 1995a). These two events act in combination to establish four unique cell identities among the eight AB descendants in the 12-cell embryo (Fig. 1A). These four cell fates are distributed along the A/P axis as four pairs

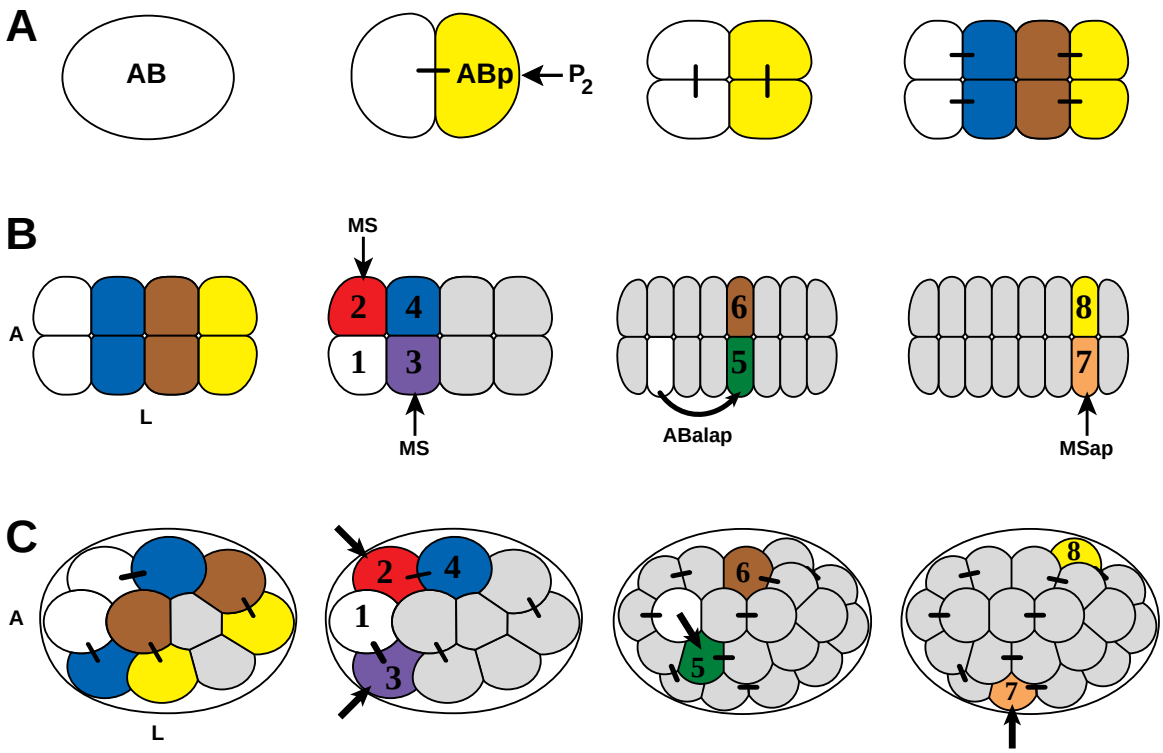

Fig. 1. Five events generate diversity among AB descendants. (A) Schematized dorsal view of AB descendants through the first three sequential AB divisions. Sister cells are indicated by black bars. Anterior is to the left and right to the top. Each color and number represents a unique lineage pattern. First, at the 4-cell (2 AB-cell) stage of embryogenesis, P_2 induces ABp (yellow) to develop differently from its sister, ABa (white). Second, from the 4 AB-cell to the 8 AB-cell stage, a developmentally asymmetric cell division occurs. The division of AB granddaughters causes the anterior granddaughters of ABa and ABp (white and brown, respectively) to develop differently from the posterior granddaughters (blue and yellow, respectively). (B) Schematized dorsal view of AB descendants with bilateral homologues directly apposed at the 12-cell (8-AB-cell) stage (columns 1 and 2), the 24-cell stage (column 3) and the 28-cell stage (column 4). Anterior is to the left and right to the top. At the beginning of the 12-cell stage, bilateral homologues are identical and each pair of bilateral homologues is unique (first column) (Hutter and Schnabel 1994; Gendreau et al., 1994; Hutter and Schnabel 1995). Three sequential inductions establish left/right asymmetry among AB descendants. Events are depicted as reported in Hutter and Schnabel (1995b). By the end of the 12-cell stage, MS has induced left/right asymmetry in ABa descendants; MS induces ABara (no. 2; red) to be different from its bilateral homologue, ABala (no. 1; white), and ABalp (no. 3; purple) to be different from its bilateral homologue, ABarp (no. 4; blue) (column 2) (Hutter and Schnabel 1994). At the 24-cell stage, ABalap induces ABplaa (no. 5; green) to be different from its bilateral homologue, ABpraa (no. 6; brown) (column 3) (Hutter and Schnabel, 1995b). At the 28-cell stage, an MS descendant induces ABplpa (no. 7; orange) to be different from its bilateral homologue, ABprpa (no. 8; yellow) (column 4). (C) Representation of dorsal view of embryo at 12-cell (8-AB-cell) stage (columns 1 and 2), 24-cell (16-AB-cell) stage (column 3), and 28-cell (16-AB-cell) stage (column 4). Interactions are depicted as in B.

of equivalent L/R homologues; there are apparently no L/R differences in cell fate among AB descendants at this point.

Three successive inductive interactions subsequently establish L/R differences between AB descendants derived from bilaterally symmetric cell lineages (Fig. 1B,C). The first of these inductions breaks bilateral symmetry between the two pairs of ABa-derived blastomeres at the 12-cell (8-AB-cell) stage: the MS cell induces the ABara (no. 2 in Fig. 1B) and ABalp (no. 3) blastomeres, causing them to develop differently from their bilateral homologues, the ABala (no. 1) and ABarp (no. 4) blastomeres, respectively (Fig. 1B) (Gendreau et al., 1994; Hutter and Schnabel, 1994). The second induction occurs between AB descendants and breaks bilateral symmetry in the anterior ABp lineage; the ABalap blastomere has been reported to induce the ABplaa blastomere (no. 5) to be different from its bilateral homologue, the ABpraa blastomere (no. 6; Fig. 1B) (Hutter and Schnabel, 1995b). Finally, the last induction breaks bilateral symmetry in the posterior ABp lineage: MSap has been reported to induce ABplpa (no. 7) to be different from its bilateral homologue, ABprpa (no. 8; Fig. 1B; Hutter and Schnabel, 1995b). The second and third inductions were proposed to occur at the 24- and 28-cell (16 AB-cell) stage, respectively (Hutter and Schnabel, 1995b). However, the lineage alterations caused by these inductions appear at a later stage, suggesting that the inductions themselves may actually occur between descendants of the previously implicated blastomeres (see Discussion).

Three of the inductions that generate diversity among AB descendants break equivalencies between bilateral homologues. In each case, by altering cell contacts, the normally uninduced bilateral homologues can become induced. (Hutter and Schnabel, 1994; Moskowitz et al., 1994; Wood, 1991). This observation demonstrates not only that the bilateral pairs of blastomeres are initially equivalent, but also suggests that cell-cell contact with the signaling cells is necessary and sufficient for all three inductions (Wood, 1991; Hutter and Schnabel, 1994; Moskowitz et al., 1994).

Two of the five events that establish asymmetries within the early AB lineage, the induction of ABp fate by P_2 and the induction of L/R differences in ABa descendants by MS, require maternally contributed GLP-1 (Hutter and Schnabel, 1994; Mello et al., 1994; Moskowitz et al., 1994). Certain weak *glp-1* alleles disrupt only the latter interaction, whereas stronger *glp-1* alleles disrupt both inductions (Hutter and Schnabel, 1994; Mello et al., 1994; Moskowitz et al., 1994). This paper focuses on the molecular requirements for the last two inductions, which generate the L/R asymmetry between ABp descendants.

Induction of ABplaa requires the Lag pathway

Genes required for the two inductions that generate L/R asymmetry within the ABp lineage have not been reported. The first of these interactions, the intra-AB induction of ABplaa, causes ABplaaa, the anterior daughter of ABplaa, to generate six head epidermal cells on the left side; only the fate of ABplaaa appears to be affected by this interaction (Hutter and Schnabel, 1995). In the absence of this induction, ABplaaa generates only nervous system and programmed cell deaths, like its normally uninduced bilateral homologue, ABpraaa (Hutter and Schnabel, 1995b). A failure of ABplaa to be induced might be expected to result in a lack of epidermal cells and a defect in morphogenesis on the left side of the head. Interestingly, animals carrying mutations in the Lag pathway genes display a twisted nose (Lambie and Kimble, 1991), raising the possibility that the induction of ABplaa may require these genes. We examined *lin-12(q269) glp-1(q231)* larvae and found that the direction of the twisted heads was always to the left side (11 out of 11 animals examined), consistent with a possible failure to specify left-sided epidermal cells.

We further examined the possibility that the Lag pathway is required for induction of ABplaa by observing the embryonic epidermis in Lag mutant embryos. The monoclonal antibody MH27 recognizes a component of the *C. elegans* adherens junctions and outlines all epidermal cells (Waterston, 1988). We analyzed the epidermal pattern of *lin-12(q269) glp-1(q231)*, *lag-1(q385)* and *lag-2(q387)* mutant embryos by observing MH27 staining (Materials and Methods; Fig. 2). In every case (>25 embryos of each genotype; see Materials and Methods), left-side-specific defects in the epidermal pattern were observed in the head region of mutant embryos (Fig. 2B-D). The defects observed in Lag mutant animals were specific to the region of the epidermis normally derived from ABplaaa. A wild-type MH27 pattern was always observed on the right side of mutant embryos, consistent with the proper specification of the bilaterally analogous lineage, which apparently requires no induction (Fig. 2A) (Moskowitz et al., 1994; Hutter and Schnabel, 1995).

The epidermal pattern of Lag mutants indicated that at least some of the epidermal cells normally derived from ABplaaa are absent or misplaced. To test directly whether these cells were generated in these mutants, we examined the expression of a marker specific for certain epidermal cells. Of six epidermal cells generated by ABplaaa in response to the induction, two are specialized epidermal 'seam' cells, which can be distinguished from other epidermal cell types by expression of a seam cell-specific construct, SCM (see Materials and Methods). Animals in which the ABplaa induction is prevented would be expected to lack two epidermal seam cells and produce eighteen rather than the wild-type total of twenty seam cells. In fact, embryos homozygous for a chromosomal deficiency that deletes both *lin-12* and *glp-1* express SCM in no more than 18 cells, suggesting that the Lag pathway may be required for the correct specification of two epidermal seam cells (R. Terns and J. Rothman, unpub-

Table 1. Quantitation of seam cells in Lag animals

Genotype	Number of cells expressing seam cell marker SCM	
	Left side*	Right side†
wild type	10 (>100)	10 (>100)
lin-12(q269) glp-1(q231)	8 (24); 7 (1)	10 (25)
lag-1(q476)	8 (18)	10 (18)
lag-2(q387)	8 (22)	10 (22)

Wild-type and homozygous mutant embryos carrying the SCM construct were stained with anti-β-galactosidase antibodies to detect seam cells and monoclonal antibody MH27 to reveal the overall epidermal pattern (see Materials and Methods). The number of embryos in each numerical class is presented in parentheses.

*Number of cells expressing the SCM construct on the left side of each embryo.

†Number of cells expressing the SCM construct on the right side of each embryo.

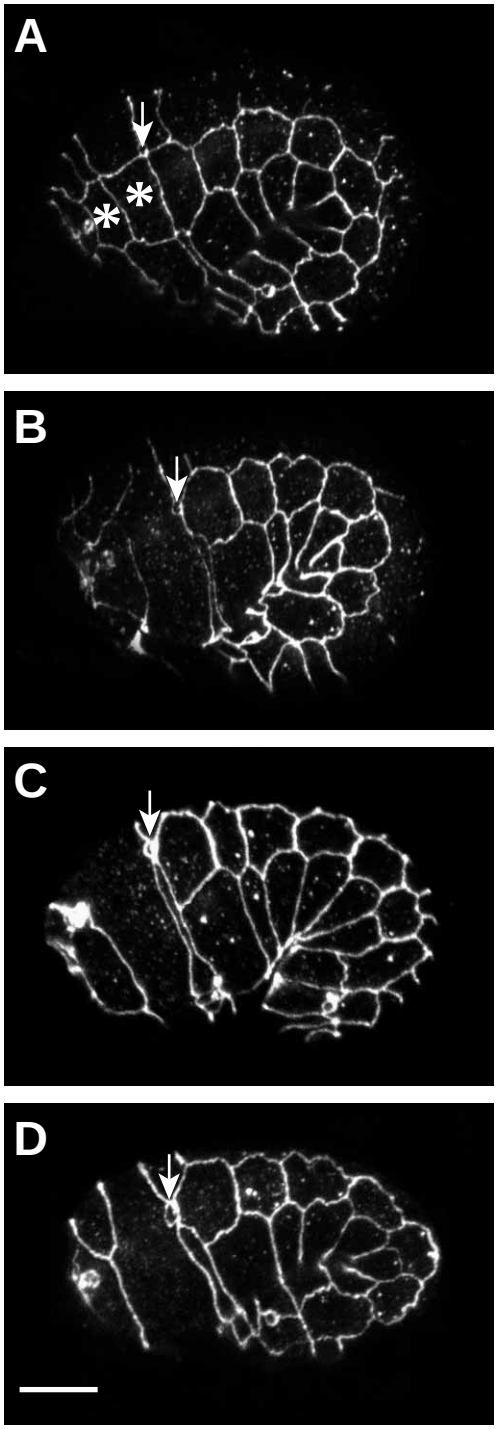

Fig. 2. *Lag* mutations cause left-side-specific epidermal defects. (A-D) Immunofluorescence micrographs of *lin-12*(*q269*) *glp-1*(*q231*) (A,B), *lag-1*(*q385*) (C) and *lag-2*(*q387*) (D) embryos. Anterior is to the left and dorsal to the top. Embryos were stained with antibody MH27, which recognizes a component of the *C. elegans* adherens junction and outlines all epithelia, including the epidermis and rectum. Embryos from heterozygous hermaphrodites were stained and homozygous mutant embryos, identified by the absence of a rectum, were analyzed (Materials and Methods). Bar, approximately 10 μm. (A) Right side of *lin-12*(*q269*)*glp-1*(*q231*) mutant embryo. The epidermal pattern appears wild type (Priess and Hirsh, 1986). Specifically, ten lateral cells can be counted, two of which, HOR and HOL (indicated by *), are anterior to the right deirid (deirid is indicated by an arrow in all panels). (B) Left side of the *lin-12*(*q269*)*glp-1*(*q231*) mutant embryo shown in A. Normally, left and right lateral rows appear identical (Hirsh and Priess, 1986). In this embryo, the two lateral cells normally present anterior to the left deirid are apparently missing. (C) Left side of a *lag-2*(*q387*) mutant embryo showing apparent absence of the two anterior-most lateral cells. The right side of this embryo appeared wild-type (not shown). (D) Left side of a *lag-1*(*q385*) mutant embryo. In this embryo the two anterior-most lateral cells are apparently missing. The right side of this embryo appeared wild type (not shown).

lished data). To assess whether the two seam cells normally derived from ABplaaa are not made in Lag mutant embryos, we counted the number of seam cells in mutant embryos as assayed by SCM expression (see Materials and Methods). In contrast to wild-type embryos, which expressed SCM in two bilateral rows of 10 nuclei each, *lin-12(q269) glp-1(q231)*, *lag-1(q385)* and *lag-2(q387)* mutant embryos expressed SCM in 8 cells on the left side and 10 cells on the right side in almost every case (Table 1). Specifically, in most cases, the two anterior-most SCM-expressing cells, i.e., those normally derived from ABplaaa, appeared to be missing from the left side of Lag animals. These results demonstrate that at least some ABplaaa-derived cells are not present in Lag animals and suggest that induction of ABplaaa fate may not occur in Lag mutants.

To analyze the fate of ABplaaa directly in the absence of zygotic *lag*-mediated signaling, we followed cell lineages in homozygous *lin-12(q269) glp-1(q231)* mutant embryos. We found that the ABplaaa lineage is transformed in *lin-12(q269) glp-1(q231)* mutant embryos (Fig. 3). Whereas the six epidermal cells normally generated by ABplaaa are born by eight sequential divisions from AB (Sulston et al., 1983), the lineages normally generating the six epidermal cells from ABplaaa did not terminate at the eighth sequential AB division. Instead, each of these cells divided at least a ninth time and the resulting survivors often took on the morphological appearance of neurons (see Materials and Methods). The transformed lineage of ABplaaa resembled the wild-type lineage of ABpraaa, its normally uninduced bilateral homologue (Fig. 3). These results demonstrate that induction of ABplaa, which establishes bilateral asymmetries between ABplaaa and ABpraaa, requires *lin-12* and *glp-1*. Furthermore, given the similarities in the Lin-12 Glp-1, Lag-1 and Lag-2 phenotypes (see above; Lambie and Kimble, 1991), they suggest that *lag-1* and *lag-2* may also participate in this induction.

Induction of ABplpa requires the Lag pathway

It has been proposed that the Lag pathway may be required for the second induction that generates bilateral asymmetries in ABp fates, the induction of ABplpa or its descendants by MSap (Hutter and Schnabel, 1995b). This induction causes ABplpapp to generate a lineage different from that of its bilateral homologue ABprpapp (Hutter and Schnabel, 1995b). Both the excretory cell and multiple rectal cells are normally generated by the induced ABplpapp. Animals in which ABplpa is not induced might therefore fail to generate an excretory cell or a functional rectum. Lambie and Kimble

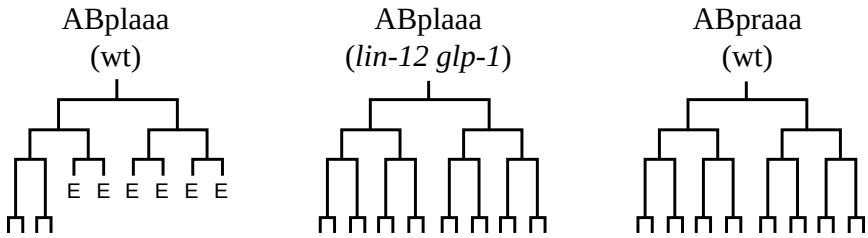

Fig. 3. The ABplaaa lineage is altered in *lin-12 glp-1* double mutant embryos. The cell lineage of ABplaaa in a *lin-12*(*q269*) *glp-1*(*q231*) embryo is shown and compared to wild-type lineages. The first and third columns show wild-type lineages (Sulston et al., 1983). The middle column shows a lineage analyzed in a single *lin-12*(*q269*) *glp-1*(*q231*) double mutant embryo. The name of the AB descendant followed is shown. Cells that differentiate as epidermal cells based on morphology are indicated by an 'E' (Moskowitz et al., 1994). For simplicity, lineages are shown from the sixth through the ninth sequential AB divisions, and cell deaths and relative timings of divisions are not shown. The lineage derived from ABplaaa in the *lin-12 glp-1* double mutant embryo is transformed to resemble the wild-type ABpraaa lineage. Similar observations were made in three other *lin-12*(*q269*) *glp-1*(*q231*) double mutant embryos (not shown).

(1991) showed that Lag animals lack both an excretory cell and a rectum, consistent with a possible role for the Lag pathway in this interaction.

We tested the possibility that the induction of ABplpa fails in Lag mutant animals by performing cell lineage analysis of ABplpapp in *lin-12(q269) glp-1(q231)* mutant embryos. We found that the differences between the ABplpapp and ABprpapp lineages, which are minor, were eliminated in *lin-12(q269) glp-1(q231)* mutant embryos. A characteristic cell death normally occurring in the ABplpapp lineage (ABplpappap) was absent in all three *lin-12(q269) glp-1(q231)* double mutant embryos analyzed. Instead of dying, this cell, like its wild-type bilateral homologue ABprpappap, divided. Furthermore, the physical asymmetry of the division of ABplpappaa, which normally generates the excretory cell, was eliminated in these three embryos and instead this cell divided symmetrically. These results imply that the excretory cell is missing in *lin-12(q269) glp-1(q231)* animals because induction of ABplpa requires *lin-12* or *glp-1*. Moreover, although one rectal cell has been shown to be specified by a *lag*-dependent lateral interaction late during embryogenesis (Bowerman et al., 1992), our results also imply that failure of the ABplpa induction is responsible for the absence of a rectum in *lin-12(q269) glp-1(q231)* animals. Finally, the similarity of the *lin-12 glp-1*, *lag-1* and *lag-2* mutant phenotypes suggest that all of the known components of the Lag signaling pathway may participate in this interaction.

Early embryonic *lag-2* expression

We tested whether LAG-2 acts directly as a signaling molecule in the inductions of the ABplaa and ABplpa lineages by characterizing the pattern of *lag-2* expression in the early embryo. Embryos carrying a *lag-2::lacZ* construct were analyzed (Henderson et al., 1994). This construct appears to be an accurate reporter of *lag-2* expression (Henderson et al., 1994; see Materials and Methods). Expression of β-galactosidase was assayed by immunofluorescence analysis using anti-β-galactosidase antibodies (see Materials and Methods).

We observed expression of β-galactosidase in descendants of both ABalap and MSap, the cells previously implicated as the signaling cells in the inductions of ABplaa and ABplpa. Reproducible β-galactosidase was first observed in all four ABala granddaughters at the 55-cell (late 32 AB-cell) stage in embryos expressing the *lag-2::lacZ* reporter (Fig. 4A; see Materials and Methods). β-galactosidase was not detected in any other cells of the AB lineage at this stage in development (Fig. 4A). Expression of β-galactosidase was also observed in MSap descendants; at the 87-cell (64 AB-cell) stage, β-galactosidase was observed in MSapa and MSapp, the daughters of MSap (Fig. 4B; see Materials and Methods). β-galactosidase expression in both the ABala and MSap lineages persists for at least two rounds of cell division (not shown). This observation may reflect actual expression of the *lag-2* gene, rather than a perdurance of β-galactosidase protein, because an engineered β-galactosidase with a short half-life was used in the construction of the translational fusion (Henderson et al., 1994). We did not observe *lag-2* expression in ABalap and MSap, the proposed signaling cells of the ABplaa and ABplpa inductions, respectively (Hutter and Schnabel 1995). Instead, we first detect *lag-2* expression at later stages, in descendants of these cells (see Discussion).

Early embryonic *lin-12* expression

We tested the possibility that LIN-12 acts directly as a receptor in the *lag*-dependent inductions of ABplaa and ABplpa by characterizing the early embryonic expression of a *lin-12* reporter construct. Embryos carrying *lin-12::lacZ*, previously shown to be an accurate reporter of *lin-12* expression (Wilkinson et al., 1994) (see Material and Methods), were analyzed.

We observed expression of β-galactosidase in a large domain of AB-derived cells in embryos carrying the *lag-2::lac-Z* construct (Fig. 4C). Reproducible β-galactosidase expression was first observed in 24 cells at the 55-cell (32-AB-cell) stage in embryos carrying the *lin-12::lacZ* construct (Fig. 4C; see Materials and Methods); variable staining was observed at the 28-, 46- and 50-cell stages. The positions of the β-galactosidase-expressing cells suggested that all 24 expressing cells at the 55-cell stage were AB-derived. Consistent with this suggestion, we found that laser-ablation of AB in the 2-cell embryo eliminated all β-galactosidase expression at a time corresponding to the wild-type 55-cell (32-AB-cell) stage (not shown). In intact transgenic embryos, β-galactosidase expression persisted through at least one round of cell division (not shown). Again, because an engineered β-galactosidase with a short half-life was used in the construction of the translational fusion, this observation may reflect continued expression of the *lin-12* reporter (Wilkinson et al., 1994). We did not observe reporter expression in either ABplaa or ABplpa, the cells previously proposed to receive inductive interactions. However, *lin-12* expression was observed in the descendants of both of these cells. *lin-12*-expressing cells

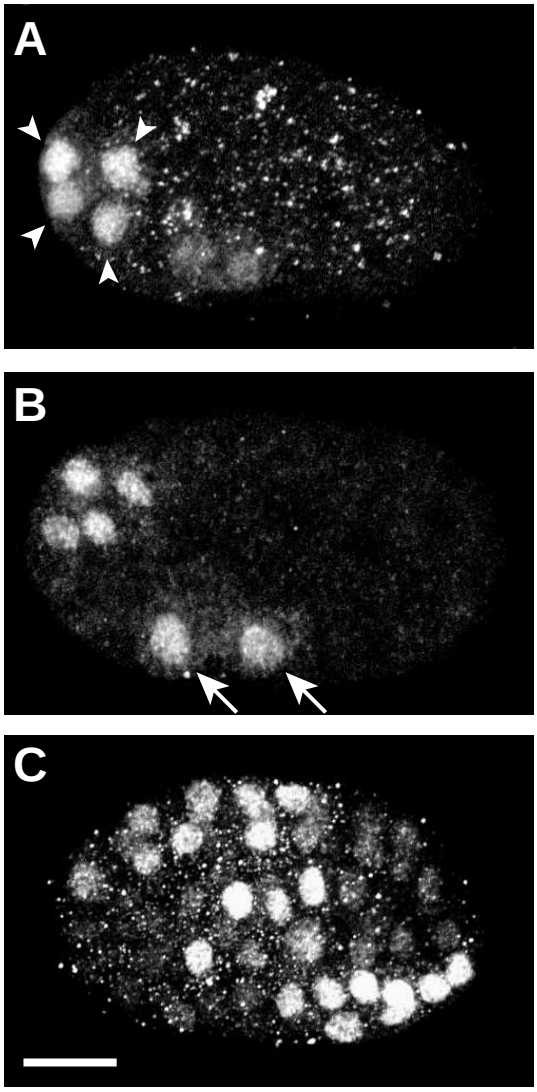

Fig. 4. *lag-2* and *lin-12* expression in the early embryo. All panels are immunofluorescence micrographs. Bar, approximately 10 μm. (A) *lag-2*::*lacZ* expression at the 55-cell stage. Shown is a *lag-2*::*lacZ* transgenic embryo stained with anti-β-galactosidase antibodies in a left-side view with anterior to the left. β-galactosidase expression is also observed in the four ABala granddaughters (designated by arrowheads), visible at the anterior of the embryo. Weak β-galactosidase expression is observed in the two daughters of MSap (MSapa and MSapp) on the ventral side of the embryo. (B) *lag-2*::*lacZ* expression at the 87-cell stage. Shown is a *lag-2*::*lacZ* transgenic embryo stained with anti-β-galactosidase antibodies in a left-side view with anterior to the left. β-galactosidase expression is observed in the eight ABala great-granddaughters, four of which are visible in the anterior of the embryo in this micrograph. Robust β-galactosidase expression is also observed in the two daughters of MSap (MSapa and MSapp; designated by arrows) on the ventral side of the embryo. (C) *lin-12*::*lacZ* expression at the 55-cell stage. Shown is a *lin-12*::*lacZ*; *smg-1* transgenic embryo stained with anti-β-galactosidase antibodies in a dorsal view with anterior to the left. Robust β-galactosidase expression is observed in 24 cells.

included ABplaaa and ABplpapp, whose lineages are directly altered by the two *lin-12 glp-1*-mediated inductions (see Discussion).

Maternal GLP-1 activity is required to repress *lag-2* expression

We have demonstrated that *lag-2* is expressed in the early embryo in a restricted group of AB-derived cells (Fig. 4A,B). Comparison of the *lag-2* expression pattern with the AB fate map (Fig. 1; see also Fig. 8) shows that *lag-2* expression is absent from all AB-derived cells that are influenced by maternal GLP-1-mediated signaling. Previous studies have shown that activation of LIN-12 can down-regulate *lag-2* expression cell autonomously in certain cells of the somatic gonad (Wilkinson et al., 1994). The expression pattern of *lag-2* in the early embryo raised the possibility that maternal GLP-1 activity might similarly repress zygotic *lag-2* expression in the early embryo. This hypothesis was tested by preventing maternal GLP-1-mediated inductions both by cell ablation and genetically, and assaying *lag-2* expression in the embryo (Fig. 5).

We found that elimination of the *glp-1*-dependent induction of ABara by ablation of MS, the signaling cell, derepressed *lag-2* expression in ABara descendants. When MS was laser-ablated before GLP-1-dependent induction of ABara occurs, both the ABala granddaughters and the ABara granddaughters expressed the *lag-2* reporter construct (15/15 embryos; Fig. 5B). To ensure that the ectopic expression of *lag-2* in ABara descendants was a result of eliminating the MS signal and not a side-effect of the laser microsurgery, *lag-2* expression was assayed in embryos in which MS was killed late in its cell cycle, after the induction of ABara had occurred (Mango et al., 1994; Hutter and Schnabel, 1994). In these control ablations, the *lag-2* reporter constructs were never expressed in ABara descendants, while ABala descendants expressed the reporter normally (11/11 embryos; not shown).

The ectopic *lag-2* expression observed when the maternal GLP-1-dependent induction of ABara was prevented by laser-ablation of MS suggests a role for maternal GLP-1 in the repression of *lag-2* expression in the early embryo. The requirement for maternal GLP-1 in the repression of zygotic *lag-2* expression was tested by analyzing the expression of the *lag-2* reporters in several different *glp-1* mutants (Fig. 5C,D). Certain weak *glp-1* alleles, including *glp-1(e2142ts)*, specifically disrupt the MS induction of ABa descendants without interfering with the GLP-1-dependent induction of ABp (Kodoyianni et al., 1992; Hutter and Schnabel, 1994). In embryos derived from *glp-1(e2142ts)* homozygous mothers and raised at the non-permissive temperature, *lag-2*::*lacZ* expression was observed in the descendants of both ABala and ABara and in no other AB-derived cells (Fig. 5C). Thus, maternal GLP-1 is required to repress *lag-2* expression in ABara descendants, consistent with the results of the laser-ablation experiment.

Certain strong *glp-1* alleles, including *glp-1(q224)*, eliminate both maternal GLP-1-dependent inductive interactions (Hutter and Schnabel, 1994; Moskowitz et al., 1994). In embryos derived from *glp-1(q224)* homozygous mothers and raised at non-permissive temperature, *lag-2*::*lacZ* expression was observed in 16 AB descendants at the 55-cell (late 32 AB-cell) stage; expression was apparent in the ABala granddaughters and 12 other AB-derived cells (Fig. 5D). Thus, half of the AB descendants express *lag-2* at this stage of development. The AB fate map in embryos derived from homozygous *glp-1(q224)* mothers is greatly simplified compared to that of wild-type embryos: each AB granddaughter divides to

produce one blastomere with a wild-type ABala-like fate, and one with a wild-type ABarp-like fate (Hutter and Schnabel, 1994; Moskowitz et al., 1994). Thus, the ABala-like and ABarp-like fates are each reiterated four times, once in the normal position and three times in ectopic positions (Hutter and Schnabel, 1994; Moskowitz et al., 1994). The positions of the ectopic *lag-2*-expressing nuclei in *glp-1(q224)* embryos suggest that they are derived from the three ectopic ABala-like cells. Similar results were obtained with another strong *glp-1* allele, *glp-1(q231)* (not shown). These results demonstrate that maternal *glp-1* activity is required to repress the expression of *lag-2*. They also suggest that the asymmetric division of each AB granddaughter restricts the ability to express *lag-2* to one of its daughters (see Discussion).

Activation of maternal GLP-1 is sufficient to repress *lag-2* expression

ABala does not normally receive maternal GLP-1 signaling (Hutter and Schnabel, 1994; Moskowitz et al., 1994) and, as we have shown, its descendants are the only AB-derived cells to express *lag-2* in the early embryo (Fig. 4A). To further investigate the effect of GLP-1 signaling on *lag-2* expression, we asked whether maternal activation of GLP-1 is sufficient to repress *lag-2* expression in the ABala lineage. Our strategy was to provide activated GLP-1 ectopically to ABala and analyze *lag-2* expression in ABala descendants; this was achieved by laser-induced fusion (Schierenberg, 1984) of ABala with ABara, a cell that is induced by MS- and GLP-1-dependent signaling (see Materials and Methods). As the fused cell maintains contact with MS, both nuclei within the resulting dikaryon are influenced by MS-dependent activation of GLP-1. The subsequent tetrapolar division of the fused cell creates two cells with nuclei derived from the ABala nucleus and two with nuclei derived from the ABara nucleus. Fusions were performed in a strain carrying the *lag-2*::*lac-Z* reporter and *lag-2* expression was assayed by examining β-galactosidase expression.

We found that GLP-1 activation in the dikaryon was sufficient to repress *lag-2* expression in descendants of the ABala nucleus (Fig. 6). In the wild-type embryo, β-galactosidase is observed in both ABala and MS descendants (Fig. 6A). However, no β-galactosidase was observed in ABala descendants when ABala and ABara were fused (12/12 embryos; Fig. 6B). (MS descendants, not directly involved in the fusion, expressed the *lag-2*::*lac-Z* fusion normally providing an internal control for marker expression; Fig. 6B). To control for the possibility that the fusion event damaged ABala and ABara, thereby preventing expression of the *lag-2*::*lac-Z* marker in their descendants, activation of GLP-1 in the dikaryon was prevented in a group of control embryos by ablating MS. When ABala and ABara were subsequently fused, the *lag-2*::*lac-Z* fusion was expressed in all descendants

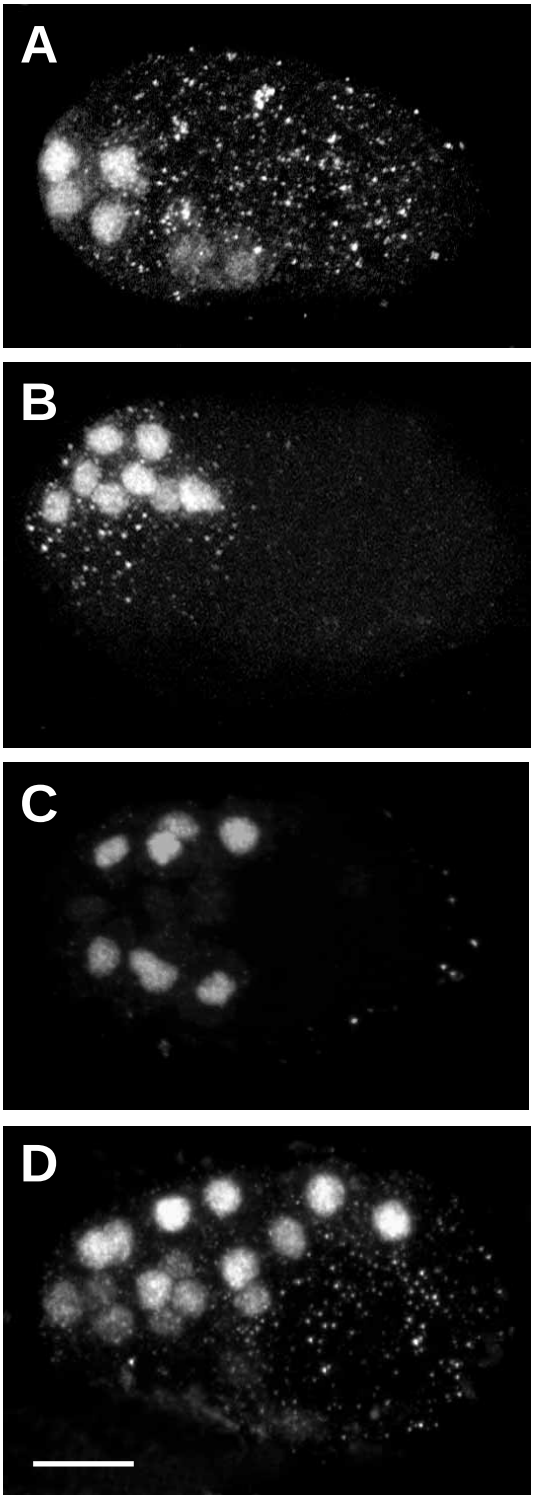

Fig. 5. Zygotic *lag-2* expression is de-repressed in *glp-1* mutants. All images are immunofluorescence micrographs of 55-cell *lag-2*::*lacZ* transgenic embryos stained with anti-β-galactosidase antibodies. Bar, approximately 10 μm. (A) Wild-type embryo, shown in a left-side view with anterior to the left. β-galactosidase expression is observed in all four ABala granddaughters. (B) MS-ablated embryo, shown in a left-side view with anterior to the left. β-galactosidase staining is observed in all four ABara granddaughters and all four ABala granddaughters. (C) *glp-1*(*e2142ts*) embryo grown at 20°C, shown in a dorsal view with anterior to the left. β-galactosidase expression is observed in all four ABara granddaughters and all four ABala granddaughters. A similar observation was made in embryos grown at 25°C. (D) *glp-1*(*q224ts*) embryo grown at 20°C, shown in a left-side view with anterior to the left. β-galactosidase expression is observed in 16 AB-derived cells, including all four ABala granddaughters. A similar observation was made in embryos grown at 25°C.

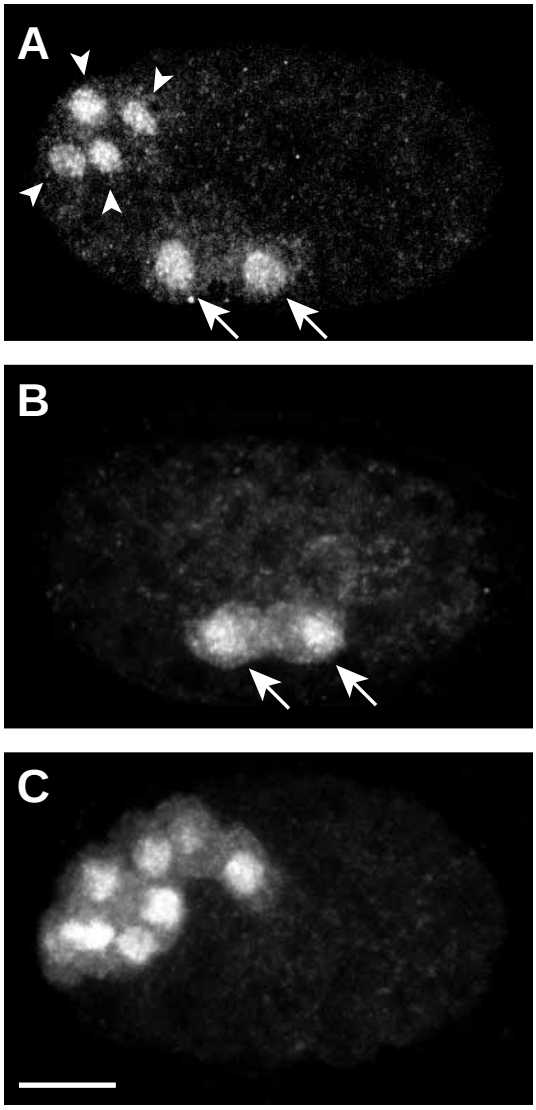

Fig. 6. Zygotic *lag-2* expression can be repressed by maternal GLP-1 signaling. All images are immunofluorescence micrographs of *lag-2*::*lac-Z* transgenic embryos. All embryos are shown in a left-side view with anterior to the left. Bar, approximately 10 μm. (A) Wild-type embryo is shown at the 87-cell stage. β-galactosidase expression is observed in ABala descendants (designated by arrowheads) and the MSap daughters (designated by arrows). (B) Embryo in which ABala and ABara were fused is shown at the 55-cell stage. No β-galactosidase is observed in AB descendants. β-galactosidase expression is observed in the MSap daughters (designated by arrows). (C) Embryo in which MS was ablated and ABala and ABara were fused is shown at the 55-cell stage. β-galactosidase expression is observed in all four ABara granddaughters and all four ABala granddaughters. No β-galactosidase fluorescence is observed from the ablated MS.

of the ABala/ABara dikaryon at the 55-cell (late 32 AB-cell) stage in 8/11 embryos (Fig. 6C). This finding demonstrates that the fusion *per se* did not prevent *lag-2* expression, and is consistent with our finding that both ABala and ABara descendants express *lag-2* when induction by MS is blocked (Fig. 4B). We conclude that activated maternal GLP-1 is sufficient to repress *lag-2* expression.

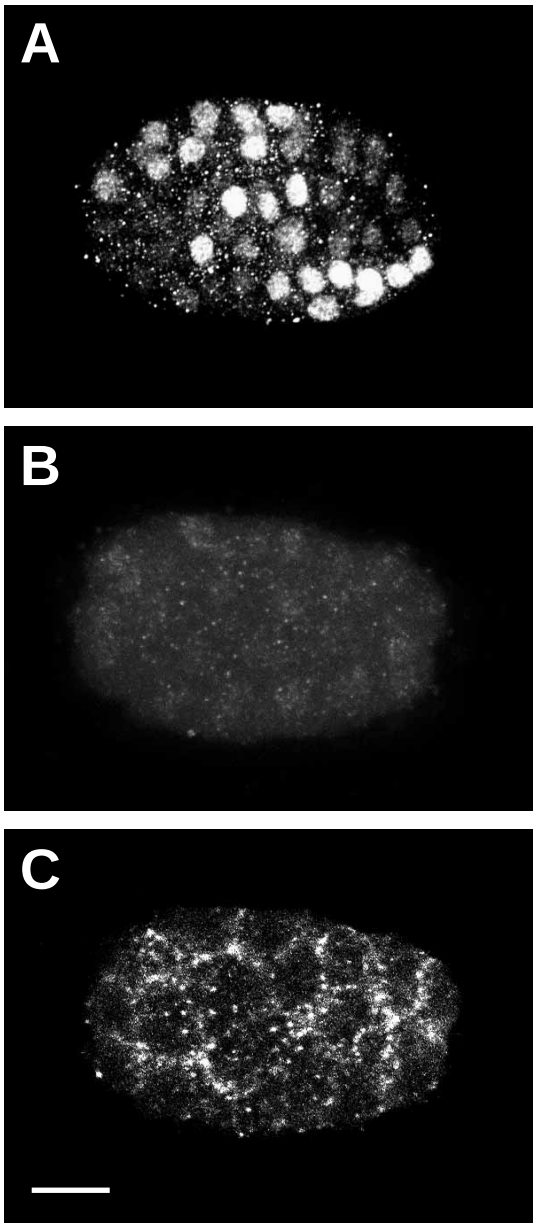

Fig. 7. Zygotic *lin-12* expression requires maternal GLP-1 signaling. All images are left-side views with anterior to the left. Bar, approximately 10 μm. (A) Immunofluorescence micrograph of 55-cell *smg-1*; *lin-12*::*lacZ* embryo stained with anti-β-galactosidase antibodies. β-galactosidase expression is observed in 24 AB-derived cells. (B) Immunofluorescence micrograph of 55-cell *smg-1*; *lin-12*::*lacZ glp-1*(*q224ts*) embryos grown at 20°C and stained with anti-β-galactosidase antibodies. β-galactosidase expression is weakly detectable. A similar observation was made in embryos grown at 25°C. (C) Immunofluorescence micrograph of the *smg-1*; *lin-12*::*lacZ glp-1*(*q224ts*) embryo shown in B stained with anti-actin antibodies. Fluorescence is observed in all cells.

Maternal GLP-1 activity is required to activate *lin-12* expression

We found that zygotic *lin-12* expression may be correlated with prior activation of maternal GLP-1 in the early embryo. Specifically, 24 of the 32 AB-derived cells in a 55-cell embryo

express *lin-12*, and 24 of the 32 AB-derived cells descend from cells that receive maternal GLP-1 activation (Fig. 4C) (Hutter and Schnabel, 1994; Moskowitz et al., 1994). This correlation suggested that *lin-12* expression may be activated by the maternal GLP-1 inductions. We tested this possibility by ablating the cells that receive maternal GLP-1-dependent signaling and assaying for *lin-12*::*lacZ* expression in the descendants of the remaining cells. We found that laser-ablation of ABp, ABara and ABalp, the cells that receive maternal GLP-1-dependent signaling, eliminated all β-galactosidase expression from operated embryos at the stage corresponding to the 55-cell stage of unoperated embryos (not shown; see Materials and Methods).

Previous work by Wilkinson et al. (1994) showed that activation of the LIN-12 receptor in particular cells in the somatic gonad can activate *lin-12* transcription cell autonomously. This raised the possibility that, by analogy to this positive feedback in the somatic gonad, activation of maternal GLP-1 may be required to activate zygotic *lin-12* expression in the early embryo. To test this hypothesis, the expression of the *lin-12* reporter construct was examined in embryos lacking maternal GLP-1 activity.

Strong loss-of-function *glp-1* alleles, including *glp-1(q224ts)*, eliminate both of the known maternal GLP-1-dependent inductive events (Hutter and Schnabel, 1994; Moskowitz et al., 1994). In embryos derived from hermaphrodites homozygous for both *glp-1(q224ts)* and the *lin-12*::*lacZ* marker and raised at the non-permissive temperature, β-galactosidase expression was absent from all AB descendants at the 55-cell (late 32-AB-cell) stage (Fig. 7B); weak and variable staining could occasionally be detected at the 46-cell (early 32-AB-cell) stage (not shown). The same results were obtained with *glp-1(q231ts)*, another strong *glp-1* allele (not shown). These results imply that maternal GLP-1 activity is necessary to activate *lin-12* expression in the embryo.

DISCUSSION

We report three primary findings. First, the *C. elegans* Lag signaling pathway (including the *lin-12*, *glp-1*, *lag-1* and *lag-2* genes) appears to be required zygotically for two early embryonic inductions. Second, the *lag-2* gene is expressed in presumptive inducing cells while the *lin-12* gene is expressed in presumptive receiving cells of these interactions. Third, *lag-2* and *lin-12* expression is regulated by maternal GLP-1-mediated signal transduction, marking a maternal-to-zygotic transition in gene expression in the early *C. elegans* embryo.

Requirement for the *lag* genes in early embryonic inductions

Previous work has demonstrated a requirement for the maternal LNG receptor, GLP-1, in two early embryonic induction events that specify the fates of AB-derived embryonic blastomeres. Here we demonstrate that *lin-12*, *glp-1*, *lag-1* and *lag-2*, all components of the Lag pathway, are apparently required zygotically for two successive induction events that specify later embryonic cell fates in the ABp lineage. These inductions, together with the maternal GLP-1-dependent induction of ABa descendants, are responsible for the major L/R differences in the AB lineage. Later L/R differences between certain pairs of cells are promoted by lateral interactions, of which at least two require zygotic Lag components (Greenwald et al., 1983; Bowerman et al., 1992). Thus, most, if not all, L/R differences in the AB lineage may be established by LNG-mediated signaling.

The finding that the same constellation of defects is observed in *lin-12 glp-1*, *lag-1* and *lag-2* mutant animals implicates the Lag cascade of ligand, receptors and downstream effector in both inductions of ABp descendants. However, the two induced blastomeres generate lineages that are entirely distinct from one another. Therefore, the Lag signaling cascade apparently does not act alone to control the fate of ABp-derived blastomeres. Instead, the zygotic Lag signal appears to act in combination with the underlying identities of ABp descendants to specify distinct fates.

The LNG-mediated induction of ABplaaa fate demonstrates that apparently identical embryonic cell fates can be established by different mechanisms. ABplaaa, an induced cell, and ABarpap, an uninduced cell, are analogues; i.e., they generate identical lineages patterns, albeit on opposite sides of the embryo (Sulston et al., 1983). The ABplaaa fate is specified by two sequential Lag-mediated inductions (induction of ABp through maternal GLP-1 and induction of ABplaaa fate through zygotic LIN-12 and GLP-1), whereas ABarpap fate is generated by an LNG-independent mechanism (this work; Hutter and Schnabel, 1994; Mello et al., 1994; Moskowitz et al., 1994). In the absence of the first or second induction, ABplaaa gives rise to a different cell lineage (this work; Hutter and Schnabel, 1994; Moskowitz et al., 1994). Thus, zygotic Lag-mediated induction of ABplaaa appears to act by canceling out the effect of the earlier induction of ABp, reverting ABplaaa to a more primary fate rather than generating a unique fate. Sequential LNG-mediated interactions apparently act antagonistically in this case to toggle cells between two alternate developmental states rather than synergistically to establish a novel state.

Correlation of *lag-2* and *lin-12* expression with their roles in embryonic inductions

We propose that the two zygotic Lag-mediated inductions of ABp descendants occur at the 55- and 87-cell stage rather at the 24- and 28-cell stage as suggested by Hutter and Schnabel (1995), and are therefore inductions of ABplaaa and ABplpapp per se, and not their ancestors. This suggestion is based on the stage at which both *lin-12* and *lag-2* expression and the induced cell lineage alterations are observed. LIN-12 and LAG-2 appear to mediate both induction events (Figs 2, 3). Reproducible *lin-12* expression is first observed in the receiving cells at the 55-cell (32-AB-cell) stage (Fig. 4). Reproducible *lag-2* expression first appears in ABala descendants at the 55-cell (32-AB-cell) stage and in MSap descendants at the 87-cell (64-AB-cell) stage (Fig. 4). Furthermore, only the lineages of ABplaaa and ABplpapp, and not their sisters or cousins, are altered by these inductions (Hutter and Schnabel, 1994). The lineage alterations occur at the 55-cell (32-AB-cell) stage in the ABplaa lineage and at the 87-cell (64-AB-cell) stage in the ABplpa lineage, and therefore correlate with the time that ligand expression appears in each case. We therefore propose that ABalapp directly induces ABplaaa fate and an MSap daughter directly induces ABplpapp fate.

LNG-mediated transcriptional feedback and regulation of *lag-2* and *lin-12* expression pattern in the early embryo

The transcriptional regulation of *lin-12* and *lag-2* by LNG receptor activity during post-embryonic development has been previously reported. Wilkinson et al. (1994) proposed that a transcriptional feedback mechanism, in which LIN-12 activity regulates both *lin-12* and *lag-2* expression, mediates a lateral interaction that specifies the gonadal AC and VU cell fates. *lag-2* and *lin-12* are initially expressed in both the uncommitted AC and VU precursor cells. When LIN-12 receptor activity rises in one cell, *lin-12* transcription is activated and *lag-2* transcription is repressed in that cell (Wilkinson et al., 1994). The other cell of this interacting pair thereby experiences a lower level of LIN-12 activation (the result of reduced LAG-2 ligand expressed by its neighbor), resulting in opposite changes of *lin-12* and *lag-2* expression (Wilkinson et al., 1994). Thus, the difference in LIN-12 activation between these neighboring cells is amplified by this LNG-mediated transcriptional feedback until one of the two cells expresses only *lin-12* and becomes the VU, while the other expresses only *lag-2* and becomes the AC. This model provides a paradigm for the molecular mechanism of lateral interactions among members of an equivalence group and provides a precedent for the transcriptional regulation of the *lag-2* ligand and *lin-12* receptor by the activity of a LNG receptor.

We propose that the zygotic expression of *lin-12* and *lag-2* in the early embryo is also regulated by the activity of an LNG receptor, maternal GLP-1. Our work demonstrates that activation of maternal GLP-1 in an embryonic blastomere

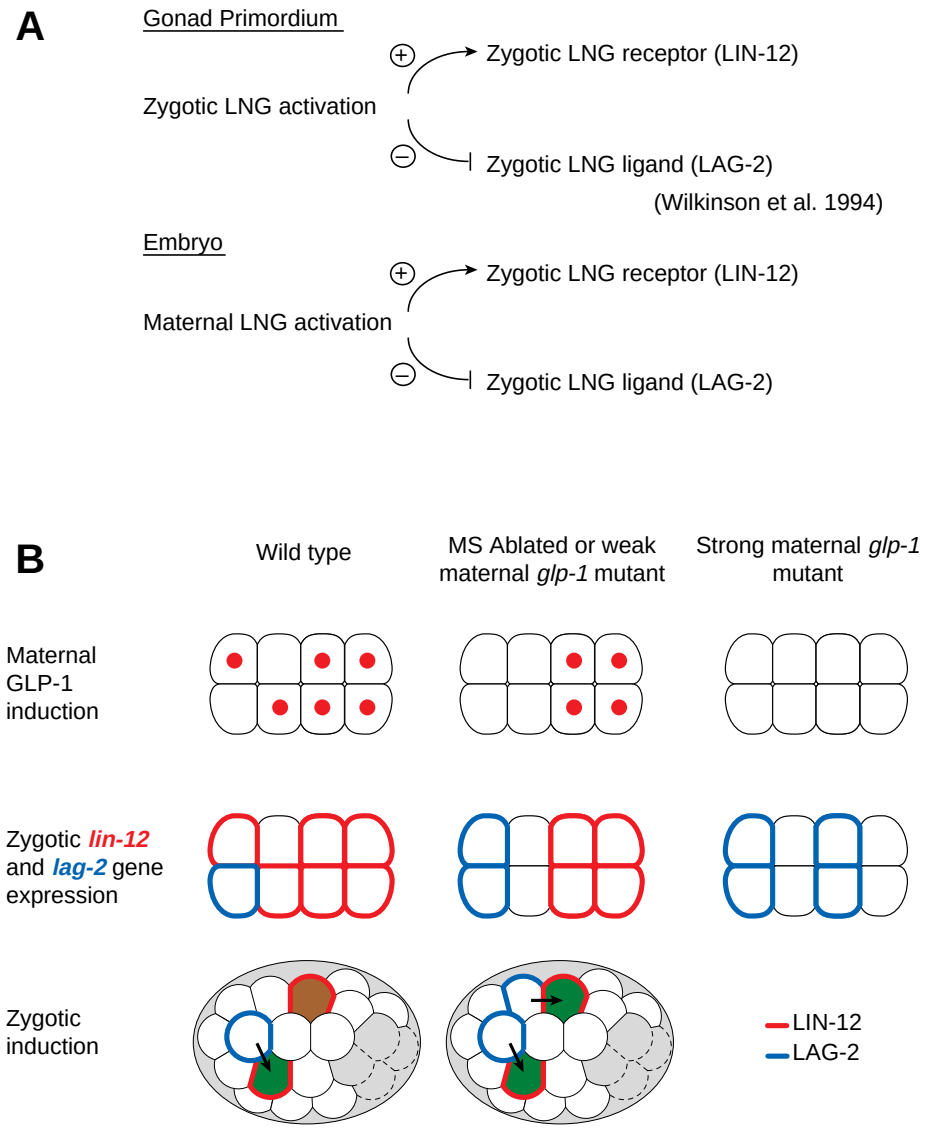

Fig. 8. Sequential LNG-mediated inductive events are organized by transcriptional regulation of *lag-2* and *lin-12* expression. (A) Model of transcriptional feedback in the somatic gonad proposed by Wilkinson et al. (1994) is shown in the top panel: LIN-12 activity positively regulates *lin-12* expression and negatively regulates *lag-2* expression. Our model of transcriptional regulation in the early embryo is shown in the bottom panel: maternal GLP-1 activity positively regulates *lin-12* expression and negatively regulates *lag-2* expression. (B) Model of organization of zygotic LNG-mediated embryonic inductions by maternal GLP-1 signaling. The first and second rows depict schematized dorsal view of AB descendants with bilateral homologues directly apposed at the 12-cell (8-AB-cell) stage following the convention in Fig. 1. In the first row, cells with red dots have been influenced by maternal GLP-1 induction. In the second row, the predicted fate map of *lin-12* and *lag-2* gene expression is shown: cells with red outlines generate *lin-12*-expressing cell and those with blue outlines generate *lag-2*-expressing cells. The observed pattern of *lin-12* and *lag-2* gene expression is consistent with this model in every case tested. The third row shows representations of dorsal views of embryos with the known intra-AB zygotic induction events. Inductions are depicted at the 24-cell stage for simplicity. Column 1: in the wild-type case, one *lag-2*-expressing cell (ABalapp) is juxtaposed with one ABp-derived *lin-12*-expressing cell (ABplaaa) such that ABplaaa becomes induced. Column 2: in the MS ablation or weak maternal *glp-1* mutant, de-repression of *lag-2* expression in ABara descendants causes the juxtaposition of *lag-2*-expressing cells (ABalapp and ABarapp) with ABp-derived *lin-12*-expressing cells (ABplaaa and ABpraaa) on both sides of the embryo. Intra-AB inductions occur bilaterally (Hutter and Schnabel, 1995b). This model provides a molecular description for the indirect effects of MS ablation on the ABp lineage reported elsewhere (Hutter and Schnabel, 1995b). Column 3: a strong maternal *glp-1* mutant results in the absence of zygotic *lin-12* and de-repression of zygotic *lag-2* expression. No zygotic inductions occur.

causes repression of *lag-2* expression and activation of *lin-12* expression in the descendants of that cell (Figs 6, 7). Significantly, this is observed for both maternal GLP-1-mediated inductions, the induction of ABp by P_2 and the inductions of ABara and ABalp by MS, although these inductions are distinct in time, space and outcome. It should be noted that the effects of LNG transcriptional regulation appear to occur several cell divisions after GLP-1-mediated signaling in the early embryo, while they are observed directly within responding cells in the somatic gonad. However, the events take place over a similar time course in both the early embryo and the somatic gonad; in fact, this transcriptional regulation may occur over a shorter time interval in the early embryo than in the somatic gonad (this work; Wilkinson et al., 1994).

LNG regulation of *lin-12* and *lag-2* expression potentiates subsequent inductive interactions in the embryo, whereas it mediates a single lateral specification event in the somatic gonad (this work; Wilkinson et al., 1994). Thus, LNG-mediated regulation of LNG gene expression is not biased to a particular mechanism of intercellular signaling (i.e. inductive versus lateral). Instead, this single molecular mechanism appears to have been recruited for distinct mechanisms of cell fate determination based on the developmental constraints of the early embryo versus the gonad primordium.

LNG-mediated transcriptional regulation can explain the entire *lin-12* expression pattern and most of the *lag-2* expression pattern in the early embryo. However, LNG-mediated transcriptional feedback cannot account for the absence of *lag-2* expression in the ABarp lineage, as this lineage is not influenced by any known LNG-mediated signaling event. Therefore, some mechanism other than LNG signaling is capable of preventing *lag-2* expression in the ABarp lineage. The ABala and ABarp fates are determined by the asymmetric division of the AB granddaughters (Gendreau et al., 1994; Hutter and Schnabel, 1995a). This event is unmasked when maternal GLP-1 is removed from the early embryo; in the absence of maternal GLP-1-mediated inductions, each AB granddaughter divides to generate an ABala-like cell and an ABarp-like cell (Hutter and Schnabel, 1994; Moskowitz et al., 1994). One of the ramifications of this asymmetric division appears to be the restriction of the capacity to express *lag-2* to the descendants of the ABala-like daughter. This regulatory process appears to be distinct from the GLP-1-dependent repression of *lag-2* expression.

LNG transcriptional regulation in the early embryo provides an example of a maternal-to-zygotic transition in *C. elegans*. The first two LNG-mediated inductions use maternally transcribed products, while the latter two use zygotically transcribed products. LAG-1, a homologue of the Notch effector Su(H), may provide a direct link between maternal GLP-1 signaling and zygotic *lin-12* transcriptional control (Christensen et al., 1996; this work). LAG-1 has recently been implicated in the transduction of the maternal GLP-1-mediated induction events: reducing maternal *lag-1* activity results in maternal-effect lethality and embryonic phenotypes resembling maternal *glp-1* loss-of-function phenotypes (V. Kodoyianni and J. Kimble, personal communication). Recent findings suggest that LAG-1 can directly interact with the GLP-1 intracellular domain (Roehl et al., 1996). Furthermore, the *lin-12* promoter contains several functional LAG-1 binding sites (Christensen et al., 1996). Together, these findings suggest that maternal LAG-1 may be directly involved in the activation of zygotic *lin-12* expression in response to maternal GLP-1 activation.

Zygotic LNG-mediated inductions are organized by maternal LNG signaling

lin-12 mediates sequential cell fate specification events in the *C. elegans* somatic gonad. *lin-12* is required cell autonomously for the specification of the VU fate by a lateral interaction and, subsequently, for the induction of the π fate in VU descendants (Newman et al., 1995). Newman et al. (1995) have suggested that *lin-12* expression is maintained in the VU lineage, establishing competence for the sequential *lin-12*-mediated cellular interactions. LNG transcriptional feedback may be used to maintain *lin-12* expression in the VU lineage and more generally to establish competence for sequential LNG-mediated inductions in other contexts.

We find that regulation of *lin-12* and *lag-2* expression by maternal LNG signaling not only establishes competence for LNG-mediated embryonic inductions, but spatially organizes these inductions as well. The importance of maternal LNG signaling for localizing subsequent zygotic LNG-mediated inductions is underscored by the cell fate transformations observed in MS-ablated embryos (Figs 5 and 8). The elimination of the inductive signal from MS has an indirect effect on the ABp lineage: ABp descendants become bilaterally induced (Hutter and Schnabel, 1994; Moskowitz et al., 1994). In such embryos, *lag-2* is not repressed on the right side (in ABara descendants) and is therefore expressed bilaterally. This places *lag-2*-expressing cells in contact with ABp-derived bilateral homologues on both sides of the embryo (Fig. 8). Thus, the failure of LNG-mediated repression of *lag-2* expression provides a molecular explanation for the bilateral induction of ABp descendants observed in this case (Fig. 8). Maternal LNG-mediated transcriptional regulation of zygotic LNG genes therefore plays an essential role in the spatial restriction of zygotic LNG-mediated embryonic induction events. The spatial organization of sequential inductions by transcriptional regulation may prove to be a general facet of LNG-mediated induction events.

We are indebted to Sam Henderson, Judith Kimble, Kevin Fitzgerald, and Iva Greenwald for providing reagents that made this work possible. Some of the strains used in this work were obtained from the *Caenorhabditis* Genetics Center, which is funded by the National Center for Research Resources of the National Institutes of Health. We thank Marcus Bosenberg, Henry Roehl, Voula Kodoyianni and Judith Kimble for sharing unpublished results and John Mimikakis, Steve Gendreau, and Judith Kimble for critical reading of the manuscript. I. M. was supported by an NIH Cell and Molecular Biology predoctoral training grant, a Procter and Gamble Fellowship, a Steenbock Fellowship, and the University of Wisconsin MD/PhD program. This work was supported by a grant from the NIH (GM 48137), a Searle Scholars Award from the Chicago Community Trust, and a Shaw Scientists Award from the Milwaukee Foundation to J. H. R.

REFERENCES

Albertson, D. G. (1984). Formation of the first cleavage spindle in nematode embryos. *Dev. Biol.* **101**, 61-72.

Artavanis-Tsakonas, S., Matsuno, K. and Fortini, M. E. (1995). Notch signaling. *Science* **268**, 225-232.

Austin, J. and Kimble, J. (1987). glp-1 is required in the germ line for regulation of the decision between mitosis and meiosis in C. elegans. *Cell* **51**, 589-99.

Austin, J. and Kimble, J. (1989). Transcript analysis of glp-1 and lin-12, homologous genes required for cell interactions during development of C. elegans. *Cell* **58**, 565-71.

Avery, L. and Horvitz, H. R. (1989). Pharyngeal pumping continues after laser killing of the pharyngeal nervous system of C. elegans. *Neuron* **3**, 473-85.

Bailey, A. M. and Posakony, J. W. (1995). Suppressor of hairless directly activates transcription of enhancer of split complex genes in response to Notch receptor activity. *Genes Dev.* **9**, 2609-2622.

Bowerman, B., Tax, F. E. Thomas, J. H. and Priess, J. R. (1992). Cell interactions involved in development of the bilaterally symmetrical intestinal valve cells during embryogenesis in *Caenorhabditis elegans*. *Development* **116**, 1113-22.

Brenner, S. (1974). The genetics of Caenorhabditis elegans. *Genetics* **77**, 71-94.

Christensen, S. K. V., Bosenberg, M., Friedman, L. and Kimble, J. (1996). *lag-1*, a gene required for *lin-12* and *glp-1* signaling in *Caenorhabditis elegans*, is homologous to human CBF1 and *Drosophila* Su(H). *Development* In press.

Coffman, C. R., Skoglund, P. Harris, W. A. and Kintner, C. R. (1993). Expression of an extracellular deletion of Xotch diverts cell fate in Xenopus embryos. *Cell* **73**, 659-71.

Evans, T. C., Crittenden, S. L., Kodoyianni, V. and Kimble, J. (1994). Translational control of maternal glp-1 mRNA establishes an asymmetry in the C. elegans embryo. *Cell* **77**, 183-194.

Fitzgerald, K., Wilkinson, H. A. and Greenwald, I. (1993). glp-1 can substitute for lin-12 in specifying cell fate decisions in *Caenorhabditis elegans*. *Development* **119**, 1019-1027.

Gendreau, S. B., Moskowitz, I. P., Terns, R. M. and Rothman, J. H. (1994). The potential to differentiate epidermis is unequally distributed in the AB lineage during early embryonic development in C. elegans. *Dev. Biol.* **1664**, 770-781.

Greenwald, I. (1985). lin-12, a nematode homeotic gene, is homologous to a set of mammalian proteins that includes epidermal growth factor. *Cell* **43**, 583-90.

Greenwald, I. S., Sternberg, P. W. and Horvitz, H. R. (1983). The lin-12 locus specifies cell fates in Caenorhabditis elegans. *Cell* **34**, 435-44.

Hartenstein, A. Y., Rugendorff, A., Tepass, U. and Hartenstein, V. (1992). The function of the neurogenic genes during epithelial development in the *Drosophila* embryo. *Development* **116**, 1203-20.

Hartenstein, V. and Posakony, J. W. (1990). A dual function of the Notch gene in Drosophila sensillum development. *Dev. Biol.* **142**, 13-30.

Henderson, S. T., Gao, D., Lambie, E. J. and Kimble, J. (1994). *lag-2* may encode a signaling ligand for the GLP-1 and LIN-12 receptors of *C. elegans*. *Development* **120**, 2913-2924.

Hird, S. N. and White, J. G. (1993). Cortical and cytoplasmic flow polarity in early embryonic cells of *Caenorhabditis elegans*. *J. Cell Biol.* **121**, 1343-1355.

Hope, I. A. (1991). 'Promoter trapping' in *Caenorhabditis elegans*. *Development* **113**, 399-408.

Hutter, H. and Schnabel, R. (1994). glp-1 and inductions establishing embryonic axes in *C. elegans*. *Development* **1207**, 2051-2064.

Hutter, H. and Schnabel, R. (1995a). Specification of anterior-posterior differences within the AB lineage in the *C. elegans* embryo: a polarising induction. *Development* **121**, 1559-1568.

Hutter, H. and Schnabel, R. (1995b). Establishment of left-right asymmetry in the *Caenorhabditis elegans* embryo: a multistep process involving a series of inductive events. *Development* **121**, 3417-3424.

Kodoyianni, V., Maine, E. M. and Kimble, J. (1992). Molecular basis of loss-of-function mutations in the glp-1 gene of Caenorhabditis elegans. *Mol. Biol. Cell* **3**, 1199-1213.

Lambie, E. J. and Kimble, J. (1991). Two homologous regulatory genes, *lin-12* and *glp-1*, have overlapping functions. *Development* **112**, 231-40.

Lecourtois, M. and Schweisguth, F. (1995). The neurogenic suppressor of hairless DNA-binding protein mediates the transcriptional activation of the enhancer of split complex genes triggered by Notch signaling. *Genes Dev.* **9**, 2598-2608.

Mango, S. E., Maine, E. M. and Kimble, J. (1991). Carboxy-terminal truncation activates glp-1 protein to specify vulval fates in *Caenorhabditis elegans*. *Nature* **352**, 811-5.

Mango, S. E., Thorpe, C. J., Martin, P. R., Chamberlain, S. H. and Bowerman, B. (1994). Two maternal genes, *apx-1* and *pie-1*, are required to distinguish the fates of equivalent blastomeres in the early *Caenorhabditis elegans* embryo. *Development* **120**, 2305-2315.

Mello, C. C., Draper, B. W. and Priess, J. R. (1994). The maternal genes apx-1 and glp-1 and establishment of dorsal-ventral polarity in the early C. elegans embryo. *Cell* **77**, 95-106.

Moskowitz, I. P., Gendreau, S. B. and Rothman, J. H. (1994). Combinatorial specification of blastomere identity by glp-1-dependent cellular interactions in the nematode *Caenorhabditis elegans*. *Development* **120**, 3325-3338.

Newman, A. P., White, J. G. and Sternberg, P. W. (1995). The *Caenorhabditis elegans lin-12* gene mediates induction of ventral uterine specialization by the anchor cell. *Development* **121**, 263-271.

Posakony, J. W. (1994). Nature versus nurture: asymmetric cell divisions in Drosophila bristle development [comment]. *Cell* **76**, 415-8.

Priess, J. R. and Hirsh, D. I. (1986). Caenorhabditis elegans morphogenesis: the role of the cytoskeleton in elongation of the embryo. *Dev. Biol.* **117**, 156-73.

Priess, J. R., Schnabel, H. and Schnabel, R. (1987). The glp-1 locus and cellular interactions in early C. elegans embryos. *Cell* **51**, 601-11.

Rulifson, E. J. and Blair, S. S. (1995). Notch regulates wingless expression and is not required for reception of the paracrine wingless signal during wing margin neurogenesis in *Drosophila*. *Development* **121**, 2813-2824.

Roehl, H., Bosenberg, M., Blelloch, R. and Kimble, J. (1996). Roles of the RAM and ANK domains in signaling by the C. elegans GLP-1 receptor. *EMBO J* (in press).

Schierenberg, E. (1984). Altered cell-division rates after laser-induced cell fusion in nematode embryos. *Dev. Biol.* **101**, 240-5.

Schnabel, R. (1991). Cellular interactions involved in the determination of the early *C. elegans* embryo. *Mech. Dev.* **34**, 85-99.

Seydoux, G. and Greenwald, I. (1989). Cell autonomy of lin-12 function in a cell fate decision in C. elegans. *Cell* **57**, 1237-45.

Sulston, J. E. and Hodgkin, J. (1988). Methods. In *The Nematode* Caenorhabditis elegans (ed. W. B. Wood et al.), pp. 587-606. Cold Spring Harbor Laboratory, NY: Cold Spring Harbor Press.

Sulston, J. E., Schierenberg, E. White, J. G. and Thomson, J. N. (1983). The embryonic cell lineage of the nematode Caenorhabditis elegans. *Dev. Biol.* **100**, 64-119.

Sulston, J. E. and White, J. G. (1980). Regulation and cell autonomy during postembryonic development of Caenorhabditis elegans. *Dev. Biol.* **78**, 577-97.

Tax, F. E., Yeargers, J. J. and Thomas, J. H. (1994). Sequence of *C. elegans lag-2* reveals a cell-signalling domain shared with Delta and Serrate of *Drosophila*. *Nature* **368**, 150-4.

Waterston, R. (1988). Muscle. In *The Nematode Caenorhabditis elegans*. pp. 281-335. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Weston, K., Yochem, J. and Greenwald, I. (1989). A Caenorhabditis elegans cDNA that encodes a product resembling the rat glutathione S-transferase P subunit. *Nucleic Acids Res.* **17**, 2138.

Wilkinson, H. A., Fitzgerald, K. and Greenwald, I. (1994). Reciprocal changes in expression of the receptor lin-12 and its ligand lag-2 prior to commitment in a C. elegans cell fate decision. *Cell* **79**, 1187-1198.

Wood, W. B. (1991). Evidence from reversal of handedness in C. elegans embryos for early cell interactions determining cell fates. *Nature* **349**, 536-8.

Yochem, J. and Greenwald, I. (1989). glp-1 and lin-12, genes implicated in distinct cell-cell interactions in C. elegans, encode similar transmembrane proteins. *Cell* **58**, 553-63.

Yochem, J., Weston, K. and Greenwald, I. (1988). The *Caenorhabditis elegans lin-12* gene encodes a transmembrane protein with overall similarity to *Drosophila Notch*. *Nature* **335**, 547-50.

(Accepted 30 August 1996)