Cell fate specification during *Caenorhabditis elegans* male tail development

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I dedicate this thesis

to the memory of my father, R. Eliot Chamberlin,

to the legacy of my grandfather, Ralph V. Chamberlin,

to the ghost of Calvin B. Bridges.

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Abstract

The cells of the specialized mating structures of the nematode *Caenorhabditis elegans* adult male tail develop from sex-specific divisions of postembryonic blast cells. One male-specific blast cell, B, is the precursor to all the cells of the copulatory spicules. Both cell interactions and autonomous fate specification mechanisms are utilized in the B lineage to specify fate.

During development the anterior daughter of B, B.a, generates four distinct pairs of cells. Cell ablation experiments indicate that the cells of each pair respond to positional cues provided by other male-specific blast cells. F and U promote anterior fates, Y.p promotes some posterior fates, and the B.a progeny promote posterior fates. The cells within each pair may also interact.

The *lin-3*/*let-23* signalling pathway, identified for its function in *C. elegans* hermaphrodite vulval induction, mediates the signal from F and U. Reduction-of-function mutations in *lin-3* (EGF-like signal), *let-23* (receptor), *sem-5* (adaptor), *let-60* (ras), or *lin-45* (raf) disrupt the fates of the anterior cells, and mimic F and U ablation. In addition, ectopically expressed *lin-3* disrupts the fates of the posterior cells, and can promote anterior fates even in the absence of F and U.

A genetic screen of over 9000 mutagenized gametes recovered 22 mutations in 20 loci that disrupt fate specification in male tail lineages. Seven of these mutations may represent new genes that play a role in male tail development.

The first division of the B cell is asymmetric. The gene *vab-3* is required for specification of B.a fates, and it may represent a factor whose

activity is localized to the B.a cell via the gene *lin-17*. *lin-17* acts both at the first division of the B cell and at specific other cell divisions in the lineage.

Table of Contents

Chapter 1

Cell fate specification in development: Cell lineage, cell interactions and an introduction to *Caenorhabditis elegans* male tail development

I. Introduction

A basic question of developmental biology is how a single cell divides to produce two cells that are different from each other. In general, the fates of the two cells can be different if either a factor or other information is distributed unequally between the daughters (autonomous specification) or the subsequent environment is different for the daughters (conditional specification). To understand these basic building blocks of development, it is necessary to understand the cellular and molecular processes that underlie these two general mechanisms. What sort of factors are unequally distributed, and how is their distribution established? What factors provide the extracellular cues that make one environment different from another? How do cells interpret these differences to produce different outcomes? Finally, during development of a multicellular organism, how do different steps and mechanisms of fate specification coordinate to produce a wide variety of differentiated fates?

This review discusses aspects of cell fate specification in several systems, including *C. elegans*, *Drosophila*, and *Xenopus*. I have focused on two issues in developmental biology: (1) the stepwise specification of fate and the interplay of different mechanisms in the development of multicellular organisms, and (2) the integration and coordination of multiple signals that can be involved in fate specification. This review concentrates on a few examples for which there is a genetic and/or molecular handle on the factors that specifically function to mediate cell interactions and fate specification.

The integral relationship between autonomous and conditional fate specification

In general, when fate specification is mediated by a cell interaction, there is a signalling source (cell or group of cells), and a population of cells that are competent to respond to the signal. Because of signal localization or other mechanisms, only a subset of those cells capable of responding actually receive the signal and respond appropriately. Such mechanisms of fate specification can make a developmental process more robust, as they result in the production of the appropriate cells or structures in the appropriate position even if there are slight variations in the position of precursor cells. An important component of this event, however, is that the responding cells must be competent to receive and interpret the signal. In other words, they are not strictly naive, but already have the "fate" of a responding cell: they possess the receptor(s), downstream machinery, and transcription factors necessary for the response. In a developing multicellular organism where a subset of cells are capable of responding to a particular signal but others are not, such restrictions of potential must have occurred by yet another (generally earlier) fate specification event. It is likely that multicellular development comprises dozens of such sequential steps that involve both autonomous and conditional mechanisms.

C. elegans embryonic development

During the first four cleavages of the development of the *C. elegans* embryo, a series of unequal, stem cell-like divisions give rise to the six founder cells of the major embryonic lineages: AB, MS, E, C, D, and P4 (Fig.

1). These cells differ from each other in size, in the tempo of their cell cycle, in the number of progeny, and in the differentiated fates of their progeny (Sulston, et al., 1983). Early embryogenesis has been studied using mutations that result in maternal effect lethality, and cell ablation and blastomere isolation experiments.

Establishing asymmetry in the first division

The first division of the zygote (P0) is asymmetric, producing the larger, anterior AB cell, and the smaller, posterior P1 cell. In addition to the difference in size, germ line specific granules (P granules) are unequally distributed between the two cells (Strome, 1983). After fertilization the P granules are distributed throughout the ooplasm, but they subsequently become localized (or protected from degradation) in the posterior part of the cell. In each cell division, the P granules are localized to the P daughter, such that they are eventually segregated into P4, the germline precursor.

The phenomena of normal development suggest that asymmetric cell division, combined with the localization of specific factors, may play an important role in the first division, and genetic analysis is consistent with this hypothesis. One class of mutations that disrupt the first asymmetric division are mutations in the *par* genes (cell partitioning: *par-1* - *par-5*; Kemphues, et al., 1988). In embryos derived from *par* mutant mothers the normally asymmetric first division is symmetric. The first cleavage results in cells of approximately equal size, the P granules remain distributed throughout the ooplasm, and the cleavages of both daughters are synchronous. Thus the *par* genes are required for establishing anterior/posterior polarity in the embryo, or mediating the subsequent

asymmetric cell divisions and localization of asymmetrically distributed factors in a general way.

The *par* genes are required to globally establish asymmetry in the first (an possibly subsequent) cell divisions of the embryo, and thus indirectly mediate proper localization of specific factors required for fate specification. Another set of genes is required for the specific localization of factors. *mex-1* represents a gene of this class. The first cell division in embryos from *mex-1* mutants is asymmetric. However, using cell lineage analysis and antibodies against markers for cells from the MS lineage, Mello, et al. (1992) determined that *mex-1* is required for proper restriction of MS fate potential to the P1 cell (precursor of MS). The function of *mex-1* is discussed further below.

Localization of MS potential to the EMS blastomere

The MS blastomere is the precursor to the cells of the posterior pharynx and a set of body wall muscles (Sulston, et al., 1983). These differentiated fates can be identified using specific antibodies (Priess and Thomson, 1987; Miller, et al., 1983). In addition, the structures of the posterior pharynx can be identified by morphology even in embryos that fail to undergo elongation. Priess and colleagues have used these tools in genetic screens for maternal effect lethal mutations that alter the number of cells expressing these markers and thus disrupt specification of MS fate. Three genes identified in such screens are *skn-1*, *mex-1*, and *pie-1*. Genetic analysis suggests that *mex-1* and *pie-1* act in sequential cell divisions to localize the activity of *skn-1* to the EMS blast, and that *skn-1* encodes a factor essential for MS specification.

skn-1 (skinhead)

Mutations in *skn-1* result in an absence of pharyngeal and intestinal cells, and eliminate MS derived muscle (Bowerman, et al., 1992). Direct lineage analysis indicated that in embryos derived from *skn-1* mothers the MS and E blastomeres produced cell lineages and differentiated fates (e.g., hypodermal cells) like the daughters of AB. The *skn-1* gene encodes a novel protein product with a domain similar in sequence to the DNA binding motif found in the bZIP class of transcription factors. Antibody studies indicate that SKN-1 is localized to P2 and EMS nuclei during normal embryogenesis (Bowerman, et al., 1993).

pie-1 (pharynx in excess)

Mutations in *mex-1* and *pie-1* cause phenotypes superficially opposite from those caused by mutant *skn-1*: embryos from mutant mothers have extra pharyngeal and muscle cells. In *pie-1* mutants both presumptive EMS and P2 behave like the normal EMS cell, producing lineages like EMS and differentiated intestinal cells, pharyngeal cells, and muscle. *pie-1; skn-1* double mutants produce none of these fates; presumptive EMS and P2 behave like P2 blast cells. Thus, in normal development, *pie-1* is required to restrict the activity of *skn-1* to the EMS blast cell. Since SKN-1 is present in the P2 blastomere, this regulation must occur at the level of protein function.

mex-1 (muscle in excess)

mex-1 mutants produce an even more striking phenotype: all four granddaughter cells of presumptive AB, in addition to MS, divide and produce progeny like the normal MS blastomere. As in *pie-1* mutants, these

fates require *skn-1*. Defects in *mex-1* and *pie-1* are additive; in *mex-1; pie-1* double mutants the four grandprogeny of presumptive AB behave like MS and both presumptive EMS and P2 behave like a normal EMS. These data suggest that one function of *mex-1* is to localize MS potential to the P1 cell at the first division. The persistence of SKN-1 in the nuclei of presumptive ABa and ABp blastomeres in *mex-1* mutants is consistent with this model. Both *mex-1* and *pie-1* are also required for normal germ cell specification.

Cell interactions in the early embryo

The sibling of the MS cell is E, the precursor to all of the cells of the intestine. Blastomere recombination experiments suggest that contact with P2 is necessary and sufficient to induce E fate in the EMS blastomere (Goldstein, 1992; 1993). Early removal of P2 results in failure of presumptive E to divide with the long cell cycle of the normal E cell and the absence of intestinal markers in the differentiated progeny. In contrast, pairing of P2 with EMS can induce these features of E fate, and they are induced in the daughter of EMS that lies next to P2 even if P2 is removed and placed on the side of EMS opposite to its normal position. Placing P2 adjacent to the daughters of AB does not induce E fate. Thus, during normal development, potential to produce E fate is restricted to EMS, and the P2 cell induces E fate on the side of EMS that it touches. Although it is not known what factors restrict E fate to the EMS blastomere, mechanisms in addition to *mex-1* must play a role because absence of maternal *mex-1* is not sufficient to produce E-like progeny from the grandprogeny of AB adjacent to P2.

Blastomere rearrangement and cell ablation experiments have identified the general regulatory capacity of the embryo as well as specific cell interactions. The first experiment that hinted at the potential for fate regulation in the embryo comes from the work of Priess and Thomson (1987). By micromanipulation of the division of AB such that ABa and ABp change places, they showed that these two cells are initially equivalent. Animals in which these cells are rearranged are indistinguishable from unmanipulated animals. Furthermore, by ablating the EMS blastomere they determined that at least one set of normal ABa progeny (the cells of the anterior pharynx) require an interaction with EMS (or its progeny) for proper fate specification. Mutations in *glp-1* result in a phenotype similar to EMS ablated animals (Priess, et al., 1987), and this gene likely plays a role in the cell interaction that makes ABa different from ABp. This interaction is also why *skn-1* mutants lack the entire pharynx rather than just the posterior portion.

A series of cell ablation experiments by Schnabel (1991) have uncovered additional cell interactions. He used cell lineage analysis as well as antibody staining to suggest that the early embryo is highly interactive, and that there are at least two major interactions, followed by several minor interactions, that occur at the eight cell stage and later. First, MS is necessary specifically for the normal development of AB progeny ABalp and ABara. When MS or EMS (also ablated by Priess and Thomson, 1987) is ablated, these lineages are disrupted (termed primary interaction 1, or I1). Likewise, ablation of C or the precursor P2 disrupts the lineages of ABarp, ABpla, and ABpra (I2). For each of these interactions these are the cells (and their progeny) that contact the MS and C cells (and progeny). For the EMS

interaction, these experiments extend the work of Priess and Thomson, as they demonstrate that not just presumptive pharynx cells are specified by interactions, but that the fate specification is at the level of specific precursors. In general these experiments hint at the complexity of cell interactions required for normal development.

Recent work of Schnabel (1994) that identifies cell interactions required for normal muscle fate specification in the EMS lineage confirms some of the secondary interactions identified during cell ablation experiments. Specifically, the results suggest that the EMS cell inherits the potential to produce muscle cells. However, the presence of P2 and ABp can suppress this potential, i.e., P2 and ABp, or their progeny, interact with EMS, or its progeny, to inhibit muscle cell fates. A second, overlying interaction from ABa or its progeny counters this inhibitory interaction to promote muscle fates.

C. elegans vulval development

During *C. elegans* vulval development, the anchor cell (AC) in the gonad produces a signal that induces three of six hypodermal cells (P3.p - P8.p) termed vulval precursor cells (VPCs) to divide and produce vulval tissue (reviewed in Sternberg, 1993). The most proximal cell (P6.p) produces a 1° lineage, and the next nearest cells (P5.p and P7.p) produce 2° lineages (the uninduced VPC lineage is 3°). Thus an important step in vulval development is the cell interaction between the AC and the VPCs. The specification of the AC so that it is competent to produce the signal, and the VPCs so that they are competent to respond to the signal, as well as to execute the proper response, are also essential (Fig. 2).

Generation of the signalling cell: AC specification

In both *C. elegans* hermaphrodites and males the cells Z1 and Z4 are the precursors of all of the cells of the somatic gonad. In a given hermaphrodite, either the cell Z1.ppp or the cell Z4.aaa will be the AC; the other cell will be the ventral uterine precursor cell VU3. These two cells comprise an equivalence group. The cells have equivalent potential, and fate specification is mediated by a lateral interaction between the two cells that ensures that only one cell adopts the AC fate. If either cell is ablated, the remaining cell invariably differentiates as the AC, indicating that AC represents the 1° fate for this equivalence group. The lateral interaction between Z1.ppp and Z4.aaa is mediated by *lin-12*. Gain-of-function mutations in *lin-12* (*lin-12(d)*) result in both cells adopting the VU fate. These animals are thus missing an AC and subsequently the interaction between the AC and the VPCs is disrupted. Loss-of-function mutations (*lin-12(0)*) result in both cells adopting the AC fate.

Generation of the responding cells: VPC specification

There are two aspects of fate specification for the VPCs that I will consider here. The first requirement is the proper generation of the Pn.p cells from the Pn precursors. The second is the differential specification of the Pn cells (and subsequently the Pn.p cells) in the central body region (P3.p - P8.p; the VPCs) from the more terminal Pn cells.

Generation of the Pn.p cells

There are at least two requirements for the proper generation of Pn.p cells. First the Pn cells must divide. Second, they must divide asymmetrically to produce an anterior neuroblast and a posterior hypodermal blast. Many genetic mutants -- considered to have a "generation Vul" phenotype -- have been identified that fail to produce Pn.p cells (Ferguson and Horvitz, 1985; Ferguson, et al., 1987). One gene identified by such mutations is *lin-26*. A reduction-of-function mutation in *lin-26* results in both Pn.a and Pn.p cells behaving as a neuroblast like the normal Pn.a cell. Thus the function of *lin-26* is to make the Pn.p cells different from the Pn.a cells. Loss-of-function mutations in *lin-26* are lethal. Additional analysis of *lin-26* suggests that it functions in many tissues to specify hypodermal fates (M. Labouesse, personal communication).

Regional specification of Pn.p cells to become VPCs

At hatching there are twelve P cells that migrate to the ventral cord and form a linear anterior/posterior array. The twelve P cells produce similar lineages, but there are distinct variations in specific lineages that correspond to anterior/posterior position. Important for this discussion is the fact that only the Pn.p cells in the central body region (P3.p - P8.p) are VPCs because only these cells can produce vulval tissue. Although normally only the three cells proximal to the AC (P5.p - P7.p) will produce vulval tissue, the other three have the potential if any of P5.p - P7.p are removed (Sulston and White, 1980) or if they are exposed to excessive or ectopic signal (Hill and Sternberg, 1992; Hill, et al., in preparation). *lin-39* plays an important role in the specification of this potential. Mutations in *lin-39* result in a Vul animal

(Clark, et al., 1993). Although P3.p - P8.p cells are present, the Pn cells in the central body region behave like their anterior neighbors. Therefore, *lin-39* functions to make the cells in the central body region different from their anterior neighbors. *lin-39* encodes a homeobox-containing protein and is a member of the *C. elegans* homeotic complex.

The cell interaction between the AC and the VPCs

Ablation of the gonadal AC results in the absence of vulval tissue, whereas ablation of the entire gonad except the AC can result in normal vulval development (Kimble, et al., 1979). The AC signal (an epidermal growth factor (EGF)-like protein encoded by the gene *lin-3* (Hill and Sternberg, 1992)) is both necessary and sufficient to promote the VPCs to initiate vulval development. Genes that are necessary for the response to *lin-3* include *let-23* (receptor) (Aroian, et al., 1990), *sem-5* (adaptor) (Clark, et al., 1992), *let-60* (ras) (Han and Sternberg, 1990), and *lin-45* (raf) (Han, et al., 1993). Reduction-of-function mutations in any of these genes result in a Vulvaless (Vul) phenotype where all six VPCs may produce hypodermis at the expense of vulval tissue. Gain-of-function mutations in *let-60* (Beitel, et al., 1990) and over-production of LIN-3 (Hill and Sternberg, 1992) result in a Multivulva (Muv) phenotype in which all six VPCs may produce vulval tissue. Loss-of-function mutations at another locus, *lin-15*, also result in a Muv phenotype (Ferguson and Horvitz, 1985; Huang, et al., 1994). Genetic epistasis experiments (Ferguson, et al., 1987, and references above) indicate that these genes act together in a signal transduction pathway.

Subsequent steps that occur in the VPCs

Lateral signalling

A second component of pattern formation during vulval development is the establishment of the 2°1°2° pattern of vulval fates. During normal development this pattern results from the coordination of proximity to the AC ("dose" of AC signal) and a lateral interaction among the induced VPCs that acts to ensure that two adjacent cells do not adopt the 1° fate. This lateral interaction is mediated by the gene *lin-12*, and is discussed in more detail below.

Sublineage execution and a subsequent role in cell interactions

Part of the 1° and 2° vulval fates is the execution of the proper lineage associated with the fate. The gene *lin-11* is required for execution of the normal 2° fate (Ferguson and Horvitz, 1985; Ferguson, et al., 1987; Freyd, et al., 1990). The normal 2° lineage is asymmetric: the daughter proximal to the vulval opening produces three progeny, whereas the more distal daughter produces four progeny. In *lin-11* mutants both daughters behave like a normal distal daughter and, among other characteristics, produce four progeny. *lin-11* encodes a protein with a homeodomain and a LIM domain (a cysteine rich motif likely involved in binding metals), and may represent a transcription factor whose activity is asymmetrically distributed between the two 2° daughters.

Two other genes are required for proper execution of the 2° fate: *lin-17* and *lin-18* (Ferguson and Horvitz, 1985; Ferguson, et al., 1987). *lin-17* has a function in mediating the asymmetric cell division of many cells (Sternberg and Horvitz, 1988), whereas *lin-18* function is more specific to the 2° vulval

lineages. However, mutations in both result in a similar lineage defect in that the 2° lineage of P7.p, but not P5.p, is primarily disrupted, with the asymmetry of the P7.p 2° lineage reversed. Cell ablation experiments in *lin-12* mutants (in which all VPCs adopt 2° fates) suggest that cells in the hermaphrodite gonad other than the AC provide a signal that orients the P7.p 2° lineage toward the vulval opening, and that *lin-18* is required for this interaction (W. Katz and P. Sternberg, in preparation).

An interaction is also mediated by VPCs that adopt the 1° fate. They provide positional information for proper migration and branching of the HSN neuronal axons (Garriga, et al., 1993).

Anterior/posterior segmental patterning during Drosophila embryogenesis

The early stages of Drosophila embryogenesis involve the division of nuclei in a cytoplasmic syncytium (reviewed by Lawrence, 1992). Early fate specification primarily utilizes gradients of diffusible maternal factors. These maternal factors activate a hierarchy of zygotic genes in a manner that sequentially subdivides the embryo into more precise domains. The gap genes and pair rule genes (Nüsslein-Vollhard and Wieschaus, 1980) establish the initial metameric pattern of the embryo prior to cellularization. After cellularization the distribution of pair rule genes establishes gene expression, but cell interactions subsequently maintain and elaborate the expression and function of the segment polarity class of genes.

Analysis of three genes suggests that cell interactions are involved in establishing anterior/posterior patterning in the Drosophila cellular blastoderm: *wingless* (*wg*), *engrailed* (*en*), and *hedgehog* (*hh*) (reviewed in Woods and Bryant, 1992; Fig. 3). Non-autonomy of gene function can be an

important indicator of the presence of cell interactions involved in fate specification, and both *wg* and *hh* act in a non-autonomous fashion. Mutant *wg* tissue in a heterozygous background can develop with a normal phenotype, indicating that normal *wg* from adjacent cells can rescue the mutant phenotype (Baker, 1988). In contrast, *hh* behaves non-autonomously in what is termed a "domineering" manner (Mohler, 1988). In other words, patches of *hh* mutant cells in a heterozygous background can cause an abnormal phenotype in both wild type and mutant tissue. *wg* encodes a secreted, diffusible protein, and *hh* encodes a transmembrane protein. *en* encodes a homeodomain protein (Poole, et al., 1985), but it may be involved in specifying the cell interactions mediated by *en* expressing cells, as well as additional functions.

At the cellular blastoderm stage, each parasegment comprises a stripe, four cells in diameter (reviewed by Akam, 1987). *wg* is expressed in the cells anterior to each parasegmental border (Baker, 1987), and *en* and *hh* are expressed adjacent in the cells posterior to each parasegmental boundary (DiNardo and O'Farell, 1987; Mohler and Vani, 1992). This expression pattern is established by the prior expression of pair rule genes. However, the proper maintenance of these patterns depends on the reciprocal activity of each of the genes (Bejsovec and Martinez Arias, 1991; Martinez Arias, et al., 1988). Specifically, *wg* and *en* expression is initiated and then lost in embryos mutant for the other gene. Likewise, *hh* expression is initiated and then lost in *en* mutants (Mohler and Vani, 1992).

One model for the cell interactions following cellularization (Woods and Bryant, 1992) proposes that *wg* is secreted from *wg*-expressing cells in vesicles (a process that requires the *armadillo* protein; Gonzales, et al., 1991),

and is necessary for the maintenance of *en* expression. *hh* acts to promote *wg* expression in cells immediately anterior to *en* expressing cells, possibly via the gene *patched* (*ptc*; Ingham, et al., 1991). In *ptc* mutants supernumerary cells express *wg*, so the normal interaction may involve negative regulation of *ptc* activity. Recent studies that use transgenic *wg* under control of a heat shock promoter rule out the possibility that *wg* acts as a morphogen in establishing fates within each segment (Sampedro, et al., 1993), and subsequent models ascribe to *wg* primarily the function of establishing or "sealing" the parasegmental boundaries (Lawrence and Sampedro, 1993). Genetic studies of *hh* using a temperature sensitive allele suggest that *hh* has two functions: the initial reciprocal induction function with *wg* in immediate anterior cells, and a later function that acts at a distance over several cell diameters (Heemskerk and DiNardo, 1994). In this second function *hh* specifies distinct fates in a dose-dependent manner, and thus may act as a morphogen. The key function of *wg* may be in the demarcation and maintenance of the segmental boundary, with the segmental gradient established by *hh*.

Specification of segmental identity across germ layers in Drosophila embryos

In addition to subdividing the embryo into repeated segmental units along the anterior/posterior axis, zygotic genes establish the distinct identity of each parasegment. The parasegmental identity is established by the genes of the Antennapedia and Bithorax Complexes (Ant-C and BX-C): the prototypes of the homeotic complexes in other metazoans (reviewed in Lewis, 1978; Peifer, et al., 1987; McGinnis and Krumlauf, 1992). The expression of the genes of the Ant-C and BX-C is under the control of earlier acting nuclear

proteins involved in establishing the metameric pattern, but also maternal genes that convey relative anterior/posterior position, and interactions among themselves. Of particular interest for this review, however, is the characterized interaction between cells of the visceral mesoderm that express specific genes of the BX-C and the underlying endodermal cells (reviewed by Bienz, 1994).

The genes *Ubx* and *abd-A* are normally expressed in parasegment 7 (ps7) and parasegments 8-12 (ps8-ps12), respectively, of the visceral mesoderm (Tremml and Bienz, 1989). The underlying endoderm exhibits few morphologically distinct characters. However, three reproducible constrictions are apparent, and expression of the homeotic gene *labial* (*lab*) coincides with the middle constriction (Diederich, et al., 1989). Study of the requirement of *Ubx* and *abd-A* mesodermal expression for proper endodermal *lab* expression suggests a role for cell interactions between the two germ layers. This interaction is likely mediated by *wg* and *decapentaplegic* (*dpp*) (Immergluck, et al., 1990; Panganiban, et al., 1990; Reuter, et al., 1990). First, *dpp* expression in ps7 and *wg* expression in ps8 require the respective activities of *Ubx* and *abd-A*. *dpp* RNA and protein are absent from the visceral mesoderm in *Ubx* mutants. Likewise, *wg* protein is absent from the visceral mesoderm in *abd-A* mutants. Both *Ubx* and *dpp* are required for *lab* expression in the midgut, and *Ubx*, *dpp*, and *lab* are required for normal formation of the middle gut constriction. *wg* expression in ps8 also modulates *lab* expression, as *wg* mutants show reduced midgut *lab* expression (discussed below). Thus, in this system, the expression of a transcription factor in the visceral mesoderm mediates expression of a specific signalling molecule, which, in turn, induces underlying endoderm to adopt the

appropriate regional fate. This fate includes the expression of a second transcription factor and the formation of the middle gut constriction.

Neurogenesis and bristle patterning in Drosophila larvae

Cell fate specification in the embryonic neuroectoderm and development of the adult bristles and sensory hairs requires many of the same genes and developmental processes (reviewed by Woods and Bryant, 1992; Ghysen, et al., 1993). In this review, I will focus on these genes as they function in cell fate specification during the development of the bristles and hairs of the adult integument (Fig. 5).

Specification of neuroblast fate

The restriction of fate potential that results in single, regularly spaced bristle precursor cells (and subsequently their respective bristles) results from the specification of patches of cells that have the potential to become neuroblasts (proneural clusters) and a lateral interaction that ensures that adjacent cells do not adopt the neuroblast fate. The genes in the *achaete-scute* complex (AS-C) are required to establish the equivalence group, and bristles are absent in AS-C mutants (reviewed by Ghysen and Dambly-Chaudiere, 1988; Ghysen, et al., 1993). The AS-C genes encode transcription factors of the basic helix-loop-helix class (bHLH). *ac* and *sc* are expressed in identical cell clusters in the third instar wing, and the expression of these genes likely defines the proneural clusters (Cubas, et al., 1991; Romani, et al., 1989). However, they likely do not represent all of the required components, as ectopic expression of *ac-sc* does not induce bristle formation outside of the proneural cluster region (Rodriguez, et al., 1990). The products of

extramacrochaete (*emc*) and *hairy* (*h*) act as negative regulators of *ac* and *sc*, respectively, and of proneural cluster formation. *emc* is expressed in a pattern essentially complementary to *ac* and *sc*, but how the expression patterns of these genes are initially established is unclear.

Once the proneural clusters are established, the cells within the clusters have equivalent potential to adopt the neuroblast (NB) fate (termed sensory organ precursor (SOP) or sensory mother cells (SMC) in the larva). A lateral interaction among these cells results in exactly one cell adopting the NB fate. Mutations in several genes, including *Notch* (*N*) and *Delta* (*Dl*), can result in a cluster of bristles forming at the sites where single bristles are seen in wild type (Dietrich and Campos-Ortega, 1984; Parks and Muskovitch, 1993). These genes act in a lateral interaction pathway such that all cells produce a signal that promotes non-NB fate (encoded by *Dl*) and the receptor for the signal (encoded by *N*). The unstable situation that results is resolved such that one cell "wins," producing more signal, and inhibits neighboring cells from making signal and adopting the NB fate (Heitzler and Simpson, 1991). Concurrent or subsequent to the outcome of the lateral interaction, *ac-sc* (and *scabrous*) expression declines in all other cells of the proneural cluster except the NB.

Specification of fates in the neuroblast lineage

The bristle NB subsequently undergoes a stereotyped cell division pattern to produce four different cells and fates: the sensory neuron and the accessory cells (bristle or hair, socket and sheath of the bristle (Bodmer, et al., 1989; reviewed by Posakony, 1994)). Cell interactions, combined with the localization of specific gene products, play an important role in fate

specification within this lineage. First, loss of *N* or *Dl* function in the NB results in bristle loss and causes all four cells to adopt the fate of the sensory neuron (Hartenstein and Posakony, 1990; Parks and Muskovitch, 1993). Partial reduction-of-function mutations can result in both daughters of the NB producing a neuron and a sheath cell, so *N* and *Dl* act at both cell divisions in the lineage. Three additional genes are required: *Hairless* (*H*), *suppressor of Hairless* (*Su(H)*), and *numb*. These genes also function at each division of the lineage to specify fate. *Su(H)* mutations result in defects similar to the *N* and *Dl* defects (Schweisguth and Posakony, 1992), whereas loss of *H* or *numb* function results in opposite defects (Bang, et al., 1991; Uemura, et al., 1989). These genes likely act together to ensure that the two daughter cells from each division adopt fates different from each other. The *numb* protein is localized and asymmetrically distributed during cell division (Rhyu, et al., 1994), so the execution of the normal lineage involves both asymmetric distribution of factors and cell interactions. It is not clear whether the cell interactions mediated by *N* and *Dl* mediate the asymmetric polarization of the precursor cell(s), or if the asymmetrically distributed information is amplified and reinforced by lateral interactions between siblings.

Cell fate specification and cell interactions during Xenopus embryogenesis

Although *Xenopus* embryos do not show an invariant cell lineage, in undisturbed embryos specific regions routinely represent the precursors of specific tissues, allowing the elucidation of an embryonic fate map (reviewed by Gurdon, et al., 1989; Kimelman, et al., 1992). *Xenopus* eggs are maternally polarized along the animal/vegetal axis. The vegetal portion of

the embryo contributes to the endoderm, the equatorial portion contributes to the mesoderm, and the animal portion contributes to the ectoderm and neuroectoderm. There is additional localization of fates as well. For instance, mesoderm comprises a diverse group of differentiated fates, and these fates do not arise in a homogeneous manner among the cells at the equator. The dorsal/ventral axis of the embryo is established orthogonally to the animal/vegetal axis, with the dorsal region established opposite the site of sperm entry. I will consider two examples of cell interactions in Xenopus that have been studied in detail: mesoderm induction and induction of the body axis by the cells of the blastopore lip (Spemann organizer activity; Fig. 6). The complexities of the multiple signal integration and the relationship between the two signals is discussed below.

Mesoderm induction is defined by experiments that combine embryonic animal caps (usually precursor to neuronal and epidermal fates) with vegetal tissue (precursor to endodermal cell fates) (e.g., Gurdon, et al., 1985; reviewed by Kimelman, et al., 1992). The presence of the vegetal cells can induce mesodermal fates in the animal caps. Thus, the initial animal/vegetal asymmetry in the oocyte provides the distinction of animal (epidermal) and vegetal (endodermal) fates, and an inductive interaction promotes mesodermal fates at the midline. However, there is a distinct difference between the vegetal cells from the presumptive dorsal region and the cells from the ventral region in the type of mesodermal fates that can be induced, so a polarization of the vegetal region must exist. Such polarization could result from a single signal from the vegetal pole that acts as a morphogen and is maximally active in the presumptive dorsal region. In contrast, there could

be two signals: a generalized vegetal signal, and a localized signal that induces dorsal mesoderm (Slack, et al., 1987; Smith, 1989; Woodland, 1989).

The Spemann organizer was identified in 1924 by the transplantation of the dorsal blastopore lip from one amphibian into another, where the transplanted tissue was able to induce a secondary body axis in which cells of the host make up a large amount of the secondary axis tissue (reviewed in Cooke, 1989; Kimelman, et al., 1992). Thus cell signalling mediates establishment of the dorsal/ventral and anterior/posterior axes.

Recent work has identified some of the molecules that mediate these cell interactions. The key molecules include the FGF class of molecules (bFGF, aFGF, XeFGF), the TGF-β class (activin, XTC-MIF), and Wnt-1 class (Xwnt-8, *noggin*). The function of these molecules has been primarily established by treating tissues with purified protein, so the nature of the specific endogenous gene that mediates the signal may not yet be defined. Nevertheless, the activity associated with the purified molecules indicates that they are likely interacting with an endogenous signalling mechanism.

A basic FGF (bFGF) class of molecule is likely responsible for the generalized mesoderm inducing function of vegetal cells. Addition of bFGF to cultured animal caps can promote differentiation of ventral mesodermal tissues (Kimelman and Kirschner, 1987; Slack, et al., 1987). However, concentration of bFGF may also play a role in fate specification, as animal caps with injected bFGF RNA can form some dorsal mesodermal tissues (Kimelman and Maas, 1992). The *in vivo* function of bFGF molecules is corroborated by the observation that bFGF transcripts and proteins are present in the *Xenopus* egg and embryo. In addition, a dominant negative mutant form of the bFGF receptor can disrupt normal development,

producing tadpoles that lack posterior and trunk regions, and reduced amounts of certain mesodermal tissue (Amaya, et al., 1991).

Activin is a member of the TGF-β class of proteins, and is a potent inducer of mesoderm. Addition of activin to cultured animal caps can induce both dorsal and ventral mesodermal tissues in a dose-dependent manner (Green, et al., 1990). The function of the dorsal mesoderm to act as the Spemann organizer can also be induced in animal caps by activin. Animal caps cultured with activin can organize a secondary axis when transplanted into host embryos (Cooke, 1989).

wnt proteins in *Xenopus* do not serve as mesodermal inducing agents, but rather as modulators involved in both the induction of dorsal mesodermal fates and in the conveyance of body axis information. Injection of *wnt* RNA into oocytes or cells on the ventral side of cleavage stage embryos can induce a secondary body axis (McMahon and Moon, 1989). *wnt* also acts in concert with factors that induce mesoderm to change the responsiveness of animal cap cells (Christian, et al., 1992). *wnt* alone can not induce mesodermal differentiation. However, in the presence of *wnt* animal caps can respond to bFGF by producing dorsal mesodermal tissues. In contrast, animal caps without *wnt* treated to the same concentration of bFGF produce mainly ventral mesodermal tissues. *noggin* is not a *wnt* gene, but it may represent the endogenous *wnt* activity. It is present both maternally and zygotically, and zygotic transcription is localized to the dorsal mesodermal region (Smith and Harland, 1992). It functions both to promote dorsal fates and as a Spemann organizer (Smith and Harland, 1992; Smith, et al., 1993).

Fate specification and the integration of multiple signals

For two cells in which fate specification is mediated by cell interactions, a single positional cue may be sufficient to make one cell different from the other. However, study in several systems suggests that cells are exposed to a number of extracellular cues that are integrated concurrently to specify fate. Thus, the study of cell interactions during development is not only the study of how a cell responds to a single signal, but how it responds to a variety of signals, integrating them to produce a specific outcome.

C. elegans vulval development

The primary cell interaction of vulval development is the AC signal mediated by the *lin-3* / *let-23* signalling pathway. As suggested above, however, this interaction is not the only event involved in proper pattern formation during vulval development. There are at least two other components -- the negative regulation mediated by a group of genes including the *lin-15* locus and the lateral interaction mediated by *lin-12* -- that also play important roles (see Fig. 2). Since these three components of pattern formation coordinate to produce the normal pattern of fates it is of interest to understand how the responding cells integrate the three positional cues.

Loss-of-function (including molecular null) mutations in *lin-15* result in a Muv phenotype (Ferguson and Horvitz, 1985, 1989; Huang, et al., 1994). Genetic analysis suggested that *lin-15* is a complex locus with two independently mutable activities, A and B. Mosaic analysis suggests that normal *lin-15* activity in either the VPCs or the AC is not sufficient for the

wild type phenotype (i.e., *lin-15* act in a cell non-autonomous fashion; Herman and Hedgecock, 1990). *lin-15* has been proposed to act in the hypodermis surrounding the VPCs, hyp7. Molecular analysis indicates that the two functions of *lin-15* correspond to two distinct but coordinately transcribed transcriptional units. *lin-15* is one member of a group of genes that also reflect these two activities. Animals bearing a mutation in either a class A or a class B gene are phenotypically wild type. Animals homozygous mutant for both a class A and a class B gene are Muv. Genetic epistasis experiments suggest that *lin-15* acts in parallel and antagonistically to *lin-3* to negatively regulate *let-23*. *lin-3; lin-15* double mutants are Muv, indicating that *lin-3* is not necessary for the effect observed in *lin-15* mutants (i.e., *lin-15* does not act by regulating or localizing *lin-3* activity). *let-23; lin-15* double mutants are Vul, indicating that *lin-15* acts to regulate the activity of *let-23*. Transgenic extrachromasomal arrays of *lin-3* that behave as a gain of *lin-3* function are similarly blocked by mutation in *let-23* (Hill and Sternberg, 1992). The effects of both *lin-3* and *lin-15* are thus integrated into the same pathway at the *let-23* receptor. *lin-15* A and B proteins likely act, either directly or indirectly, to negatively regulate *let-23* receptor activity in the absence of the specific, localized signal.

The integration of the AC signal and the lateral interaction is less straightforward. However, the two pathways act coordinately to specify fate, rather than one pathway directly regulating the other as in the *lin-3*/*let-23* pathway and *lin-15*. In *lin-12(0)* mutants 2° vulval fates are absent, but the VPCs are still responsive to the AC signal (*lin-3*) (Greenwald, et al., 1983). Thus *lin-12* is not necessary for the function of the AC signal, and when both pathways are not functional all cells adopt the 3° fate. Lateral interaction is

not necessarily essential for 2° fates, as 2° fates can be recovered from isolated VPCs with the AC present (Sternberg and Horvitz, 1986) or following a brief pulse of *lin-3* from a heat shock promoter (Hill, et al., in preparation). In *lin-12(d)* mutants all six VPCs adopt the 2° fate, even in the absence of the AC (which is generally absent in *lin-12(d)* animals because of the earlier AC/VU decision). Thus the activated *lin-12* is sufficient to bypass the requirement for the AC signal to promote vulval fates. Nevertheless, in rare *lin-12(d)/lin-12(0)* animals that have an AC, the cells are still responsive the AC signal, and P6.p will produce a 1° lineage (Sternberg and Horvitz, 1989). Transgenic overexpression of *lin-3* in otherwise normal animals can also override the normal lateral interaction and promote adjacent 1° cells (R. Hill, pers. comm.). Finally, in experiments where both pathways are artificially activated (overexpression of *lin-3* in *lin-12(d)/lin-12(0)* animals), adjacent 1° fates are possible, although at a much lower frequency than when *lin-12* is not mutant (R. Hill, pers. comm). Although in experimental conditions both pathways can dominate in the formation of vulval fates, the two pathways may normally act in parallel to result in the reproducible pattern of fates required for vulval development. In the normal dose of AC signal, *lin-12* may play an important role in amplifying the differences between the proximal P6.p cell and the more distal cells.

Drosophila endoderm induction

The expression of *Ubx* and *dpp* in the visceral mesoderm during *Drosophila* development is required for normal endodermal development, including expression of *lab* in the midgut. However, the spatial regulation of expression is complex (see Fig. 4). *wg* expressed in ps8 of the visceral

mesoderm functions to modulate *lab* expression. In *wg* mutants the gradient of *lab* expression, with the highest concentration near the posterior, is reduced (Immergluck, et al., 1990). *Ubx* and *dpp* are also required for normal *wg* expression. Thus, in this system, signals act sequentially (*dpp* -> *wg*) and in parallel (*dpp/wg* -> *lab*) to induce regional specification. Signalling feedback is also involved in producing the normal gene expression pattern. For instance, both *dpp* and *wg* are required for normal *Ubx* expression (Panganiban, et al., 1990). They also mediate feedback to reinforce the normal pattern of gene expression in the signalling cells.

Xenopus mesoderm induction and body axis formation

Traditional models of mesoderm induction and body axis formation suggest that functionally distinct processes and molecules mediate each step (see Fig. 6.A). However, the complex developmental functions associated with recently characterized molecules suggest that (1) although the two processes are temporally distinct, they are likely not functionally independent, and may use many of the same molecules, and (2) multiple molecules may act in concert to mediate mesoderm induction and body axis formation, and production of a variety of differentiated fates (Kimelman, et al., 1992; see Fig. 6.B). The synergistic properties of *wnt* and bFGF in the promotion of mesoderm suggests that these two signals may act in parallel to promote mesodermal fates, with *wnt* activity localized to the presumptive dorsal region of the embryo. An additional signal ("X", possibly mediated by an activin) may also act in parallel in a partially redundant way with bFGF because bFGF is neither necessary nor sufficient for normal anterior structures such as head structures (Amaya, et al., 1991; Christian, et al.,

1992). One component of the model is that this activity normally functions together with *wnt*. Recent work suggests that these signals may indeed require synergistic function, as a dominant negative FGF receptor blocks full activity of activin in promotion of mesodermal fates (Cornell and Kimelman, 1994; however, this experiment does not rule out that FGF and activin pathways may act in series). Thus two generalized signals acting in concert with a localized *wnt* activity induce mesoderm, as well as specify both relative dorsal/ventral and anterior/posterior fate based on the concentration of *wnt* activity. Head specification occurs at the domains of highest *wnt* activity combined with "activin X" activity, trunk specification at domains with moderate *wnt* and bFGF activity, and tail specification at domains with only bFGF activity.

An additional cell interaction occurs during mesoderm induction among the presumptive mesodermal cells. This positive lateral interaction (termed the community effect) is required for specification of mesodermal fates, and may act in parallel to the mesodermal inducing signals (Gurdon, et al., 1984; Gurdon, 1988; Gurdon, et al., 1993). The community effect likely functions in mesodermal cells to create homogeneity within cell populations, but may also demarcate subpopulations of cells within a larger group. In this function the community effect acts like the lateral interaction in vulval development: it allows cells that detect subtle differences in the activity of other signals to amplify those differences resulting in a discontinuity between cell or tissue types.

Tissue culture

Most of the molecules proposed to act in providing parallel signal information are often thought to function directly as signals in the traditional sense. However, models that suggest that some of the key players may actually function in more mundane cell biological roles (e.g., the proposed function of *wg* to "seal" the parasegmental boundaries during embryogenesis; Lawrence and Sampedro, 1993) may be more accurate. Such molecules, while perhaps less exciting, are no less important in cell interactions, especially as modulators of primary signalling factors. Indeed, it is clear that the extracellular matrix can play a critical role in what factors a cell is exposed to, how the cell perceives those factors, and how it is capable of responding (reviewed in Adams and Watt, 1993). As one example, embryonal carcinoma (EC) cells grown on laminin or fibronectin layers respond to activin A or bFGF mitogenically, whereas EC cells grown on plastic require such factors for viability, but do not respond with cell division (Schubert and Kimura, 1991).

Developing cells are exposed to a variety of extracellular cues, and the autonomous components of those cells (e.g., presence of receptors) allow the specific response of the cells to these cues. Signals in the traditional sense (e.g., diffusible factors that induce different fates) make up some of these cues. However, as work continues in the direction of how cells integrate multiple signals, the role of extracellular matrix molecules in modulating cell interactions *in vivo* may become more apparent.

II. The *C. elegans* male tail as a model system to study specific developmental questions

Male tail development provides a reproducible background in which to study cell fate specification. The male specific blast cells, particularly the B cell, produce fairly complex lineages, likely requiring several fate specification steps. These lineages thus offer a good starting point to study the stepwise specification of fate and the interplay of different mechanisms important in development of multicellular organisms. Furthermore, my experiments indicate (Chapter 2) that multiple cell interactions are important for proper development of the B.a(l/r)xx cells. Thus, this lineage is also useful to study the integration and coordination of multiple signals involved in fate specification. In these studies I have used the experimental tools of cell ablation and genetics, coupled with direct observation of cell lineage as an assay of fate. In this section I review the development of the male tail as an introduction to the experimental system.

C. elegans as a system to study developmental biology

C. elegans provides a useful system for genetic analysis and the direct and precise analysis of development. The ability to visualize cell nuclei and their divisions, combined with the reproducibility of the lineage among animals, has allowed the complete description of the normal cell division pattern and the differentiated fates of all somatic cells (Sulston and Horvitz, 1977; Sulston, et al., 1980; 1983). The cell lineage in *C. elegans* is essentially invariant. However, because the extracellular environment for most developing cells is as constant as the ancestry, the correlation of cell division

and fate does not necessarily indicate that fate specification is autonomous. Although asymmetric cell divisions and segregated factors likely are important for some cell divisions (e.g., the first division of the zygote (Strome, 1983); the first division of the male B cell (Sternberg and Horvitz, 1988)), cell interactions also play an important role in cell fate specification. For example, the normal lineage includes several cells that have the potential to adopt more than one fate (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; Sulston, et al., 1983). In addition, embryonic blast cell rearrangement (Priess and Thomson, 1987; Wood, 1991) and isolation experiments (Schierenberg, 1987; Goldstein, 1992; 1993) as well as embryonic (Schnabel, 1991; 1994; Bowerman, et al., 1992) and postembryonic cell ablation experiments (Sulston and White, 1980; Kimble, 1981; Chisholm and Hodgkin, 1989) have identified instances of cell interactions that specify cell fate. The invariant cell lineage, rather than indicating a limited repertoire of fate specification mechanisms, offers a reproducible background to study the interplay of autonomous and conditional mechanisms.

Cell lineage as a marker for cell fate

In *C. elegans* the fate of precursor cells includes the cell lineage (number, timing and axes of divisions) and the terminal differentiated fates of the progeny produced by those divisions. Although both criteria are important to understanding the fate of a precursor cell, for both cell ablation and genetic studies I have focused on the former. In other analyses of development in *C. elegans*, cell lineage has proven to be an accurate indicator of fate choice (e.g., Horvitz, et al., 1983; Kenyon, 1986; Sternberg and Horvitz, 1986; Schnabel, 1991; Bowerman, et al., 1992).

Review of the anatomy and normal development of the *C. elegans* male tail

At hatching, male and hermaphrodite *C. elegans* are morphologically similar. Sexual dimorphism develops postembryonically as different blast cells divide following sex-specific lineages (Sulston and Horvitz, 1977). The *C. elegans* male tail consists of several structures (description and data of Sulston and Horvitz, 1977 and Sulston, et al., 1980): the fan, the hook, the postcloacal sensilla, and the spicules (Fig. 7).

The fan comprises eighteen bilaterally symmetric ray sensilla laminated between two layers of cuticle. The cells of the rays derive from sex-specific divisions during the development of the lateral hypodermis cells V5, V6, and T.

The hook and the associated sensillum lie directly anterior to the cloacal opening. The cells of the hook sensillum derive from sex-specific divisions during the development of the ventral hypodermal cells P10 and P11.

The postcloacal sensilla are paired structures that reside posterior to the cloaca. They are not associated with a morphologically distinct structure as is the hook sensillum. The cells of the postcloacal sensilla derive primarily from Y, a male specific blast cell (Fig. 8). In hermaphrodites, the cell that is the male Y cell differentiates as a neuron (PDA). In males, the cell divides asymmetrically, producing an anterior daughter (Y.a) that differentiates as a neuron as the mother cell does in hermaphrodites, and a posterior daughter (Y.p) that is the precursor to ten cells of the postcloacal sensilla. Other male

specific blast cells are F and U that produce several male-specific interneurons, and B (discussed below). In general, the male-specific blast cell lineages include one progeny that functions similar to the mother cell in hermaphrodites, and several other male-specific neurons and hypodermal cells. The work in this thesis focuses primarily on the development of the male-specific blast cells.

The two spicules lie within the cloaca. The spicules are sensilla; each includes two neurons with processes that run the length of the spicule to the tip where they are open to the environment. The other cells of the spicules are socket and sheath cells that provide structure and support. The sheath cells secrete a specialized cuticle that is harder and more refractile than the body cuticle. The spicules are attached proximally to protractor and retractor sex muscles. During mating, after the male locates the hermaphrodite vulva, the spicules are inserted into the vulval opening. The structure of the spicules helps to anchor the male cloaca at the vulval opening, and the neurons of the spicules coordinate sperm transfer (K. Liu and P. W. Sternberg, in preparation).

All of the cells of the spicules derive from the male B cell (Fig. 8). In hermaphrodites this cell is part of the dorsal rectal epithelium, and progeny of B.p retain this function during male postembryonic development. B.a divides to produce the cells of the spicules, as well as some proctodeal cells, two neurons of the postcloacal sensilla, and four cell deaths. The B cell lineage consists of three different stages: early divisions, migration, and late divisions. The early divisions take place primarily in the second larval stage (L2), and result in ten progeny. A short-range migration of the eight B.a progeny occurs in the late L2 stage. The late round of divisions begins in the

mid-L3 stage. The first division of B is asymmetric and along an approximately anterior/posterior axis. The larger anterior cell (B.a) is the precursor of all cells of the spicules. B.a divides along a left/right axis, establishing the bilateral symmetry of the spicules. This symmetry is broken (during migration) and then re-established (during the late divisions) in the progeny of these cells. B.al and B.ar each divide twice to produce a ring of eight cells, one cell thick. These cells then migrate to form a ring of four cells, two cells thick. The medial migration of the two dorsal (**pp**) and ventral (**aa**) cells exhibits natural variation. In both cases, either the right or the left cell can adopt the more anterior position, while the other cell adopts the more posterior position. The cell that adopts the anterior position will produce a different lineage than the cell that adopts the posterior position. The other four B.a progeny migrate invariantly. The **ap** cells adopt the anterior position (one on the left, one on the right), and the **pa** cells adopt the posterior position.

Cell interactions during the development of the male-specific blast cells

The variability of position and fate of specific **aa** and **pp** cells suggested that each pair represents an equivalence group. Sulston and White (1980) carried out a series of ablation experiments to better understand the interactions involved in fate specification of these pairs. Their experiments showed that following ablation of either the left or the right **aa** cell, the remaining **aa** cell will migrate to the midline and produce an α lineage. This establishes α fate as primary (1°) and β fate as secondary

(2°). Even if the targeted cell is migrating to the anterior position when it is ablated, the remaining cell will produce an α lineage. Similar experiments with the **pp** cells could not establish 1° or 2° fate in the γ/δ pair. However, the variability of the B lineage from animal to animal, combined with these preliminary cell ablation experiments, suggested that cell interactions may play a role in establishing the fate of these cells.

Additional information about cell interactions during development of the male-specific blast cells comes from the work of Chisholm and Hodgkin (1989). Work with the gene *mab-9* (see below) suggested a possible interaction between B and the F and U cells. Indeed, ablation of the B cell prior to division resulted in disruption of F and possibly U lineages. Specifically, the asymmetric divisions of F (such as the division of F.(l/r)) were reversed about 50% of the time, i.e., their orientation became random. The data for U are less conclusive as the U lineage only shows evidence of asymmetry in some animals. Thus the B cell, or its progeny, provide a positional cue to the F and U cells that orients the asymmetric cell divisions.

Genetic analysis of the development of the male-specific blast cells

Genes that play roles in the development of the male-specific blast cells have been identified both directly, by identifying mutations that disrupt male tail development, and indirectly, by characterizing pleiotropic effects of mutations disrupting fate specification in other tissues. Most of the genes analyzed to date play a role in the development of the B cell (summarized in Fig. 10). None of the genes functions in a truly sex-specific manner; rather, their function in male tail development reflects their role in a developmental

process that is sex-specific. Although only a few genes have so far been identified, they provide a glimpse of the genetic complexity in the development of the male-specific blast cells.

Specification of blast cells

mab-9 (male abnormal)

Mutations in *mab-9* were identified on the basis of the abnormal male tail phenotype they confer (Hodgkin, 1983). Lineage analysis identified several defects in mutant males: F, U, and B lineages were disrupted (Chisholm and Hodgkin, 1989). The B lineage defect is consistent with the interpretation that B is transformed to Y fate. The F and U lineage defects reflect the disruption of the B cell, but also may indicate a function of *mab-9* that makes F different from U. Analysis of *mab-9* hermaphrodites indicated that the hermaphrodite B cell also adopts a fate like the hermaphrodite Y cell. Thus the function of *mab-9* is to specify B fate and make it different from Y. Its function is not sex-specific, but the sex-specific differences in development make the defect more obvious in males than in hermaphrodites. Since the B and Y cells are neither sisters nor lineal homologues, the reason the function of only a single gene is necessary to distinguish them is not straightforward. However, they are neighbors, so *mab-9* may play a role in establishing regional differences.

egl-5 (egg-laying defective)

One gene that likely plays a role in the regional specification of fate is *egl-5*. *egl-5* is part of the *C. elegans* homeotic complex (Kenyon and Wang, 1991). Mutations in *egl-5* disrupt the fate of many cells in the tail region of the worm, including B (Chisholm, 1991). However, the B lineage defect in

egl-5 males does not appear to be a transformation of one fate to another, and thus the specific function of *egl-5* in the B lineage is unclear.

lin-12

One of the functions of the gene *lin-12* is to specify the fate of Y, and make it different from its lineal homolog, the neuron DA9. The function of *lin-12* is discussed further below.

Establishing the first asymmetric cell division of B

lin-17

lin-17 was originally identified for a subtle defect in vulval development (Ferguson and Horvitz, 1985; Ferguson, et al., 1987). However, further analysis revealed that *lin-17* functions in multiple postembryonic lineages (Sternberg and Horvitz, 1988). The common theme among the *lin-17* defects is that specific asymmetric divisions become symmetric. In the male tail, *lin-17* is required for proper development of T, P10/11, and B. Normally, the initial division of B is asymmetric. Not only does B.a produce a different cell lineage from B.p, but the B.a cell is larger than the B.p cell. In *lin-17* mutants the B.a and B.p cells are generally equal in size, and both cells produce an early lineage that is similar to the normal B.a lineage.

lin-44

Like *mab-9*, mutations in *lin-44* were identified on the basis of male tail defects (Herman and Horvitz, 1994). *lin-44* is required for the asymmetric division of the B and T cells. Unlike *lin-17*, mutations in *lin-44* do not result in symmetric cell divisions, but rather a reversed polarity of the asymmetric division. For example, normally the nucleus of B.a is always larger than B.p. However, in *lin-44* mutants, B.a was larger in only 20% of

animals scored, the cells were equal in size in 8% of animals, and B.p was larger than B.a in 71% of animals (Herman and Horvitz, 1994). *lin-17 lin-44* double mutants display the *lin-17* phenotype, suggesting that *lin-17* is necessary to establish the asymmetric division, while *lin-44* is necessary to orient the division.

Fate specification in the later lineage

lin-12

Mutations in *lin-12* disrupt fate specification in a variety of postembryonic lineages (Greenwald, et al., 1983). The two classes of mutations in *lin-12* -- gain-of-function (*lin-12(d)*) and loss-of-function (*lin-12(0)*) -- result in opposite phenotypes. In general, if the normal fates of two cells are A and B, in *lin-12(d)* mutants both cells adopt the A fate, whereas in *lin-12(0)* mutants both cells adopt the B fate. In several cases the two cells involved are known to interact to ensure that each cell adopts a fate different from the other. Thus *lin-12* may mediate a lateral interaction (lateral inhibition). The molecular similarity between *lin-12* and other genes involved in cell interactions (e.g., *glp-1* in *C. elegans* and *Notch* in *Drosophila*) is consistent with the function of *lin-12* in cell interactions. In one *lin-12*-mediated fate specification process (an interaction between two cells in the hermaphrodite gonad that results in a choice between anchor cell fate and a ventral uterine fate (the AC/VU decision)) mosaic analysis indicates that *lin-12* acts in a cell-autonomous manner (Seydoux and Greenwald, 1989).

Mutations in *lin-12* disrupt the B and Y lineages in males. In *lin-12(d)* mutants there are two Y-like cells, whereas in *lin-12(0)* mutants there are no Y cells. *lin-12* likely mediates precursor fate specification between the Y cell

and its lineal homologue neuron DA9. However, there is no evidence that these two cells interact to specify fate, as cell ablation experiments have not uncovered any fate regulation for these cells (Greenwald, et al., 1983; Sulston, et al., 1983). In the B lineage *lin-12* plays two roles: distinguishing B.pa from B.pp and δ from γ in the **pp** pair. In *lin-12(d)* animals, both B.pa and B.pp produce a lineage like the normal B.pa cell; in *lin-12(0)* animals both cells produce a lineage like the normal B.pp cell. In contrast, *lin-12(d)* mutants are normal in the B.a lineage. Only in *lin-12(0)* mutants do both **pp** cells produce a γ lineage rather than an anterior γ lineage and a posterior δ lineage. Although this exception was originally thought to be due to the possibility that the *lin-12(d)* mutation was not strong enough to produce the opposite transformation, my results (see Chapter 3) suggest that this exception is due to additional cell interactions. Nevertheless, the lineage defect in *lin-12(0)* mutants also hints at the possible cell interactions in B lineage specification.

III. Overview of the thesis

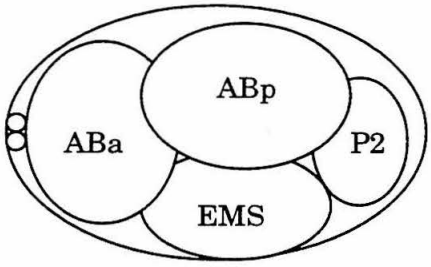
My work has centered on three issues: (1) the cell interactions that establish fate among the eight intermediate B.a progeny, (2) fate specification in the unequal cell divisions of both the B cell and specific cell divisions late in the lineage, and (3) the identification and initial characterization of new genes that may function at additional steps in the lineage. In Chapter 2, I describe cell ablation experiments that identify five distinct cell interactions required for proper fate specification in the eight intermediate B.a progeny. Experiments in which multiple signalling sources are removed together also

address how these positional cues are integrated. In Chapter 3 I describe experiments that indicate that the genes in the *lin-3/let-23* signalling pathway mediate one of these positional cues from F and U. I extend the experiments of Greenwald, et al. (1983) in analyzing the function of *lin-12* in lateral interactions between cells of equivalent potential. I also extend the signal integration experiments of Chapter 2 with double mutant and cell ablation experiments that address the integration of both *lin-15* and the Y.p positional cue into the *lin-3/let-23* signalling pathway. In Chapter 4, I describe a genetic screen for mutants with abnormal male tail development. This screen has identified additional genes that play a role in fate specification in the B lineage. In Chapter 5 I describe experiments that characterize the function of one of these genes, *vab-3*. vab-3 represents a candidate factor distributed asymmetrically at the initial division of B that is necessary for B.a fates. Experiments also identify its interaction with *lin-17* at the first division of B, and identify additional functions for *lin-17* in specific other asymmetric cell divisions in the B lineage.

Figures

Figure 1. Fate specification in *C. elegans* early embryogenesis. A. Relative positions of blast cells at the four cell stage. B. Early embryonic lineage. Cell interactions and genes that mediate specific steps of fate specification are indicated. The *par* genes mediate the initial asymmetric cell division of the zygote. *mex-1* and *pie-1* act sequentially to localize *skn-1* activity to EMS. Multiple cell interactions also occur among the early blast cells to specify fate (see text).

A.


ABp
ABa
P2
EMS

B.

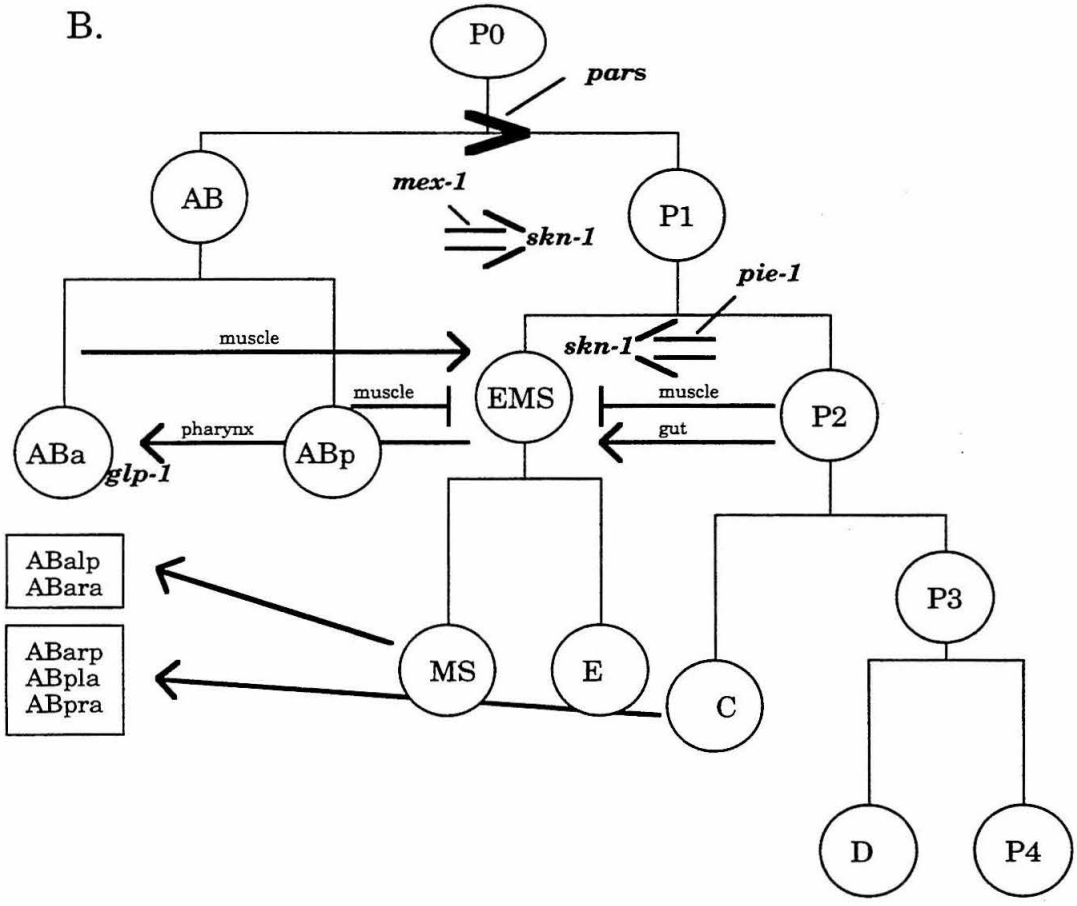

P0
pars
AB
mex-1
skn-1
P1
pie-1
skn-1
muscle
muscle
EMS
muscle
P2
pharynx
gut
ABa
glp-1
ABp
ABalp
ABara
ABarp
ABpla
ABpra
MS
E
C
P3
D
P4

Figure 2. Genes and cell interactions involved in *C. elegans* vulval development. A. Two gonadal precursor cells interact to produce a single anchor cell (AC) and a ventral uterine cell (VU). B. Regional specification by *lin-39* and proper development of the P cells are required for the production of vulval precursor cells (VPC). C. The AC produces a signal that overrides negative regulation by *lin-15* and induces vulval fates in the more proximal VPCs. The induced VPCs also interact with each other to establish the 2°1°2° pattern of fates. D. *lin-11* is required for the execution of 2° fate, and the non-AC gonad produces a signal that orients the 2° lineage of P7.p toward the vulval opening. 1° fates promote proper branching and positioning of the hermaphrodite specific neuron (HSN) process.

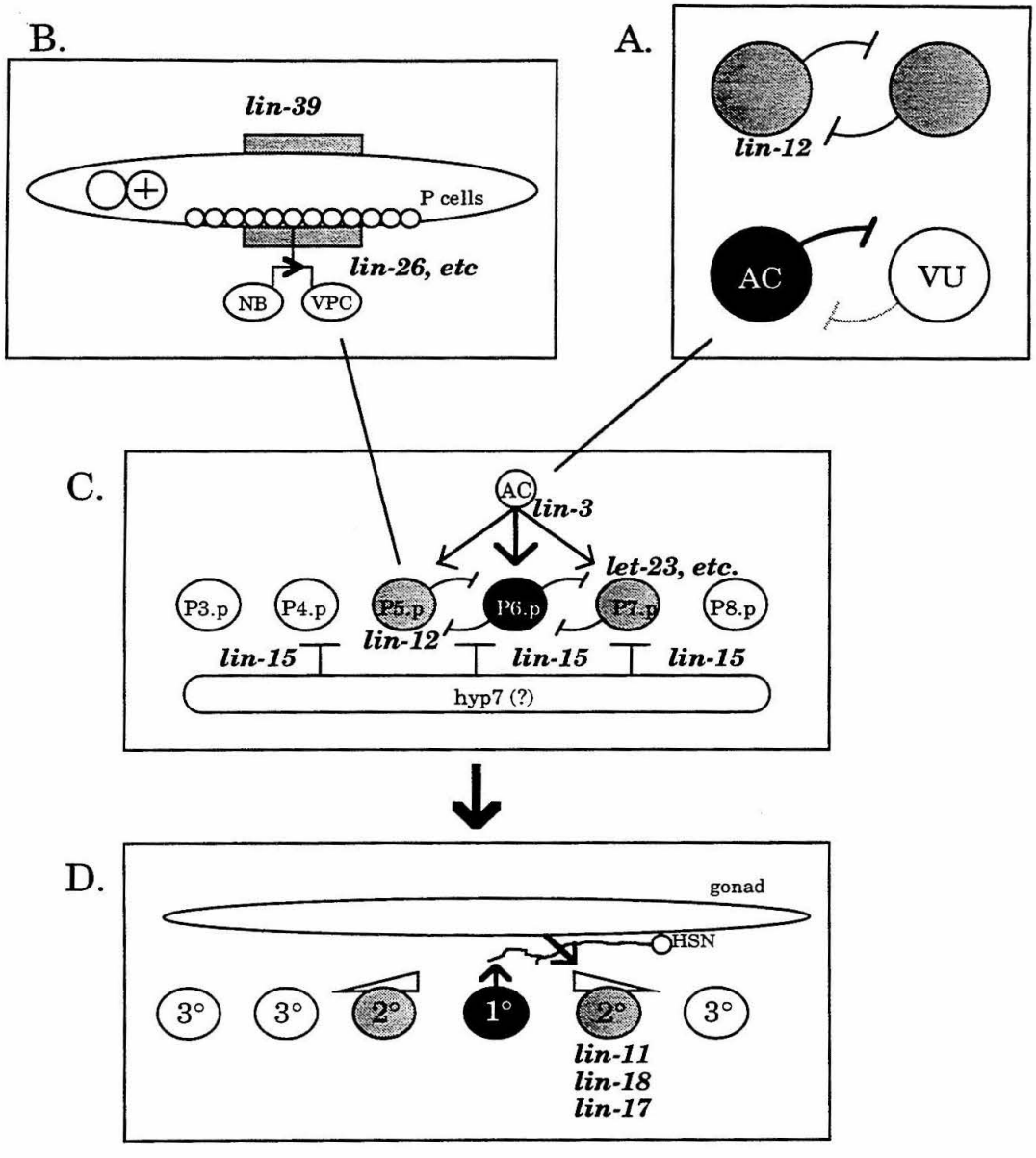
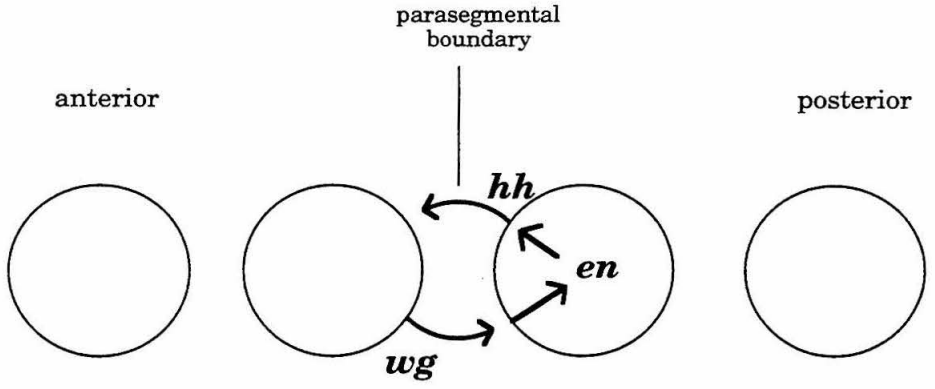

B.
lin-39
P cells
lin-26, etc
NB
VPC
A.
lin-12
AC
VU
C.
AC
lin-3
let-23, etc.
P3.p
P4.p
P5.p
P6.p
P7.p
P8.p
lin-12
lin-15
lin-15
lin-15
hyp7 (?)
D.
gonad
HSN
3°
3°
2°
1°
2°
3°
lin-11
lin-18
lin-17

Figure 3. Anterior/posterior patterning within each parasegmental unit in the *Drosophila* epidermis (after Woods and Bryant, 1992; Lawrence and Sampedro, 1993). A. *wg*, *hh*, and *en* act reciprocally to reinforce expression patterns, and to establish or maintain the parasegmental boundary. B. *hh* may act in a dose-dependent manner to specify different fates.

A.



B.

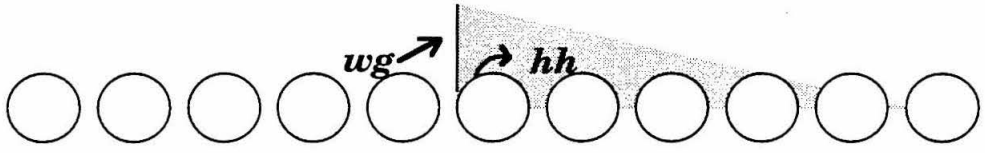


Figure 4. Regional specification of the *Drosophila* endoderm: the interaction between the visceral mesoderm and the midgut (after Bienz, 1994). *Ubx* expression is required for *dpp* expression, and *abd-A* expression is required for *wg* expression in specific regions of the visceral mesoderm. *dpp* and *wg* are also necessary for proper expression of *lab* in the midgut, and the middle gut constriction. The pattern of expression of these genes is reinforced and maintained by an elaborate series of feedback interactions.

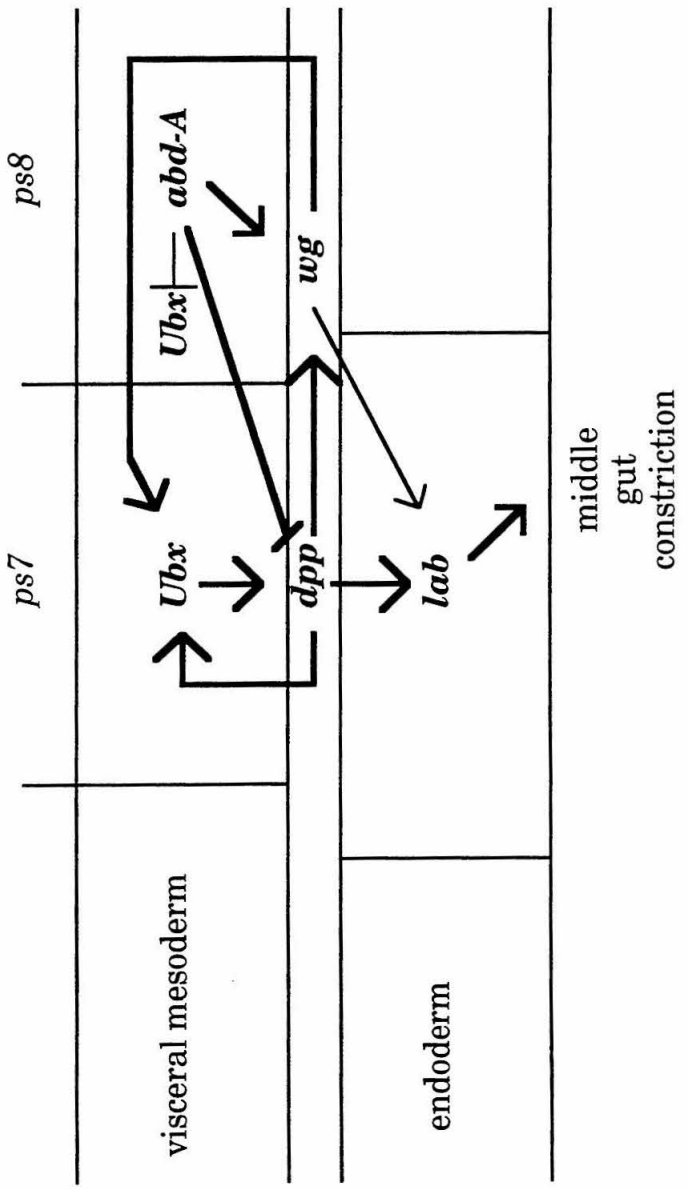

ps7
ps8
visceral mesoderm
endoderm
Ubx
abd-A
Ubx
wg
dpp
lab
middle
gut
constriction

Figure 5. Patterning of sensory organ precursors and the production of bristle sensilla in the *Drosophila* epidermis (after Ghysen, et al., 1993; Posakony, 1994). A. The expression of *ac* and *sc* (and their regulators) establish the proneural cluster, a group of cells competent to adopt the neuroblast (NB) fate. B. A lateral interaction among cells within the proneural cluster ensures that only one cell adopts the NB fate. C. The NB (or sensory organ precursor, SOP) executes a stereotyped lineage to produce the four fates of the sensory organ: the socket (So), the shaft (bristle, Br), the sheath (Sh), and the sensory neuron (N). Both cell interactions and localized factors play a role in fate specification in this lineage.

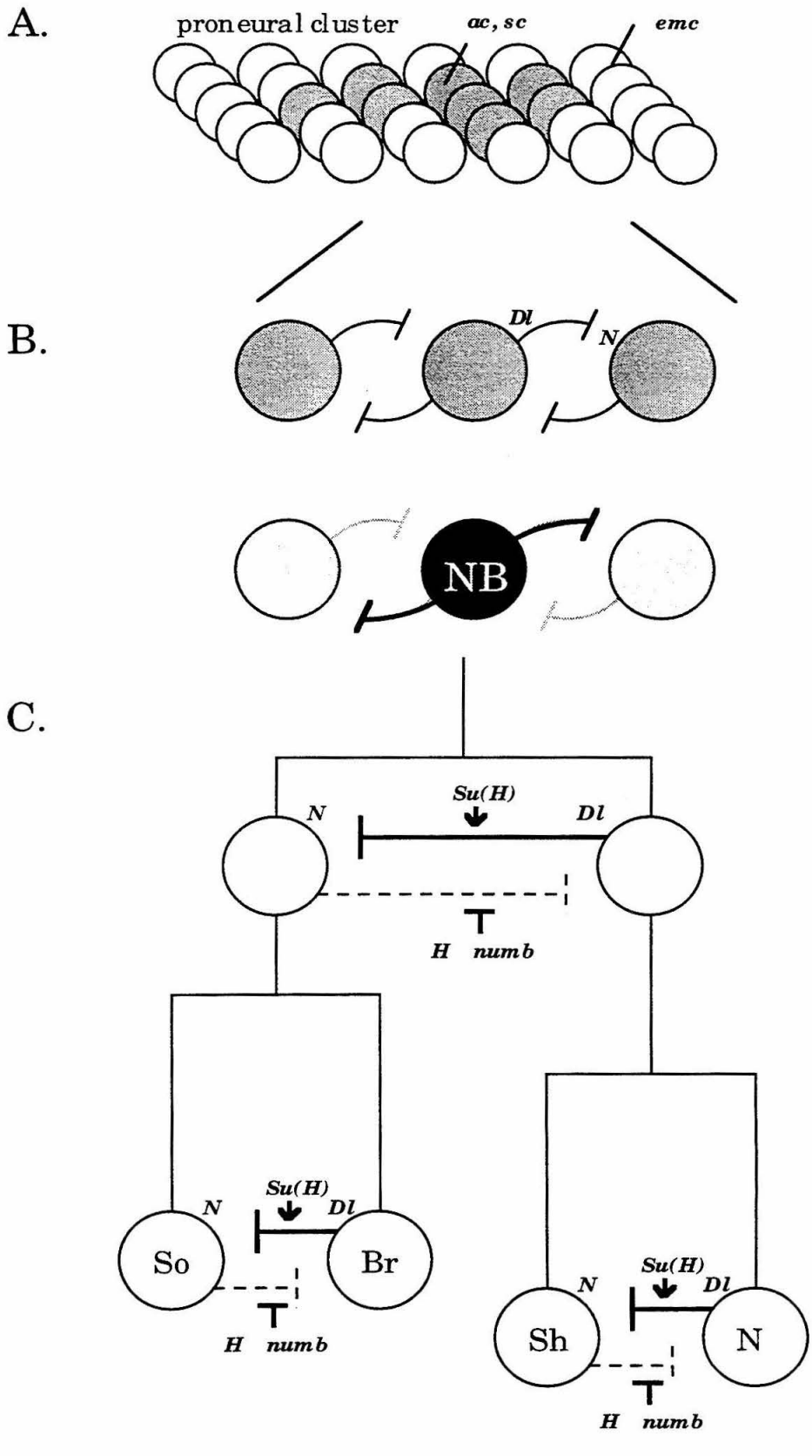

A.
proneural cluster
ac, sc
emc
B.
Dl
N
NB
C.
Su(H)
N
Dl
H numb
So
Br
Sh
N

Figure 6. Cell interactions in the *Xenopus* embryo. A. Transplantation and tissue recombination experiments define three distinct activities required for early *Xenopus* development: A vegetal activity the induces ventral mesoderm (VV), a vegetal activity that induces dorsal mesoderm (DV), and an organizer activity (O). B. Synergistic model of mesoderm induction and body axis specification (after Kimelman, et al., 1992). O = organizer, D = dorsal mesodermal fates, V = ventral mesodermal fates, A = anterior mesodermal fates (head, eye cups), M = middle mesodermal fates (trunk), P = posterior mesodermal fates (tail). Secondary interactions may also take place emanating from the organizer to promote notochord, which promotes more ventral mesodermal structures.

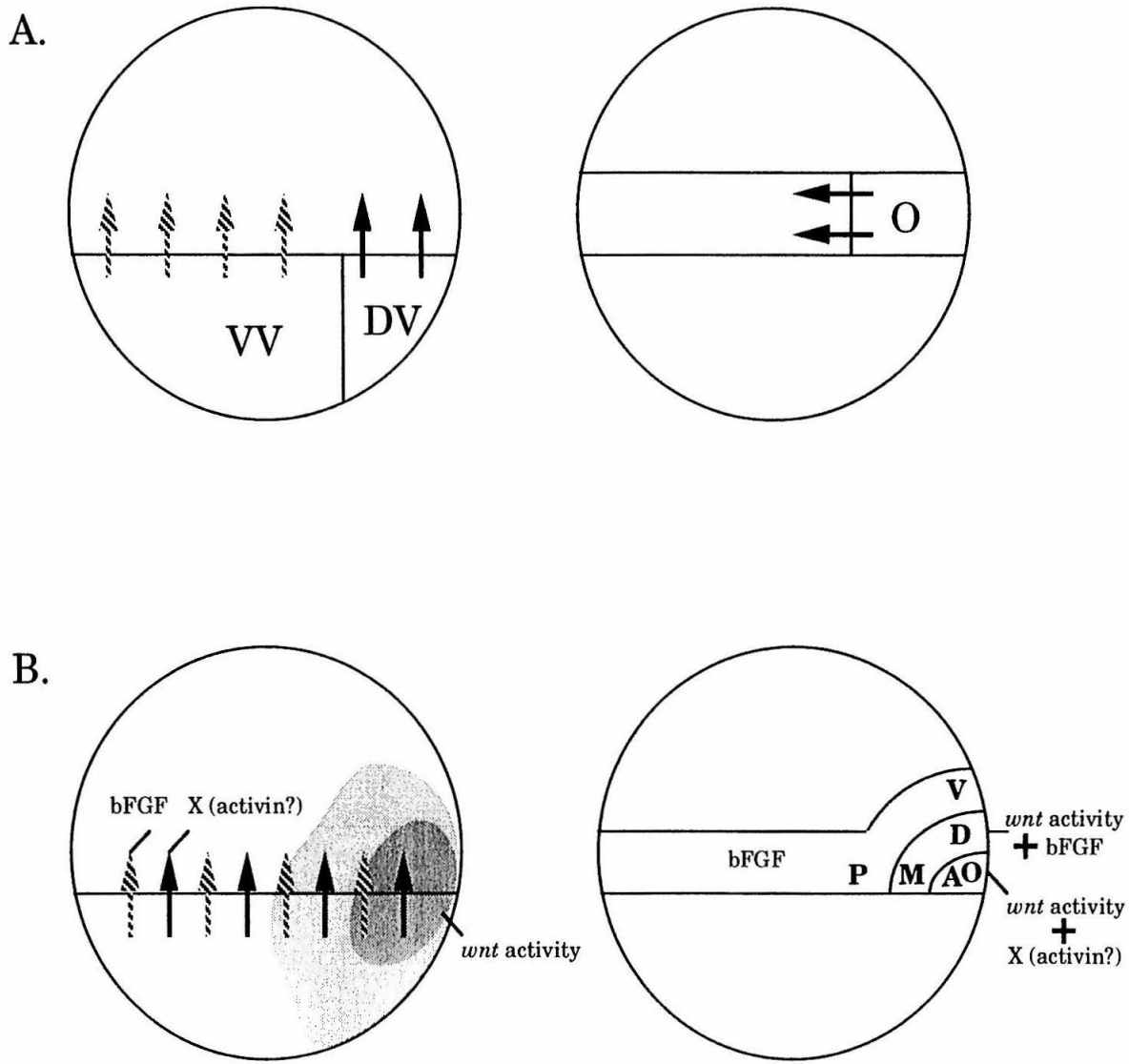

A.
VV
DV
O
B.
bFGF
X (activin?)
wnt activity
bFGF
V
D
P
M
AO
wnt activity
+ bFGF
wnt activity
+
X (activin?)

Figure 7. *C. elegans* male tail, illustrating the rays, spicules, and hook and postcloacal sensilla (after Sulston, et al., 1980; image scanned and edited by K. Liu).

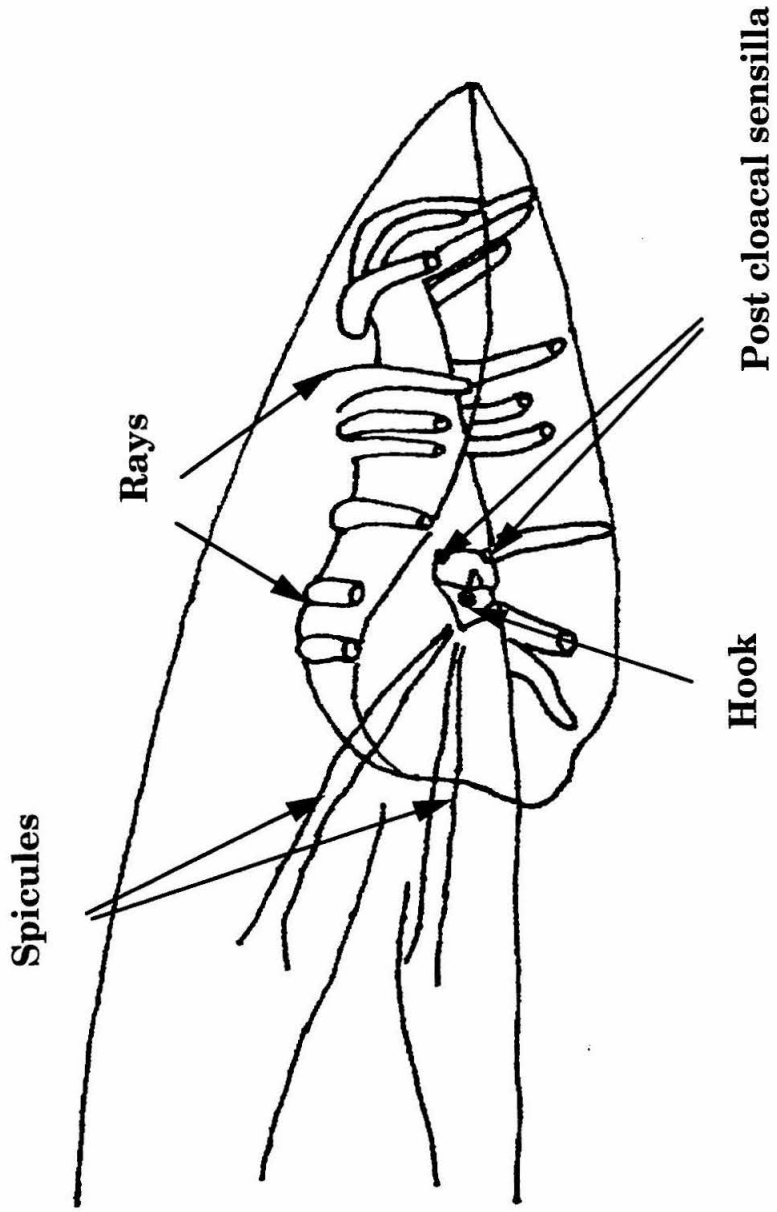
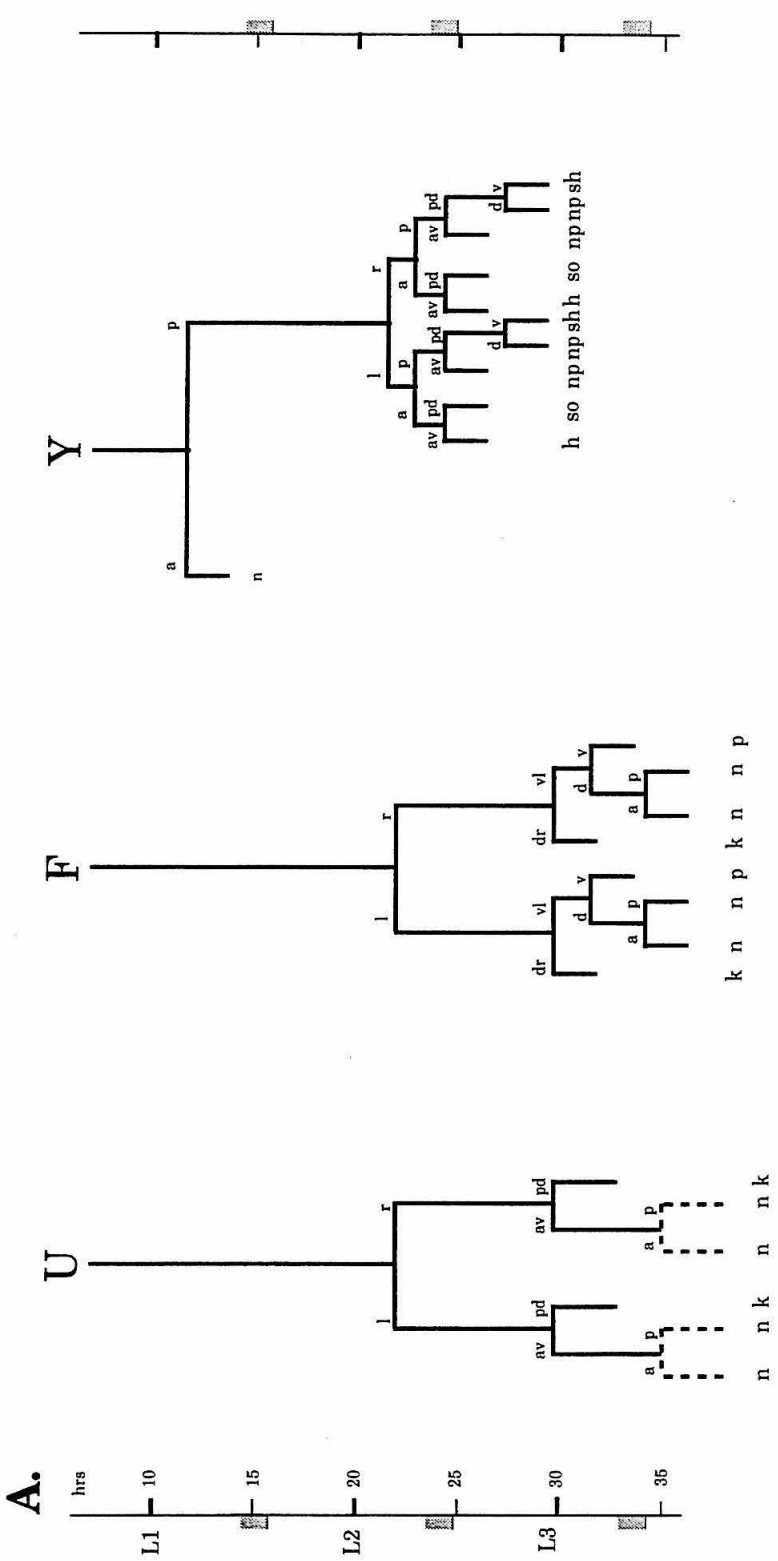

Post cloacal sensilla
Rays
Hook
Spicules

Figure 8. U, F, Y, and B lineages in wild type males, with the differentiated fates of each (after Sulston, et al., 1980). n = neuron, ns = neuron of the spicule, np = neuron of the postcloacal sensilla, so = socket cell, sh = sheath cell, h = hypodermal (associated with post cloacal sensilla), p = proctodeal cell, k = cell associated with the death of another cell ("killer" cell; proctodeal).


A.
hrs
10
15
20
25
30
35
L1
L2
L3
U
F
Y
n n k
n n k n n k
k n n p k n n p
h so npnpshh so npnpsh

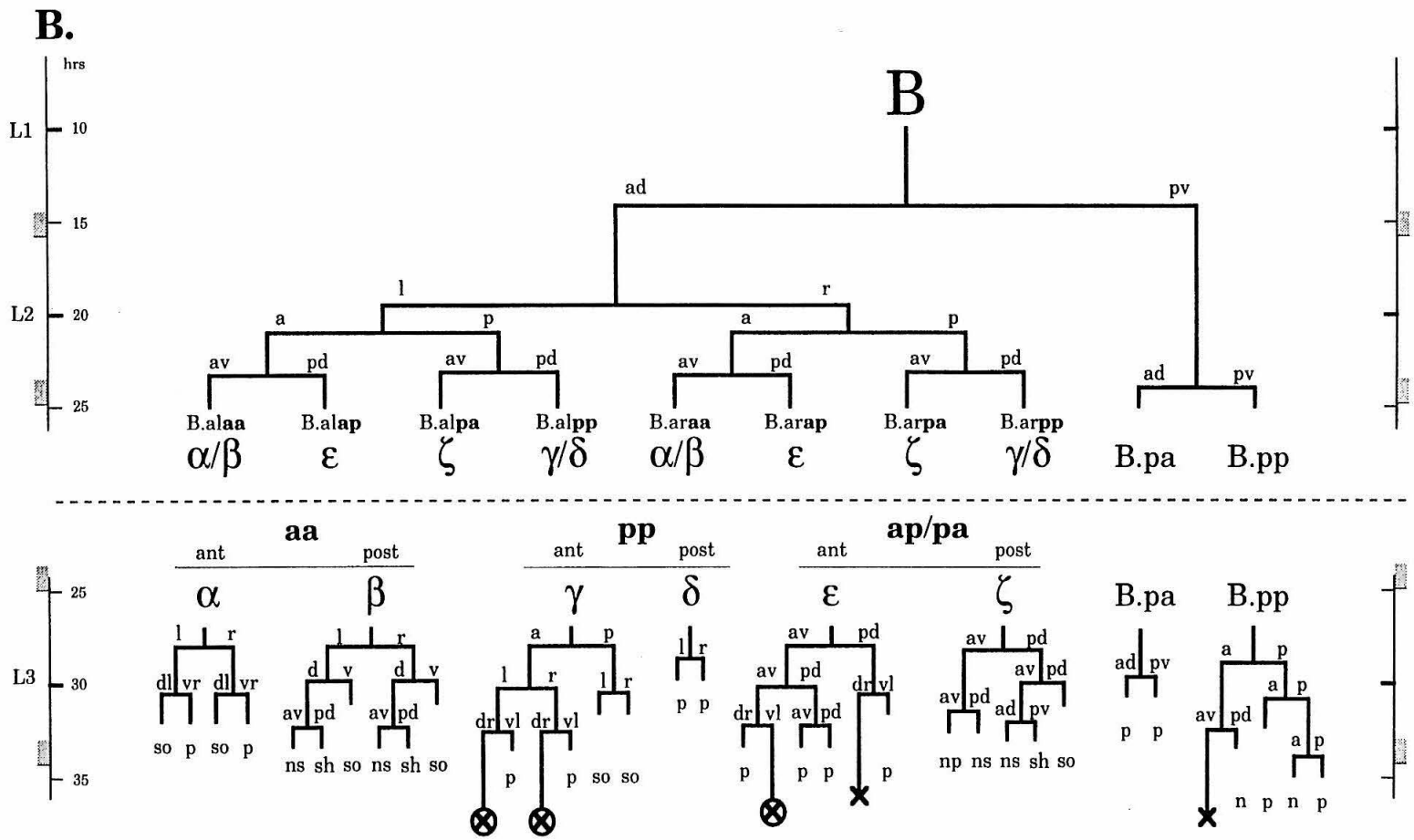
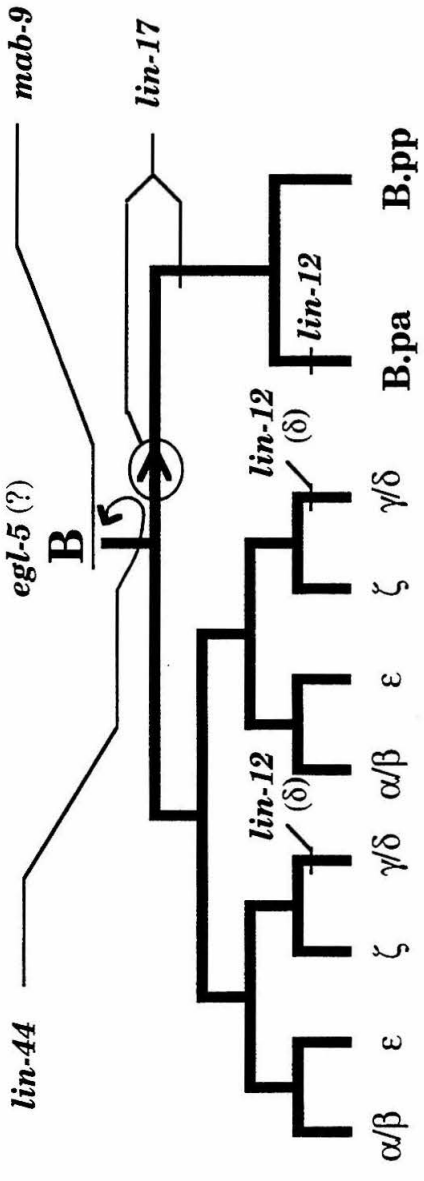

B.
hrs
L1
10
15
L2
20
25
B
ad
pv
l
r
a
p
a
p
av
pd
av
pd
av
pd
av
pd
ad
pv
B.alaa
B.alap
B.alpa
B.alpp
B.araa
B.arap
B.arpa
B.arpp
B.pa
B.pp
α/β
ε
ζ
γ/δ
α/β
ε
ζ
γ/δ
aa
pp
ap/pa
ant
post
α
β
γ
δ
ε
ζ
B.pa
B.pp
L3
25
30
35
so p so p
ns sh so ns sh so
p p so so
p p p p
np ns ns sh so
p p
n p n p

Figure 9. Genes required for normal development of the B cell.


mab-9
lin-17
egl-5 (?)
B
lin-44
lin-12 (δ)
lin-12
lin-12 (δ)
lin-12
α/β
ε
ζ
γ/δ
α/β
ε
ζ
γ/δ
B.pa
B.pp

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Chapter 2

Multiple cell interactions are required for fate specification during male spicule development in *Caenorhabditis elegans*

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SUMMARY

The B blast cell divides postembryonically in *C. elegans* males to produce 47 progeny that include all of the cells of the copulatory spicules. During the early development of the B lineage, the anterior daughter of B, B.a, generates eight cells. These cells migrate to form four pairs of cells that flank the developing cloaca (ventral, dorsal, and two identical lateral pairs). For each pair, the more anterior cell produces a distinct lineage ('anterior fate') from the posterior cell ('posterior fate'). For the ventral and dorsal pairs, either cell can migrate to the anterior position and produce the anterior lineage, and the other cell migrates posterior and produces the posterior lineage (Sulston and Horvitz, 1977, *Dev. Biol.* 56, 110-156). The migration is variable, although the resultant fate pattern is invariant. In the two lateral pairs, both the migration and fate pattern are invariant. Using a laser microbeam to selectively ablate neighboring cells we have found that the cells of the lateral pair also respond to positional cues. For all four pairs other male-specific blast cells provide extracellular cues. In general, F and U promote anterior fates, Y promotes some posterior fates, and the B.a progeny promote posterior fates. Several of these cues are redundant. By ablating combinations of cells we have deduced how these signals may act in concert to specify the fates of the B.a progeny. We propose that fate specification in these pairs depends on three general classes of extracellular cues: positional cues, modulators of positional cues, and lateral signals. The B lineage thus provides an opportunity to study with single cell resolution the integration of multiple intercellular signals.

Key words: *C. elegans*, cell lineage, cell interactions, cell fate specification, redundancy, pattern formation

INTRODUCTION

The fate of a cell can be specified by information provided by the cell's precursors and by signals from the cell's neighbors. The experimental study of cell lineage (patterns of cell division and their relationship to cell fate) can provide clues about these mechanisms of fate specification and their interrelationship. The ability to visualize cell nuclei and their divisions in *C. elegans*, combined with the reproducibility of the lineage among animals, has allowed the complete description of the normal cell division pattern and the differentiated terminal fates (Sulston and Horvitz, 1977; Sulston et al., 1980, 1983). The cell lineage in *C. elegans* is essentially invariant. However, because the extracellular environment for most developing cells is as constant as the ancestry, the correlation of cell division and fate does not indicate that fate specification is completely autonomous. Indeed, cell interactions likely play an important role in cell fate specification. For example, the lineage includes several instances of cells that have the potential to adopt more than one fate (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; Sulston et al., 1983). In addition, embryonic blast cell rearrangement (Priess and Thomson, 1987; Wood, 1991) and isolation experiments (Schierenberg, 1987; Goldstein, 1992), as well as embryonic (Bowerman et al., 1992) and postembryonic cell ablation experiments (Sulston and White, 1980; Kimble, 1981; Chisholm and Hodgkin, 1989) have identified instances of cell interactions that specify cell fate. The invariant cell lineage, rather than indicating a limited repertoire of fate specification mechanisms, offers a reproducible background to study the interplay of autonomous and conditional mechanisms.

To study the components of fate specification, we have focused our attention on the postembryonic lineage of the male B cell. The B cell is one of four male-specific blast cells (B, U, F and Y) that divide in males, but not in hermaphrodites, and produce some of the cells of the specialized mating structures of the male tail. The male B cell, for instance, produces all of the cells of the copulatory spicules (Fig. 1). The B lineage (Fig. 2) includes examples of autonomous as well as conditional fate specification. For example, the B cell undergoes asymmetric cytokinesis in its first division, which has been studied as a possible example of autonomous fate specification (Sternberg and Horvitz, 1988). Later in the lineage, two pairs of B progeny cells exhibit natural variation (Sulston and Horvitz, 1977). In each pair, one cell adopts the anterior position and fate, and the other cell the posterior position and fate. Although which cell adopts the anterior position varies from one animal to the next, the resultant pattern of anterior and

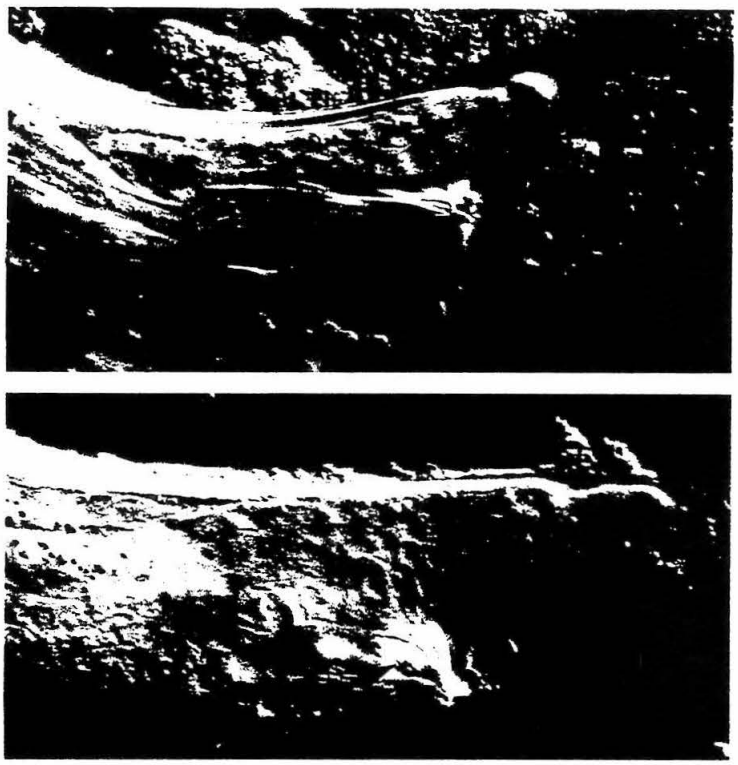

Fig. 1. Comparison of the adult male spicule in intact (A) and F^-U^- (B) animals. Arrows point to the left spicule. Nomarski photomicrographs, left lateral view (anterior left, ventral down). (A) In intact animals the spicules are long and straight. (B) In F^-U^- animals the spicules are short and crumpled due to disruption of the cell lineage that produces the spicule cells. Scale bar, 20 μm.

posterior fates is always the same. Since the fate reflects the cell's position rather than its ancestry, this represents an example where extracellular cues likely play a role in fate specification.

In this study we use cell ablation to identify cells that provide specific extracellular cues. Ablation experiments also allow us to characterize the interaction of these cues, and to deduce their roles in fate specification. We focus on four pairs of B progeny cells that represent three distinct pair types. We have identified multiple extracellular cues that specify fate choice in each pair. These fall into three general classes (see Fig. 17): positional cues (such as inducers and inhibitors), modulators of positional cues (active or passive), and lateral signals. We present evidence that multiple extracellular cues may act in parallel, as well as in series, to specify cell fate(s). Having established extracellular components of fate and the developmental potentials of these cells, we also consider the interplay of autonomous and extracellular cues in producing the fates of the B cell lineage.

MATERIALS AND METHODS

Strains

Methods for culturing and handling *C. elegans* have been described by Brenner (1974) and Sulston and Hodgkin (1988). The strain **CB1490** *him-5(e1490)* provides a convenient source of normal males, and we established it as the 'wild type' background for all ablations. The mutation *him-5(e1490)* increases loss and nondisjunction of the X chromosome, with little effect on the five autosomes (Hodgkin et al., 1979). Consequently, *him-5* mutant hermaphrodites (XX) segregate about 40% male (XO) self progeny.

Cell nomenclature

Nomenclature follows the standard of Sulston and Horvitz (1977). The proper names for the B.a progeny at the 10 cell stage (Fig. 3E and Legend) are B.a(l/r)aa, B.a(l/r)ap, B.a(l/r)pa, and B.a(l/r)pp. Collectively, we refer to the cells as B.a(l/r)xx, where x refers to both daughter cells. We use (l/r) to indicate both the left and the right members of a pair. When we refer to a single set in the text we have removed the common prefix and shortened the names to **aa**, **ap**, **pa**, and **pp**. The intention is to increase readability, hopefully without the confusion often associated with the renaming of cells. We consider this simply a shorthand way of referring to the cells, and use the full, proper name in any instance that the shortened name might prove ambiguous. In general, **aa**, etc, refers to two cells - both the left and the right - except where explicitly noted.

Cell lineage and laser ablation

Cell nuclei divisions were directly observed in live animals under Nomarski optics as described by Sulston and Horvitz (1977). Except where noted, the lineage of all B progeny cells was followed in each animal to completion. We have used the cell lineage - the axes, timing, and number of cell divisions - as an assay of cell fate. Therefore, although most animals were observed from the first divisions of the B.a(l/r)xx cells (early-mid L3 larval stage)

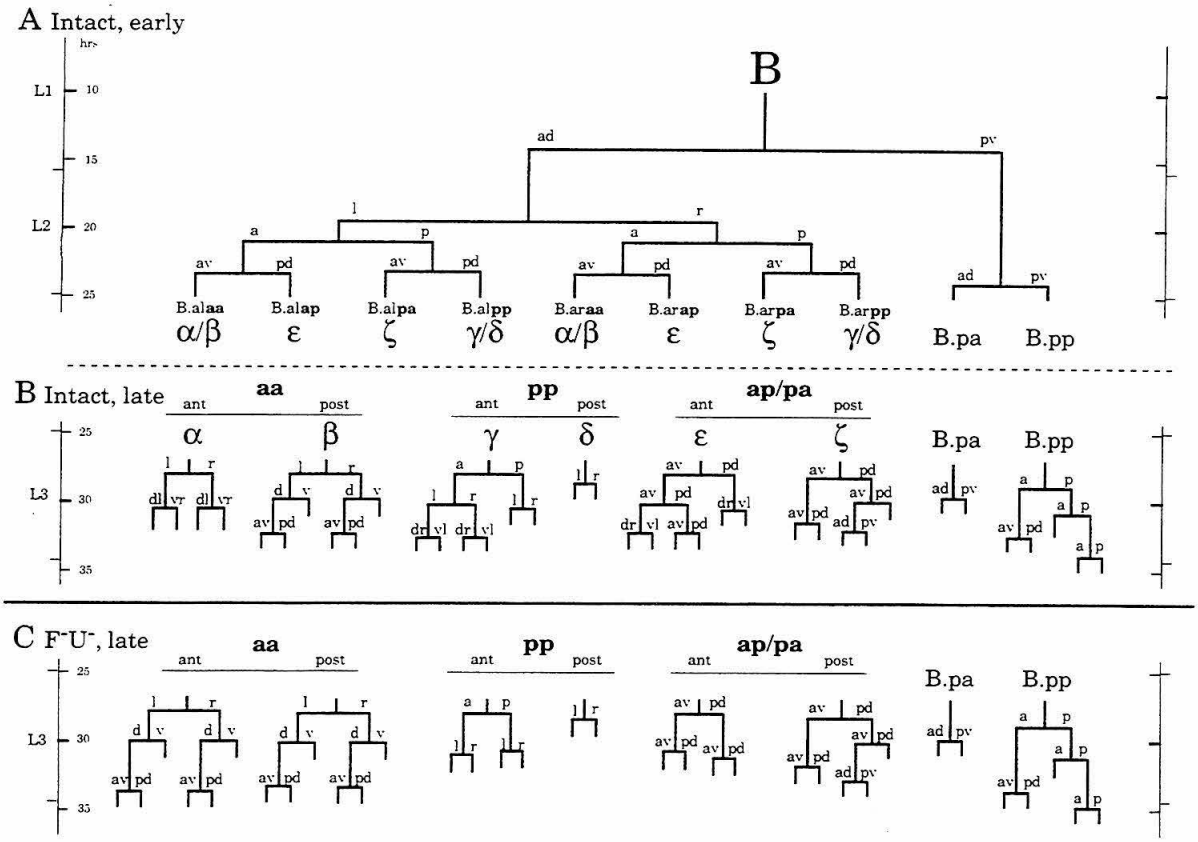

Fig. 2. B lineage in intact and ablated animals. Division axes are shown: anterior (a), posterior (p), left (l), right (r), dorsal (d), ventral (v). (A) Early divisions in the intact male. Lineage chart constructed from lineage and observation of anatomy of intact *him-5(e1490)* males. (B) Late divisions in the intact male. Lineage chart constructed from unperturbed lineages observed in B.p⁻, F⁻, or U⁻ animals. (C) Late divisions in F⁻U⁻ animal. Lineage chart constructed from F⁻U⁻ animal 128 (see Table 1B.1). Note that lineages of presumptive α, γ, and ε cells (anterior cells: ant) are disrupted. Early B divisions are normal in ablated animals. Division axes and time scale are standardized to Sulston et al. (1980). Cell deaths and terminal fates are not shown. The terminal fates are indicated in Table 8. The α, β, δ and ζ lineage patterns are all distinct. γ and ε lineages both produce 6 progeny in a 2+4 pattern (2 progeny from one daughter, 4 from the other), but they differ in division axes. β lineages produce 6 progeny in a 3+3 pattern.

through L3 molt, cells generally were not followed to the time when programmed cell deaths are observed in intact animals. Since the fates of B.p progeny were not disrupted by any ablation, we have used the timing of B.p divisions to correlate abnormal to normal lineages. Outside of the B progeny, the divisions of P11.p, P10.p and P9.p provide additional non-disrupted timepoints. Although complete lineages were not obtained for these cells, we generally followed at least two division cycles in these cells.

Laser killing of cells was performed by the method of Sulston and White (1980), using the laser microbeam system and procedure of Avery and Horvitz (1987) and Sternberg (1988), except that the laserbeam was passed through a circular variable attenuator (gift of Newport Corporation), and animals were anesthetized on pads of 5% agar in water, containing 5 μM (rather than 10 μM) sodium azide. In general, F, U, and Y.p were ablated at the stage when B had divided to produce two cells (late L1 or early L2 stage). B.a progeny were ablated during mid to late L2 stage, generally soon after the targeted cells were generated. Many animals required ablation of cells at different stages of development. In these cases the animals underwent two to three rounds of anesthetic, surgery, recovery, and development. In all cases, animals that appeared unhealthy following treatment were discarded. The ablation of the targeted cells was confirmed the following day when the animals were prepared for lineage analysis.

For technical reasons, surgeries were performed on one day, and lineages followed the next. At 20°C only 5-10 hours separate the time of surgery and the re-initiation of B.a(l/r)xx cell division. Consequently, after a recovery and development period (1-6 hours, depending on the nature of the surgery) animals were shifted from 20°C to 10°C overnight to slow further development. Such shifts could disrupt temperature-sensitive aspects of the cell interactions or other developmental processes. However, (1) all of the experimental animals were treated the same, so our experiments are internally consistent, (2) normal lineages are observed in many ablation backgrounds (e.g., B.p⁻, see Table 1C.2), and (3) many ablations specifically disrupt a subset of fates, with the remaining fates essentially normal. Because normal lineages are possible, and

abnormal lineages are specific to certain ablation backgrounds, we believe the temperature shift does not fundamentally affect our results. All lineages were observed at 20°C.

RESULTS

Summary of spicule development

The spicules are a pair of structures that flank the male cloaca (Fig. 1). Electron microscope reconstruction by Sulston et al. (1980) indicate that the two spicules are sensilla covered with a hard, sclerotic cuticle. Each spicule includes two presumptive sensory neurons. Each neuron has a process that runs down the length of the spicule to the tip, where it is open to the environment. The neuronal processes are surrounded by socket and sheath cells, which provide structure and support. Each spicule is anchored by extensor and retractor muscles. During copulation the male inserts his spicules into the hermaphrodite vulva, where they anchor the male cloaca at the vulval opening and possibly aid in sperm transfer.

The B cell divides to produce all of the cells of the spicules. The B cell lineage consists of three distinct stages: early divisions, migration, and late divisions (Figs 2, 3 and 4; original observations of Sulston and Horvitz, 1977; Sulston et al., 1980). The early divisions take place primarily in the second larval stage (L2), and result in 10 progeny. A short range migration of the eight B.a progeny occurs in the late L2 stage. The late round of divisions begin in the mid-L3 stage, ultimately producing 47 progeny. The first division of B is asymmetric and along an approximately anterior/posterior axis (Figs 3A, 4A). The larger anterior cell (B.a) is the precursor of all cells of the spicules. B.a divides along a left/right axis, establishing the bilateral symmetry of the spicules. This symmetry is broken (during migration) and then re-established (during the late divisions) in the progeny of these cells. B.al and B.ar each divide twice to produce a ring of eight cells, one cell thick (Fig. 3D). These cells then migrate. The medial migration of the two dorsal (**pp**) and ventral (**aa**) cells exhibits natural variation. In both cases, either the right or the left cell can adopt the more anterior position, while the other cell adopts the more posterior position. The cell that adopts the anterior position will produce a different lineage than the cell that adopts the posterior position (compare anterior α and γ lineages to posterior β and δ lineages in Fig. 2). The other four B.a progeny migrate invariantly. The **ap** cells adopt the anterior position (one on the left, one on the right), and the **pa** cells adopt the posterior position. The end result is a ring of cells, two cells thick (Figs 3E, 4B). It is convenient to refer to animals with the B progeny in this configuration as the '10 cell stage,' since in addition to the eight progeny of B.a, B.p has divided once.

The **aa** and **pp** pairs represent equivalence groups (Sulston and Horvitz, 1977; Sulston et al., 1980). In general, cells in an equivalence group have equivalent potential, but they adopt different fates after interacting with each other, after receiving positional cues, or both. Because of the variability from animal to animal, one must distinguish between lineal ancestry and fate choice when referring to cells in an equivalence group. Within the **aa** pair, the fate of the ante-

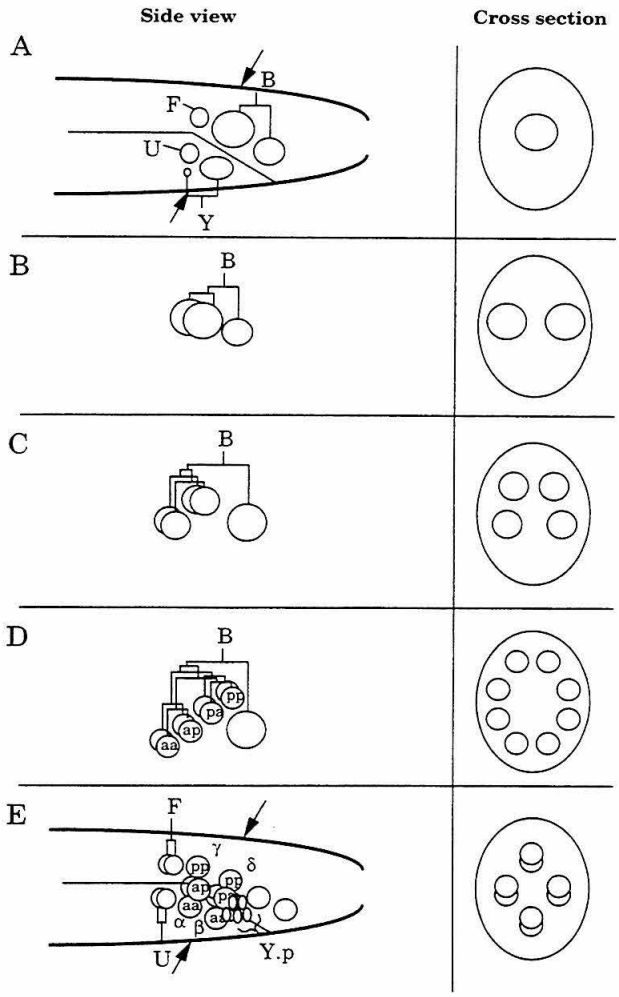

Fig. 3. The early divisions of B. Left lateral view and cross section of B.a at one (A), two (B), four (C), eight (D) cell stage, and after migration (E). Arrows indicate approximate plane of cross section. B.a divides along a left-right axis to initially establish the bilateral symmetry of the spicules (B). B.a produces eight progeny that form a ring of cells, a single cell thick (D). These cells migrate to form a ring of cells, two cells thick (E). It is convenient to refer to animals with the B progeny in this configuration as the '10 cell stage' since in addition to the eight progeny of B.a, B.p has divided once. F, U, and Y.p also divide during the early B divisions. F and U lie anterior to B, dorsal and ventral to the rectum, respectively (A). These cells divide once during the early B lineage, so that when the B.a(l/r)xx cells have migrated to their anterior/posterior positions, F.l and F.r lie next to presumptive γ, and U.l and U.r lie next to presumptive α (E). These cells further divide during the late B divisions (Sulston et al., 1980). Y divides asymmetrically prior to the first division of B, producing a neuron and the Y.p blast cell (A). During the early B lineage, Y.p divides once to establish left/right symmetry. Further divisions result in a cluster of five cells on each side. The position of these clusters is more dorsal and lateral than the original position of Y (E).

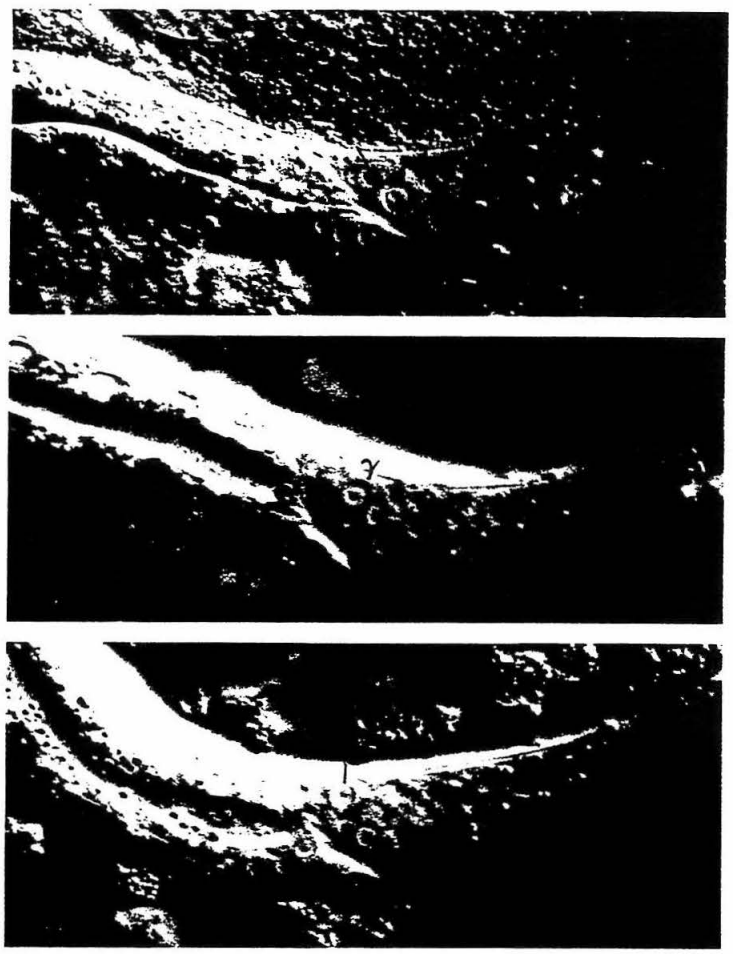

Fig. 4. Nomarski photomicrographs of early divisions of the B cell. (A) Arrangement of male-specific blast cells in the early L2 stage male. B and Y have each divided once (compare with Fig. 3A). (B1.2) Arrangement of B progeny at the 10 cell stage in early L3 male, medial (B.1) and left lateral (B.2) view of same animal. F and U have each divided once. Division of Y.p(l/r)pp is the final cell division in the Y lineage. The remaining Y.pl progeny are slightly ventral, anterior, and lateral to the dividing Y.plpp cell (compare with Fig. 3E). Scale bar, 20 μm.

rior cell is termed α, and the posterior cell, β. Likewise, the anterior cell in the **pp** pair is γ, and the posterior cell, δ (Sulston and Horvitz, 1977). Sulston and White (1980) showed that after ablation of either the left or right **aa** cell, the remaining **aa** cell will migrate to the midline and produce an α lineage. This establishes the α fate as primary (1°) and the β fate as secondary (2°). Even if the targeted cell is migrating to the anterior position when it is ablated, the remaining cell will produce an α lineage, indicating either that position promotes the α fate, or that the fate of the **aa** cell is still flexible after migration is initiated. Similar experiments with the **pp** cells could not establish 1° or 2° fate in the γ/δ pair.

We will show (Section II.B) that the **ap** and **pa** cells also respond to positional cues. In intact animals the fates of these cells are invariant. Our experiments indicate that there are distinct anterior and posterior fates, normally associated with the **ap** and **pa** cells, respectively. To distinguish between lineal ancestry and fate choice for these cells, we have assigned the Greek letter ε to the lineage normally associated with the **ap** cells (the 'anterior' fate), and the letter ζ to the lineage normally associated with the **pa** cells (the 'posterior' fate).

For the B.a(l/r)xx cells, lineal heritage, cell position, and fate (subsequent lineage) are distinct characteristics. There are thus three distinct groups of B.a progeny. The **aa** cells represent one group, the ventral pair. Likewise, the **pp** cells comprise the dorsal pair. There are two lateral pairs (one left, one right), each comprising one **ap** and one **pa** cell. We consider the two laterally symmetrical pairs to be identical. We refer to the ventral, lateral, and dorsal groups as the **aa**, **ap/pa**, and **pp** pairs, respectively. Within each group there is a distinct anterior and a distinct posterior position. For instance, in intact animals the **ap** cells always adopt the anterior position in each lateral group. Finally, there are six distinct fates (α, β, γ, δ, ε, ζ). γ indicates the lineage normally associated with the anterior cell in the dorsal group. Experimentally, however, this lineage is not restricted to the anterior position, although it *is* restricted to the dorsal group. Since these names represent observed

lineages as well as conceptual fates, we use the term γ *lineage* when referring to an observed result (literally, a γ-like lineage), and γ *fate* when discussing interpretations. We use the term 'presumptive γ' to refer to the cell in the anterior position of the dorsal group, regardless of the lineage.

Interpretation of cell lineage data

The fate of precursor cells includes the cell lineage (number, timing and axes of divisions) and the terminal differentiated fates of the progeny produced by those divisions. Although both criteria are important to understanding the fate of a precursor cell, we have focused on the first. In the B lineage, the precise differentiated fates of the progeny are difficult to assess because (1) many of the precursors produce a similar set of differentiated cell types, (2) nuclear morphology (often used to identify neurons) is difficult to establish unambiguously due to the dense packing of cells in the tail, and (3) we currently lack suitable molecular markers that distinguish among the fates. In other analyses of postembryonic development in *C. elegans*, cell lineage has proved to be an accurate indicator of fate choice (e.g., Horvitz et al., 1983; Kenyon, 1986; Sternberg and Horvitz, 1986). In addition, we have observed transformation of terminal differentiation (cell death) in two animals where **pa** produced ε lineages (data not shown). In this example, the transformation of cell lineage correlates with the transformation of differentiated cell fate. Thus, we believe that the number, timing, and axes of cell divisions generally reflect fate choice for a postembryonic blast cell. We cannot rule out the hypothesis that the terminal fates are distinct from the cell lineage, or that fate transformations are from B progeny to another cell type. However, we have limited our discussion to transformations within B cell fates. For simplicity, we initially hypothesize that the choice of α versus β, γ versus δ, and ε versus ζ fates is specified in the B.a(l/r)xx cells. We discuss the validity of these assumptions at the end of each sub-section of Section III.

We often observe full transformation from one fate to another following cell ablation. However, we also observe a variety of abnormal lineages, and we have named the common ones (Fig. 5). A rigorous interpretation of the fate represented by abnormal lineages is sometimes difficult. In these cases we use the number of progeny as an indicator of fate as it is the most objective of cell lineage criteria. In the **pp** pair, for instance, we refer to the production of more progeny as reflecting an anterior-like (γ) state, and the production of fewer progeny a posterior-like (δ) state. We rely on the criterion of progeny number only in cases where the

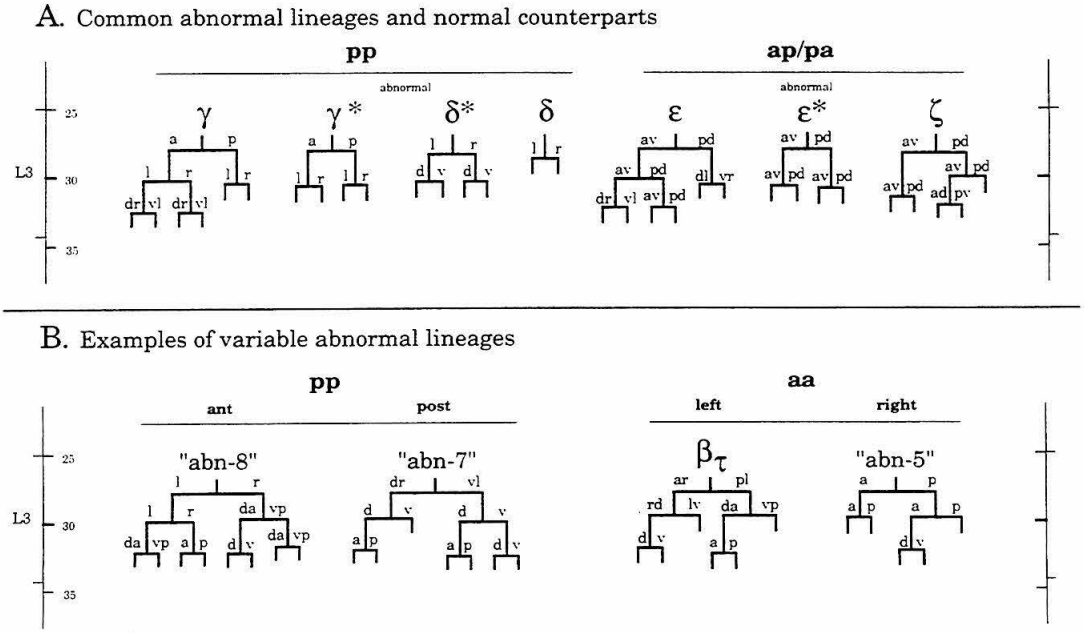

Fig. 5. Abnormal lineages observed in experimental animals. (A) Common abnormal lineages. Three abnormal lineages have been observed frequently and thus have been named (γ*, δ*, ε*). γ* and δ* are both intermediate between γ and δ, but differ in the division axes. γ, δ, ε, and ζ lineages are from Fig. 2. Timing of divisions in abnormal lineages can be variable. The lineage charts for γ*, δ*, ε* were constructed from abnormal lineages observed in three or more affected animals. (B) Examples of other abnormal lineages. **pp** lineages from **aa**⁻**ap**⁻**pa**⁻ animal 275 (Table 1E.1). These lineages are representative of 'abnormal proliferative' lineages. The axes of division vary from animal to animal. **aa** lineages from F⁻U⁻Y.p⁻ animal 203 (Table 1G). In this animal, the two **aa** cells remained left/right. The left **aa** cell illustrates β_τ. Although the axes are different, the division pattern is the same as a normal β lineage (3+3: compare with Fig. 2). We consider β_τ lineages to be similar to normal β lineages. The right **aa** cell is an example of an abn-5 lineage. Regardless of division axes, any cell that produces 5 progeny in a 2+3 pattern is considered abn-5.

observed lineages do not clearly suggest a fate. Consideration of the data in these terms has allowed the extraction of general interpretations from ablations that result in a mixture of abnormal lineages.

Table 1 includes the results from the 150 B lineages that we have followed in this study. Much of the data from this Table are also summarized in simpler Figures and Tables. We have divided the Results into four sections. First, we characterize interactions of the other male-specific blast cells (F, U, Y) on the fate of B.a progeny. This identifies anterior (from F/U) and posterior (from Y) positional cues that promote anterior and posterior fates. Second, we characterize the extracellular cues that the other B.a progeny provide for each of the three cell pairs. In general, the other B.a progeny act to promote posterior fate within each pair, providing a distinct posterior positional cue. Third, we investigate the relationship among the identified cues. These experiments allow us to establish modulatory and antagonistic relationships among the cues. We also investigate lateral interactions between cells in each pair, the 'ground state' within each pair, and the possible units of fate specification for each pair. Fourth, we consider what may distinguish the cells in one pair from the cells in another.

I. The male-specific blast cells (F, U, and Y) provide positional cues for the three B.a progeny groups

We examined the effect of the other male-specific blast cells on the B progeny because they are close neighbors. The results of these ablations are summarized in Fig. 6. In intact animals, F and U lie anterior to B, such that F is dorsal and U is ventral to the rectum (see Figs 3, 4). Y lies ventral to B, but the progeny of Y.p are situated more dorsal and posterior than the precursor. Thus, F, U, Y.p and their progeny are positioned anterior or posterior, and immediately next to the B cell and its progeny.

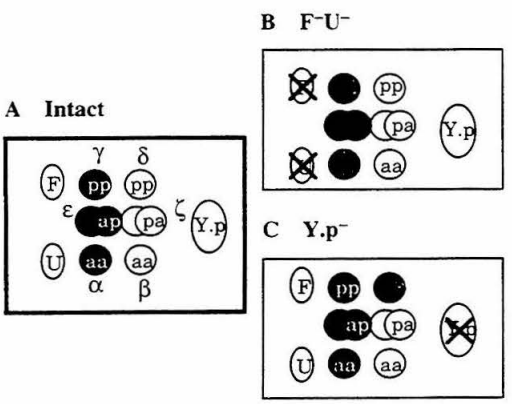

Fig. 6. Illustration of effects of F/U and Y.p ablation on the fates of the eight B.a progeny. F, U, Y.p are drawn to show the approximate position of F, U, and Y.p progeny when B is at the 10 cell stage. B.p progeny are not included in this Figure. Anterior is to the left, ventral down. (A) Intact. Black circles indicate anterior fates. White circles indicate posterior fates. (B) Ablation (indicated by X) of F and U disrupts anterior fates γ, ε, and α (shaded circles). In some animals presumptive γ (the anterior **pp** cell) is transformed to δ and presumptive α is transformed to β. (C) Ablation of Y.p disrupts posterior fate, δ (shaded circles). Data of Table 1B,C.

A. F and U promote anterior fates

Ablation of F and U disrupts the lineages of presumptive α, γ, and ε, but not β, δ and ζ (summarized in Fig. 6B; data summary in Table 2; photographs in Fig. 7; data of Table 1B). Presumptive α cells (i.e., the positionally anterior **aa** cell) produce extra divisions, in many cases with the timing, axes, and number of divisions similar to those seen in a normal β lineage. Specifically, presumptive α lineages were abnormal in all seven animals examined, and in 3/7 animals the presumptive α cells produced a complete β-like lineage. Presumptive γ cells, which normally produce six progeny, produce truncated lineages. 3/7 produced four progeny, and 2/7 produced only two. Although the timing and axis of the single division of these cells was slightly irregular, we provisionally interpret these as γ to δ transformations. Finally, 3/7 animals (5/14 sides) had truncated ε lineages (ε*, and one abn-5). In these animals the **ap** cells produced four rather than the usual six progeny.

The strong disruption of anterior fates is only apparent if both F and U are ablated (Table 1B.2,3). Ablation of only F or U results in essentially normal lineages. One abnormal γ lineage was observed in four F^- animals, and one abnormal α lineage was observed in four U^- animals. In these cases, the fate is disrupted in the anterior cell that is normally closest to the progeny of the ablated cell. Thus, F and U are partially redundant in their ability to promote anterior fate. In addition, the lineage disruption is specific to the absence of F and U, and is not likely the non-specific result of cell damage or nearby debris.

Although F/U ablation disrupts anterior fates for all four pairs of cells, the results are variable, and the extent of the defect is not the same for the different pairs. Nevertheless, fates of the anterior cells but not fates of the posterior cells are disrupted in these animals. We conclude that F and U, or their progeny, are necessary to promote normal anterior fates in each of the four pairs of cells. In the absence of F and U, these cells produce abnormal lineages, and in some cases adopt the fate normally associated with their posterior neighbors.

B. Y.p promotes some posterior fates

Ablation of Y.p disrupts presumptive δ lineages (summarized in Fig. 6C; data summary in Table 2; photographs in Fig. 8; data of Table 1C). Presumptive δ cells divide an extra round, producing up to four progeny. Although the number of progeny increases, and thus might be considered a partial transformation of δ to γ fate, the first division retains the axis and approximate timing associated with the δ lineage. Therefore, we conclude only that Y.p is required for proper δ fate. The fate of the presumptive β cell was disrupted in 1/7 $Y.p^-$ animals examined, which may reflect a minor role for Y.p in the **aa** pair (see Section III.A.1). However, Y.p is not sufficient to specify β (see Section II.A). No abnormalities in the lineages of the anterior cells (α, γ, or ε) were observed (7 animals (6 for ε)), nor was the ζ lineage disrupted (12 sides).

The progeny of B.p lie posterior to the four pairs of B.a

Table 1. Summary of lineages observed in experimental animals

		aa		pp		ap		pa		
	animal no.	Ant	Post	Ant	Post	Left	Right	Left	Right	B.p(a/p)
A.	**Intact**	α	β	γ [s]	δ [t]	ε	ε	ζ	ζ	normal (n)
	no. of progeny	4	6	6	2	6	6	5	5	
B. (1)	**F⁻U⁻**									
	124	abn-5	β	δ_τ	δ	ε	ε			n
	125	abn-5	β	δ	δ	ε	ε			n
	128	β	β	γ*	δ	ε*	ε*			n
	144	β	β	γ*	δ	ε	abn-5			n
	148	abn-5	β	γ	δ	ε*	ε*			n
	151	β	β	γ*	δ	ε	ε			n
	216	β_τ (l)	abn-5 (r)	γ	δ	ε	ε			n
(2)	**F⁻**									
	142	α	β	γ*	δ	ε	ε			n
	145	α	β	γ	δ	ε	ε			n
	146	α	β	γ	δ	ε	ε			n
	152	α	β	γ	δ	ε	ε			n
(3)	**U⁻**									
	140	abn-5	β	γ	δ	ε	ε			n
	141	α	β	γ	δ	ε	ε			n
	143	α	β	γ	δ	ε	ε			n
	155	α	β	γ	δ	ε	ε			n
C. (1)	**Y.p⁻**									
	166	α	β	γ	δ*	n.d.	n.d.	n.d.	n.d.	n
	172	α	β_τ	γ	δ*	ε	ε			n
	175	α	abn-5	γ	abn-3 [s]	ε	ε			n
	177	α	β	γ	abn-3 [s]	ε	ε			n
	179	α	β	γ	abn-3 [o]	ε	ε			n
	224	α	β	γ	δ	ε	ε			n
	227	α	β	γ	δ*	ε	ε			n
(2)	**B.p⁻**									
	479	α	β	γ	δ	ε	ε			----
	480	α	β	γ	δ	ε	ε			----
	481	α	β	γ	δ	ε	ε			----
(3)	**Y.p⁻ B.p⁻**									
	167	α	β	γ	abn-3 [s]	ε	ε			----
	170	α	β	γ	δ*	ε	ε			----
	171	n.d.	n.d.	γ	δ*	n.d.	n.d.	n.d.	n.d.	----
D. (1)	**B.a(l/r)ap⁻ B.a(l/r)pa⁻ B.a(l/r)pp⁻**									
	243	α	α	----	----	----	----	----	----	n
	244	α	α	----	----	----	----	----	----	n
	245	α	α	----	----	----	----	----	----	n
	246	α	β	----	----	----	----	----	----	n
	445	α	abn-5	----	----	----	----	----	----	n
	450	α	α	----	----	----	----	----	----	n
(2)	**B.a(l/r)p⁻**									
	241	α	β	----	----	ε	ε	----	----	n
	242	α	β	----	----	ε	ε	----	----	n
(3)	**B.a(l/r)ap⁻ B.a(l/r)pp⁻**									
	247	α	β	----	----	----	----		ε	n
	279	α	β	----	----	----	----	n.d.	n.d.	n
	280	α	abn-5	----	----	----	----	n.d.	n.d.	n
E. (1)	**B.a(l/r)aa⁻ B.a(l/r)ap⁻ B.a(l/r)pa⁻**									
	261	----	----	abn-5 (l) [s]	abn-7 (r) [s]	----	----	----	----	n
	262	----	----	abn-8 (lv) [s]	abn-4 (rd) [s]	----	----	----	----	n
	275	----	----	abn-8 [t]	abn-7 [o]	----	----	----	----	n

Table 1. Continued

	animal no.	aa Ant	aa Post	pp Ant	pp Post	ap Left	ap Right	pa Left	pa Right	B.p(a/p)
(2)	**B.a(l/r)ap⁻ B.a(l/r)pa⁻**									
	234	α	β_τ	abn-7 [o]	δ	----	----	----	----	n
	235	α	β	abn-7 [s]	abn-7 [s]	----	----	----	----	n
	388	α	β	abn-8 [s]	abn-3 [o]	----	----	----	----	n
	402	α	β	abn-8 [s]	δ	----	----	----	----	n
	478	α	β	abn-6 [s]	δ*	----	----	----	----	n
(3)	**B.a(l/r)a⁻**									
	238	----	----	γ	δ	----	----	ε	ε	n
	239	----	----	γ	δ	----	----	ε	ε	n
	240	----	----	γ	δ	----	----	ε	ε	n
	389	----	----	γ	δ	----	----	ε	abn-4	n
	454	----	----	γ	δ	----	----	ε	ε	n
(4)	**B.a(l/r)aa⁻ B.a(l/r)pa⁻**									
	256	----	----	γ* (r)	δ (l)	ε	ε	----	----	n
	258	----	----	γ*	δ	ε	ε	----	----	n
	259	----	----	γ*	δ	ε	ε	----	----	abn (.pp->.pa)
	260	----	----	γ*	δ	(ε)	ε	----	----	n
(5)	**B.a(l/r)pa⁻**									
	282	α	β	δ (l)	δ (r)	ε	ε	----	----	n
	283	α	β	γ*	δ	ε	ε	----	----	n
	284	α	β	γ	δ	ε	ε	----	----	n
	285	α	β	γ*	δ	ε	ε	----	----	n
	390	α	β	δ	δ	ε	ε	----	----	n
F. (1)	**B.a(l/r)a⁻ B.a(l/r)pp⁻**									
	251	----	----	----	----	----	----	ε	ε	n
	252	----	----	----	----	----	----	ε	ε	n.d.
	257	----	----	----	----	----	----	ε	ε	n
	399	----	----	----	----	----	----	ε	ε	n
(2)	**B.a(l/r)p⁻ B.a(l/r)aa⁻**									
	422	----	----	----	----	ε*	ε	----	----	n
	423	----	----	----	----	ε	ε	----	----	n
G.	**F⁻ U⁻ Y.p⁻**									
	161	α_τ (l)	β_τ (r)	γ	δ*	ε*	ε*	ε	ε	n
	181	abn-5 (l)	abn-7 (r)	γ	δ*	ε	ε	ε	ε	n
	182	β_τ (l)	α_τ (r)	abn-5 [s]	abn-3 [o]	ε*	ε*	ε	ε	n
	187	α	β	γ	δ	ε*	ε	ε	ε	n
	203	β_τ (l)	abn-5 (r)	γ	abn-3 [t]	abn-5	ε*	ε	ε	n
H. (1)	**F⁻ U⁻ B.a(l/r)ap⁻ B.a(l/r)pa⁻ B.a(l/r)pp⁻**									
	395	abn-5	β_τ	----	----	----	----	----	----	n.d.
	397	α_τ (r)	β_τ (l)	----	----	----	----	----	----	n
	400	$\beta\tau$	β_τ	----	----	----	----	----	----	n
	415	abn-5 (l)	abn-5 (r)	----	----	----	----	----	----	n
	449	α_τ (r)	β_τ (l)	----	----	----	----	----	----	n
	453	α_τ (r)	abn-7 (l)	----	----	----	----	----	----	n
(2)	**F⁻ U⁻Y.p⁻ B.a(l/r)ap⁻ B.a(l/r)pa⁻ B.a(l/r)pp⁻**									
	406	α_τ (r)	β_τ (l)	----	----	----	----	----	----	n
	416	α	β_τ	----	----	----	----	----	----	n
	444	α_τ (l)	abn-6 (r)	----	----	----	----	----	----	n
	448	α_τ (l)	β_τ (r)	----	----	----	----	----	----	n
	455	α_τ (l)	abn-5 (r)	----	----	----	----	----	----	n
	458	α_τ (l)	abn-5 (r)	----	----	----	----	----	----	n

Table 1. Continued

	animal no.	aa Ant	aa Post	pp Ant	pp Post	ap Left	ap Right	pa left	pa right	B.p(a/p)
I. (1)	**Y.p⁻ B.a(l/r)pa⁻**									
	446	α	β	γ	δ^{w}	ε	ε	----	----	n
	447	α	abn-5	γ	δ^{w}	ε	ε	----	----	n
	451	α	β_{τ}	γ	abn-5 [t]	ε	ε	----	----	n
	452	α	β_{τ}	γ	δ^{w}	ε	ε	----	----	n
(2)	**F⁻ U⁻ B.a(l/r)pa⁻**									
	286	abn-6	β_{τ}	δ	δ^{w}	ε	ε^{w}	----	----	n
	391	α	abn-5	δ	δ	ε	ε	----	----	n
	392	α	β	abn-3 [s]	δ	ε	ε	----	----	n
	393	abn-5	β	δ (l)	δ (r)	ε^{w}	ε	----	----	n
	394	α	β	γ^{w}	δ	abn-5	ε^{w}	----	----	n
	396	abn-5	β	γ^{w}	δ^{w}	ε	ε	----	----	n
(3)	**F⁻ U⁻ B.a(l/r)ap⁻ B.a(l/r)pa⁻**									
	263	abn-5	abn-6	γ^{w}	δ	----	----	----	----	n
	264	α	β_{τ}	γ^{w}	δ	----	----	----	----	n
	398	abn-5 (l)	α_{τ} (r)	γ_{τ} (r) [s]	γ^{w} (l)	----	----	----	----	n
	412	α	β	γ_{τ}	δ	----	----	----	----	n
(4)	**F⁻ U⁻ B.a(l/r)aa⁻ B.a(l/r)ap⁻ B.a(l/r)pa⁻**									
	417	----	----	γ	γ^{w}	----	----	----	----	n
	420	----	----	γ^{w}	δ	----	----	----	----	n
(5)	**F⁻ U⁻ Y.p⁻ B.a(l/r)pa⁻**									
	405	abn-5 (l)	β_{τ} (r)	γ_{τ} (l) [t]	$\delta^{w}{}_{\tau}$ (r) [o]	ε^{w}	ε^{w}	----	----	n
	407	abn-6	abn-5	γ	δ^{w}	ε	ε^{w}	----	----	n
	456	α	β_{τ}	γ	δ^{w}	abn-5	ε^{w}	----	----	n
(6)	**F⁻ U⁻ Y.p⁻ B.a(l/r)ap⁻ B.a(l/r)pa⁻**									
	413	α	β_{τ}	γ^{w}	abn-6 [s]	----	----	----	----	n
	414	α	β_{τ}	γ	abn-5 [t]	----	----	----	----	n
	418	α_{τ} (l)	abn-5 (r)	abn-7 (r) [s]	abn-5 (l) [o]	----	----	----	----	n
	421	β_{τ} (l)	β_{τ} (r)	abn-7 [t]	δ^{w}	----	----	----	----	n
(7)	**F⁻ U⁻ Y.p⁻ B.a(l/r)aa⁻ B.a(l/r)ap⁻ B.a(l/r)pa⁻**									
	419	----	----	abn-6 [s]	abn-6 [o]	----	----	----	----	n
J. (1)	**F⁻ U⁻ B.a(l/r)p⁻**									
	253	α	abn-7	----	----	ε	ε^{w}	----	----	n
	254	α	β	----	----	ε^{w}	ε	----	----	n
(2)	**F⁻ U⁻ Y.p⁻ B.a(l/r)a⁻**									
	404	----	----	abn-6 (l) [o]	γ_{τ} (r) [o]	----	----	[illegible]	[illegible]	n
	409	----	----	γ	δ^{w}	----	----	[illegible]	[illegible]	n
	410	----	----	γ	δ^{w}	----	----	[illegible]	[illegible]	n
	411	----	----	γ (l)	$\delta^{w}{}_{\tau}$ (r)	----	----	[illegible]	[illegible]	n
	459	----	----	γ	abn-3 [s]	----	----	[illegible]	[illegible]	n
(3)	**F⁻ U⁻ Y.p⁻ B.a(l/r)p⁻**									
	461	n.d.	n.d	----	----	ε	ε	----	----	n
K. (1)	**B.a(l or r)⁻**									
	147 (l⁻)	α		γ		ε		[illegible]		n
	157 (l⁻)	α		δ		n.d.		n.d.		n
	164 (l⁻)	α		γ		ε		[illegible]		n
	153 (r⁻)	α		γ		ε		[illegible]		n
	156 (r⁻)	abn-5		γ		ε		[illegible]		n

Table 1. Continued

	animal no.	aa Ant	aa Post	pp Ant	pp Post	ap Left	ap Right	pa Left	pa Right	B.p(a/p)
(2)	**F⁻ U⁻ B.a(l or r)⁻**									
	168 (l⁻)	β_τ		δ		ε^*		abn-4		n
	180 (l⁻)	β_τ		δ^*		ε		ζ_τ		n
	159 (r⁻)	abn-6		γ^*		ε^*		ζ		n
	160 (r⁻)	β_τ		δ		ε		ζ		n
	162 (r⁻)	β_τ		δ		ε		ζ		n
	165 (r⁻)	β_τ		δ		abn-5		ζ		n
	169 (r⁻)	β		δ		ε		ζ		n
(3)	**F⁻ U⁻ Y.p⁻ B.a(l or r)⁻**									
	174 (l⁻)	β_τ		γ^*		ε		ζ		n
	213 (l⁻)	β_τ		abn-3 [s]		ε		ζ		n
	173 (r⁻)	α_τ		γ^*		ε		ζ		n
	176 (r⁻)	β_τ		δ^*		ε		ζ		n
L. (1)	**F⁻ U⁻ Y.p⁻ B.a(l/r)p⁻ B.a(l or r)a⁻ B.a(l or r)ap⁻**									
	457 (l⁻)	α_τ		----		----		----		abn (damaged)
	460 (l⁻)	α_τ		----		----		----		n
	463 (l⁻)	β_τ		----		----		----		n
	464 (r⁻)	α_τ		----		----		----		n
	465 (r⁻)	α_τ		----		----		----		n
(2)	**F⁻ U⁻ Y.p⁻ B.a(l/r)a⁻ B.a(l or r)p⁻ B.a(l or r)pa⁻**									
	466 (l⁻)	----	----	abn-5 [s]		----		----		n
	467 (l⁻)	----	----	γ_τ [s]		----		----		n
	468 (l⁻)	----	----	abn-6 [s]		----		----		n
	469 (r⁻)	----	----	γ_τ [s]		----		----		n
	462 (r⁻)	----	----	abn-8 [s]		----		----		abn (damaged)
(3)	**F⁻ U⁻ Y.p⁻ B.a(l/r)a⁻ B.a(l/r)pp⁻**									
	470	----	----	----	----	----	----	ζ	ζ	n
	471	----	----	----	----	----	----	ζ	ζ	n
	472	----	----	----	----	----	----	ζ	ζ	n
	473	----	----	----	----	----	----	ζ	ζ_τ	n
(4)	**F⁻ U⁻ Y.p⁻ B.a(l/r)p⁻ B.a(l/r)aa⁻**									
	474	----	----	----	----	ε_τ	ε_τ	----	----	n
	475	----	----	----	----	ε_τ	abn-7	----	----	n
	476	----	----	----	----	ε_τ	abn-8	----	----	n
	477	----	----	----	----	ε_τ	ε_τ	----	----	n

Each line represents the observed lineages for one animal. Each animal is identified by a unique number. A dash indicates that the cell was ablated in that animal. n indicates normal B.p lineage. n.d. indicates that the cell was present, but not followed to completion of the lineage. abn-*n* indicates that the lineage was abnormal, but produced *n* progeny. If the cells of a pair failed to migrate, the side is indicated in parentheses (e.g., left is (l), left ventral is (lv)). Canonical γ^*, δ^*, and ε^* lineages, and examples of some other abnormal lineages, are illustrated in Fig. 5. The number of progeny produced by each cell in the inact animal is listed in Section A for reference. For ablations in Sections K and L we have grouped bilaterally symmetrical experiments together, and indicate the actual cell ablated in parentheses after the number of the animal (e.g., in K.1 147(l⁻) is B.al⁻).

Cell lineages are characterized by the number, axes, and timing of divisions. Of these, number of divisions is the most objective criterion. Our analyses suggest that although first division axis may reflect cell state in the **pp** pair, it appears not to be predictive in the **aa** pair (it was never fundamentally disrupted in the **ap/pa** pairs). Thus, we have utilized the symbol τ (as in β_τ), which indicates that the cell division pattern was topologically that lineage, although the division axes were skewed. For instance, if an **aa** cell fails to migrate, it will often divide along an anterior/posterior axis. However, if both anterior and posterior cells produce three progeny, the lineage is β_τ because of the 3+3 pattern of progeny, regardless of the division axis (illustrated in Fig. 5). Similarly, γ_τ indicates a 4+2 pattern of progeny, but abnormal division axes. Note that all lineages within a class of lineages (e.g., abn-5) are topologically the same (2 progeny from one daughter, 3 from the other, or 2+3; see Fig. 5). Specifically, abn-3 are (1+2), abn-4 (2+2), abn-5 (2+3), abn-6 (3+3 in a **pp** lineage, 2+4 in an **aa** lineage), abn-7 (3+4), abn-8 (4+4). In general, abnormal lineages from cells that migrate properly retain the axes associated with the normal lineage (l-r, or transverse [t] for presumptive α, β, and δ, a-p or sagittal [s] for presumptive γ, av-pd for presumptive ε and ζ). If **aa** cells fail to migrate, the initial axis is generally a-p. If many B.a neighbors are absent, an **ap** or **pa** axis is often a-p. Although it is not fully predictive, the first axis of division differs in γ lineages compared with δ lineages, and it may reflect cell state in the **pp** pair. Because the cell lineage can be variable, this axis is indicated for the abnormal **pp** cell lineages in brackets. [s] sagittal, [t], transverse, [o], oblique. γ and γ^* lineages are [s], δ and δ^* lineages are [t]. Not every division of the left **ap** lineage was observed in G.4, animal 260.

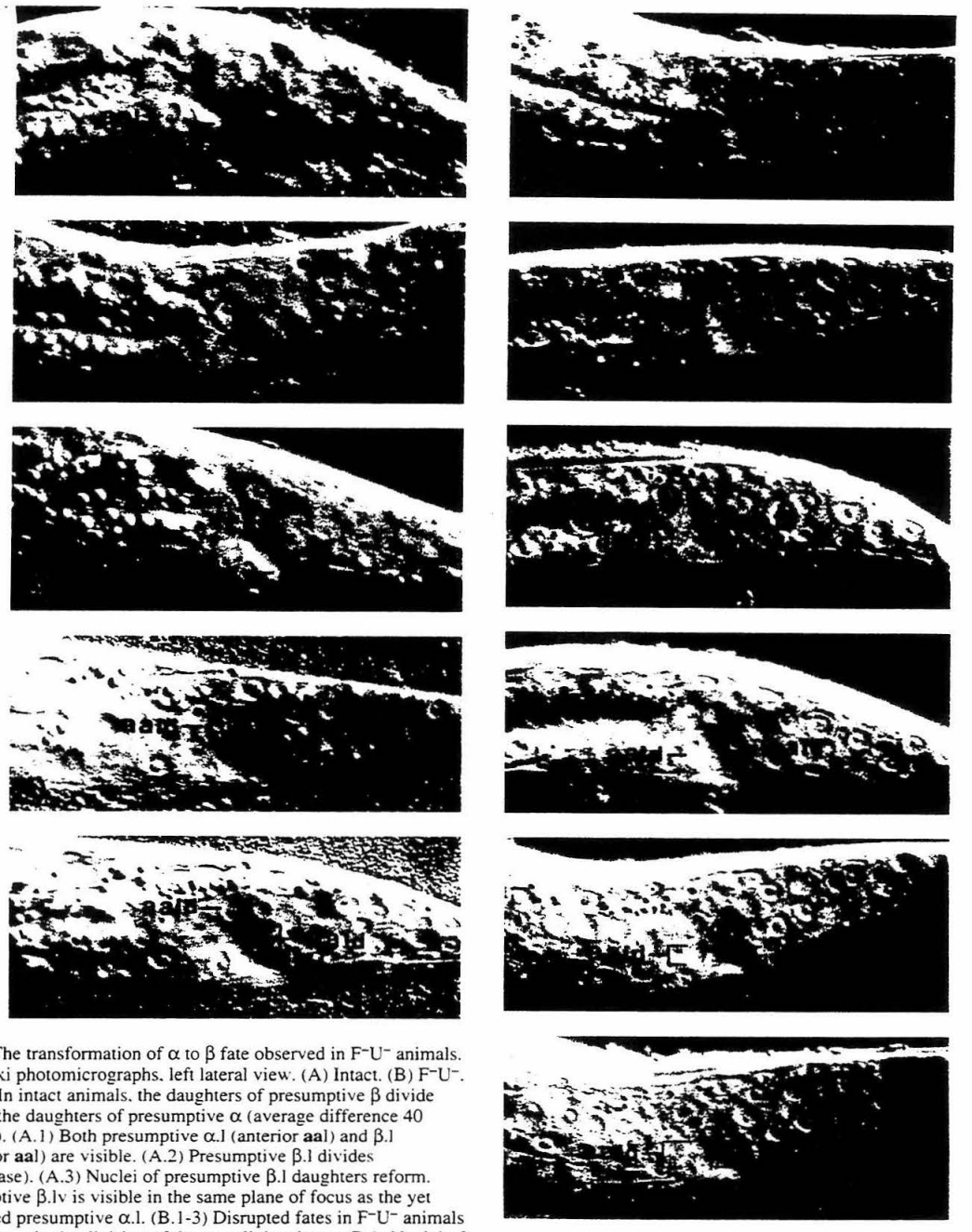

Fig. 7. The transformation of α to β fate observed in F⁻U⁻ animals. Nomarski photomicrographs, left lateral view. (A) Intact. (B) F⁻U⁻. (A.1-3) In intact animals, the daughters of presumptive β divide prior to the daughters of presumptive α (average difference 40 minutes). (A.1) Both presumptive α.l (anterior **aa**l) and β.l (posterior **aa**l) are visible. (A.2) Presumptive β.l divides (metaphase). (A.3) Nuclei of presumptive β.l daughters reform. Presumptive β.lv is visible in the same plane of focus as the yet undivided presumptive α.l. (B.1-3) Disrupted fates in F⁻U⁻ animals are apparent in the division of the **aa** cell daughters. (B.1) Nuclei of anterior and posterior **aa**l cells (presumptive α.l and β.l, respectively) are apparent. (B.2) Both cells begin to divide. (B.3) Nuclei of daughters of both presumptive α.l and β.l reform. Presumptive α.ld and β.ld are indicated. (A.4,5) In intact animals, β.(l/r)d divide. (A.4) Metaphase of presumptive β.ld. The nucleus presumptive α.ld is also labeled. (A.5) The daughter nuclei of presumptive β.ld reform. Presumptive α.ld remains intact. (B.4-6) Both presumptive α.(l/r)d and β.(l/r)d divide in transformed F⁻U⁻ animals. (B.4) Presumptive β.ld metaphase. (B.5) Presumptive α.ld anaphase. Daughter nuclei of presumptive β.ld are reforming. (B.6) Daughter nuclei of both presumptive α.ld and β.ld are visible in this plane of focus. Scale bar, 20 μm.

Table 2. Summary of lineage disruptions that result from F/U and Y.p ablation

	aa				pp				ap/pa			
	Anterior		Posterior		Anterior		Posterior		Anterior		Posterior	
	α		β		γ		δ		ε		ζ	
Intact	all÷	(4)‡	all	(6)	all	(6)	all	(2)	all	(6)	all	(5)
$F^- U^-$	0/7	(5.4)	7/7	(6)	2/7	(4)	7/7	(2)	9/14	(5.4)	14/14	(5)
F^-	4/4	(4)	4/4	(6)	3/4	(5.5)	4/4	(2)	8/8	(6)	8/8	(5)
U^-	3/4	(4.2)	4/4	(6)	4/4	(6)	4/4	(2)	8/8	(6)	8/8	(5)
$Y.p^-$	7/7	(4)	6/7	(5.9)	7/7	(6)	1/7	(3.3)	12/12	(6)	12/12	(5)

Data from Table 1B.C. Ablation of F and U together disrupts anterior (α, γ, ε) fates, but removal of only F or U generally results in normal lineages. Ablation of Y.p disrupts posterior (δ and rarely β) fates. ÷Normal lineages/lineages followed: ‡average number of progeny in parentheses.

progeny, and thus might also promote posterior fates. However, ablation of B.p does not disrupt the fates of B.a progeny. In addition, ablation of B.p and Y.p does not appreciably enhance the effect of ablation of Y.p alone (Table 1C.2.3). We conclude that Y.p or its progeny is necessary to promote normal posterior fate in the **pp** pair.

C. Summary of male-specific blast cell effects

We have demonstrated that the male-specific blast cells F, U, and Y (or their progeny) provide at least some of the positional cues that specify anterior versus posterior fates in the **aa**, **pp**, and **ap/pa** pairs. The disruptions and fate transformations resulting from ablation are variable, and not complete. We cannot distinguish whether this is a result of the limitations of cell ablation techniques in general, whether it is a result of the methods we have used, whether it reflects an inherent variability of the system, or whether other cells provide additional positional cues. Nevertheless, we have found that distinct cell fates are disrupted, and in some cases complete transformation of fate is observed.

II. Activity of B.a(l/r)xx cells on the cell pairs

At the 10 cell stage, many of the neighbors of the cells of each pair are other B.a progeny. We have investigated the interactions that occur among the B.a progeny. For sim-

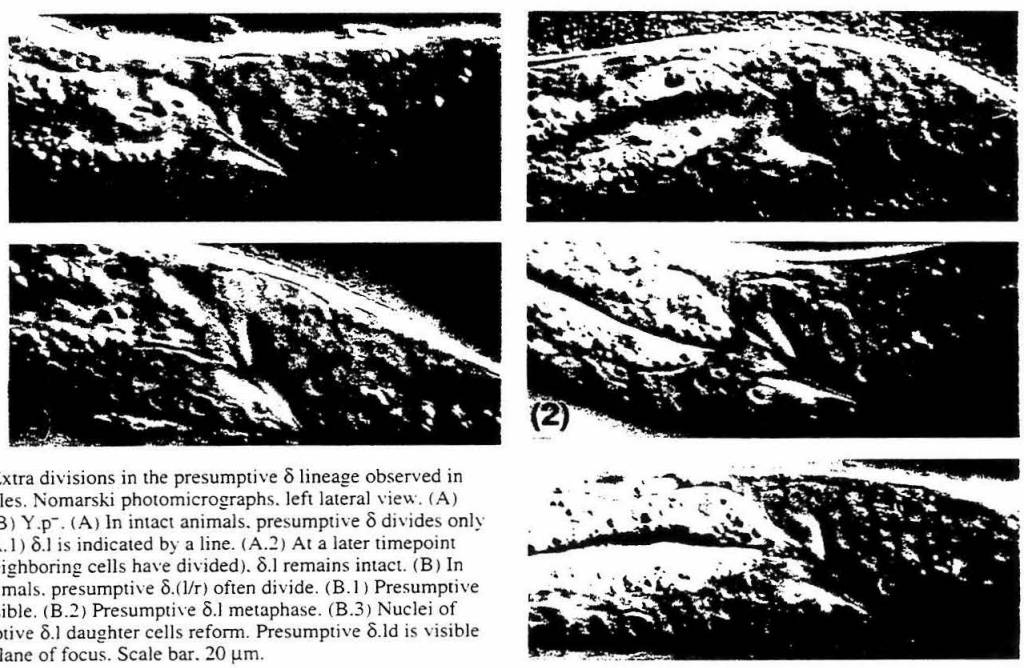

Fig. 8. Extra divisions in the presumptive δ lineage observed in $Y.p^-$ males. Nomarski photomicrographs, left lateral view. (A) Intact. (B) $Y.p^-$. (A) In intact animals, presumptive δ divides only once. (A.1) δ.l is indicated by a line. (A.2) At a later timepoint (after neighboring cells have divided), δ.l remains intact. (B) In $Y.p^-$ animals, presumptive δ.(l/r) often divide. (B.1) Presumptive δ.l is visible. (B.2) Presumptive δ.l metaphase. (B.3) Nuclei of presumptive δ.l daughter cells reform. Presumptive δ.ld is visible in this plane of focus. Scale bar, 20 μm.

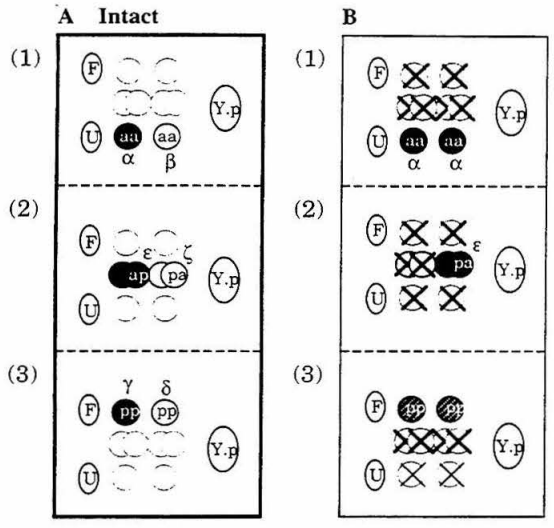

Fig. 9. Schematic comparison of the effects of removal of the other B.a progeny on each of the three cell pairs. In general, the posterior cell of the pair adopts a more anterior-like fate (anterior left, ventral down). (A) Intact. Black circles represent anterior fates, white circles represent posterior fates. (B) Experimental. (B.1) Posterior **aa** cells adopt α (anterior) fate in **ap⁻pa⁻pp⁻** animals. (B.2) **pa** cells adopt ε (anterior) fate in **aa⁻ap⁻pp⁻** animals. (B.3) Both anterior and posterior **pp** cells produce abnormal proliferative lineages (indicated by shading; example in Fig. 5B) in **ap⁻pa⁻aa⁻** animals. The effect is also seen if only **ap** and **pa** are ablated. Data of Table 1D.E.F.

plicity, we present the data for each cell pair separately. These results are summarized in Fig. 9.

A. Ventral group (**aa** cells)

To characterize any influences on the **aa** pair from the other six B.a progeny, we first isolated the pair by ablation of **ap**, **pa**, and **pp** (Table 3; data of Table 1D.E.2). If anterior cues from F/U and possible posterior cues from Y.p are sufficient to promote normal α and β fates in the **aa** pair,

Table 3. Summary of lineage disruptions in the aa cell pair that result from ablation of other B.a progeny

	aa			
	Anterior		Posterior	
	α		β	
Intact	all†	(4)‡	all	(6)
ap⁻pa⁻pp⁻	6/6	(4)	1/6	(4.5)
pa⁻pp⁻	2/2	(4)	2/2	(6)
ap⁻ pp⁻	3/3	(4)	2/3	(5.7)
ap⁻pa⁻	4/4	(4)	4/4	(6)

Data from Table 1D.E.2. Removal of **ap/pa/pp** cells results in the disruption of posterior (β) fate. In general, presumptive β cells adopt an α fate. However, if any other pair of cells remain (**ap⁻pa⁻**, **ap⁻pp⁻**, or **pa⁻pp⁻** [B.a(l/r)a⁻]), the presumptive β will be essentially normal. †normal lineages/lineages followed: ‡average number of progeny in parentheses.

Table 4. Summary of lineage disruptions in the ap/pa pairs that result from ablation of other B.a progeny

	ap		**pa**	
	Anterior		Posterior	
	ε		ζ	
Intact	all†	(6)‡	all	(5)
aa⁻ap⁻ pp⁻	X		0/8	(6)
aa⁻ap⁻	X		3/10	(5.5)
ap⁻ pp⁻	X		1/2	(5.5)
aa⁻ pa⁻pp⁻	3/4	(5.5)	X	
aa⁻ pa⁻	8/8	(6)	X	
pa⁻pp⁻	4/4	(6)	X	

Data from Table 1D.E.F. Each animal has two **ap/pa** pairs, which are considered independently for this Table (i.e., 10 **aa⁻ap⁻** represents 5 animals). Removal of **aa/ap/pp** cells results in the disruption of **pa** fate. The **pa** cells generate ε lineages. If other pairs remain (**aa-ap-** [B.a(l/r)a-], or **ap-pp-**), the **pa** cells will sometimes generate a ζ lineage, and sometimes an ε lineage. Removal of **aa/pa/pp** (or a subset thereof) generally does not disrupt the fate of **ap** cells. X indicates that the cell is absent. †normal lineages/lineages followed: ‡average number of progeny in parentheses.

then **aa** cells isolated from the other B.a progeny should produce normal lineages. However, we find that the other B.a progeny are necessary for proper specification of fate. Specifically, ablation of all of the other B.a progeny results in truncation of the lineage of the presumptive β cell (5 of 6 animals). Since the axes and timing, as well as number of progeny of these cells resembles those associated with a normal α lineage, we consider this to be a β to α transformation. Thus the B.a progeny promote posterior (β) fate in the **aa** pair. The presence of any pair is sufficient to promote normal β lineages. Ablation of **ap** and **pa**, or **ap** and **pp**, or **pa** and **pp** (B.a(l/r)p⁻) generally results in normal lineages from the presumptive α and β cells. Thus the cell pairs are redundant in their ability to promote β fate.

B. Lateral groups (**ap/pa** cells)

The other B.a progeny act to promote posterior ζ fate in the **pa** cells (Fig. 10; Table 4; data of Table 1D.3.E.3.F). Ablation of the other six B.a progeny causes **pa** cells to produce ε lineages. If one other pair of B.a progeny remain, the **pa** cells will sometimes produce an ε lineage, and other times a ζ lineage (**aa⁻** and **ap⁻** (B.a(l/r)a⁻); **ap⁻** and **pp⁻** lineages; **aa⁻ pp⁻** by examination of anatomy only (data not shown)). We conclude that the other B.a progeny act to promote posterior fate in the **pa** cells. Consistent with this result, the ε fate of **ap** does not depend on the presence of other B.a progeny.

C. Dorsal group (**pp** cells)

Ablation of all other B.a progeny (**aa**, **ap**, and **pa**) results in abnormal lineages produced by both the presumptive γ and the presumptive δ cells (Table 5; data of Table 1E). These abnormal lineages are novel in that they can result in up to eight progeny, which is more than is produced by

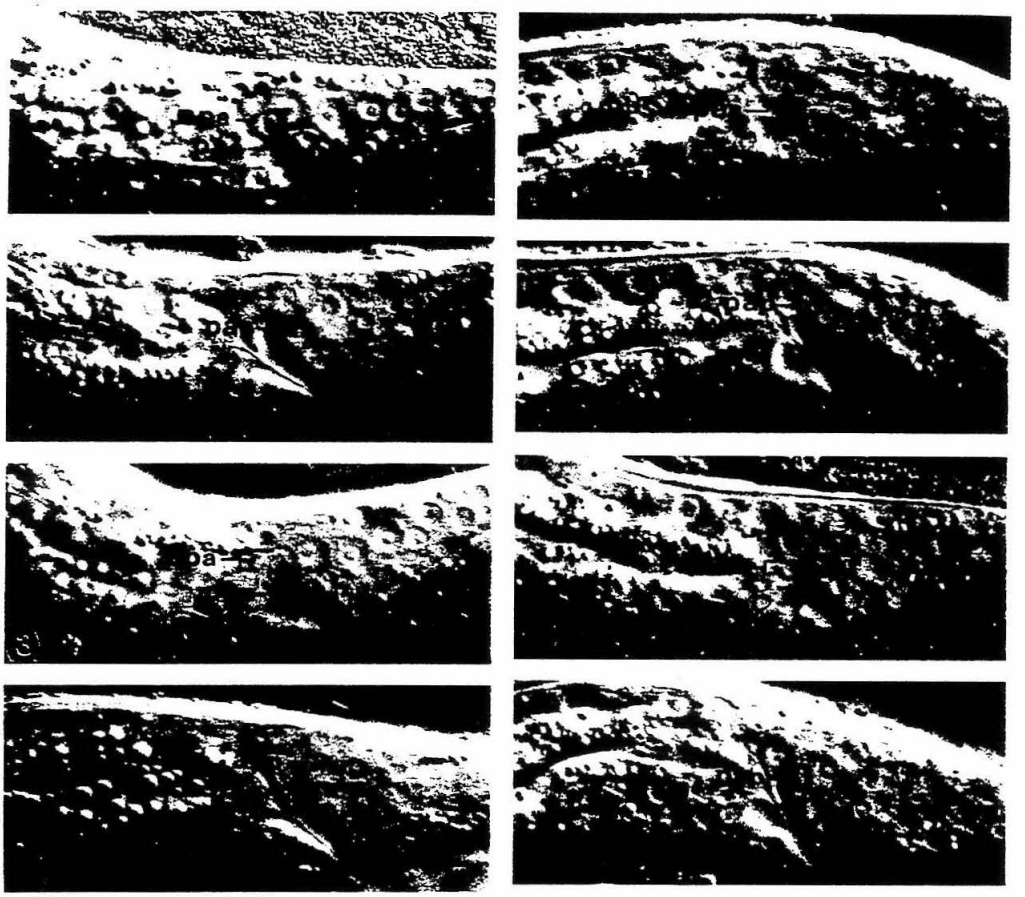

Fig. 10. The transformation of **pa** to ε fate in the absence of other B.a progeny. As with **aa** fates (Fig. 7), differences between ε and ζ lineages are apparent in the timing of division of the progeny of **ap** and **pa**. The anteroventral daughter of ε divides prior to the posteriodorsal daughter. In intact animals (A), **ap** produce ε lineages, and **pa** produce ζ lineages. In an ε lineage, the **ap**a (A.1) cell is larger than **ap**p (A.2), and divides first (A.3,4). In a ζ lineage, the **pa**p (A.2) cell is larger than **pa**a (A.1), and divides first (A3,4). (B) In the absence of other B.a progeny, **pa** will adopt ε fate. This transformation sometimes occurs if a subset of other B.a progeny are absent, as shown in this B.a(l/r)a$^-$ (**aa**$^-$**ap**$^-$) animal (see Table 1E.3). In this animal, **pa**a (B.1) is larger than **pa**p (B.2), and divides first (B.3,4). Both daughters of **pa**a divide, consistent with an ε lineage (not shown). Nomarski photographs are of left lateral (A.1,3; B.1,3) and left medial (A.2,4; B.2,4) focal planes. Scale bar, 20 μm.

either normal γ or δ lineages (example in Fig. 5). For simplicity they can be thought of as γ lineages with γ.p behaving like γ.a (see below). These abnormal lineages are also observed when only **ap** and **pa** are ablated. The results of experiments that ablate B.a progeny in different combinations indicate that either **ap** or **pa** is sufficient to prevent the abnormal proliferative lineages, and thus the pairs are redundant. We propose that the **ap** and **pa** cells play two roles in γ/δ fate specification. One role is to promote proper execution of the γ lineage. This function ensures that a specified γ has six progeny, in a 4+2 pattern (4 progeny from one daughter, 2 from the other, see Table 1 legend). The other role is to promote posterior (δ) fate. Thus in the absence of **ap**/**pa**/(**aa**), presumptive δ adopts a more anterior-like fate. However, because of the second function of the **ap**/**pa**/(**aa**) cells, neither presumptive γ nor presumptive δ properly executes the γ lineage. We present further evidence for this two step fate specification model in Section III.C.

Removal of **ap** and **aa** (by ablation of the precursors B.a(l/r)a) results in normal γ and δ lineages. In some of these animals the **pa** cells produce ε lineages (see above; Table 1E.3). Thus, the **pa** cells are sufficient to promote normal γ and δ lineages, and this function is independent of their own fate. Removal of **aa** and **pa** (or **pa** alone) results in truncation of presumptive γ lineages, similar to the lineages in an F$^-$U$^-$ animal. Thus, the **pa** cells appear to play a role in two distinct processes. Together with the **ap** cells, the **pa** cells act to inhibit extra proliferation in both presumptive γ and δ. Alone, they function to increase

Table 5. Summary of lineage disruptions in the pp cell pair that result from ablation of other B.a progeny

	pp			
	Anterior		Posterior	
	γ		δ	
Intact	all†	(6)‡	all	(2)
aa⁻ap⁻pa⁻	0/3	(7)	0/3	(6)
ap⁻pa⁻	0/5	(7.2)	2/5	(3.6)
aa⁻ap⁻	5/5	(6)	5/5	(2)
aa⁻ pa⁻	0/4	(4)	4/4	(2)
pa⁻	1/5	(3.6)	5/5	(2)

Data from Table 1E. Removal of **aa/ap/pa** cells results in the disruption of anterior (γ) and posterior (δ) fate. Both cells generally undergo abnormal proliferative lineages, producing up to 8 progeny (example in Fig. 5B). A similar effect is seen if only **ap/pa** cells are ablated. Removal of **aa/ap** cells (B.a(l/r)a⁻) results in essentially normal lineages. However, removal of **pa** (alone or with **aa**) disrupts presumptive γ fate. In these animals, presumptive γ undergoes truncated lineages similar to those seen in F⁻U⁻ animals. †normal lineages/lineages followed; ‡average number of progeny in parentheses.

proliferation of presumptive γ. Although they promote γ fate in both (or γ and δ fate), the effects are opposite on the extent of cell division (Table 5).

D. Summary of B.a progeny interactions

We conclude that the cells in each of the three types of B.a(l/r)xx cell pairs respond to cues provided by the other B.a progeny. For all pairs, the other B.a progeny act to promote the posterior fate. Specifically, **ap**, **pa**, and **pp** promote β fate in the **aa** pair, and **aa**, **ap**, and **pp** promote ζ fate in the **ap/pa** pairs. B.a progeny ablation results in abnormal lineages in both cells of the **pp** pair. However, as discussed above, one function of **aa**, **ap**, and **pa** might be to promote posterior (δ) fate in the **pp** pair.

For each pair the interaction is unique. Any other pair of B.a progeny is sufficient to promote posterior fate (β) in **aa** cells, whereas a single pair is not always sufficient to promote posterior fate (ζ) in **pa** cells. In the **pp** pair, only the **pa** cells are sufficient to promote both γ and δ fates. At least one set of **ap** or **pa** cells is required to promote posterior fate (δ). The **pa** cells have two distinct roles. Ablation of the **pa** cells alone reduces the progeny of presumptive γ, whereas ablation of both the **ap** and **pa** cells increases the progeny of presumptive γ, compared to normal. These roles of the **pa** cells are discussed in Section III.C.

III. Interactions among identified positional cues

The ablation experiments described so far indicate that each pair responds to several positional cues. For each pair, most of these have distinct effects. To understand how the cues might combine to specify fate in each group, we examined animals in which we ablated two or more 'signals.' Such ablations can indicate the regulatory relationship among the cues and the nature of signal integration in the responding cells. We refer to ablation of single components (e.g., F and U) as a 'single' ablation, and ablation of two components (e.g., F and U, **ap** and **pa** and **pp**) as a 'double' ablation, even though multiple cells are removed.

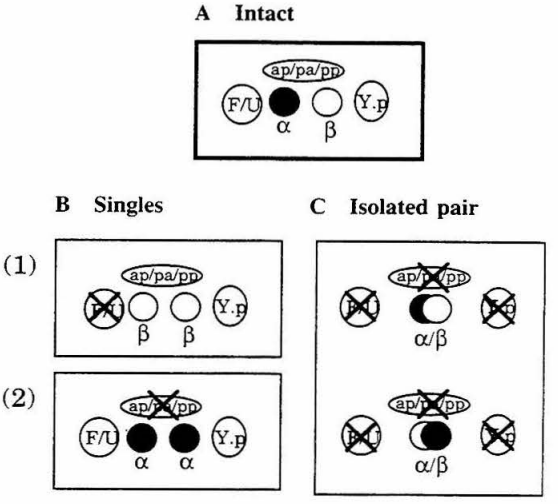

Fig. 11. Schematic summary of the effects of ablation of multiple positional cues on the **aa** cell pair. (B.1) Ablation of F and U results in both **aa** cells adopting the posterior (β) fate (white circle). (B.2) Ablation of other B.a progeny results in both **aa** cells adopting the anterior (α) fate (black circle). (C) In the absence of all identified positional cues, **aa** cells fail to migrate, but often produce one α-like and one β-like lineage. Anterior left, ventral down. Data of Table 1B.1:D.1 (B), and H.2 (C).

A. Ventral group (**aa** cells)

Two major components of fate specification have been identified for the **aa** pair. F and U provide anterior positional cues because ablation of F/U disrupts presumptive α fate and results in (partial) transformation of α to β. The other B.a progeny, **ap/pa/pp**, promote posterior fate. Ablation of these cells disrupts presumptive β fate and results in transformation of β to α. In addition, Y.p may play a minor role in providing posterior cues. To understand the interplay of these cues, we have followed the cell lineages of animals in which two or all three of these components have been ablated (key experiments summarized in Fig. 11).

1. F/U and Y.p

Analysis of ablations of F/U and Y.p is complicated by the fact that in most animals the **aa** cells remain left/right rather than migrate to anterior/posterior positions as they do in intact animals. Thus *positionally* there is no 'presumptive' α or β. Since neither F/U nor Y.p ablation alone results in such high frequency of failure to migrate, it suggests that both F/U and Y.p (in the presence of all B.a progeny) are sufficient to promote normal migration. Thus, although Y.p ablation alone has only a minor effect on fate specification (see Table 2), it appears to play a role in anterior/posterior patterning. Despite the abnormal positioning, one can follow the lineages of the cells, and interpret them in terms

of the lineages that those cells would normally produce. However, the division axes of the abnormally positioned cells are usually abnormal.

The **aa** cells in F⁻U⁻Y.p⁻ animals can produce both α- and β-like lineages, although some abnormal lineages are also observed (2/5 animals abnormal, Table 1G). In each of the three animals without abnormal lineages, one of the two **aa** cells produced an α-like lineage, and the other produced a β-like lineage. Since α lineages were observed in 3/5 F⁻U⁻Y.p⁻ animals while no α lineages were observed in 7 F⁻U⁻ animals, the removal of Y.p may partially counteract the absence of F and U in specification of fate. Thus although single ablation of Y.p results in only a minor disruption of fate in the **aa** cells, ablation of Y.p together with F/U indicates that the **aa** cells can respond to Y.p cues.

2. F/U, and **ap/pa/pp**

Ablation of the two primary components of **aa** fate specification, F/U and **ap/pa/pp**, does not clearly resemble one or the other single ablation (Table 1H.1). As in the F⁻U⁻Y.p⁻ double ablation, the **aa** cells tend to remain side by side, and some abnormal lineages are observed. In contrast to the single ablation of **ap/pa/pp**, we observe many β-like lineages. Since the β to α transformations that occur upon **ap/pa/pp** ablation are dependent on the presence of F and U, one of the roles of the other B.a progeny may be to modulate (inhibit, regulate, or otherwise localize) the F/U activity. However, the B.a progeny must also have an active role of their own, antagonistic to F/U, since the double ablation does not simply resemble the single ablation of F/U.

3. F/U, Y.p, and **ap/pa/pp**

Removal of all three components (F/U, Y.p, **ap/pa/pp**) isolates the **aa** pair from all characterized components of fate specification (Table 1H.2). The majority of the cells remain left/right (and thus first division axes are a-p rather than l-r), but both α-like and β-like lineages are observed. In each animal, one cell produces four progeny suggesting it is α-like, and the other cell produces six (or sometimes five) progeny, often in the 3+3 pattern associated with the β fate. Thus patterning of the two cells is apparent, although the cells do not migrate properly and the extracellular cues that we have identified are absent. There might be other factors that interact with the **aa** cells to promote their fates. However, since the **aa** cells remain side by side, these factors are unlikely to be providing anterior-posterior positional cues. A more satisfactory explanation is that the two **aa** cells interact with each other. In the presence of the cues provided by the other cells this lateral interaction may act to reinforce the positional information, to ensure the result of one cell with each fate. However, in the absence of those cues the cells can interact to establish one cell adopting an α-like fate, and one a β-like fate.

4. Interaction between **aa** cells

a. B.al⁻ or B.ar⁻ background

To further characterize the possible role of interaction between the two **aa** cells in promoting fate, we have carried out a series of experiments in which a single **aa** cell remains in different ablation backgrounds (Table 1K). We eliminated one of the **aa** cells by ablating either the precursor B.al or B.ar. These ablations also eliminate all of the other B.a progeny on one side. Although we have evidence that the other B.a progeny can play a role in α/β fate specification, in pairwise ablations their activity is redundant. In addition, all B.al⁻ or B.ar⁻ animals have the same ablation background, so comparison among these experiments can provide some information about the contribution of interaction between **aa** cells in the specification of fate.

Ablation of B.al or B.ar leaves one **aa** daughter, but the other positional cues (from F/U, Y.p, and potentially from the remaining B.a progeny) are intact. Four of five of the **aa** cells in this case produce an α lineage (one abnormal). This result is consistent with the results of Sulston and White (1980), who found that if a single **aa** cell is ablated, the remaining cell will produce an α lineage. We find that ablation of F and U in a B.al⁻ or B.ar⁻ animal results in the single **aa** cell producing a β lineage. Therefore, it is F and U that promotes α fate in the single **aa** cell. In the absence of F and U, the remaining B.a progeny and Y.p promote the β fate. If both F/U and Y.p are ablated in a B.al⁻ or B.ar⁻ background, the remaining **aa** cell still usually adopts the posterior fate (Table 1K.3), although the presence of the remaining B.a progeny is apparently not always sufficient to promote β fate. We have not distinguished whether this is because Y.p is absent, or because the full set of B.a progeny is not present. Nevertheless, in general, single **aa** cells can respond to positional cues.

b. Isolated B.a(l or r)aa

We isolated single **aa** cells in five animals by removing F/U, Y.p, and all of the B.a progeny except for one of the **aa** cells (Table 1L.1). In four cases this cell underwent an α-like lineage, and in one case it underwent a β-like lineage. Thus in the absence of all identified cues, **aa** cells generally adopt α fate. However, because of the variable results, we have not established α as the 'ground state'. Another possibility is that the 'isolated' **aa** cell chooses between α and β fate stochastically.

5. Summary of experiments in the ventral group

Multiple cell interactions play a role in fate specification in the **aa** pair. F/U, **ap/pa/pp**, and Y.p contribute external cues to distinguish anterior and posterior fates, as well as to promote proper migration of the cells to an anterior/posterior orientation. This role in promotion of migration is apparent only if more than one component is removed, and is therefore redundant. The presence of any two of the three components is generally sufficient to promote normal migration, whereas the presence of any one is not. Double ablation experiments suggest that one role of the **ap/pa/pp** cells may be to localize the activity of F and U. Isolation of the **aa** pair suggests that the two **aa** cells interact to specify fate or ensure that the cells each adopt a different fate. Experiments that leave a single **aa** cell indicate that single cells can respond to the identified positional cues, and that interaction between the **aa** cells is not required for the adoption or execution of α or β fate. Isolation experiments, however, did not identify a 'ground state' for **aa** cells.

Our observations may be consistent with the hypothesis that α and β lineages represent distinct precursor cell fates. The first division axis may not be critical since essentially

normal α and β division patterns are observed even if the **aa** cells remain side-by-side and the first division is along an anterior/posterior axis. Even if such lineages (α_τ, β_τ) are considered normal, we have observed several other types of abnormal lineages produced by presumptive α and β cells. Thus, other interpretations of the data are possible. However, we reject the simple hypothesis that the fate is independently specified in the **aa** cell daughters. Specifically, in one type of abnormal **aa** lineage ('abn-5'), the **aa** cell generates one daughter that produces 2 progeny (like α), and one that produces 3 progeny (like β). These lineages are sometimes observed in animals where the ablation results in the **aa** cells remaining lateral, rather than migrating to the midline. Since the first axis of division in these cells is usually anterior/posterior, as opposed to the normal left/right, this places one daughter of each cell in the anterior position (normally occupied by α.(l/r)), and the other daughters in the posterior position (β.(l/r) environment). Nevertheless, lineages with both **aa** cells executing abn-5 lineages (e.g., 2 progeny from the anterior daughter, 3 progeny from the posterior daughter) are rare (1/64 of all animals with abnormal lineages; 1/19 of animals where **aa** cells remain left/right). In addition, many abn-5 lineages show β-like timing in the division of both **aa** daughters, but fail to execute the final division on one side, rather than showing α-like timing in the daughter with α-like divisions and β-like timing in the daughter with β-like divisions. It is possible that the abn-5 lineages might simply be abortive β lineages. In addition to these intermediate lineages, we also observe lineages in which an **aa** cell produces more than six progeny or an abnormal pattern of six progeny (4+2 rather than 3+3). These lineages, which represent 14% of abnormal **aa** lineages, suggest that the progeny of the **aa** cells may be somewhat responsive to extracellular cues.

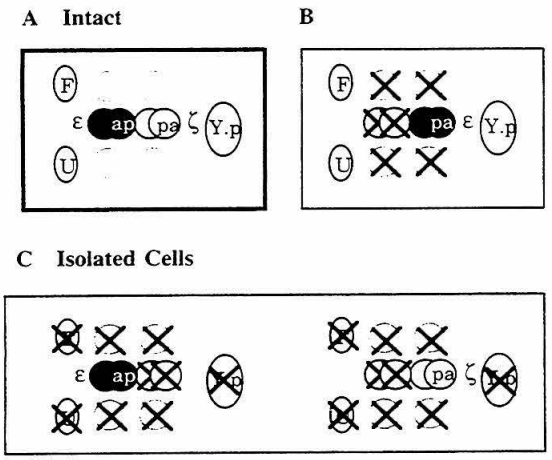

Fig. 12. Schematic summary of the effects of ablation of multiple positional cues on the **ap/pa** cell pairs. In intact animals, **ap** cells always adopt ε fate, and **pa** cells adopt ζ fate (A). If other B.a progeny are ablated, **pa** cells adopt ε fate (B). This transformation of fate is dependent on the presence of F/U. In the absence of identified positional cues, **ap** cells will usually adopt ε fate, and **pa** cells will adopt ζ fate. Anterior left, ventral down. Data of Table 1F.1 (B) and L.3.4 (C).

B. Lateral groups (**ap/pa** cells)

A summary of the interactions affecting the **ap/pa** pairs is shown in Fig. 12. Ablation of F and U can sometimes disrupt the fate of the anterior **ap** cells. Ablation of all of the B.a progeny except for the **pa** cells transforms these cells from their normal ζ fate to ε fate (see Fig. 9B.2). We observed no disruption of fates in these pairs after ablation of Y.p. The $F^-U^-Y.p^-$ animals have a slight but not significant enhancement of the F^-U^- effect on the **ap** cells (compare Table 1B.1 with G). However, the fate of the **pa** cells is still not appreciably disrupted.

How do these positional cues interact with the cues from the other B.a progeny? The ζ to ε transformation observed in $\mathbf{aa^-ap^-pp^-}$ (B.a(l/r)a^- B.a(l/r)pp^-) animals is dependent on the presence of F and U (Table 1L.3). Although we have not examined F^-U^-B.a(l/r)a^-B.a(l/r)pp^- animals specifically, Y.p does not appear to play a role in **pa** fate specification. $F^-U^-Y.p^-$B.a(l/r)a^-B.a(l/r)pp^- ablation isolates the **pa** cells (one on each side), and these cells produce ζ lineages. Thus in the absence of F and U, **pa** cells do not require the other B.a progeny to adopt the ζ fate. In contrast, an $F^-U^-Y.p^-$B.a(l/r)p^-B.a(l/r)aa^- ablation isolates the **ap** cells, and these cells usually produce ε lineages (Table 1L.4).

Both the **ap** and the **pa** cells exhibit plasticity in fate specification. Indeed, since the **pa** cells can adopt the fate normally associated with **ap**, the pairs have some characteristics that might indicate that they form equivalence groups. However, in the absence of all identified cells that influence **pa** and **ap** cell fate, the **ap** and the **pa** cells exhibit distinct fate differences. Thus, either there are as yet unidentified positional cues that somehow distinguish the two cell types, or these cells have distinct fate potentials due to their lineal history.

For the **ap** and **pa** cells, many ablations that result in a disruption of normal fates result in a complete transformation of cell fate. In intact animals, differences between ε and ζ lineages are apparent soon after division of **ap** and **pa** cells (see Fig. 10). Under experimental conditions, 85% of **ap/pa** cells with disrupted fate produced either ε or ε* lineages (24/46 were ε*, 15/46 were ε). Since ε* lineages are not observed in intact animals, ε* may be an abnormal ε (or ζ) lineage, rather than a distinct 'fate' of its own. However, transformations from ζ to ε lineages in **pa** cells are apparent in the first division, and are generally complete transformations. The early evidence of fate choice (size and division timing of **ap/pa** cell daughters), along with the high percentage of fate disrupted cells that produce one of three distinct lineages, is consistent with a hypothesis of early commitment to one of a defined set of potential fates. However, it may also simply reflect the relatively simple role of positional cues in the specification of the **ap/pa** fates.

C. Dorsal group (**pp** pair)

We have identified four distinct activities that are involved in the specification of fate in the **pp** pairs: F/U, Y.p, **pa**, **ap/pa/(aa)**. Ablation of either F/U or the **pa** cells results in

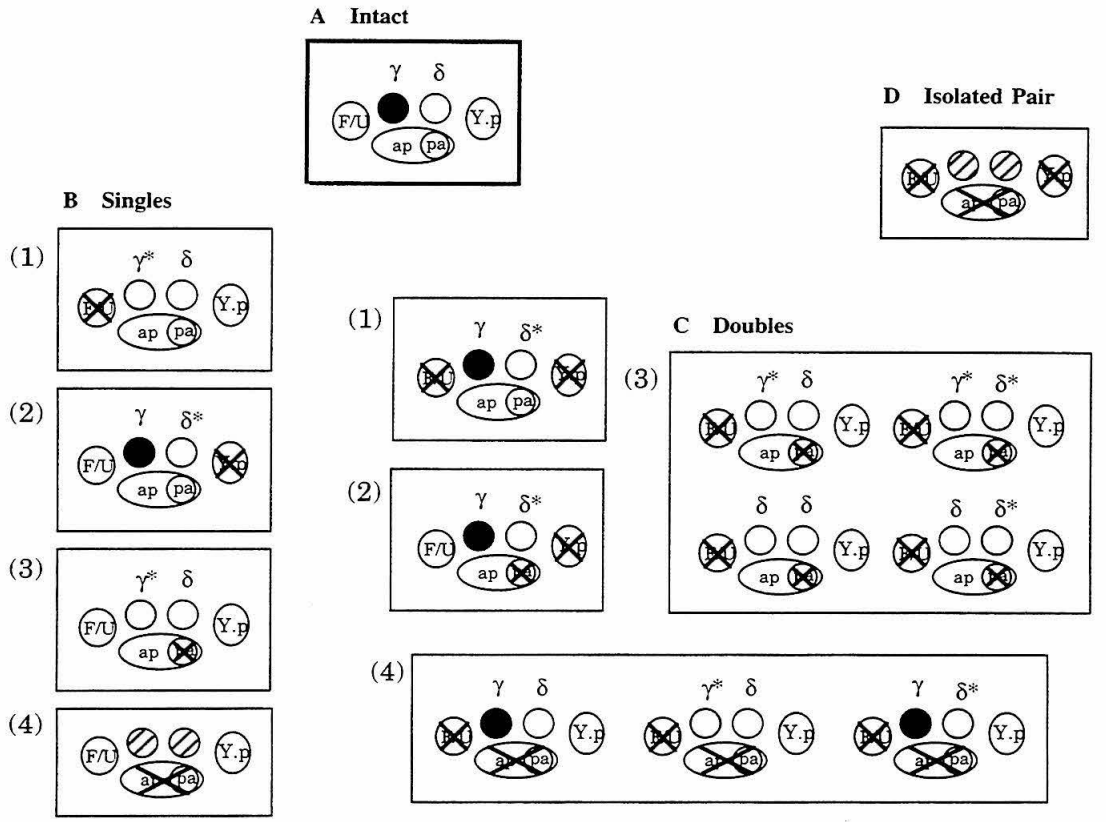

Fig. 13. Schematic summary of the effects of ablation of multiple positional cues in the **pp** cell pair. (B.1) Ablation of F/U results in truncated anterior (presumptive γ) lineages. (B.2) Ablation of Y.p results in extra divisions of posterior (presumptive δ) cells. (B.3) Ablation of **pa** cells results in truncated anterior lineages. (B.4) Ablation of **ap/pa/(aa)** results in abnormal proliferative lineages in both anterior and posterior **pp** cells. (C.1) Ablation of F/U and Y.p resembles Y.p ablation, although presumptive γ lineages may also be disrupted. (C.2) Ablation of **pa** and Y.p resembles Y.p ablation. (C.3) Ablation of F/U and **pa** randomizes the polarity of pattern. (C.4) Ablation of F/U and **ap/pa/(aa)** results in variable lineages, but reinstatement of pattern polarity. (D) Removal of all four identified positional cues (F/U, Y.p, **pa**, **ap/pa/(aa)**) results in abnormal proliferation of both presumptive γ and δ. Anterior left, ventral down. Data of Table 1B.1, C.1, E (B); G. 1.1-4 (C); 1.6.7 (D).

truncation of presumptive γ lineages, so we infer that these cells promote anterior fate in some way. Ablation of Y.p results in extra divisions of presumptive δ, and ablation of **ap/pa/(aa)** results in extra divisions of both presumptive γ and δ. Thus Y.p and **ap/pa/(aa)** promote posterior fate, or otherwise inhibit proliferation. To understand how each of these four components exerts its effect, we have followed the B cell lineage in animals after ablation of two, three, or all four components (Fig. 13).

1. F/U and Y.p

F/U and Y.p provide positional information from outside of the B.a progeny group (Fig. 13B.1,2). Ablation of both F/U and Y.p results in extra divisions of the presumptive δ cell, and occasionally abnormal presumptive γ lineages (Fig. 13C.1; data of Table 1G). Thus, the double ablation resembles the single Y.p ablation (although the fate of presumptive γ is not always normal). Therefore, one of the roles of F/U might be to counteract (modulate) the posterior-promoting activity of Y.p on presumptive γ. This activity of F/U is required only in the presence of Y.p. However, while F and U likely have other roles (see below), these experiments indicate that F and U are not necessary for a normal γ lineage. In $F^-U^-Y.p^-$ animals, in contrast to the **aa** cells, the **pp** cells still migrate to their normal anterior/posterior positions. Furthermore, the fact that the anterior cell generally produces a γ lineage and the posterior cell a δ* lineage indicates that removal of both identified positionally anterior and posterior sources of cues is not sufficient to eliminate anterior/posterior patterning in this pair.

2. Y.p and **pa**

Ablation of the **pa** cells results in truncation of presump-

tive γ lineages, and ablation of Y.p results in extra divisions of the presumptive δ cell (Fig. 13B.2.3). In the **pp** pair, the double Y.p⁻ **pa**⁻ animals resemble Y.p⁻ animals (Fig. 13C.2; data of Table 1 I.1). Thus, one of the roles of the **pa** cells is to inhibit Y.p activity from influencing the fate of the presumptive γ cell. This function is only required if Y.p is present. In addition, this experiment suggests how the positionally posterior **pa** cells can act to promote an anterior fate. The position of the **pa** cells (see Figs 3E, 4B.2) suggests this role may be 'passive.' Specifically, the **pa** cells may physically block the activity of Y.p from reaching the presumptive γ cell. This proposed mechanism does not require that the **pa** cells be biochemically distinct from the other B.a progeny, although it also does not rule out this possibility.

3. F/U and **pa**

Single ablations of either F/U or **pa** result in intermediate and variable disruption of presumptive γ lineages (Fig. 13B.1.3). To establish whether complete transformations from the γ to the δ fate could be achieved by removal of both components, we characterized F⁻U⁻**pa**⁻ animals (Fig. 13C.3; data of Table 1 I.2). While this double ablation does not result in an increased frequency of complete γ to δ transformations, the normal pattern of anterior/posterior polarity is disrupted. In either F⁻U⁻ or **pa**⁻ animals, the fate of presumptive δ is not disrupted, whereas presumptive γ lineages are often truncated. Thus, two patterns are observed: normal polarity and apolar. In contrast, double ablation of F/U and **pa** can result in a fate disruption of both presumptive γ and δ cells. In addition, we observe all possible classes of pattern: normal polarity, apolar, and reversed polarity.

4. F/U and **ap/pa/(aa)**

Single ablation of **ap/pa/(aa)** results in novel lineages and abnormal proliferation of presumptive γ and δ, whereas ablation of F/U results in truncated lineages produced by the presumptive γ cell (Fig. 13B.1.4). The double ablation of F/U and **ap/pa** or **ap/pa/aa** (considered together as **ap/pa/(aa)**) has allowed us to determine whether the excessive proliferation results in part from inappropriate modulation of F/U information. In F⁻U⁻**ap**⁻**pa**⁻(**aa**⁻) animals, lineages of presumptive γ and δ do not closely resemble those seen in either the single F⁻U⁻ or **ap**⁻**pa**⁻(**aa**⁻) animals (Fig. 13C.4; data of Table 1 I.3.4). Proliferative lineages are not observed. Although many of the lineages are slightly abnormal, we only observe the more common lineages: normal γ and δ, and γ*/δ*. However, the presence of Y.p is not sufficient to ensure δ fate in the posterior cell. Disruption of both anterior and posterior fates is observed, although not in the same animal. In Section II.C we proposed that the **ap/pa/(aa)** cells have two functions: promotion of posterior fate (δ) and promotion of proper execution of the γ lineage. The double ablation result indicates that F/U may likewise act at two steps: promotion of anterior fate (γ) and execution of γ lineage. We hypothesize that one role of the F/U activity for γ fate execution is to promote **pp** proliferation. The **ap/pa/(aa)** cells counteract this activity, thus localizing it to γ.a (or γ.a(l/r)). Since removal of both F/U and **ap/pa/(aa)** results in more normal lineages than either single ablation, we conclude that F/U and **ap/pa/(aa)** act antagonistically and in parallel on the same process(es).

One consistent characteristic among F⁻U⁻**ap**⁻**pa**⁻(**aa**⁻) animals is that the fate of the positionally anterior **pp** cell is relatively more 'anterior' than the fate of the positionally posterior **pp** cell; the pair exhibits polarity of pattern. In other words, anterior/posterior polarity, which is lost in F⁻U⁻**pa**⁻ animals, is regained by the additional ablation of the **ap** (compare Fig. 13C.3 with C.4). Y.p, the posterior-promoting cell, can promote polarity in the **pp** pair in the absence of F/U and **ap/pa/(aa)**. Since polarity can be disrupted in F⁻U⁻**pa**⁻ animals, the presence of **ap** must somehow counteract this activity of Y.p.

5. F/U, Y.p, and **ap/pa/(aa)**

We have removed all of the identified components that specify fate in the **pp** pair (Fig. 13D). As with the **aa** pair, this isolation may allow identification of potential interaction between **pp** cells. The normal lineages and patterning observed in F⁻U⁻**ap**⁻**pa**⁻(**aa**⁻) animals are lost with the removal of Y.p (Table 1 I.6.7). A variety of proliferative lineages are observed from both presumptive γ and δ. Although there is no appreciable difference between presumptive γ and presumptive δ, proliferation may be less than in **ap**⁻**pa**⁻(**aa**⁻) animals. Thus, without the identified cues, no polarity or evidence for interaction between the **pp** cells is readily apparent. However, the lineages are abnormal and variable enough that they are not easily interpreted.

A difference between the **aa** and **pp** cells is that the **pp** cells generally migrate to anterior/posterior positions even in the absence of the identified extracellular cues. Although this may indicate that anterior/posterior patterning cues for the **pp** cells may still be present, it may also reflect a difference in the physical environments within which the two pairs reside. The ventral area (**aa** environment) is relatively smaller and more crowded than the dorsal area (**pp** environment). Thus, in the absence of positional cues the **aa** cells might not be able to migrate medially, whereas the **pp** cells can. We cannot, however, rule out the possibility that additional cues exist for **pp** cells.

6. Interaction between **pp** cells

a. B.al⁻ or B.ar⁻ background

Isolation of both **pp** cells did not provide direct evidence that they interact with each other to specify their fates. However, we have also characterized the lineages of single **pp** cells obtained after ablation of the precursor B.al or B.ar (Table 1K). Four of five single **pp** cells obtained by ablation of B.al or B.ar produced a γ lineage, whereas one produced a δ lineage. Thus, interaction between **pp** cells is not essential to produce either normal γ or δ fates. In these animals, it is possible that choice between the two fates is related to the relative anterior-posterior positioning of the single **pp** cell in the normal **pp** environment. Adoption of the γ fate, however, is dependent on the presence of F and U. Remaining **pp** cells in F⁻U⁻ [B.al⁻ or B.ar⁻] animals produce δ lineages (5/7) or γ*/δ* lineages (2/7). Likewise, the δ fate is dependent on the presence of Y.p. Single **pp** cells in F⁻U⁻Y.p⁻ [B.al⁻ or B.ar⁻] animals produce γ*/δ* (3/4 γ*/δ*; 1/4 abn-3) lineages. We do not believe this

effect is merely the result of fewer neighbors (or more debris), because even single isolated **pp** cells can produce γ lineages (see below). These observations are consistent with the proposed roles of F/U and Y.p in promoting γ and δ fates, respectively.

Loss-of-function mutations in *lin-12*, a gene known to play a role in the interactions between cells in other equivalence groups, result in δ to γ fate transformations (Greenwald et al., 1983). This observation implies that the **pp** cells do interact, by analogy to other *lin-12*-dependent equivalence groups. Although isolation of the two **pp** cells did not offer evidence to support this hypothesis, comparison of F⁻U⁻Y.p⁻ to F⁻U⁻Y.p⁻[B.al⁻ or B.ar⁻] animals is suggestive. Specifically, the single **pp** cells in F⁻U⁻Y.p⁻[B.al⁻ or B.ar⁻] animals do not produce γ lineages (0/4 are γ), whereas in F⁻U⁻Y.p⁻ animals, where both **pp** cells are present, the anterior cell generally will produce a γ lineage (4/5). Although these animals also differ in the total number of B.a progeny present, we suggest that the γ fate in the F⁻U⁻Y.p⁻ animals may result from interaction between the two **pp** cells. If the **pp** cells do interact, it is possible that the interaction is not apparent in **ap⁻pa⁻(aa⁻)** animals because the **ap/pa/(aa)** cells are necessary either for this interaction to occur, or for all aspects of the fates to be properly executed.

b. Isolated B.a(l or r)pp

After ablation of F/U, Y.p, and all B.a progeny except a single **pp** cell, we observed γ-like lineages in two of five animals, and abnormal proliferative lineages in three of five animals (Table 1L.2). We conclude that a **pp** cell requires positional cues to adopt the correct fate. Although the isolated fate can be γ-like, extracellular cues are apparently required to reliably ensure that the fate is properly executed. As is the case for isolated **aa** cells, there is no clear 'ground state' for the **pp** cells.

7. Summary of experiments in the dorsal group

The results of our removal of identified components of fate specification for the **pp** pair suggest how some of the cues may interact. The **pa** cells likely promote anterior fate by inhibiting or localizing the activity of Y.p, and the **ap** cells likewise may inhibit or localize the activity of F/U. One of the roles of F and U is to counteract Y.p. However, F and U have additional roles in the promotion of fate. For instance, comparison of **ap⁻pa⁻(aa⁻)** to F⁻U⁻**ap⁻pa⁻(aa⁻)** animals, both of which have Y.p intact, suggests that F and U play a role in producing the abnormal, proliferative lineages. Likewise, although F and U are not required for the production of γ lineages in F⁻U⁻Y.p⁻ animals, in a [B.al⁻ or B.ar⁻] background, γ lineages are only seen if F and U are present.

In most experimental animals the cells move roughly anterior/posterior relative to each other, as in intact animals. This movement allows the additional analysis of patterning and polarity within the **pp** pair. Removal of the positionally anterior and posterior F/U and Y.p cells still results in normal polarity within the **pp** pair. Thus either additional, unidentified cues can establish this polarity, or polarity information can come from the other B.a progeny. Analysis of F⁻U⁻**pa⁻** animals suggests that the latter explanation may indeed be the case, since the pattern appears random in these animals. However, polarity is restored after removal of the **ap** cells in this background. Therefore positional cues are not absent in the F⁻U⁻**pa⁻** animals, but they are either inhibited or somehow equally balanced. There is no obvious polarity in F⁻U⁻Y.p⁻**ap⁻pa⁻(aa⁻)** animals. However, the abnormality of the resultant lineages makes it difficult to score polarity.

Although there is some evidence for lateral interactions between the **pp** cells, the ablation results are less conclusive in the **pp** pair than in the **aa** pair. Experiments that include a single **pp** cell suggest that interaction between the **pp** cells is not necessary for the specification of γ and δ fates, and isolation of the **pp** pair does not provide evidence that the homologues interact. However, in F⁻U⁻Y.p⁻ animals γ lineages are observed if both **pp** cells remain, but not if only one remains, suggesting that the two **pp** cells may have the potential to interact.

In contrast to our initial hypothesis, we believe that specification of **pp** lineages involves two steps based on (1) the nature of the γ*/δ* lineages, and (2) the existence of the abnormal proliferative lineages. The aspects of **pp** cell fate that we have used in our analysis are the axis of **pp** cell division (sagittal versus transverse; see Table 1 legend) and the number of progeny produced by each **pp** cell. Considering these criteria, δ* lineages include characteristics of both γ and δ lineages: the initial division is transverse (δ-like), but each cell divides again. Thus the **pp** division axis can be uncoupled from the number of progeny produced. Similarly, γ* lineages have an initial sagittal division (like normal γ lineages), but only two rounds of division. In abnormal proliferative lineages the **pp** cell produces up to eight progeny. Although the first division of an abnormal proliferative lineage is often sagittal (γ-like), the axes and timing of divisions and the placement of progeny are highly variable. We propose that these abnormal types of lineages reflect two steps in lineage specification: an earlier step (possibly reflected in division axis) and a later step (reflected in proliferation). This two step process model of lineage specification distinguishes the **pp** pair because it suggests that γ and δ fates do not result from the simple choice and execution of distinct sublineages, but rather a series of decisions influenced by extracellular cues. Thus although γ and δ are distinct **pp** *fates*, they do not represent distinct *sublineages*.

IV. Differences between B.a(l/r)a and B.a(l/r)p

Our ablation results suggest that there is a fundamental difference in fate potential between the **aa** pair and the **pp** pair. For instance, single isolated **aa** cells generally produce α-like lineages (average number of progeny=4.4), whereas single isolated **pp** cells generally produce more proliferative and variable lineages (av. prog.=6.2). The cells also respond differently in the same ablation backgrounds. In addition, although the siblings of the **aa** cells (**ap**) and the siblings of the **pp** cells (**pa**) can respond to extracellular cues, the behavior of these cells when isolated from the identified cues suggests that they may also be inherently different from one another. Specifically, isolated **pa** cells produce ζ lineages, and isolated **ap** cells generally produce ε lineages, the fates associated with these cells in intact animals. Since we now

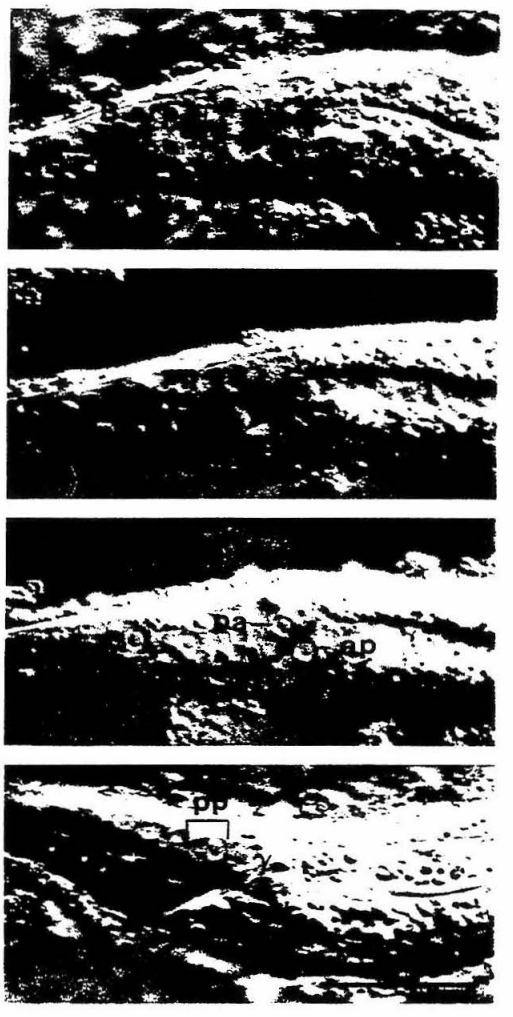

Fig. 14. Nomarski photomicrographs illustrating (A) the difference between B.arp and B.ara in timing of cell division and (B, C) the migration of the 8 B.a progeny. Anterior is to the right, ventral down (right lateral view). (A) B.arp divides prior to B.ara. (B) After both B.a(l/r)a and B.a(l/r)p have divided, B.a(l/r)aa and B.a(l/r)pp are positioned left/right prior to migration (B.1; compare with Fig. 4B.1) and B.a(l/r)ap and B.a(l/r)pa are approximately dorsal/ventral (B.2; compare with Fig. 4B.2). The cells then migrate to their anterior/posterior positions. The **pp** cells are migrating (C). In this animal, B.arpp will be γ, and B.alpp will be δ. Scale bar, 20 μm.

have a handle on some of the conditional components of fate, we can consider what may reflect an autonomous component of fate specification for these cells.

A simple event that could account for the inherent difference(s) between the **aa** and **pp** cells, and between the **ap** and **pa** cells, is that a difference in fate is established between the precursors B.a(l/r)a and B.a(l/r)p. We have

Table 6. Bilateral symmetry in α/β and asymmetry in γ/δ fates

	B.alpp fate γ	B.alpp fate δ	total	
B.alaa fate α	5	5	10	$\chi^2 = 0.18$ (1 d.f.) $p > 0.5$
β	1	11	12	
total	6	16	22	
	$\chi^2 = 4.54$ (1 d.f.) $p < 0.05$			$\chi^2 = 4.78$ (3 d.f.) $p > 0.1$

The table indicates the fate of the left cell observed through migration of the **aa** and **pp** cells. For instance, of 22 animals observed, in 11, B.alpp adopts the posterior position (δ) and B.alaa adopts the posterior position (β) (implicitly, B.arpp adopts the anterior position (γ) and B.araa adopts the anterior position (α) in these animals). χ^2 calculated for **pp** and **aa** fates independently, and dependently. Fate choice in the **aa** pair is random. Fate choice in the **pp** pair is skewed. B.alpp was observed to adopt the posterior position 73% of the time. Fate choice in the **aa** pairs is independent of **pp** choice.

closely examined the behavior of these cells to identify whether there are any observable differences that would be consistent with this hypothesis. Analysis of the timing of the division of the B.a(l/r)a and B.a(l/r)p cells suggests that the two pairs of cells, although morphologically similar, have acquired observable differences by this stage. Specifically, B.a(l/r)p always divide prior to B.a(l/r)a (mean time difference=16 minutes (n=22), Fig. 14A). Although in rare cases (3/44 sides) a posterior cell divides at about the same time as the anterior cells, they never divide after the anterior cells. One component of cell state - timing of division - clearly differs between the B.a(l/r)a and B.a(l/r)p cells, and thus the precursors of the **aa** and **pp** cells are distinct. These results are consistent with the observations of Sulston and Horvitz (1977) and Sulston et al. (1980).

The animals examined for timing of B.a(l/r)a and B.a(l/r)p divisions were also observed through the migration of the B.a(l/r)xx cells (Fig. 14 B, Table 6). Our results suggest that although both left and right **pp** cells can adopt the anterior position, the probabilities are not equal: B.arpp adopted the anterior position (and γ fate) 16/22 times (73%). The biological relevance of this apparent bias is not clear. **aa** cells adopted anterior and posterior positions randomly, and there is no correlation between anterior/posterior choice adopted by the **aa** pair and the **pp** pair.

DISCUSSION

I. Multiple cell interactions specify B.a(l/r)xx fates: a model

We have characterized fate specification in three distinct

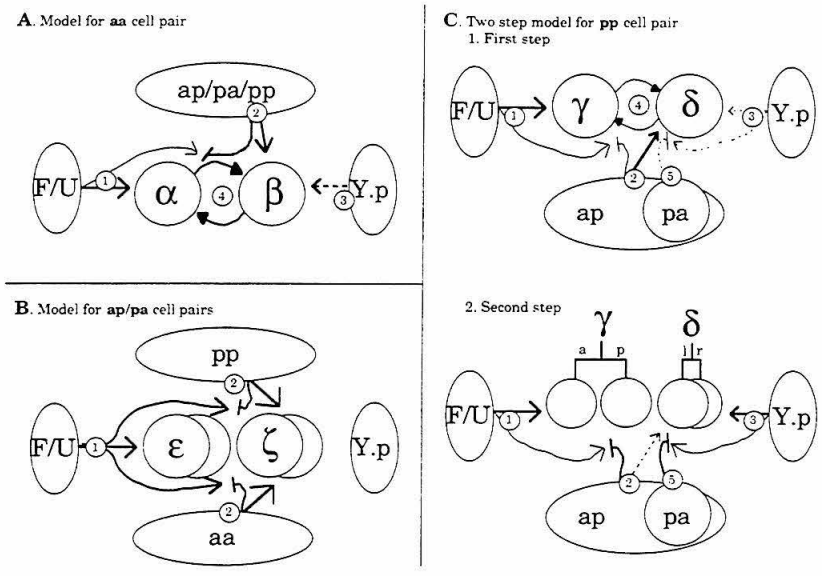

Fig. 15. A model for fate specification in the three cell pair types (anterior left, ventral down). Refer to text for more complete discussion. Positional cues and lateral interactions indicated with arrows. Modulatory interactions indicated with bars. Dashed lines indicate a weaker effect. In general the B.a progeny are indicated to have two effects (function both as a positional cue and a modulator). This may reflect their potential role as 'insulators' as well as providers of an active positional cue. The relative requirement for each function may be different in each of the three pairs. (A) In the **aa** pair, F/U (1), B.a progeny (2), and possibly Y.p (3) provide positional cues. The other B.a progeny (2) modulate F/U information (shown as blocking F/U cue). The two **aa** cells also interact with each other (4). This model does not take into account the possibility of later interactions required for maintenance of fate choice. (B) In the **ap/pa** pairs, F/U (1) and possibly the other B.a progeny (2) provide positional cues. The B.a progeny may modulate or localize cues from F/U (2). (C) A two step model for the **pp** pair. In the first step, F/U (1) and the B.a progeny (2) provide positional cues. The B.a progeny may act to modulate F/U activity (2). The two **pp** cells may also interact with each other (4). We envision that the second step of fate specification occurs in the progeny of the **pp** cells. In this step, F/U (1) and Y.p (3) (and possibly the B.a progeny (2)) provide positional cues. The other B.a progeny regulate F/U (2) and Y.p (5) information, and F/U may modulate Y.p information (not included in Fig.). Note that some components of the two steps may temporally or functionally overlap. As drawn, the models indicate that interactions take place at the B=10 cell stage for the **aa** pair (A) and the **ap/pa** pairs (B). This is consistent with the data, although the possibility that interactions occur later cannot be ruled out. Likewise, the timing of fate specification in the two step model for **pp** cells (C) is consistent with the data, but not the only possibility. We have interpreted the results in terms of promotion and transformation of distinct fates. However, a more conservative interpretation which considers only whether a lineage is disrupted (such as in Tables 3, 4, and 5) yields a similar model. We have not ruled out the possibility that the function(s) that we have ascribed to F, U, and Y.p may actually be due to a subset of progeny of these cells. Although this is possible, it would not change our interpretation of how fate is specified in the eight B.a progeny. In addition, we have not ruled out the possibility that the 'activities' we have identified are mediated by multiple gene products or biochemical mechanisms. The different functions of a particular cell might be mediated by different genes, or a single function might be mediated by multiple processes. These issues, as well as the validity of the current model, may be addressed by genetic analysis of the system. Many of the ablations result in spicule abnormalities (for example, see Fig. 1; other data not shown), indicating that the system is amenable to mutant isolation and characterization.

pairs of cells generated in the B cell lineage of the *C. elegans* male: **aa**, **ap/pa**, and **pp**. Our results suggest that multiple cell interactions (positional cues, their modulators, and lateral interactions) specify fate in the B.a(l/r)xx pairs. Although **aa** fates (α/β) and **ap/pa** fates (ε/ζ) may result from one specification step, **pp** fates (γ/δ) require a two-step specification process. Fig. 15 illustrates a possible interpretation of how fate is specified by extracellular cues in the three B.a(l/r)xx cell pairs.

aa pair (Fig. 15A): For the **aa** pair, we envision that the two cells of the pair interact to establish a pattern of one α-like and one β-like cell by a lateral signalling mechanism (labelled (4); analogous to anchor cell specification in the *C. elegans* hermaphrodite: Seydoux and Greenwald, 1989). Positional cues overlay this interaction so it is skewed to always form the same anterior/posterior pattern. **ap/pa/pp** (and Y.p) act to generally promote posterior (β) fate (2, 3), and F and U act to override this effect and locally promote α fate (1). The integration of these two types of cues could be entirely within the **aa** cells themselves, although the **ap/pa/pp** cells may also act to modulate or localize the F/U signal.

ap/pa pairs (Fig. 15B): There are two identified positional cues that act in the **ap/pa** pairs. F and U act to promote ε fate in both **ap** and **pa** (1), and the other B.a progeny act to promote ζ fates in **pa** (2). Since 'isolated' **ap** and **pa** cells adopt ε and ζ fates, respectively, one part of F/U function may be to counteract the activity of the other B.a progeny. In addition, the B.a progeny cells may act to inhibit or localize the activity of F and U (2). We cannot conclude whether the **ap** and **pa** cells interact actively.

pp pair (Fig. 15C): We propose two distinct steps in γ/δ fate specification, which may correspond to specification in **pp** cells, and in the **pp** cell daughters. In the first step, F/U promote anterior fate (1) and **ap/pa/(aa)** promote posterior fate (2). The **ap/pa/(aa)** cells may also act to modulate or localize F/U activity at this step. The two **pp** cells may also interact with each other (4). Although the initial predilec-

tion for γ or δ fate may be specified in the first step, proper execution of the fates requires a second step. In the second step, F and U promote anterior fate (1), and Y.p promotes posterior fate (3). The **ap** and **pa** cells act to modulate or localize these two potential cues (2, 5). The role of the **pa** cells is to prevent Y.p activity from extending inappropriately to the anterior **pp** cell. Both the **ap** and **pa** cells act to localize F/U activity to the anterior daughter (or granddaughters) of presumptive γ. We envision that these functions may be achieved by physically preventing the activities from reaching the inappropriate cells ('insulation'), although a variety of more active blocking or localization mechanisms are not excluded. F and U also appear to have a role in modulating cues produced by Y.p (not drawn in Fig.). This could be either direct or indirect. In the latter case, F and U might promote γ fate to the exclusion of δ fate. Although the two steps of **pp** fate specification are distinct, some of their components may temporally or functionally overlap.

II. Properties of the identified extracellular cues

The relationship among the cues

Our model results in part from interpretation of lineages observed in multiply ablated animals. These experiments can address the specific nature of an interaction. For example, with respect to the **pp** cell fates, the result of double Y.p **pa** ablation resembles that of single Y.p ablation. The order of action of these two cues depends on interpretation. Although the Y.p activity can be interpreted to act before the **pa** activity, we prefer the simpler interpretation that **pa** is a negative modulator of Y.p activity. This interpretation places the activity of Y.p after, or concurrent with, the activity of the **pa** cells, and does not require that the **pa** cells be biochemically distinct from the **ap** cells or from the other B.a progeny.

The activities produced by F/U and the B.a progeny may act in parallel. This can be illustrated in the **pp** pair, where the lineages in F⁻U⁻**ap**⁻**pa**⁻(**aa**⁻) animals are distinct from those seen in either single F⁻U⁻ or **ap**⁻**pa**⁻(**aa**⁻) animals. Specifically, ablation of F/U results in truncated lineages from presumptive γ, and ablation of **ap/pa/(aa)** results in abnormal proliferative lineages for both presumptive γ and δ. The double F/U **ap/pa/(aa)** ablation results in abnormal lineages less extreme than in either of the single ablations. The abnormal proliferation is not apparent, but the cells generally exhibit more 'anterior-like' fates than in F/U ablations, and the fate of presumptive δ cells can be disrupted. If the F/U cue and **ap/pa/(aa)** cue acted in a linear pathway, we would expect the effect produced by one ablation to be epistatic to the other, as in Y.p⁻ **ap**⁻ animals. If the activities acted independently but at distinct steps, or on different cells, we might expect an additive effect in the doubly ablated animals. Since the double ablation more closely approximates to normal patterning than either single ablation, it suggests the activities may act in parallel and antagonistically on the same process (for example, see Kenyon, 1986).

Active extracellular cues in the specification of B.a(l/r)xx fates

Although many of the identified 'activities' may actually represent multiple biochemical products, it is useful to establish the minimum number of *active* products proposed by the model. We infer that a particular cue is active if the following two criteria are met: (1) ablation of the cell(s) that provides it results in fate disruption, and (2) double ablation experiments indicate that it can act independently of other cues. Of the five distinct interactions that specifiy fate in the B.a(l/r)xx cells, we propose that at least four are active: cues from F/U, B.a progeny, Y.p, and lateral interactions (Fig. 15). (1) The activity of the F/U cue is suggested by the parallel function of F/U and B.a progeny in the **aa** and **pp** pairs, by its ability to promote ε fate in **pa** cells, and by its ability to promote the abnormal proliferative lineages in **pp** cells. (2) The activity of the B.a progeny cue is suggested by the parallel function of F/U and B.a progeny in the **aa** and **pp** pairs. In the **ap/pa** pair, only the modulatory function of the other B.a cells may normally be required. (3) The activity of the Y.p cue is suggested by its interaction with other activities (e.g., F/U), and by its ability to promote the pattern of **pp** cells in F⁻U⁻**ap**⁻**pa**⁻(**aa**⁻) animals. (4) A lateral interaction is suggested in the **aa** cells in the patterning of fates in F⁻U⁻Y.p⁻**ap**⁻**pa**⁻**pp**⁻ animals. (5) The role of the **pa** cells in promoting γ fate is not independent of Y.p. Thus it might not represent an active process.

Redundancy of the extracellular cues

Our ablation experiments have revealed three instances of redundancy in extracellular cues. F and U are embryonic sister cells, and despite different lineages, they produce many common progeny types, including similar neurons (the EF and DX male specific neurons) and cells that play a role in the death of neighboring cells (Sulston et al., 1980). F and U behave similarly in their interactions with the B.a(l/r)xx cells, thus in terms of 'F/U activity' we consider them to be duplicate cells with identical function.

The B.a progeny sets provide the second example of redundancy. It involves a relatively large but specifically characterized set of cells that are present (but not essential) as a group. Like F and U, they are lineally related. Although these cells share many characteristics, distinct differences in B.a(l/r)xx precursors and in terminal fates are evident during development of B.a. The redundancy is best seen in the **aa** pair, where any pair of the six other B.a cells is sufficient to specify β fate. However, the 'B.a progeny' cue acts distinctly on each of the three cell pairs. Any pair of B.a(l/r)xx cells is not always sufficient to promote posterior fate in the **pa** cells, and the six cells are not equivalent in their action on the **pp** pair. We envision that the B.a progeny may serve more than one role (for instance, an active role plus a passive, 'insulating' role). Thus this redundancy may reflect redundancy of position (neighboring cells are present, regardless of fate) as well as redundancy of a common positional cue. The relative requirement for, or responsiveness to, these two distinct functions may differ among the three types of cell pairs.

The cues involved in **aa** anterior-posterior patterning are distinct. Ablation of F/U, for instance, results in a different effect on **aa** fate than ablation of Y.p or **ap/pa/pp** (compare Fig. 6 and Table 2 with Fig. 9 and Table 3). Therefore, the redundancy of this system is not a common activity

Table 7. Summary of cells that provide positional cues and the fates they promote

Cell(s)	Promote fate: aa α	aa β	pp γ	pp δ	ap/pa ε	ap/pa ζ
F/U	++	–	++	–	+	–
Y.p	–	+/–	–	++	–	–
pa	–	–	++	–	–	×
ap/pa/(aa)	×÷	×÷	++	++	×	×
ap/pa/pp	–	++	×	×	×	×
aa/ap/pp	×	×	×	×	×	++

The role of a cell in promotion of a particular fate is inferred from the effects observed following cell ablation. ++, strongly promotes fate. +, weakly promotes fate. +/–, may play a minor role in promoting fate. –, does not play a role in promoting fate. x, cell is absent. ÷If they are present, the **aa** cells are not affected.

Table 8. Differentiated fates of B progeny

Precursor	Differentiated Fates: N	N.Sup	Proct	Death
α		2	2	
β	2	4		
γ		2	3	1
δ			2	
ε			5	1 + (1)÷
ζ	3	2		

N, neuron; N. Sup, neuronal support cell (socket or sheath cell); Proct, proctodeal cell; Death, cell that undergoes programmed cell death. For example, a β lineage produces 6 progeny: 2 neurons and 4 neuronal support cells (2 socket and 2 sheath cells). ÷Each ε lineage includes one invariant and one conditional death (either the left or the right cell will die, but not both). Thus combined, the ε lineages produce a total of 3 cell deaths. Data of Sulston et al. (1980).

shared by the three components, but rather different pieces of information about anterior-posterior position. For instance, if **ap/pa/pp** cells are present, either F/U (anterior cue) or Y.p (posterior cue) provides sufficient information about the environment for proper migration. The '2 of 3' aspect of this system may reflect a precise sensitivity of the cells to the positional cues. Alternatively, it may result because the **ap/pa/pp** cells modulate or localize the F/U and Y.p cues. Specifically, if only one of F/U or Y.p is present, then the modulatory role of the **ap/pa/pp** cells is required to establish a gradient of F/U or Y.p cue. If both F/U and Y.p are present, the **ap/pa/pp** activity is not necessary because the F/U 'anterior' and Y.p 'posterior' cues are distinct, and can establish asymmetry in the **aa** environment.

The permissive nature of the extracellular cues

In general, removal of several distinct cells or groups of cells can disrupt specific B.a progeny fates (Table 7). For instance, disruption of presumptive γ lineages results from ablation of F/U, **pa**, or **ap/pa/(aa)** (see Figs 6, 9; Tables 2, 5). Likewise, presumptive δ lineages are disrupted by ablation of either Y.p or **ap/pa/(aa)**. All of these cues are not entirely necessary for a specific fate, however. For instance, 2/5 **pp** cells isolated from all identified positional cues produced γ-like lineages (Table 1L.2). **pp** cells thus retain the potential to adopt γ fate. Nevertheless, the integration of multiple extracellular cues, combined with the cells' inherent potential, is required for proper, consistent execution of **pp** cell fate.

Is any particular cue *necessary* and/or *sufficient* to specify any given fate? Y.p is necessary, though not sufficient, for δ fate. F/U is necessary, and possibly sufficient, for ε fate in **pa**, though clearly not in **ap**. No cells or combination of cells is entirely necessary or sufficient for α, β, and γ fates. We thus consider most of the interactions in terms of 'promotion' of one fate choice over another.

The differences of the responses from the three cell pairs suggest that the extracellular cues promote specific choices between neuroectoblast fates rather than promoting proliferation or the production of a specific differentiated cell type *per se*. For example, F and U act to increase proliferation in the case of **pp** and **ap/pa** cells (promote γ and ε), but decrease proliferation in the case of **aa** cells (promote α) (see Fig. 2). Likewise, F and U do not appear to promote specific precursor or differentiated cell types. In intact animals, there are three general cell types in the progeny of B.a: neurons, neuronal support cells, and proctodeum (epidermis) (Sulston et al., 1980). However, neurons, for instance, arise at several positions in the lineage rather than from a single neuroblast precursor (Table 8). Even if one considers neurons and support cells as a common 'neuronal' type, β and ζ are neuronal precursors, δ and ε are proctodeal precursors, and α and γ are 'mixed,' producing both neuronal and proctodeal progeny. In addition, ablation of F and U disrupts the more neuronal lineage in the **pp** pair, but the more proctodeal lineage in the **ap/pa** pairs.

III. Equivalence groups and specification of the pairs

An equivalence group is a set of identical cells that are equally capable of executing a shared set of fates (Sulston and White, 1980; Kimble, 1981; Blair and Weisblat, 1984; Shankland and Weisblat, 1984; Doe and Goodman, 1985; Nishida and Satoh, 1989). These cells have equivalent developmental potential, and extracellular cues dictate the eventual fate. Are the **aa**, **ap/pa**, and **pp** pairs equivalence groups? The **aa** pair and the **pp** pair both exhibit natural variation in fate. Ablation experiments of Sulston and White (1980) indicate that the cells of the **aa** pair also exhibit a hierarchy of fates, with α 1°, and β 2°. Thus, these cells likely represent an equivalence group in the strict sense. In contrast, similar experiments with the **pp** pair were unable

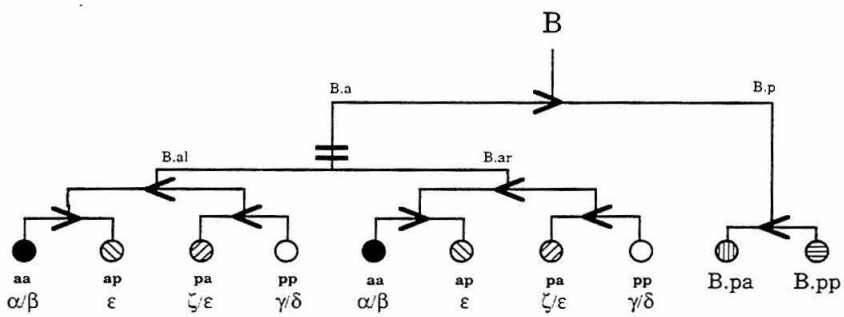

Fig. 16. Lineage illustrating the asymmetries in the B lineage necessary to produce the eight B.a progeny types. = represents equational division: > or < represent non-equational divisions, but do not necessarily indicate that the daughter cells are of different size. In the case of the division of B, however, cytokinesis is unequal, producing a larger anterior (B.a) and smaller posterior (B.p) cell. Although positional cues specify fate choice within, for example, the **pp** pair, the **aa** and **pp** cells are distinct. Thus additional components of fate specification (indicated abstractly by >s or <s) must also play a role. Since differences are apparent in the precursors of these cells (B.a(l/r)a and B.a(l/r)p), some of these components may be autonomous to the B.a(l/r)xx cells.

to establish a fate hierarchy. Although in the first step of **pp** fate specification the cells may be equivalent, our results indicate that there are two steps in the specification of γ and δ fates. Thus it is impossible to interpret the fates in simple terms of 1° and 2° fates.

The **ap**/**pa** pairs exhibit replacement regulation, a characteristic often associated with cells in an equivalence group (e.g., Sulston and White, 1980). Specifically, **pa** cells will produce ε lineages in the absence of the other B.a progeny. However, under experimental conditions the **ap** cells have never produced ζ lineages. Although both cells respond to positional cues, F and U are required to promote the transformation to ε fate in **pa**, whereas the **ap** cells can produce normal ε lineages in the absence of F and U. Our experiments do not completely rule out the ability of **ap** to adopt ζ fate. Nevertheless, after isolation of the cells by removal of all identified positional cues, the **ap** cells adopt their normal ε fate, and the **pa** cells the ζ fate. Thus, the **ap** and **pa** may not be functionally equivalent. **pa** may have the potential to adopt both ε and ζ fates, whereas **ap** could be restricted to the ε fate. If this is the case, then these cells represent an example of replacement regulation without equivalent potential.

What is the relationship among the three pairs? We propose that each B.a(l/r)xx cell is competent to make a particular choice of fate (α versus β, γ versus δ, ε versus ζ), and this competence results from an earlier specification event. First, the cells of a given pair (e.g., **aa**) respond to specific cell ablations (e.g., $Y.p^-$) with different intensity than the cells in the other pairs (e.g., **pp**). Second, the behaviors of **aa**, **pa**, **ap**, and **pp** cells in 'isolated' backgrounds are distinct (see Results, Section III). Third, in general there are no obvious examples of a cell of one pair that adopts the fate normally associated with another pair. Although some lineages share some superficial characteristics, we do not believe that any disrupted lineages ever represent transformation of fate potential from one pair to another. Since the three cell pairs appear to be different, distinct fate specification events may take place in the early progeny of B to distinguish, for instance, the **aa** pair from the **pp** pair (Fig. 16). These distinctions might be autonomously specified. Division of B.a establishes the left/right symmetry of the spicules, and produces two apparently identical daughters. This equational division produces two cells that each produce a set of four progeny. To obtain four distinct progeny types, both rounds of division must be asymmetric, i.e., give rise to different progeny types. Although, as yet unidentified cell interactions may be responsible for these distinctions, the difference in the timing of B.a(l/r)p division compared with B.a(l/r)a indicates that these precursors of the B.a(l/r)xx cells already have distinct cell states.

IV. Signal integration: three general types of cell interactions

We have demonstrated that the B.a(l/r)xx cell fates are specified by three distinct types of cell interactions: (a) positional cues, (b) modulators of positional cues, and (c) lateral cues (Fig. 17). Positional cues are unidirectional, or at least the cells producing them are not responsive to direct feedback as a result of exposure to the cue (in contrast to lateral cues). Active 'signals' - so called inducers and inhibitors - represent positional cues. Modulators act to localize or otherwise modify the activity or effect of a positional cue. Their unique characteristic is that this function is dependent entirely on the presence of the positional

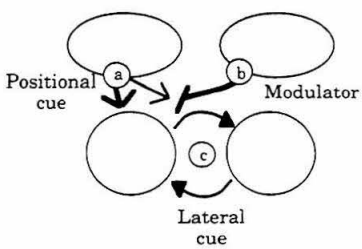

Fig. 17. Three general types of intercellular signals. (a) Positional cues: inducers, inhibitors. These cues provide unidirectional positional information. (b) Modulators: active or passive. Modulators act on positional cues. Their function is dependent on the presence of the positional cue. (c) Lateral signals. These signals act reciprocally among cells of equivalent potential, and include a feedback mechanism. See text for additional discussion.

cue. Modulators can be active (e.g., production of an enzyme that degrades or modifies the positional cue) or passive (e.g., insulators that physically block another cell's access to the positional cue). Lateral cues are reciprocal (at least initially) between cells of equivalent potential, and include a feedback mechanism. Feedback can be inhibitory, and thus result in the amplification of a discrepancy between the cells (Seydoux and Greenwald, 1989; Heitzler and Simpson, 1991), or excitatory, and thus result in the amplification of a particular effect in both (all) cells. In general, complexity may arise from the fact that there may be multiple positional cues, all acting in parallel, as well as multiple modulators, and so on. In addition, the actual units of specification may be layered so that fates may result from a series of discrete specification steps.

In the B.a(l/r)xx cells, we observe examples of cues that act in series as well as in concert at the same step of fate specification. The cues involved in the first and second steps of **pp** cell fate specification act in series. Modulators, in contrast, act at the same step as the activities they modulate. In addition, some activities (e.g., those produced by F/U and B.a progeny) may act in parallel and antagonistically on the same process, and thus act at the same step. Lateral interactions may also be concurrent with the positional cues in the B lineage. Since in both **aa** and **pp** cells all fates (α/β and γ/δ) are observed in animals with just a single cell of the pair present, lateral interactions are not necessary for any fate. Thus, in this case the normally precise pattern of fate is unlikely to result from the promotion of one fate (e.g. anterior) followed by lateral interactions to promote the other (e.g. posterior).

Although a single positional cue might be sufficient to specify fate distinctions, the integration of the three types of cues, combined with redundancy, may result in a more robust fate specification mechanism. The use of multiple signals is observed in several systems. For instance, *Xenopus* mesoderm induction requires multiple positional cues from vegetal cells (reviewed by Kimelman et al., 1992) and the 'community effect' (Gurdon et al., 1984; Gurdon et al., 1993), a potential excitatory lateral interaction. Similarly, vulval induction in *C. elegans* hermaphrodites likely involves at least two positional cues and a lateral interaction (reviewed by Horvitz and Sternberg, 1991). In some systems one cue may predominate (for example, lateral signalling during anchor cell specification: Seydoux and Greenwald, 1989) and be both necessary and sufficient for a particular fate. However, technical constraints may also limit the characterization of the true complexity of fate specification in many systems. We expect that complex integration of these three types of cell interactions may be common in other tissues and organisms.

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Table 1 enlargement. These are the data in Table 1 pages B-9 - B-12.

		aa		pp		ap		pa		
	animal #	ant	post	ant	post	left	right	left	right	B.p(a/p)
A.	**Intact**	α	β	γ[s]	δ[t]	ε	ε	ζ	ζ	normal (n)
	# of progeny	4	6	6	2	6	6	5	5	
B. (1)	**F⁻U⁻**									
	124	abn-5	β	δ_τ	δ	ε	ε	ζ	ζ	n
	125	abn-5	β	δ	δ	ε	ε	ζ	ζ	n
	128	β	β	γ*	δ	ε*	ε*	ζ	ζ	n
	144	β	β	γ*	δ	ε	abn-5	ζ	ζ	n
	148	abn-5	β	γ	δ	ε*	ε*	ζ	ζ	n
	151	β	β	γ*	δ	ε	ε	ζ	ζ	n
	216	β_τ (l)	abn-5 (r)	γ	δ	ε	ε	ζ	ζ	n
(2)	**F⁻**									
	142	α	β	γ*	δ	ε	ε	ζ	ζ	n
	145	α	β	γ	δ	ε	ε	ζ	ζ	n
	146	α	β	γ	δ	ε	ε	ζ	ζ	n
	152	α	β	γ	δ	ε	ε	ζ	ζ	n
(3)	**U⁻**									
	140	abn-5	β	γ	δ	ε	ε	ζ	ζ	n
	141	α	β	γ	δ	ε	ε	ζ	ζ	n
	143	α	β	γ	δ	ε	ε	ζ	ζ	n
	155	α	β	γ	δ	ε	ε	ζ	ζ	n
C. (1)	**Y.p⁻**									
	166	α	β	γ	δ*	n.d.	n.d.	n.d.	n.d.	n
	172	α	β_τ	γ	δ*	ε	ε	ζ	ζ_τ	n
	175	α	abn-5	γ	abn-3 [s]	ε	ε	ζ	ζ	n
	177	α	β	γ	abn-3 [s]	ε	ε	ζ	ζ	n
	179	α	β	γ	abn-3 [o]	ε	ε	ζ	ζ	n
	224	α	β	γ	δ	ε	ε	ζ	ζ	n
	227	α	β	γ	δ*	ε	ε	ζ	ζ	n

		aa		pp		ap		pa		
	animal #	ant	post	ant	post	left	right	left	right	B.p(a/p)
(2)	**B.p⁻**									
	479	α	β	γ	δ	ε	ε	ζ	ζ	----
	480	α	β	γ	δ	ε	ε	ζ	ζ	----
	481	α	β	γ	δ	ε	ε	ζ	ζ	----
(3)	**Y.p⁻ B.p⁻**									
	167	α	β	γ	abn-3 [s]	ε	ε	ζ	ζ	----
	170	α	β	γ	δ*	ε	ε	ζ	ζ	----
	171	n.d.	n.d.	γ	δ*	n.d.	n.d.	n.d.	n.d.	----
D. (1)	**B.a(l/r)ap⁻ B.a(l/r)pa⁻ B.a(l/r)pp⁻**									
	243	α	α	----	----	----	----	----	----	n
	244	α	α	----	----	----	----	----	----	n
	245	α	α	----	----	----	----	----	----	n
	246	α	β	----	----	----	----	----	----	n
	445	α	abn-5	----	----	----	----	----	----	n
	450	α	α	----	----	----	----	----	----	n
(2)	**B.a(l/r)p⁻**									
	241	α	β	----	----	ε	ε	----	----	n
	242	α	β	----	----	ε	ε	----	----	n
(3)	**B.a(l/r)ap⁻ B.a(l/r)pp⁻**									
	247	α	β	----	----	----	----	ζ	ε	n
	279	α	β	----	----	----	----	n.d.	n.d.	n
	280	α	abn-5	----	----	----	----	n.d.	n.d.	n
E. (1)	**B.a(l/r)aa⁻ B.a(l/r)ap⁻ B.a(l/r)pa⁻**									
	261	----	----	abn-5 (l) [s]	abn-7 (r) [s]	----	----	----	----	n
	262	----	----	abn-8 (lv) [s]	abn-4 (rd) [s]	----	----	----	----	n
	275	----	----	abn-8 [t]	abn-7 [o]	----	----	----	----	n

		aa		pp		ap		pa		
	animal #	ant	post	ant	post	left	right	left	right	B.p(a/p)
(2)	**B.a(l/r)ap⁻ B.a(l/r)pa⁻**									
	234	α	β_τ	abn-7 [o]	δ	----	----	----	----	n
	235	α	β	abn-7 [s]	abn-7 [s]	----	----	----	----	n
	388	α	β	abn-8 [s]	abn-3 [o]	----	----	----	----	n
	402	α	β	abn-8 [s]	δ	----	----	----	----	n
	478	α	β	abn-6 [s]	δ*	----	----	----	----	n
(3)	**B.a(l/r)a⁻**									
	238	----	----	γ	δ	----	----	ε	ζ	n
	239	----	----	γ	δ	----	----	ζ	ζ	n
	240	----	----	γ	δ	----	----	ε	ε	n
	389	----	----	γ	δ	----	----	ε	abn-4	n
	454	----	----	γ	δ	----	----	ε	ε	n
(4)	**B.a(l/r)aa⁻ B.a(l/r)pa⁻**									
	256	----	----	γ* (r)	δ (l)	ε	ε	----	----	n
	258	----	----	γ*	δ	ε	ε	----	----	n
	259	----	----	γ*	δ	ε	ε	----	----	abn (.pp->.pa)
	260	----	----	γ*	δ	(ε)	ε	----	----	n
(5)	**B.a(l/r)pa⁻**									
	282	α	β	δ (l)	δ (r)	ε	ε	----	----	n
	283	α	β	γ*	δ	ε	ε	----	----	n
	284	α	β	γ	δ	ε	ε	----	----	n
	285	α	β	γ*	δ	ε	ε	----	----	n
	390	α	β	δ	δ	ε	ε	----	----	n
F. (1)	**B.a(l/r)a⁻ B.a(l/r)pp⁻**									
	251	----	----	----	----	----	----	ε	ε	n
	252	----	----	----	----	----	----	ε	ε	n.d.
	257	----	----	----	----	----	----	ε	ε	n
	399	----	----	----	----	----	----	ε	ε	n

	animal #	aa ant	aa post	pp ant	pp post	ap left	ap right	pa left	pa right	B.p(a/p)
(2)	**B.a(l/r)p⁻ B.a(l/r)aa⁻**									
	422	----	----	----	----	ε*	ε	----	----	n
	423	----	----	----	----	ε	ε	----	----	n
G.	**F⁻ U⁻ Y.p⁻**									
	161	α_τ (l)	β_τ (r)	γ	δ*	ε*	ε*	ζ	ζ	n
	181	abn-5 (l)	abn-7 (r)	γ	δ*	ε	ε	ζ	ζ_τ	n
	182	β_τ (l)	α_τ (r)	abn-5 [s]	abn-3 [o]	ε*	ε*	ζ	ζ	n
	187	α	β	γ	δ	ε*	ε	ζ	ζ	n
	203	β_τ (l)	abn-5 (r)	γ	abn-3 [t]	abn-5	ε*	ζ	ζ	n
H. (1)	**F⁻ U⁻ B.a(l/r)ap⁻ B.a(l/r)pa⁻ B.a(l/r)pp⁻**									
	395	abn-5	β_τ	----	----	----	----	----	----	n.d.
	397	α_τ (r)	β_τ (l)	----	----	----	----	----	----	n
	400	βτ	β_τ	----	----	----	----	----	----	n
	415	abn-5 (l)	abn-5 (r)	----	----	----	----	----	----	n
	449	α_τ (r)	β_τ (l)	----	----	----	----	----	----	n
	453	α_τ (r)	abn-7 (l)	----	----	----	----	----	----	n
(2)	**F⁻ U⁻Y.p⁻ B.a(l/r)ap⁻ B.a(l/r)pa⁻ B.a(l/r)pp⁻**									
	406	α_τ (r)	β_τ (l)	----	----	----	----	----	----	n
	416	α	β_τ	----	----	----	----	----	----	n
	444	α_τ (l)	abn-6 (r)	----	----	----	----	----	----	n
	448	α_τ (l)	β_τ (r)	----	----	----	----	----	----	n
	455	α_τ (l)	abn-5 (r)	----	----	----	----	----	----	n
	458	α_τ (l)	abn-5 (r)	----	----	----	----	----	----	n

		aa		pp		ap		pa		
	animal #	ant	post	ant	post	left	right	left	right	B.p(a/p)
I. (1)	**Y.p⁻ B.a(l/r)pa⁻**									
	446	α	β	γ	δ*	ε	ε	----	----	n
	447	α	abn-5	γ	δ*	ε	ε	----	----	n
	451	α	β_τ	γ	abn-5 [t]	ε	ε	----	----	n
	452	α	β_τ	γ	δ*	ε	ε	----	----	n
(2)	**F⁻ U⁻ B.a(l/r)pa⁻**									
	286	abn-6	β_τ	δ	δ*	ε	ε*	----	----	n
	391	α	abn-5	δ	δ	ε	ε	----	----	n
	392	α	β	abn-3 [s]	δ	ε	ε	----	----	n
	393	abn-5	β	δ (l)	δ (r)	ε*	ε	----	----	n
	394	α	β	γ*	δ	abn-5	ε*	----	----	n
	396	abn-5	β	γ*	δ*	ε	ε	----	----	n
(3)	**F⁻ U⁻ B.a(l/r)ap⁻ B.a(l/r)pa⁻**									
	263	abn-5	abn-6	γ*	δ	----	----	----	----	n
	264	α	β_τ	γ*	δ	----	----	----	----	n
	398	abn-5 (l)	α_τ (r)	γ_τ (r) [s]	γ* (l)	----	----	----	----	n
	412	α	β	γ_τ	δ	----	----	----	----	n
(4)	**F⁻ U⁻ B.a(l/r)aa⁻ B.a(l/r)ap⁻ B.a(l/r)pa⁻**									
	417	----	----	γ	γ*	----	----	----	----	n
	420	----	----	γ*	δ	----	----	----	----	n
(5)	**F⁻ U⁻ Y.p⁻ B.a(l/r)pa⁻**									
	405	abn-5 (l)	β_τ (r)	γ_τ (l) [t]	δ^*_τ (r) [o]	ε*	ε*	----	----	n
	407	abn-6	abn-5	γ	δ*	ε	ε*	----	----	n
	456	α	β_τ	γ	δ*	abn-5	ε*	----	----	n

		aa		pp		ap		pa		
	animal #	ant	post	ant	post	left	right	left	right	B.p(a/p)
(6)	**F⁻ U⁻ Y.p⁻ B.a(l/r)ap⁻ B.a(l/r)pa⁻**									
	413	α	β_τ	γ*	abn-6 [s]	----	----	----	----	n
	414	α	β_τ	γ	abn-5 [t]	----	----	----	----	n
	418	α_τ (l)	abn-5 (r)	abn-7 (r) [s]	abn-5 (l) [o]	----	----	----	----	n
	421	β_τ (l)	β_τ (r)	abn-7 [t]	δ*	----	----	----	----	n
(7)	**F⁻ U⁻ Y.p⁻ B.a(l/r)aa⁻ B.a(l/r)ap⁻ B.a(l/r)pa⁻**									
	419	----	----	abn-6 [s]	abn-6 [o]	----	----	----	----	n
J. (1)	**F⁻ U⁻ B.a(l/r)p⁻**									
	253	α	abn-7	----	----	ε	ε*	----	----	n
	254	α	β	----	----	ε*	ε	----	----	n
(2)	**F⁻ U⁻ Y.p⁻ B.a(l/r)a⁻**									
	404	----	----	abn-6 (l) [o]	γ_τ (r) [o]	----	----	ζ	ζ	n
	409	----	----	γ	δ*	----	----	ζ	ζ	n
	410	----	----	γ	δ*	----	----	ζ_τ	ζ	n
	411	----	----	γ (l)	δ^*_τ (r)	----	----	ζ	ζ	n
	459	----	----	γ	abn-3 [s]	----	----	ζ	ζ	n
(3)	**F⁻ U⁻ Y.p⁻ B.a(l/r)p⁻**									
	461	n.d.	n.d	----	----	ε	ε	----	----	n
K. (1)	**B.a(l or r)⁻**									
	147 (l-)	α		γ		ε		ζ		n
	157 (l-)	α		δ		n.d.		n.d.		n
	164 (l-)	α		γ		ε		ζ		n
	153 (r-)	α		γ		ε		ζ		n
	156 (r-)	abn-5		γ		ε		ζ		n

animal #	aa ant	aa post	pp ant	pp post	ap left	ap right	pa left	pa right	B.p(a/p)
(2) **F⁻ U⁻ B.a(l or r)⁻**									
168 (l-)	β_τ		δ		ε*		abn-4		n
180 (l-)	β_τ		δ*		ε		ζ_τ		n
159 (r-)	abn-6		γ*		ε*		ζ		n
160 (r-)	β_τ		δ		ε		ζ		n
162 (r-)	β_τ		δ		ε		ζ		n
165 (r-)	β_τ		δ		abn-5		ζ		n
169 (r-)	β		δ		ε		ζ		n
(3) **F⁻ U⁻ Y.p⁻ B.a(l or r)⁻**									
174 (l-)	β_τ		γ*		ε		ζ		n
213 (l-)	β_τ		abn-3 [s]		ε		ζ		n
173 (r-)	α_τ		γ*		ε		ζ		n
176 (r-)	β_τ		δ*		ε		ζ		n
L. (1) **F⁻ U⁻ Y.p⁻ B.a(l/r)p⁻ B.a(l or r)a⁻ B.a(l or r)ap⁻**									
457 (l-)	α_τ		----	----	----	----	----	----	abn (damaged)
460 (l-)	α_τ		----	----	----	----	----	----	n
463 (l-)	β_τ		----	----	----	----	----	----	n
464 (r-)	α_τ		----	----	----	----	----	----	n
465 (r-)	α_τ		----	----	----	----	----	----	n
(2) **F⁻ U⁻ Y.p⁻ B.a(l/r)a⁻ B.a(l or r)p⁻ B.a(l or r)pa⁻**									
466 (l-)	----	----	abn-5 [s]		----	----	----	----	n
467 (l-)	----	----	γ_τ [s]		----	----	----	----	n
468 (l-)	----	----	abn-6 [s]		----	----	----	----	n
469 (r-)	----	----	γ_τ [s]		----	----	----	----	n
462 (r-)	----	----	abn-8 [s]		----	----	----	----	abn (damaged)

animal #	aa ant	aa post	pp ant	pp post	ap left	ap right	pa left	pa right	B.p(a/p)
(3) **F⁻ U⁻ Y.p⁻ B.a(l/r)a⁻ B.a(l/r)pp⁻**									
470	----	----	----	----	----	----	ζ	ζ	n
471	----	----	----	----	----	----	ζ	ζ	n
472	----	----	----	----	----	----	ζ	ζ	n
473	----	----	----	----	----	----	ζ	ζ_τ	n
(4) **F⁻ U⁻ Y.p⁻ B.a(l/r)p⁻ B.a(l/r)aa⁻**									
474	----	----	----	----	ε_τ	ε_τ	----	----	n
475	----	----	----	----	ε_τ	abn-7	----	----	n
476	----	----	----	----	ε_τ	abn-8	----	----	n
477	----	----	----	----	ε_τ	ε_τ	----	----	n

Chapter 3

The *lin-3/let-23* pathway mediates inductive signalling during male spicule development in *Caenorhabditis elegans*

Helen M. Chamberlin and Paul W. Sternberg

SUMMARY

During *Caenorhabditis elegans* male spicule development four pairs of precursor cells establish a pattern of fates that correlates with relative anterior-posterior cell position. The F and U cells provide one of the extracellular cues that establish this pattern by promoting anterior fates. We show that the genes in the *lin-3/let-23* signalling pathway required for hermaphrodite vulval induction also mediate this F/U signal. Reduction-of-function mutations in *lin-3*, *let-23*, *sem-5*, *let-60* or *lin-45* disrupt the fate of anterior cells. Likewise, activation of the pathway with ectopically produced signal results in posterior cells inappropriately adopting the anterior fate even in the absence of F and U. We have further used this genetic pathway to begin to understand how multiple positional cues are integrated to specify cell fate. One negative regulatory pathway is represented by the *lin-15* locus. *lin-15* acts in spicule development as it does in vulval induction: as a negative regulator of *let-23* receptor activity. A second extracellular cue, from Y.p, also acts antagonistically to the *lin-3/let-23* pathway. However, this signal is integrated into the *lin-3/let-23* pathway at some step after *lin-45* raf, and is thus functionally distinct from *lin-15*. We have investigated the role of *lin-12* in forming the anterior/posterior pattern of fates. A *lin-12* gain-of-function defect is masked by redundant positional information from F and U.

INTRODUCTION

Cell interactions play a central role in the development of many organisms. As the specific proteins and cellular processes involved in classic vertebrate inductions become identified, the underlying complexities of multiple signals that act in parallel are striking (reviewed in Kimelman, et al., 1992; Davidson, 1993). A key issue in understanding how fate is specified by multiple signals is understanding how the information of such signals is integrated in the responding cells. In *C. elegans*, the essentially invariant and precisely described cell lineage provides a reproducible background to study cell interactions. The set of characterized interactions required during the development of the male B cell indicate that it may serve as a model that combines complex signal integration with the precision of single cell analysis (Chamberlin and Sternberg, 1993).

During *C. elegans* male postembryonic development several precursor cells divide in a sex-specific manner to produce the cells of the copulatory tail. One such cell, B, is the precursor of all of the cells of the male spicules (Fig. 1.A). During male development the anterior daughter of B (B.a) divides to produce eight progeny (Fig. 2). These eight progeny make up four pairs: ventral (**aa**), dorsal (**pp**), and two identical lateral pairs (**ap/pa**). For each pair there is an anterior and a posterior fate that differ in the subsequent cell lineage produced by each cell, as well as the differentiated fates of the progeny produced by that lineage (Sulston and Horvitz, 1977; Sulston, et al., 1980). The choice of fate within each pair is responsive to extracellular cues provided by neighboring cells. Cell ablation experiments suggest that the other male-specific blast cells, or their progeny, provide distinct positional

cues, and that the eight B.a greatgrandprogeny may also interact (Chamberlin and Sternberg, 1993) (Fig. 3). For example, ablation of two other male specific blast cells called F and U disrupts the fates of positionally anterior cells. In some cases they produce the cell lineage normally associated with their posterior neighbors. In contrast, ablation of the blast cell Y.p disrupts the fate of positionally posterior cells.

To identify the genes that mediate the cell interactions required for proper development of the B cell, we have begun to characterize the role of genes required for other cell interactions in *C. elegans*. In this study we have focused on a subset of the genes in the *lin-3/let-23* signalling pathway that mediates *C. elegans* vulval induction since some mutations in these genes result in an abnormal spicule phenotype (e.g., see Aroian and Sternberg, 1991). *C. elegans* hermaphrodite vulval development requires a signal from the anchor cell (AC) in the gonad that acts on three of six epidermal blast cells termed vulval precursor cells (VPCs) (Kimble, 1981). In normal development, the three proximal VPCs produce vulval tissue, whereas the three distal VPCs produce nonspecific hypodermis. The AC signal (an epidermal growth factor (EGF)-like protein encoded by the gene *lin-3* (Hill and Sternberg, 1992)) is both necessary and sufficient to promote the VPCs to initiate vulval development. Genes that are necessary for the response to *lin-3* include *let-23* (receptor) (Aroian, et al., 1990), *sem-5* (adaptor) (Clark, et al., 1992), *let-60* (ras) (Han and Sternberg, 1990), and *lin-45* (raf) (Han, et al., 1993). Reduction-of-function mutations in any of these genes result in a Vulvaless (Vul) phenotype where all six VPCs may produce hypodermis at the expense of vulval tissue. Gain-of-function mutations in *let-60* (Beitel, et al., 1990) and over-production of LIN-3 (Hill and Sternberg, 1992) result in a

Multivulva (Muv) phenotype in which all six VPCs may produce vulval tissue. Loss-of-function mutations at another locus, *lin-15*, also result in a Muv phenotype. In vulval development, genetic analysis suggests that *lin-15* acts as a negative regulator of *let-23*, in parallel to *lin-3* (Ferguson, et al., 1987; Huang, et al., 1994).

MATERIALS AND METHODS

Strains

Nematode strains were cultured according to standard techniques (Brenner, 1974; Sulston and Hodgkin, 1988). Loss-of-function mutations in *lin-3*, *let-23*, *sem-5*, *let-60*, and *lin-45* are known or believed to be lethal. However, we believe the genotypes in Table 1.B. represent a reduction of normal gene function based on two criteria. First, in the male tail each allele is recessive to a wild type copy of the gene (except for *let-60(sy95dn)* and *let-60(sy100dn)*; data not shown). Second, more stringent genetic tests indicate that the alleles represent a reduction of normal function of the gene for vulval development (Aroian and Sternberg, 1991; Clark, et al., 1992; Ferguson and Horvitz, 1985; Han, et al., 1990; Han, et al., 1993). For *lin-3*, *let-23*, and *let-60* we have tested several alleles or allelic combinations in order to verify that the observed phenotypes represent the common phenotypes associated with mutation, as well as to identify genotypes that may represent severe reduction of gene function for the male tail. The extent of the lineage defects can be variable both between and within given genotypes. This may be due to the alleles retaining partial gene activity.

Mutations used are described by Brenner (1974), Hodgkin, et al. (1988), and as noted below.

Linkage Group (LG) II: *clr-1(e1745)*. *let-23(sy97, sy278, n2020)* (Aroian and Sternberg, 1991; H.M.C. and P.W.S., unpublished; S. Clark and R. Horvitz, unpublished). *unc-4(e120)*.

LG III: *lin-12(n137; n137n760)* (Greenwald, et al., 1983).

LG IV: *unc-24(e138)*. *mec-3(e1338)*. *lin-3(n378, n1058, n1059, sy53)* (Ferguson and Horvitz, 1985; Hill and Sternberg, 1992). *lin-45(sy96)* (Han, et al., 1993). *let-60(n2021, sy95dn, sy100dn, n1046gf, sy103gf)* (Beitel, et al., 1990; Han and Sternberg, 1991). *dpy-20(e1282)*. *unc-22(s7)*. *nT1[unc(n754) let]* (=*DnT1* balancer; Ferguson and Horvitz, 1985).

LG V: *him-5(e1467; e1490)* (Hodgkin, et al., 1979).

LG X: *sem-5(n1619)* (Clark, et al., 1992). *lin-15(e1763, n309, n377)* (Ferguson and Horvitz, 1985).

Extrachromasomal arrays: *syEx21(hsp::lin-3)*, *syEx23(hsp::lin-3)* (Hill, et al., 1994).

Full genotypes of animals in Tables 1, 2 and 3 are as follows: *mut-x; him-5(e1490)* doubles were constructed for *let-23(sy97)*, *let-23(sy278)*, *let-60(n2021)*, *lin-45(sy96)*, *let-60(n1046gf)*, *let-60(sy103gf)*, *lin-15(e1763)*, *lin-15(n309)*, *lin-15(n377)*, and *lin-12(n137)*. Data originally summarized in Greenwald, et al. (1983) (Tables 2 and 3) are from *lin-12(n137); him-5(e1467)* and *lin-12(n137n760); him-5(e1467)* animals. *let-23(n2020)* is *let-23(n2020) unc-4(e120); him-5(e1490)*. A male strain was maintained for *syEx21* and *syEx23*.

Other genotypes and construction:

lin-3(sy53/n1058): *unc-24(e138) mec-3(e1338) lin-3(sy53) dpy-20(e1282) / lin-3(n1058).* Construction: *unc-24(e138) mec-3(e1338) dpy-20(e1282)/+* males were crossed with *lin-3(n1058)/DnT1* hermaphrodites. Single non-Unc cross males (genotype: *unc-24(e138) mec-3(e1338) dpy-20(e1282)/lin-3(n1058)* or *+/lin-3(n1058)*) were crossed with *unc-24(e138) mec-3(e1338) lin-3(sy53) dpy-20(e1282)/DnT1* hermaphrodites. non-Dpy non-Unc animals from crosses that yield Dpy Unc progeny (indicating paternal genotype of *unc-24(e138) mec-3(e1338) dpy-20(e1282)/lin-3(n1058)*) are desired genotype.

lin-3(n378/n1059): *lin-3(n378) / unc-24(e138) lin-3(n1059) dpy-20(e1282); him-5(e1490)/+.* Construction: *lin-3(n378); him-5(e1490)* males were crossed with *lin-3(n1058)/DnT1* hermaphrodites. non-Unc male cross progeny are desired genotype.

sem-5(n1619): *clr-1(e1745)/+; sem-5(n1619).* Construction: N2 males were crossed with *clr-1(e1745); sem-5(n1619). clr-1(e1745)* suppresses the lethality associated with *sem-5(n1619)* and maternally rescues for lethality. However, the male tail defect is still observed.

let-60(dn): *let-60(sy95)/dpy-20(e1282); him-5(e1490)* or *unc-24(e138) let-60(sy100) dpy-20(e1282)/unc-22(s7); him-5(e1490).*

Double mutant strains of *let-23* or *lin-45* and *lin-15* were constructed according to standard methods (Ferguson, et al., 1987). In addition to the two mutations, double mutant strains include *him-5(e1490). let-23(sy278); lin-15(e1763)* is *let-23(sy278) unc-4(e120); him-5(e1490); lin-15(e1763).*

Cell lineage and ablation

Cell nuclei divisions in living animals were directly observed using Nomarski differential interference contrast optics as described by Sulston

and Horvitz (1977). Nomenclature follows the standard of Sulston and Horvitz (1977), with modifications of Chamberlin and Sternberg (1993). All lineages were followed from the first divisions of the B.a(l/r)xx cells (early - mid L3) through the L3 molt. Cell nuclei were destroyed by a laser microbeam as described by Avery and Horvitz (1987). F, U, and Y.p were ablated at the stage when B had divided to produce two progeny (early L2), according to the procedures in Chamberlin and Sternberg (1993). Ablation of B.a progeny was during mid to late L2 stage, soon after the targeted cells were generated. Ablation of the "B.a positional cue" for the **aa** cells in Table 2 represents the ablation of B.a(l/r)p, followed by the ablation of B.a(l/r)ap.

Heat shock induction of *lin-3* transgenes

Heat shock was carried out according to the method of Hill, et al. (1994). All animals received heat shock at late L2 larval stage. Individual animals were anaesthetized on pads of 5% agar in water containing 5 μM sodium azide, staged, and allowed to recover one hour on a standard 5 cm NGM agar petri plate seeded with OP50. Plates were then sealed with parafilm and floated in a 33°C water bath for 90 minutes.

RESULTS

The *lin-3/let-23* signalling pathway mediates the F/U signal during male spicule development

We have examined the effects of increasing and decreasing the activity of the *lin-3 / let-23* pathway using chromosomal mutations and extrachromasomal transgenes. Taken together the results indicate that

activation of the pathway is both necessary and sufficient to promote anterior fates, and it likely mediates the positional cue provided by F and U.

Mutations in some genes in the lin-3/let-23 *pathway disrupt fates of anterior cells in the male B lineage*

Some mutations in a subset of genes in the *lin-3 / let-23* signalling pathway result in an abnormal male spicule phenotype (see Fig. 1; also Aroian and Sternberg, 1991). These genes include *lin-3*, *let-23*, *sem-5*, *let-60*, and *lin-45*. In the B lineage, reduction-of-function mutations in any of these genes disrupt the lineages of the anterior cells (Table 1, B.2-10). For instance, of seven *lin-3(sy53 / n1058)* animals followed, none had a normal α or a normal γ lineage, and only one had normal ε lineages (Table 1, B.2). The phenotypes are similar to the abnormalities that result from ablation of both F and U in wild type males (Table 1, B.1). In some cases the anterior cells produce a lineage similar to their posterior neighbors. For instance, in all seven *lin-3(sy53 / n1058)* animals both **aa** cells produced β lineages.

Although the mutant phenotypes resemble the effects of F/U ablation, the F and U cells appear normal in the mutants. First, the F and U lineages are normal in mutant animals (2/2 *lin-3(sy53 / n1058)* and 2/2 *lin-45(sy96)* animals followed). Second, the linker cell in the gonad dies as in wild type animals (10/10 *lin-3(sy53 / n1058)* and 8/8 *lin-45(sy96)* animals). Since U.(l/r)p "murder" the linker cell in intact animals (Sulston and White, 1980), these U progeny are still capable of one of their normal functions.

The lineage defect in mutants is enhanced by ablation of F and U (Table 1, B.11, 12). However, ablation of F and U in mutant animals still did not result in a consistent transformation of all anterior cells to posterior fate.

In particular, although the lineages of anterior **ap** cells were disrupted, the **ap** cells did not produce ζ lineages.

Ectopic expression of the ligand, LIN-3, disrupts the fates of posterior cells

To further test the role of this pathway in the B lineage, we used a transgenic construct that includes the EGF-coding domain of *lin-3* under control of a tissue-general heat shock promoter (Hill, et al., 1994). In the B lineage, heat shock treatment of transgenic animals results in disruption of the lineage of the posterior cells (Table 1, C.1,2). In the **aa** and **ap/pa** pairs the posterior cells produce lineages normally associated with their anterior neighbors. For example, in five of six heat-shocked *syEx21* animals followed, both **aa** cells produced α lineages and both **ap** and **pa** cells produced ε lineages (Fig. 4).

In the **pp** pair, the posterior cells produce more anterior-like lineages. However, lineages of both anterior and posterior cells in this pair can be disrupted to produce up to eight progeny. Although abnormal, these lineages are consistent with our hypothesis that all four **pp** daughters can produce up to four progeny like the normal anterior daughter of γ (see Fig. 2) if the positional cue from F and U is not localized or "modulated" (Chamberlin and Sternberg, 1993). Such abnormal lineages are observed following ablation of the **ap/pa** cells in wild type (Chamberlin and Sternberg, 1993) as well as in transgenic animals.

Transgenic ubiquitous LIN-3 is also sufficient to compensate for the absence of F and U (Table 1, C.4), suggesting that activation of the *lin-3/let-23* pathway is sufficient to promote anterior fates. Gain-of-function *let-60*

mutations that result in an activated protein also disrupt posterior fate, but to a lesser extent than ubiquitous LIN-3 (Table 1, C.3).

Two activities that act antagonistically to *lin-3/let-23* are integrated at functionally distinct steps in the pathway

Mutations in lin-15 *disrupt the fate of posterior* ***pp*** *cells*

In the hermaphrodite vulva, reduction-of-function mutations in the *lin-15* locus result in a phenotype opposite from reduction-of-function mutations in the *lin-3/let-23* genes required for vulval fates. In the male tail, mutations in *lin-15* disrupt posterior fates (Table 1, D.1-3). However, even in *lin-15* null mutants the lineage defect is only observed in some animals, and only the fate of the posterior **pp** cell is usually disrupted: it divides to produce up to four progeny instead of the normal two. Thus mutations in *lin-15* result in a phenotype opposite from those seen in *lin-3/let-23* mutants, but the effect is weak. 31/63 (49%) *lin-15(n309)*, 23/55 (42%) *lin-15(n377)*, and 23/57 (40%) *lin-15(e1763)* adult males have abnormal spicule morphology, consistent with the observation that the B lineage is abnormal in only 5/13 *lin-15* mutant animals followed.

lin-15 *likely acts as a negative regulator of* let-23 *in the male B lineage*

We followed the B lineage in a key subset of double mutants to confirm the position of *lin-15* in the genetic pathway. Mutations in both *let-23* and *lin-45* block the requirement for functional *lin-15* (Table 1, D.4-6). This result is consistent with *lin-15* acting in the B lineage as it does in vulval development, where it is integrated genetically upstream of *let-23*.

The positional cue from Y.p represents a signalling pathway distinct from the lin-3/let-23 *pathway*

Y.p, or its progeny, produces a positional cue that promotes posterior fate, especially in the **pp** pair (Chamberlin and Sternberg, 1993). Ablation of Y.p disrupts the fate of the posterior **pp** cell similar to the defect observed in *lin-15* mutants (Table 1, E.1), suggesting that Y.p may be the source of a signal mediated by *lin-15*. Ablation of F, U, and Y.p together results in a disruption of both anterior and posterior **pp** cell lineages (Table 1, E.2). Ablation of Y.p in *lin-3*, *let-23*, *let-60*, or *lin-45* mutants resembles $F^-U^-Y.p^-$ animals (Table 1, E.3-6). The similar results obtained for *lin-3*, *let-23*, *let-60,* and *lin-45* mutants following Y.p ablation indicate that the positional information from Y.p is not integrated into the *lin-3*/*let-23* signalling pathway upstream of *lin-45* raf. Thus, *lin-15* and the Y.p signal are functionally distinct.

***lin-12* can mediate a lateral interaction between the** pp **cells**

Reduction-of-function mutations in *lin-12* (*lin-12(0)*) result in both **pp** cells producing γ-like lineages. In contrast, no B lineage defects are observed in animals bearing *lin-12* gain-of-function mutations (*lin-12(d)*) (Greenwald, et al., 1983). To better understand the role of *lin-12* in **pp** fate specification, we have carried out cell ablation experiments in *lin-12(d)* mutants. Our results suggest that *lin-12(d)* mutations result in the opposite transformation from *lin-12(0)* mutations if F and U are ablated (Table 2). The effect is best seen when F, U, and Y.p are removed. In wild type animals, this ablation

results in γ and γ*/δ* lineages from both **pp** cells, whereas in *lin-12(d)* animals both **pp** cells produce δ lineages (compare Table 2. f to g). The presence of F and U is sufficient to override the effect of the *lin-12(d)* mutation. An additional defect in *lin-12(0)* animals is that the presumptive Y cell is transformed to a neuronal fate similar to its lineal homolog DA9 (Greenwald, et al., 1983). Since *lin-12(0)* animals are also missing the positional cue from Y.p, at present we cannot establish if the presence of Y.p is likewise sufficient to compensate for the absence of *lin-12*.

Ablation experiments suggested that the **aa** cells may also interact with each other (Chamberlin and Sternberg, 1993). However, ablation experiments in *lin-12(d)* mutants carried out for the **aa** pair did not produce conclusive results (Table 3).

DISCUSSION

The genes in the *lin-3/let-23* signalling pathway mediate the F/U signal defined by cell ablation

Cell ablation experiments suggest that four distinct cell interactions are essential for normal anterior/posterior patterning of fates for four pairs of cells in the *C. elegans* male B lineage (Chamberlin and Sternberg, 1993). The male specific blast cells F and U mediate one of these signals that is necessary for normal anterior fates. The *lin-3* / *let-23* pathway is both necessary and sufficient to promote anterior fates, and thus likely mediates the F/U signal.

The lineage defect in *lin-3/let-23* mutants is enhanced by ablation of F and U. This result suggests that not only might the analyzed mutations not represent null mutations for male tail function, but that all signalling activity may not be eliminated from animals in which F and U have been ablated. Such residual activity may come from the debris of the ablated cells, or may come from other, unidentified sources. However, if such other sources exist they are not sufficient to promote the normal anterior fates in the absence of F and U, and likely do not play a significant role in normal development.

The role of *lin-3/let-23* in fate specification

What specific role in fate specification does the *lin-3/let-23* pathway play in *C. elegans* spicule development? Our results suggest that the same genes mediate the F/U signal for all four pairs of B.a progeny. Since mutations in a single gene of the *lin-3/let-23* pathway can result in all of the defects observed in animals with F and U ablated we can exclude the possibility that F and U produce three different signals, with one for the **aa** pair, one for the **pp** pair, and one for the **ap/pa** pairs. Nevertheless, the cellular response is distinct for each pair. In response to ectopic LIN-3, the **pp** cells produce more progeny than normal while **aa** cells produce fewer progeny. LIN-3 also does not appear to induce a specific differentiated cell type (Chamberlin and Sternberg, 1993), since neuronal and hypodermal progeny arise from both anterior and posterior blast cells (Sulston, et al., 1980). These differences among the three pairs suggest that the *lin-3/let-23* pathway promotes a particular choice among possible responses. The final outcome depends upon functional differences among the responding cells.

The role of *lin-15* in the B lineage

In the *C. elegans* vulva, loss-of-function, molecular null, mutations in *lin-15* result in all VPCs adopting vulval fates. The defect in the B lineage, in contrast, is less extreme, and less than 50% penetrant. Both vulval development and spicule development have a necessary requirement for the positive acting genes of the *lin-3 / let-23* pathway. One possible reason for this difference in the requirement for *lin-15* is that development of the B cell includes additional specification mechanisms, such as positional cues from Y.p and the other B.a progeny, that act antagonistically to the *lin-3 / let-23* pathway. Thus although *lin-15* may function biochemically the same in both developmental processes, its role is diminished in the B lineage as it is partially redundant with other activities.

Integration of multiple signals

Two activities that act antagonistically to the *lin-3 / let-23* pathway are required for normal fate specification in the dorsal **pp** pair. If *lin-15* acts in the B lineage as it does in vulval induction and is required in cells other than the responding cells (Herman and Hedgecock, 1990), then both *lin-15* and the Y.p positional cue represent extracellular cues that must be integrated by the responding cells. We have characterized the integration of both these activities relative to the *lin-3 / let-23* pathway. If the function of a gene (like *lin-15*) or a signal (like the Y.p cue) is to negatively regulate the activity of the receptor *let-23*, mutations in the receptor or any downstream gene such

as *lin-45* should render the pathway insensitive to the removal of the regulator. Consequently, the lineage defect observed in *let-23* or *lin-45* mutants with the activity removed should be the same as when the activity is intact. This is the case with *lin-15*, as *let-23; lin-15* and *lin-45; lin-15* double mutants resemble the single *let-23* and *lin-45* mutants, and the posterior **pp** cell always produces a normal δ lineage. However, ablation of Y.p in *let-23*, *let-60*, or *lin-45* mutants results in abnormal lineages similar to $F^-U^-Y.p^-$ or *lin-3* $Y.p^-$ animals. In all cases, the posterior **pp** cell produces abnormal lineages rather than the normal δ lineage. Therefore, the Y.p cue does not act by negatively regulating the *lin-3 / let-23* pathway. We propose that it represents a distinct, parallel signalling pathway that acts at the same time or later than the *lin-3 / let-23* pathway.

The positional cues from F/U and Y.p represent two of the four active cell interactions identified by cell ablation experiments (see Fig. 3.B). F and U provide an anterior positional cue (labeled 1 in Fig. 3) that is mediated by the *lin-3 / let-23* signalling pathway. Our results and the results of Greenwald, et al., (1983) suggest that, at least in the **pp** pair, the lateral interaction (4 in Fig. 3) is mediated by the gene *lin-12*. Our results specifically indicate that *lin-15* does not mediate the Y.p cue (3 in Fig. 3). However, our data do not rule out the possibility that *lin-15* plays a role in mediating the positional cue from the other B.a progeny (2 in Fig. 3). Testing of this possibility, as well as identifying the point of integration of the lateral cue and the B.a progeny cue into the *lin-3 / let-23* pathway, awaits further analysis.

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Table 1. Effects of disruption of cell interactions on the B lineage. Each line represents all of the animals observed under a specific experimental condition. Each of the pairs of B.a progeny cells (**aa**, **pp**, **ap/pa**) are indicated, along with the lineage produced by the positionally anterior and the positionally posterior member of the pair. The numbers represent the number of animals followed that produced the indicated lineage. For instance, in all seven *lin-3(sy53/n1058)* mutant animals (line B.2), both **aa** cells produced a β lineage. In some animals the left and right cells of a pair fail to migrate to anterior and posterior positions. Superscript numbers indicate the lineage associated with cells that fail to migrate, and the number of animals. Thus the **aa** cells failed to migrate in two *lin-3(sy53/n1058)* animals, and both cells produced β-like lineages. In cases where the two cells that fail to migrate produce dissimilar lineages, they are aligned in the table to most closely approximate wild type. Cases where a cell produced a lineage in which the timing and axes are consistent with a normal lineage but the axes are skewed are included as a normal lineage. "." indicates no animals produced the lineage. Data for B.1, E.1, and E.2 are from Chamberlin and Sternberg (1993). γ*/δ* and ε * are commonly observed abnormal lineages. Each results in four progeny. "abn" indicates any other abnormal lineage. "abn>4" and "abn<4" indicate abnormal with more than, or less than four progeny, respectively. The nature of the abnormal lineages is further discussed in Chamberlin and Sternberg (1993). In some cases the numbers of **aa**, **pp**, and **ap/pa** cells do not follow a 1:1:2 ratio because all cells were not followed to the completion of the lineage for some animals.

	genotype	ablations	aa						pp										ap/pa									
			ant			post			ant					post					ant				post					
			α	abn	β	α	abn	β	γ	abn>4	γ*/δ*	abn<4	δ	γ	abn>4	γ*/δ*	abn<4	δ	ε	ε*	abn	ζ	ε	ε*	abn	ζ
A. Normal																												
	wild type	intact	all	.	.	.	.	all	all	.	.	.	.	.	.	.	.	all	all	.	.	.	.	.	.	all
B. Activity of the pathway reduced																												
1		F-U-	.	4	3	.	.	7	2	.	3	.	2	.	.	.	.	7	9	4	1	.	.	.	.	14
2	*lin-3(sy53/n1058)*		.	.	7^{2}	.	.	7^{2}	.	1	5	.	1	.	.	.	.	7	2	11	1	.	.	.	.	14
3	*lin-3(n378/n1059)*		1	.	2^{1}	.	.	3^{1}	3	.	.	.	.	.	.	.	.	3	6	.	.	.	.	.	.	6
4	*let-23(sy97)*		.	1	4	.	.	5	3	.	1	.	.	.	.	.	.	4	1	7	.	.	.	.	.	8
5	*let-23(n2020)*		.	1	1	.	.	2	2	.	.	.	.	.	.	.	.	2	3	1	.	.	.	.	.	4
6	*let-23(sy278)*		.	.	4	.	1	3	2	.	2	.	.	.	.	.	.	4	.	6	1	1	.	.	.	8
7	*sem-5(n1619)*		.	1^{1}	4	.	.	5^{1}	1	.	4	.	.	.	.	.	.	5	3	6	1	.	.	.	.	10
8	*let-60(n2021)*		.	.	5^{1}	.	.	5^{1}	1	.	1	.	3^{3}	.	.	.	.	5^{3}	7	3	.	.	.	.	.	10
9	*let-60(dn)/+*		3	4	1	.	.	8	4	.	1	.	.	.	.	.	.	5	8	.	1	.	.	.	.	9
10	*lin-45(sy96)*		.	3	4^{1}	.	1	6^{1}	2	.	4	.	1	.	.	.	.	7	3	10	1	.	.	.	.	14
11	*lin-3(sy53/n1058)*	F-U-	.	1	4^{1}	.	1	4^{1}	.	.	.	.	7^{4}	.	.	.	.	7^{4}	1	9	2	.	.	.	.	12
12	*lin-45(sy96)*	F-U-	.	1	2^{1}	.	.	3^{1}	.	.	2	1	1	.	.	.	.	4	.	6	.	.	.	.	.	6
C. Activity of the pathway increased																												
1	*syEx23(hsp::lin-3)*		5^{1}	.	.	2^{1}	1	2	5	.	.	.	.	1	.	3	1	.	9	.	1	.	7	2	1	.
2	*syEx21(hsp::lin-3)*		6	.	.	5	.	1	1	4	.	.	.	.	4	1	.	.	12	.	.	.	11	1	.	.
3	*let-60(gf)*		8	.	.	1	1	6	8	.	.	.	.	.	.	3	2	3	11	.	.	.	.	.	1	10
4	*syEx21(hsp::lin-3)*	F-U-	3^{1}	.	2	1	1^{1}	3	4^{1}	1	.	.	.	.	1	3^{1}	1	.	8	1	1	.	5	1	3	1
D. Role of *lin-15*																												
1	*lin-15(n309)*		5	.	1	.	.	6	3	.	.	.	.	.	.	1	.	2	5	.	.	.	.	.	.	5
2	*lin-15(n377)*		3	.	.	.	1	2	3	.	.	.	.	.	.	2	.	1	6	.	.	.	.	.	.	6
3	*lin-15(e1763)*		7	.	.	.	.	7	7	.	.	.	.	.	.	2	.	5	14	.	.	.	.	.	.	14
4	*let-23(sy97); lin-15(n309)*		.	.	5	.	.	5	5	.	.	.	.	.	.	.	.	5	4	6	.	.	.	.	.	10
5	*let-23(sy278); lin-15(e1763)*		.	.	4^{3}	.	.	4^{3}	3	1	.	.	.	.	.	.	.	4	.	8	.	.	.	.	.	8
E. Interaction with Y.p cue																												
1		Y.p-	7	.	.	.	1	6	7	.	.	.	.	.	.	3	3	1	12	.	.	.	.	.	.	12
2		F-U- Y.p-	3^{2}	2^{2}	.	.	1^{1}	4^{3}	4	1	.	.	.	.	.	2	2	1	3	6	1	.	.	.	.	10
3	*lin-3(sy53/n1058)*	Y.p-	.	1	4	.	1	4	1	1	3	.	.	.	1	3	.	1	1	9	.	.	.	.	.	10
4	*let-23(sy97)*	Y.p-	.	3^{2}	.	.	.	3^{2}	2	.	1	.	.	.	.	3	.	.	2	2	.	.	.	.	.	4
5	*let-60(n2021)*	Y.p-	.	1	4^{1}	.	.	5^{1}	3	1	1	.	.	.	.	3	1	1	6	4	.	.	.	.	.	10
6	*lin-45(sy96)*	Y.p-	.	2	2	.	1	3	1	1	1	1	.	.	.	2	2	.	3	5	.	.	.	.	.	8

Table 2. The role of *lin-12* in the **pp** pair. In *lin-12(0)* animals, both **pp** cells adopt γ fate (b). In *lin-12(d)* animals, the **pp** cells are normal. Ablation of the positional cues from F/U and Y.p (g) uncovers the defect in *lin-12(d)* animals. Each line indicates the presence of the F/U positional cue, *lin-12* genotype, and presence of Y.p positional cue. Data in b and c (and b and c of Table 3) were originally summarized in Greenwald, et al. (1983). Data in d and f (and d and f of Table 3) are from Chamberlin and Sternberg (1993). In *lin-12(0)* animals (b), the Y cell adopts a neuronal fate and thus the Y.p cue is absent. In *lin-12(d)* animals (c, e, g) there are two Y.p-like cells. Both cells were ablated in the animals of line g. Notation is as in Table 1.

pp

	F/U	*lin-12*	Y.p	anterior γ	anterior abn	anterior δ	posterior γ	posterior abn	posterior δ
a.	+	+	+	**all**	.	.	.	.	**all**
b.	+	-	-	**3**^{2}	.	.	**2**^{2}	**1**	.
c.	+	d	+	**3**	**1**	.	.	.	**4**
d.	-	+	+	**2**	**3**	**2**	.	.	**7**
e.	-	d	+	.	.	**3**^{1}	.	.	**3**^{1}
f.	-	+	-	**4**	**1**	.	.	**4**	**1**
g.	-	d	-	.	.	**5**^{3}	.	.	**5**^{3}

Table 3. The role of *lin-12* in the **aa** pair. **aa** lineages are normal in both *lin-12(0)* and *lin-12(d)* mutants. Removal of F/U, Y.p and B.a positional cues uncovers a possible lateral interaction between **aa** cells (Chamberlin and Sternberg, 1993). Removal of F/U, Y.p and B.a positional cues in *lin-12(d)* mutants indicates that *lin-12* does not appear to play an essential role in the specification of **aa** fates even in the absence of positional cues. Notation is as in Tables 1 and 2.

aa

	F/U	*lin-12*	Y.p	B.a	anterior α	anterior abn	anterior β	posterior α	posterior abn	posterior β
a.	+	+	+	+	**all**	.	.	.	.	**all**
b.	+	-	-	+	**2**	.	.	.	.	**2**
c.	+	d	+	+	**3**	.	.	.	.	**3**
d.	-	+	-	+	$\mathbf{3}^{2}$	$\mathbf{2}^{2}$	.	.	$\mathbf{1}^{1}$	$\mathbf{4}^{3}$
e.	-	d	-	+	$\mathbf{2}^{2}$	$\mathbf{2}^{2}$	.	.	$\mathbf{2}^{2}$	$\mathbf{2}^{2}$
f.	-	+	-	-	$\mathbf{6}^{5}$	.	.	.	$\mathbf{3}^{3}$	$\mathbf{3}^{2}$
g.	-	d	-	-	**1**	$\mathbf{1}^{1}$	$\mathbf{1}^{1}$	.	**1**	$\mathbf{2}^{2}$

Figure 1. Comparison of the adult male spicules in wild type (A), *lin-3(sy53/n1058)* (B), and *syEx21(hsp::lin-3)* (C) animals. Arrow points to the left spicule. Nomarski photomicrographs, anterior left, ventral down. (A) In wild type animals the spicules are long and straight. (B, C) In both animals with *lin-3* activity reduced and *lin-3* activity increased the spicules are short and crumpled. However, the B lineage defects responsible for the morphological defect are opposite from each other. Scale = 20 μm. For reader: A = top, B = middle, C = bottom.

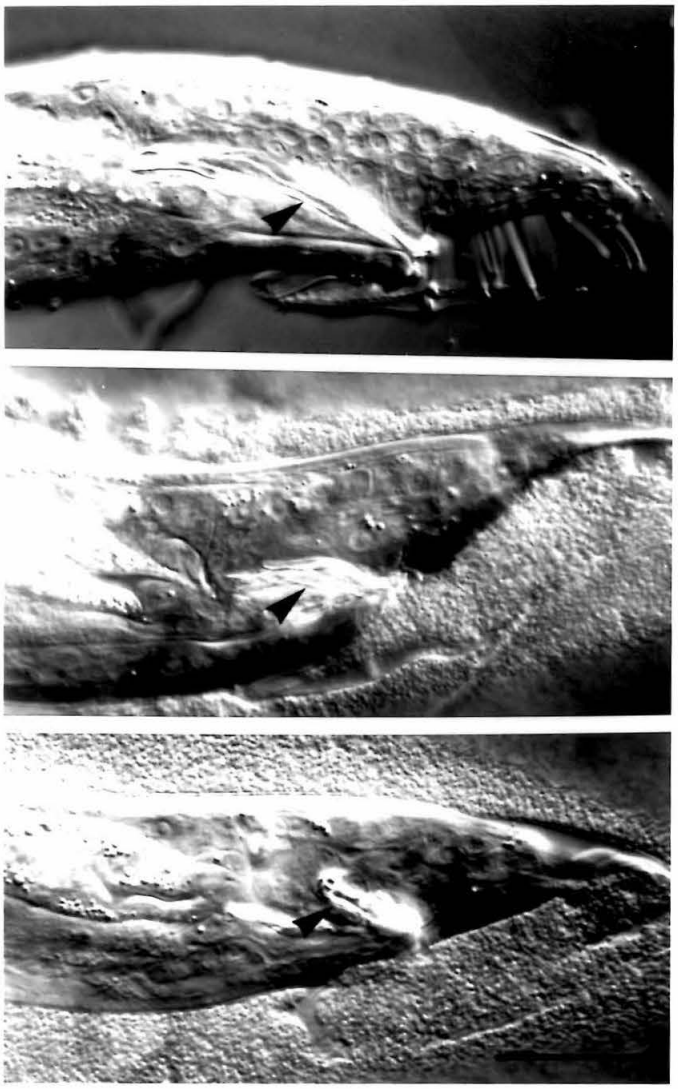


Figure 2. The lineage of the *C. elegans* male B cell, after Sulston and Horvitz (1977) and Sulston, et al. (1980). Vertical lines indicate a cell, horizontal lines indicate a cell division. Larval stage and approximate developmental time post-hatching are indicated in the left margin. Division axes are as indicated: a = anterior, p = posterior, d = dorsal, v = ventral, l = left, r = right.

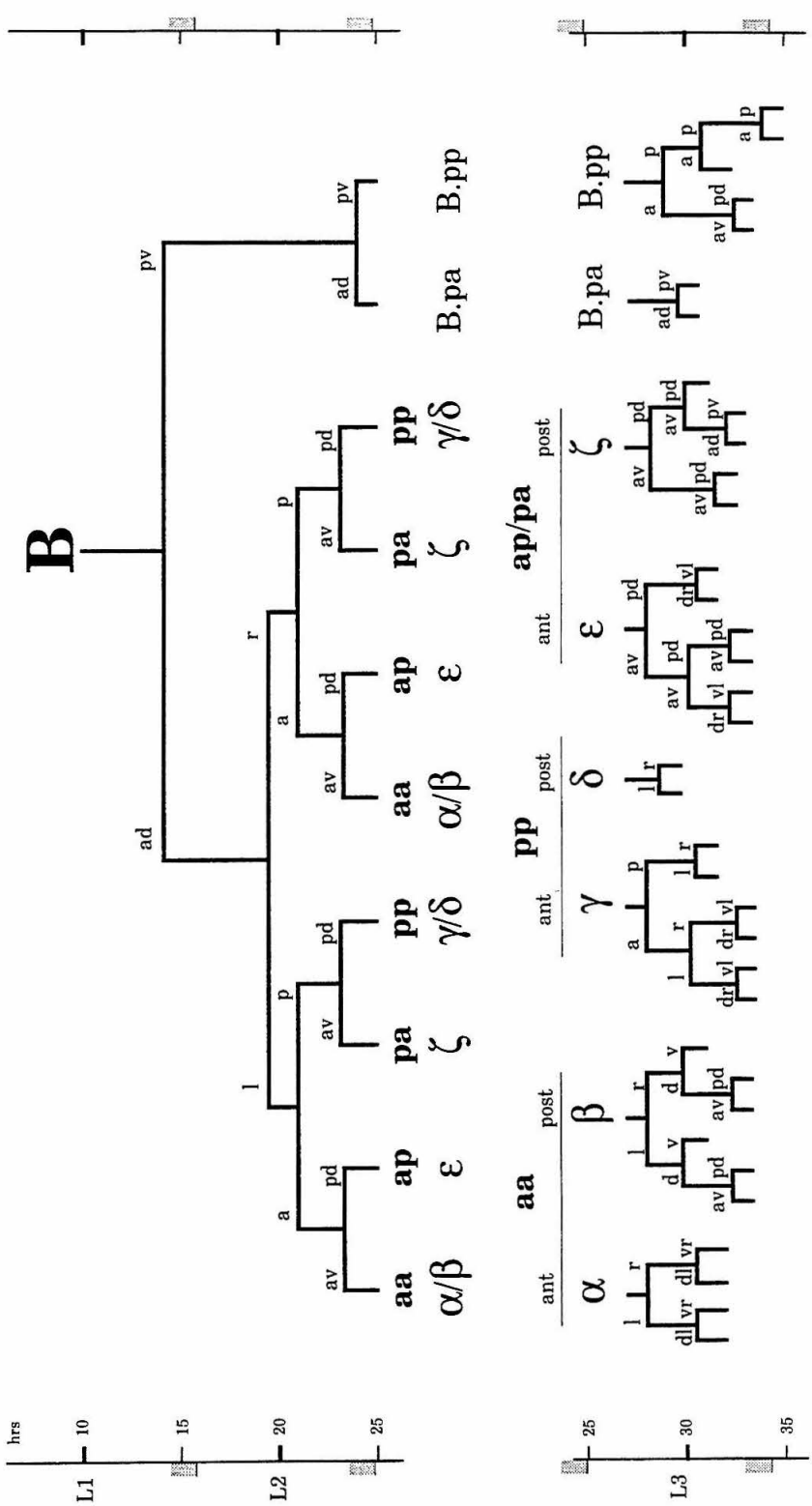
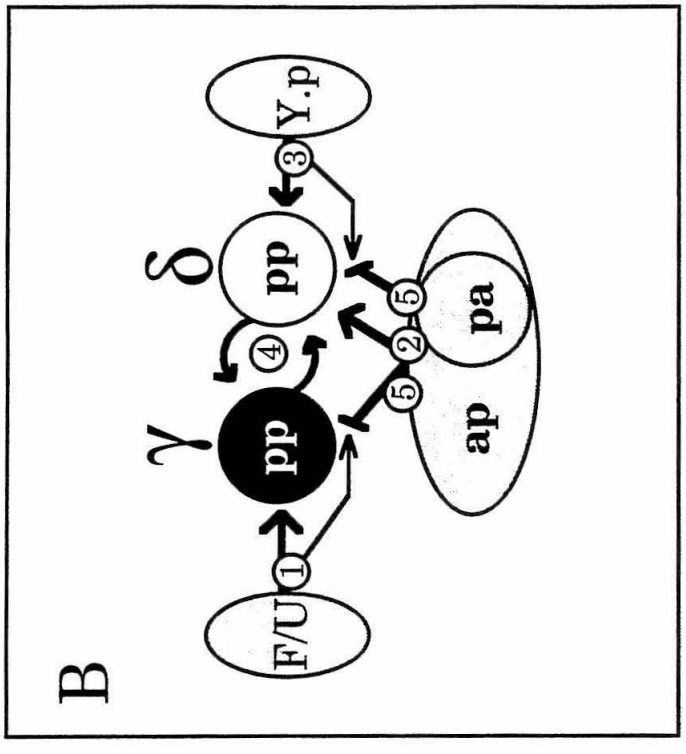

B
ad
pv
aa ap pa pp
α/β ε ζ γ/δ
B.pa B.pp
aa
ap
pa
pp
α/β
ε
ζ
γ/δ
ant
post
α
β
γ
δ
ε
ζ
ap/pa
B.pa
B.pp
hrs
L1
L2
L3

Figure 3. A. Diagram illustrating the approximate positions of the B, F, U, and Y.p progeny in a mid-L3 larval stage male (anterior left, ventral down). Arrows indicate the signal from F and U that promotes the anterior fates in the **aa**, **ap/pa**, and **pp** pairs of B.a progeny. B. The signal from F and U (1) represents one of several cell interactions that act to specify fates in the B.a progeny, as illustrated in this model for the **pp** cells (after Chamberlin and Sternberg, 1993). Other positional cues (arrows) are provided by Y.p, or its progeny (3), and the other neighboring B.a progeny (2). In addition, the neighboring B.a progeny act to prevent the F/U and Y.p cues from acting on inappropriate cells (bars, 5). This interaction may be passive (Chamberlin and Sternberg, 1993). The two **pp** cells may also interact (4) (Greenwald, et al., 1983; this work).


B
F/U
1
γ
pp
δ
4
pp
3
Y.p
5
2
5
ap
pa

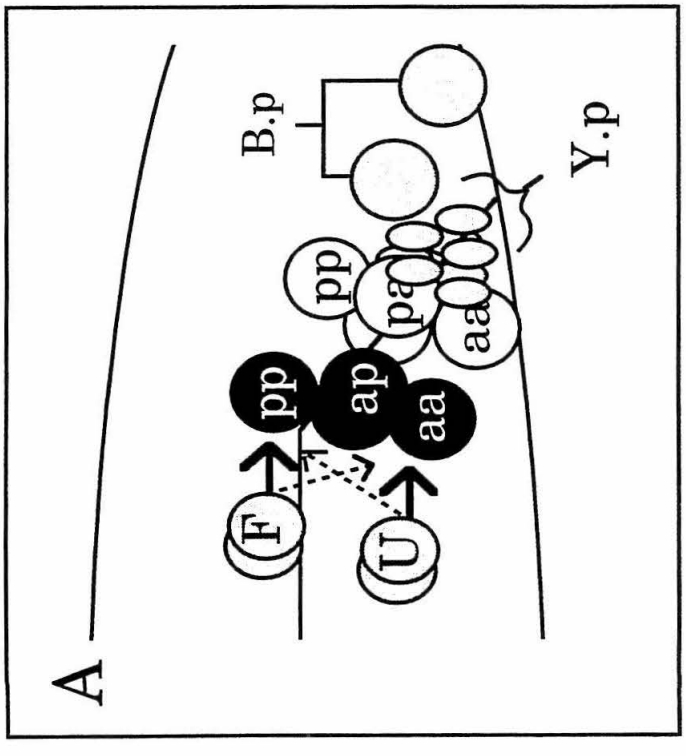

A
F
pp
U
ap
aa
pp
pa
aa
B.p
Y.p

Figure 4. Transformation of **pa** cells to ε fate in animals with ectopically produced LIN-3. Nomarski photomicrographs compare wild type to heat shocked, transgenic *syEx21* animals (anterior left, ventral down). Differences between ε and ζ lineages are apparent in the timing of division of the progeny of **ap** and **pa**. In wild type animals (A), **ap** cells produce ε lineages, and **pa** cells produce ζ lineages. In a normal ε lineage, the anteroventral daughter (**ap**a, A1; metaphase plate is visible) divides prior to the posterodorsal daughter (**ap**p, A2). In a normal ζ lineage, **pa**p divides prior to **pa**a. Ectopic LIN-3 can promote posterior cells to produce the lineages normally associated with their anterior neighbors. In such animals, the anteroventral daughters of both **ap** and **pa** (B1; metaphase plates are visible) divide prior to the posterodorsal daughters (B2), and both cells produce ε lineages. Scale = 20 μm. For reader: A = left, B = right; 1 = upper, 2 = lower.

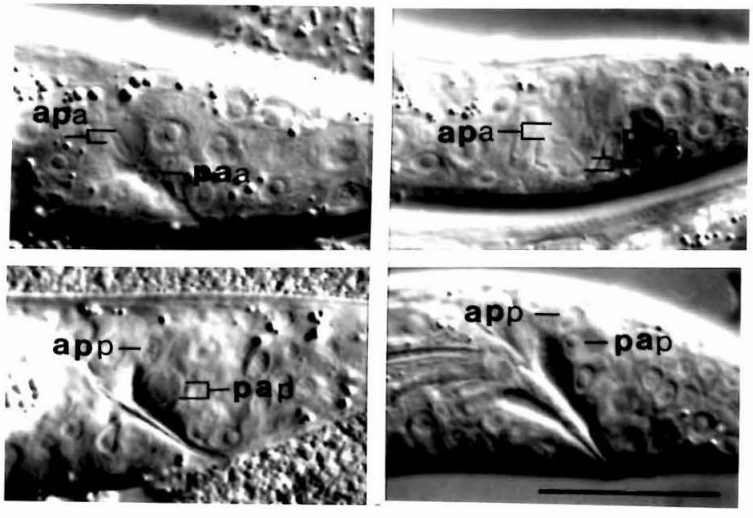
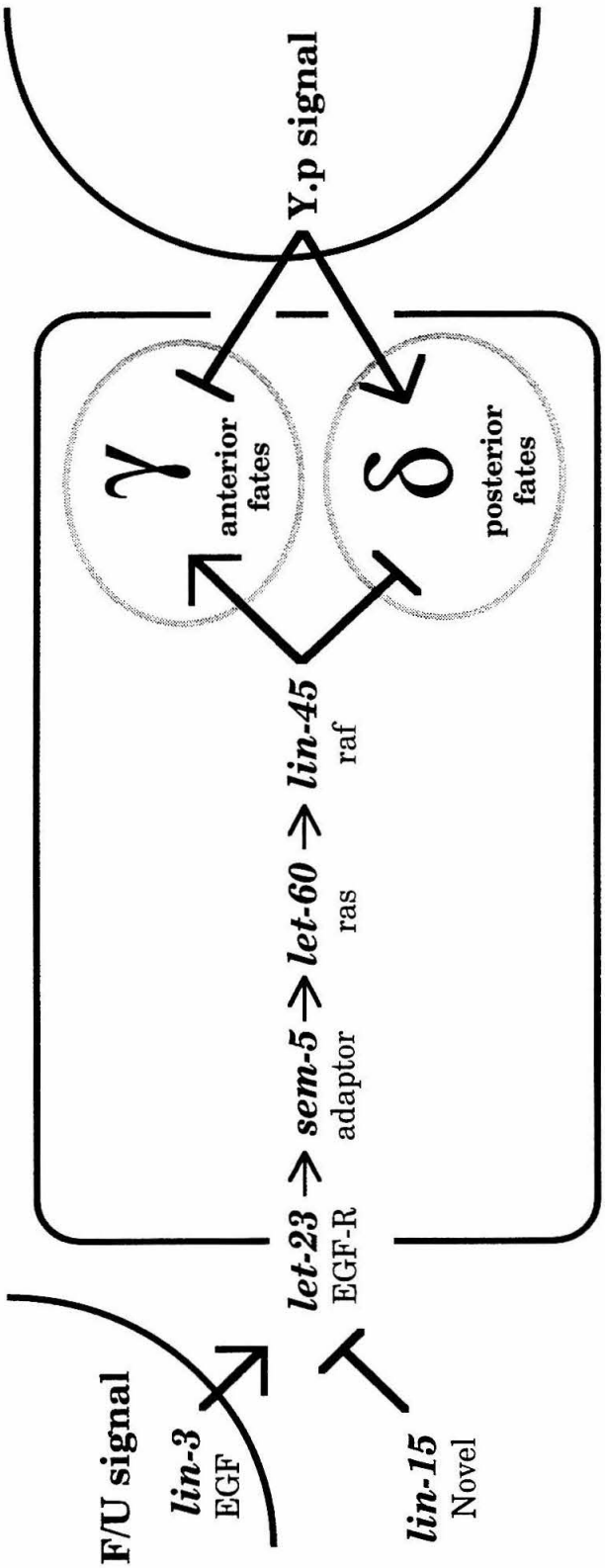

apa
paa
apa
app
pap
app
pap

Figure 5. Integration of *lin-15* and the Y.p cue into the *lin-3 / let-23* pathway. *lin-15* likely acts antagonistically and in parallel to *lin-3* as a negative regulator of *let-23* activity to specify fates in the B lineage. The Y.p signal is integrated downstream of *lin-45*, and may represent an independent signalling pathway that acts in parallel to, or possibly after, the *lin-3 / let-23* pathway. The gene order in the pathway is based on the epistasis established for these genes in hermaphrodite vulval induction.


F/U signal
lin-3
EGF
lin-15
Novel
let-23 → sem-5 → let-60 → lin-45
EGF-R
adaptor
ras
raf
γ
anterior fates
δ
posterior fates
Y.p signal

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Chapter 4

A screen for *Caenorhabditis elegans* mutants defective in lineages of male-specific blast cells

ABSTRACT

The specialized structures of the adult *C. elegans* male tail result from sex-specific cell division of blast cells during larval development. To identify genes required for the normal development of specific postembryonic lineages I screened over 9000 mutagenized gametes for mutations that result in morphologically abnormal male tails. Mutations that disrupt gross tail morphology and others that specifically disrupt the copulatory spicules were recovered. Candidate isolates were further screened to identify mutants in which the morphological defect results from abnormal cell lineages. 22 mutations identified (likely) 20 genes, of which at least seven are previously unknown to play a role in a specific developmental process. Although the screen focused on mutations that disrupt the male B lineage (the precursor to the spicules), mutations that confer F, U, Y, and V lineage defects were also recovered. These mutations provide the genetic tools to begin understanding the mechanisms involved in the postembryonic development of the male tail.

INTRODUCTION

At hatching male and hermaphrodite *C. elegans* are morphologically similar. The specialized mating structures of the adult animals -- such as the hermaphrodite vulva and the male tail -- result from the sex-specific division of postembryonic blast cells (Sulston and Horvitz, 1977; Sulston, et al., 1980). In the male tail, additional divisions of lateral epidermal blast cells V1-V6 and T, and ventral epidermal blast cells P10 and P11 produce the cells of the eighteen ray sensilla and the hook sensillum, respectively. Cells of the spicules, the postcloacal sensillum, and other male specific neurons and hypodermal cells derive from four male-specific blast cells: B, U, F, and Y. These cells divide in the male, but not in the hermaphrodite. The B cell is the precursor of all of the cells of the spicules. The B lineage produces 47 progeny that include hypodermal, neuronal and neuronal support cells, and cell death fates.

To identify genes that mediate fate specification during male tail development I have screened for mutations that result in morphologically abnormal male tails. Animals from candidate mutant lines were further screened for lineage defects. I have focused on the B lineage because (1) at least some mutations that disrupt fate specification in the B lineage result in deformed spicules or male tail (Sternberg and Horvitz, 1988; Chisholm and Hodgkin, 1989; Chisholm, 1991; Herman and Horvitz, 1994; Chamberlin and Sternberg, in preparation), (2) the B lineage is complex, with multiple specification steps, and (3) some steps in the lineage utilize asymmetric cell divisions (Sternberg and Horvitz, 1988; Herman, 1991) whereas others require cell interactions (Sulston and White, 1980; Chamberlin and

Sternberg, 1993) to properly specify fate. Thus mutations that disrupt this lineage can be recovered, and they provide a genetic handle to understand a variety of developmental processes.

In this chapter I describe the isolation and initial characterization of a set of EMS induced mutations that disrupt the B lineage and other lineages in the male tail. Some of these mutations disrupt genes already known to play a role in male tail development. Others define novel genes, or at least genes without previously characterized developmental functions. In all, 22 mutations defining at least 20 genes were isolated from a screen of over 9000 chromosome sets (Table 1), suggesting both that fate specification in the *C. elegans* male tail is genetically tractable, and that many genes are required for normal development.

MATERIALS AND METHODS

General Methods

Nematode strains were cultured according to standard techniques (Brenner, 1974; Sulston and Hodgkin, 1988).

Mutations used

Mutations described in Hodgkin, et al. (1988).

Linkage Group (LG) I: *dpy-5(e61). hIn1(unc-54(h1040)). sy258. sy316.*

LG II: *dpy-10(e128). let-23(sy97, sy278). lin-31(n301, bx31). rol-6(e187). unc-4(e120). unc-52(e444). mnC1. mnDf61.*

LG III: *dpy-1(e1). dpy-17(e164). dpy-18(e1096). egl-5(n945, sy279). lin-48(sy234). mab-5(e1293, sy173). unc-25(e156). unc-32(e189). unc-45(r450). unc-79(e1068). eDf2. nDf11. eT1.*

LG IV: *dpy-20(e1282). fem(sy240). lin-49(sy238). mec-3(e1338). unc-24(e138). unc-31(e169). DnT1. eDf18. eDf19. sy287.*

LG V: *dpy-11(e224). him-5(e1467, e1490). lin-25(e1446, sy29). eT1. DnT1.*

LG X: *let-1(mn119). let-4(mn105). let-5(mn106). let-9(mn107). lin-2(e1309). lin-15(sy307). lin-47(sy32). lon-2(e678). mab-27(sy202). unc-3(e131). vab-3(?sy66, sy281). mnDp1.*

Unknown linkage: *sy282. sy285. sy285. sy294. sy295.* K.33.3. Q.29.3.

Cell lineage analysis

Cell nuclei divisions in living animals were directly observed using Nomarski differential interference contrast optics as described by Sulston and Horvitz (1977). Nomenclature follows the standard of Sulston and Horvitz, with modifications of Chamberlin and Sternberg (1993).

Isolation of mutations

Mutant isolation consisted of a positive and a negative selection scheme. First, mutant lines that display a male tail defect (male abnormal, or Mab) were selected. Second, animals from these candidate strains were screened for the presence of a cell lineage defect (Lin). Mab non-Lin strains were excluded from further study.

Selection of two general defects identified Mab candidates: male mating defects and male morphology defects. Mutageneses were carried out according to standard conditions (Sulston and Hodgkin, 1988): worms were suspended in a solution of 50 mM Ethylmethanesulfonate (EMS) in M9 buffer for four hours at 20°C. The animals were then washed and allowed to recover for four hours. F_2 screens for mutations that confer a male mating defect were carried out by Leslie Barber (Table 2, screen 1) and Yvonne Hajdu and Katherine Liu (screen 2), using the protocol of Hodgkin (1983). Briefly, ten F_2 hermaphrodites were cloned from the F_1 progeny of mutagenized *him-5(e1490)* animals. Six F_3 males from the cloned hermaphrodites were tested together for ability to sire cross progeny with *unc-52(e444)* or *unc-31(e169)* hermaphrodites. Strains from which males sired no cross progeny were tested a second time. Strains which failed the mating test twice are candidate Copulation Defective (Cod) strains. Adult males from these lines were then observed under Nomarski optics at 1250x magnification for any tail morphology defects associated with the mating defect (Mab candidates).

Additional screens (screens 3 and 4) were carried out directly for male tail morphology defects using a Wild Makroskop. Four to five F_1 progeny were cloned from mutagenized P_0 *him-5(e1490)* hermaphrodites. The population of F_2 males from F_1 clones was screened at 200x-400x magnification for the presence of males with deformed tails. If the F_1 clone harbored a mutation that confers a male tail defect, 1/4 of self progeny males are expected to be abnormal (assuming a recessive, viable mutation with no maternal rescue). Sibling hermaphrodites were selected from strains with abnormal males in order to recover the homozygous mutation. The Mab phenotype was verified by observing adult males under Nomarski optics at

1250x magnification. Screen 3 included the balancer *DnT1* in an attempt to preferentially recover mutations on LG IV and V.

Initial analysis of mutants

Mab lines were retained as candidate strains. Either before or after back crossing to *him-5(e1490)*, early third larval stage (L3) males from each line were observed for defects in the B lineage. Normally, males at this stage have ten B progeny arranged in a stereotyped pattern around the rectum. Strains in which there were an abnormal number of B progeny at this stage were retained and comprise the "early defect" class. Strains in which there are ten B progeny, but they are arranged in abnormal positions were retained as the "migration or mid-stage defect" class. Although these animals do not directly display a lineage defect at this point, the importance of cell interactions for proper development after the ten cell stage (see Chapter 2) predicts these mutants will be lineage defective. Subsequent lineage analysis (Table 3) verified this assumption. Finally, the B lineage was followed in animals from strains in which there were ten B progeny in the normal positions. Mutants that exhibited B lineage defects under this criterion comprise the "late defect" class. Since some mutations result in a variable migration defect, in some cases the mid/late defect distinction is arbitrary.

Mapping and complementation tests

Approximate map positions of mutations and markers are illustrated in Fig. 1. Map data are listed according to the standard for data submission to acedb (a *C. elegans* data base) (J. Hodgkin, pers. comm).

lin-25(sy29): Recessive, linked to V.

sy29 e1490/+ + selected Lin: 20 Lin Him 0 Lin

e224 sy29 e1490/+ + + selected Dpy:

49 Dpy Lin Him 2 Dpy Lin 5 Dpy

Fails to complement *lin-25(e1446)* for Vul and Mab

lin-47(sy32): Linked to X.

sy32 e1309/+ + 68 WT 2 Mab 0 Egl 3 MabEgl

sy32 e151/+ + selected Unc: 16 Lin Unc 7 Unc

e1309/*sy32 e151* selected Vul, score Unc: *lin-2* 0 *sy32* 5 *unc-3*

mnDp1; sy32 e151 : *mnDp1* does not include *sy32*

sy66: Linked to X.

mab-5(sy173): Recessive, linked to III.

Fails to complement *mab-5(e1293)*.

mab-27(sy202): Recessive, linked to X.

sy202 e151/+ + selected Unc: 46 Mab Unc 2 Unc

sy202/*mn105 e151* selected Unc non-Let:

let-4 0 *sy202* 10 *unc-3*

mnDp1; sy202 e151 : *mnDp1* includes *sy202*

let-5(mn106), *let-1(mn119)*, *let-9(mn107)* escaper or

maternally rescued males do not have *sy202* phenotype.

lin-48(sy234): Recessive, linked to III.

e1 sy234/+ +; *e1490* selected Dpy: 48 Dpy Lin 14 Dpy

r450 sy234/+ +; *e1490* selected Unc: 18 Unc Lin 9 Unc

sy234 e1096/+ +; *e1490* selected Dpy: 16 Dpy Lin 15 Dpy

sy234 e156/+ +; *e1490* selected Unc: 44 Unc Lin 9 Unc

sy234 +/+ *e189; e1490* selected Unc:

12 Unc 3 sy234/+ Unc 1 Lin Unc

sy234/e1096 e156; e1490

select Unc non-Dpy: *dpy-18* 0 *sy234* 5 *unc-25*

select Dpy non-Unc: *dpy-18* 0 *sy234* 3 *unc-25*

sy234/e189 e1096; e1490

select Unc non-Dpy: *unc-32* 1 *sy234* 13 *dpy-18*

select Dpy non-Unc: *unc-32* 1 *sy234* 13 *dpy-18*

sy234/r450 e1; e1490

select Unc non-Dpy: *unc-45* 1 *sy234* 0 *dpy-1*

select Dpy non-Unc: *unc-45* 2 *sy234* 0 *dpy-1*

sy234 e156/eDf2; e138 e1338 e1282/+ + +; e1490/+:

eDf2 does not delete *sy234*

nDf11 cannot be tested. *nDf11/+* males do not mate and appear to be non-Lin-48 Mab.

lin-49(sy238): Recessive, linked to IV.

sy238/e138 e1338 e1282; e1490

select Unc non-Dpy: *unc-24* 15 *sy238* 6 *mec-3* 3 *dpy-20*

select Dpy non-Unc: *unc-24* 4 *sy238* 3 *mec-3* 4 *dpy-20*

e189 e1096/+ +; eDf18/unc-24(e138) sy238; e1490/+

eDf18 deletes *sy238*

e189 e1096/+ +; eDf19/unc-24(e138) sy238; e1490/+

eDf19 deletes *sy238*

sy240: Recessive, linked to IV.

sy258: Recessive, linked to I.

let-23(sy278): Recessive, linked to II.

sy278/e187 e120; e1490

select Rol non-Unc: *rol-6* 0 *sy278* 1 *unc-4*

select Unc non-Rol: *rol-6* 1 *sy278* 4 *unc-4*

sy278 e120/mnDf61; e1490/+: *mnDf61* deletes *sy278*

Fails to complement *let-23(sy97)*.

sy279: Recessive, linked to III.

Fails to complement *egl-5(n945)* for Mab and Egl.

sy281: Linked to X.

sy282: Not mated into.

sy285: Recessive.

sy287: Recessive, linked to IV.

sy287/e138 e1338 e1282; e1490

select Unc non-Dpy: *unc-24* 9 *sy287* 1 *dpy-20*

select Dpy non-Unc: *unc-24* 0 *sy287* 1 *dpy-20*

sy294: Recessive.

sy295: Not mated into.

sy307: Linked to X.

One locus: *sy307/+* segregates 210/898 (23%) Muv.

sy307/e151 mn119

select Unc non-Let: *unc-3* 2 *sy307* 0 *let-1*

sy316: Recessive, linked to I(?).

K.33.3: Recessive.

Q.29.3: Dominant (?). Genetically complex (?).

lin-31(bx31): Recessive, linked to II.

bx31/e128 e120; e1490

select Unc non-Dpy: *dpy-10* 0 *bx31* 3 *unc-4*

select Dpy non-Unc: *dpy-10* 0 *bx31* 7 *unc-4*

Fails to complement *lin-31(n301)* for Mab and Muv.

RESULTS

Although some of the other lineages of male specific blast cells are abnormal in the mutants, candidates were initially classified according to identified B lineage defects.

Mutants with early B lineage defects

Mutants with early B lineage defects generally displayed fewer than normal B progeny at the L3 stage. These lineage defects are commonly associated with grossly abnormal tails ("club tails") and structural instability of the tail. Often, these mutants die before reaching adulthood because they explode as larvae, or at the L4 molt into the adult stage.

sy66, *sy281* [*vab-3* ?]. *sy66* and *sy281* are linked to X and have a distinctive B lineage defect with six B progeny rather than the normal ten in the early L3 (see Chapter 5). The Y lineage is also abnormal in that Y.p appears to divide only a single time leaving two large cells of indeterminate differentiation. Both mutations occasionally confer a notched head phenotype in both males and hermaphrodites, although this phenotype is rare in *sy281* animals. The lineage defect and head morphology defect is similar to phenotypes associated with viable mutations in *vab-3* (A. Chisholm, pers. comm.). The B lineages followed in two *sy66* animals are illustrated in Chapter 5, Fig. 1.

sy279 [*egl-5*]. *sy279* males have four B cells in the L3 stage. This mutation is linked to LG III, and fails to complement *egl-5(n945)* for both the male tail defect and the egg laying defect. *egl-5* is part of the *C. elegans*

homeotic cluster. Mutations in *egl-5* disrupt a specific subset of cells in the posterior body region, including B. The B lineage defect in *egl-5* mutants has been described by Chisholm (1991).

sy282 and K.33.3. *sy282* hermaphrodites have a knobby protruding vulva, do not lay eggs, and are often sterile. The *sy282* strain was not successfully mated into after over 200 attempts. It is not temperature sensitive, nor can hermaphrodites lay eggs after passing through the dauer stage. K.33.3 hermaphrodites are Egl and difficult to mate into. They have small brood sizes, many worms die early in adulthood, and they occasionally have protruding vulvae. No chromosomal linkage for K.33.3 has been identified, although a marker for the cluster for each linkage group, as well as balancers for many of the arms, have been tested.

Mutants with migration or mid-stage defects in the B lineage

Mutations that disrupt proper specification of F and U

sy238 [*lin-49*]. *sy238* animals are sickly, and some larvae as well as adults display an abnormal "scarring" or refractile material at the junction of the intestine and the rectum. *sy238/Df* is lethal: animals arrest at or before the L4 stage (based on gonad morphology), but they are significantly smaller than wild type. They display heavy scarring at the rectum such that it is blocked. It is likely that this morphological defect blocks proper digestion and contributes to the lethality in *sy238/Df* animals. The male B lineage of *sy238* homozygotes can be disrupted in the specification of proper anterior and posterior positioning and fates of the **aa** and **pp** pairs (Table 3 B.1). The F and U cells are abnormal in *sy238* homozygous males. One or the other cell

can appear to be missing in individual males, and the lineage of the cell(s) that remains can be abnormal (Fig. 2 B). The fate of the "missing" F and U cells has not been established. However, it is reasonable that *lin-49* is required for proper specification of F and U. F and U (and their progeny in males) make up part of the rectal epithelium, and are essential for forming a connection between the intestine and the rectum (Sulston, et al., 1983; Herman, 1991). Thus *sy238/Df* is likely lethal because F and U are absent and do not perform this function. It is not yet clear if the B lineage defect associated with *sy238* results directly from the *lin-49* mutation, or if it is a secondary effect of the abnormal F and U lineages.

sy294. *sy294* mutants are sickly and can die at different stages. *sy294* hermaphrodites display an abnormal "notch" anterior to the anus. In *sy294* males the B cell produces the normal ten progeny by the L3 stage. However, all of the B.a cells remain dorsal to the rectum rather than organized around it in two rings of four cells. The U cell in *sy294* males is abnormal. It produces a lineage with the timing, axes, number of progeny, and apparent differentiated fates of Y.p (or possibly Y, Fig. 2 C). It is not clear if the B lineage defect in *sy294* males is due directly to the mutation or a secondary effect of the U lineage defect.

Q.29.3. The mutation in the Q.29.3 strain has not been successfully outcrossed. The strain appears to be genetically complex and includes at least one dominant component. Although the lineage defect in the strain has not been characterized, the fact that the strain exhibits the sickly phenotype associated with scarring at the rectum and a migration defect in the B lineage suggests that it may be another member of the class of mutations that disrupt F and U specification.

Other mutations that confer a mid-stage defect

sy285. *sy285* males exhibit a variable migration defect among the progeny of the B cell. In general, when the cells migrate to the proper positions they produce the normal lineage, but cells that migrate incorrectly can be abnormal (Table 3 B.2). In many animals all B progeny remain dorsal and posterior to the rectum. The variable lineage defect is consistent with the observation that about 20% of *sy285* males are morphologically wild type.

Mutants with late defects in the B lineage

Mutations in genes also required for normal hermaphrodite vulval development and egg laying

Egglaying defective (Egl) and Vulvaless (Vul)

sy278 [*let-23*]. In *sy278* males the lineages of the anterior **aa**, **pp**, and **ap/pa** cells are disrupted (Table 3 A.6). These cells can produce the lineage normally associated with their posterior neighbors. This is the phenotype associated with mutations in genes in the *lin-3/let-23* pathway also required for vulval induction (see Chapter 3). *sy278* maps between *rol-6* and *unc-4* on LG II, and fails to complement *let-23(sy97)* for the male tail defect (Table 4). However, *sy278* complements *sy97* for the defect in vulval development, and for lethality. Furthermore, *sy278/Df* males are Mab, but *sy278* complements for the other *let-23* functions. *sy278/Df* hermaphrodites have normal vulval development and are fertile, and *sy278/Df* animals are normal in P11/P12 specification. There is also no appreciable lethality associated with *sy278* homozygotes or hemizygotes. *sy278* likely represents a mutation in *let-23*

that disrupts male tail function but retains significant activity for the other *let-23* functions.

sy29 [*lin-25*]. In *sy29* males the lineages of the anterior B.a progeny cells are sometimes disrupted (Table 3 A.1). Hermaphrodites are also Egl and Vul. *sy29* maps between *dpy-11* and *him-5* on LG V, and fails to complement *lin-25(e1446)*. *sy29* is temperature sensitive (Simon Tuck, pers. comm.), and likely represents a hypomorphic mutation in *lin-25*.

sy258 and *sy316*. In *sy258* and *sy316* males the lineages of the anterior B.a progeny cells are sometimes disrupted (Table 3 A.5, A.9). Hermaphrodites are Egl and weakly Vul, although the reduction in vulval development is not sufficient to account for the extent of the egg laying defect. Both mutations are on LG I, and they may represent alleles of *sur-2*, a locus defined originally because mutations are suppressors of gain-of-function mutations in *let-60* (N. Singh and M. Han, pers. comm.).

sy295. A lineage defect in the anterior **aa** cell is associated with *sy295*. *sy295* hermaphrodites are Egl but not Vul. Nevertheless, attempts to cross in to the strain failed with over 200 hermaphrodites. *sy295* is not temperature sensitive, and it is not suppressed by passing worms through dauer stage.

Multivulva (Muv)

sy307 [*lin-15*]. *sy307* males have a weak spicule defect, but also have hook abnormalities, pseudovulvae, and a gonad migration defect. *sy307* hermaphrodites have excessive vulval development (multivulva or Muv). These are phenotypes associated with mutations in *lin-15* and other genes in that class (Ferguson and Horvitz, 1985; 1989). *sy307* maps to the right of

unc-3 on LG X, and segregates as a single mutation. *sy307* likely represents a mutation in the *lin-15* locus.

bx31 [*lin-31*]. *bx31* males have a variable late B lineage defect similar to *lin-31(n301)* males (Table 3 A.10, A.11). The hermaphrodites are variably Muv. *bx31* maps left of *dpy-10* on LG II, and fails to complement *lin-31(n301)* for male tail and vulval defects.

Other mutations that disrupt development of F and U

sy234 [*lin-48*]. The cells in the **aa** pair, and occasionally the anterior **pp** cell are disrupted in *sy234* males (Table 3 A.4). F and U lineages are also disrupted: both F and U divide earlier and produce more progeny than normal (Fig. 2 E). There are no obvious pleiotropies associated with *sy234*. Lineages in *sy234* animals are normal for the following cells: (1) lateral hypodermis: hermaphrodite V cell through the L2 stage (postdeirid), hermaphrodite T cell, male T and V ray sublineages (2) ventral hypodermis: hermaphrodite early P cells (Pn.a), male and hermaphrodite Pn.p cells (vulva and hook) (3) gonad: male early Z1, Z4 cells, hermaphrodite ventral uterine cells (VU), partial hermaphrodite dorsal uterine cells (DU) (4) early hermaphrodite M cell (5) hermaphrodite K cell and (6) early divisions and migration of hermaphrodite Q cells.

sy234 defines the gene *lin-48*, and maps between *dpy-1* and *unc-32* on LG III. It has not been established whether the B lineage and F and U lineage defects are independent effects of *sy234*, or if the B lineage defect is a secondary effect of the F and U lineage defect, or vice versa. However, neither lineage defect is the defect associated with simply removal of the other cell(s) (Chisholm and Hodgkin, 1989; Chamberlin and Sternberg, 1993).

sy287. *sy287* hermaphrodites are essentially normal and fertile. In the B lineage *sy287* males exhibit a defect in the **pp** pair: the cells fail to migrate to their normal anterior/posterior positions, and both cells behave like a normal posterior **pp** cell, producing a δ lineage (Table 3 A.7). The F lineage can also be abnormal, producing more progeny than normal (Fig. 2 D). The F lineage defect may be similar to that associated with *sy234*, but the B lineage defect is clearly distinct between the two.

Other mutations that confer a late defect

sy32 [*lin-47*]. Several late B lineage defects are observed in *sy32* males. The fates of the anterior cells in the **aa** and **ap/pa** pairs and the posterior cell in the **pp** pair can be disrupted (Table 3 A.2). In a lineage of the F and U cells the lineages were esentially wild type, although the polarity of one of the asymmetric divisions of F (F.r) was reversed. This is the lineage defect observed when B is ablated, so the defect may reflect a secondary effect of the B lineage defect, although this has not been verified. There are no overt pleiotropies associated with *sy32*, although it appears to result in reduced viability (not quantified). *sy32* defines the gene *lin-47*, and is linked to X, probably between the right breakpoint of *stDp2* and the left breakpoint of *mnDp1*.

sy202 [*mab-27*]. *sy202* males have only a weak B lineage defect, although the timing of all cell divisions can be abnormal (Table 3 A.3). *sy202* animals are weakly Unc. *sy202* defines the gene *mab-27*, and maps to the X LG between the left breakpoint of *mnDp1* and *unc-3*. Rare escapers or maternally rescued males mutant for essential genes in the region (*let-*

1(mn119), *let-5(mn106)*, *let-9(mn107)*) do not have the *mab-27* phenotype, and thus *sy32* is not likely a hypomorphic mutation in one of those genes.

Mutants with other lineage defects

sy173 [*mab-5*]. *sy173* males display a distinctive phenotype where the normal V rays are missing and the lateral cuticular lines (alae) extend posteriorly. *sy173* maps to LG III and fails to complement *mab-5(e1293)*. *sy175* males have abnormal spicules, but mosaic analysis (Kenyon, 1986) suggests that this defect is due to abnormal M lineage in *mab-5* mutants rather than abnormal B lineage (M progeny are required for proper morphogenesis of the spicules).

sy240 [*fem*]. *sy240* males have rudimentary tails, and some have oocytes in the gonad rather than sperm. *sy240* hermaphrodites are self-sterile, but can produce cross-progeny with N2 males. *sy240* hermaphrodites also lack sperm, suggesting that the self-sterility results from feminization. *sy240* maps to LG IV, the location of two genes involved in sex determination: *fem-1* and *fem-3*. Although complementation was not tested, *sy240* likely represents an allele of one of these two genes. Defects in the male tail lineages of *sy240* males presumably arise from (partial) sexual transformation rather than from disrupted fate specification *per se*.

DISCUSSION

A screen for mutations that disrupt cell lineages in the *C. elegans* male tail recovered 22 mutations in over 9000 mutagenized gametes screened, with a frequency of about one in 450. Of these 22 mutations, at most two loci are

represented by two mutations, and the other eighteen are distinct either by map location, phenotype, or both. The relatively small number of chromosomes screened, combined with the high number of loci represented by only a single mutation, indicate that this screen does not represent saturation for the Mab Lin phenotype. Nevertheless, it is clear that many genes can mutate to produce a Mab Lin phenotype. Many of the mutations are pleiotropic, and some represent alleles of genes previously identified for their role in other developmental processes. However, this study has identified and allowed the characterization of (1) additional functions of already known genes, (2) a tissue preferential allele of a gene (*let-23*) known to function in several tissues, and (3) possibly new genes required for development.

Several genes have previously been identified to play a role in the development of the male tail, and as expected this screen recovered mutations in some of these genes. Mutations in genes involved in sex determination can partially feminize XO animals, and *sy240* represents this class of mutation. *sy173* and *sy279* represent the genes in the *C. elegans* homeotic cluster required for proper fate specification in the posterior body region (*mab-5* and *egl-5*). Alleles of other genes known to have abnormal male tails associated with mutations (Hodgkin, 1983) were also expected (e.g., *vab-3*: *sy66* and *sy281*). Mutations in *let-23*, *lin-15*, *lin-25*, *lin-31*, and likely *sur-2* were recovered. Genes involved in vulval development are required for F and U promotion of anterior fates in the B.a progeny (see Chapter 3). Other mutations were recovered that concomitantly disrupt male tail development and vulval function (*sy282*, K.33.3, *sy295*). However, these mutations do not appear to disrupt vulval induction or development, and thus represent a different class of gene.

Among recovered mutations in genes required for vulval development, *sy278* is of particular interest because it represents a mutation that preferentially disrupts the male tail function of *let-23* but not any of the other known functions. Other screens for *let-23* alleles have required that the mutation either be lethal (Herman, 1978) or that the vulval development function be reduced (Aroian and Sternberg, 1991). *sy278* shows no appreciable lethality and complements a deficiency of *let-23* for vulval development. It represents a unique allele that could only be recovered by specifically screening for mutations that disrupt male tail development.

Several mutations likely represent novel genes, or novel alleles and functions for known genes (such as essential genes). Most detailed characterization so far has been for *sy32*, *sy202*, *sy234*, and *sy238*, which define the genes *lin-47*, *mab-27*, *lin-48*, and *lin-49*, respectively. Because of the novel lineage defects, additional genetic mapping will likely indicate that *sy285*, *sy287*, and *sy294* also represent new genes, or define new functions for existing genes. Several of these mutations are associated with "sickness" and lethality, so some of these mutations may represent hypomorphic mutations in genes with a loss-of-function lethal phenotype. For example, *sy238/Df* animals arrest as larvae, with a phenotype that likely represents an enhancement of the *sy238/sy238* phenotype. When tested, mutations such as *sy294* may behave likewise.

Many of the isolated mutants have weak male tails that are structurally unstable and prone to explosion. Of these, a novel class of mutations recovered in this screen result in a rectal "scarring" phenotype. These mutations result in misspecification of F and U cells. In addition to providing a signal for proper B development, these cells make up part of the

rectal epithelium and are required for the formation of the junction between the rectum and the intestine. Other mutations that disrupt the development of F, U, or B can be detrimental to the survival of mutant males but not hermaphrodites. This is likely because among the progeny of F, U, and B are cells that must function as the parent cell does in the young male larva or in the hermaphrodite. If the precursor is properly specified but the lineage is abnormal, these fates may be misspecified, resulting in males with damaged tails, but not hermaphrodites.

In summary, this screen for mutations that disrupt male tail development specifically associated with a lineage defect is an effective way to identify a class of developmental mutants in *C. elegans*. The direct screen for F_2 morphological mutants followed by selection of mutant lines with lineage defects prior to backcross and genetic mapping (the protocol followed in screen 4) is particularly efficient in identifying mutants in this class. These mutations represent a wide range of functions in male tail development.

Table 1. Mutations recovered in the screen for Mab Lin mutants. The mutations are subdivided according to the B lineage defect observed in mutants. Male morphology defect: spicule = only spicules are abnormal; blowout = male tails tend to be delicate, and often become damaged or explode; club tail = male tails are extremely deformed, affecting all structures. Other phenotypes: Egl = egglaying defective; p-Vul = protruding vulva; Ste = sterile; Let = lethal; Vul = vulvaless; Muv = multivulva. Link = linkage group. dom/rec = dominance or recessiveness of the mutant allele in *trans* to a wild type allele. comp/gene = Results of complementation or other tests assign the mutation to the indicated gene. Genes followed by (?) indicate that the mutation is likely in the indicated gene, but has not yet been formally tested.

B lin. defect	allele	isolation no.	male morphology defect	other phenotypes	link	dom/rec	comp/gene
early							
	sy66	709-g	spicule; blowout	notch head	X	?	*vab-3 (?)*
	sy279	G.30.2	club tail	weak Egl	III	r	*egl-5*
	sy281	I.1.3	spicule; blowout	v. weak notch head	X	?	*vab-3 (?)*
	sy282	J.4.2	club tail; blowout	Egl p-Vul; some Ste	?	?	
		K.33.3	club tail	Egl; some Let	?	r	
mid							
	sy238	A.3.3	spicule	sickly; scarring at rectum	IV	r	***lin-49***
	sy285	P.2.2	spicule		?	r	
	sy287	O.36.2	spicule		IV	r	
	sy294	N.10.4	club tail; blowout	sickly; notch at rectum; Egl	?	r	
		Q.29.3	spicule; blowout	sickly; scarring at rectum; Egl	?	d?	
late							
	sy29	241-A2	spicule	Vul	V	r	*lin-25*
	sy32	447-g	spicule		X	?	***lin-47***
	sy202	16.18.8	spicule	weak Unc	X	r	***mab-27***
	sy234	B.28.2	spicule		III	r	***lin-48***
	sy258	20.20.3	spicule	Vul	I	r	*sur-2 (?)*
	sy278	J.40.2	spicule		II	r	*let-23*
	sy295	L.27.1	spicule	Egl; some p-Vul	?	?	
	sy307	Q.45.1	weak spicule; hook, Muv	Muv	X	r	*lin-15 (?)*
	sy316	O.3.4	spiclue	Egl/Vul	I	r	*sur-2 (?)*
	bx31		spicule	Muv	II	r	*lin-31*
other							
	sy173	14.18.2	V rays missing; spicule		III	r	*mab-5*
	sy240	B.44.8	reduced tail; club tail	Ste	IV	r	*fem-1, -3 (?)*

Table 2. Screens used to isolate Mab Lin mutants. The Table indicates the source of mutations and the methods used to recover the mutations.

	Screen	Source	No. gametes	No. mutations	alleles
1	Mating	Barber	900	3	*sy29, sy32, sy66*
2	Mating	Hajdu, Liu	2100	3	*sy173, sy202, sy258*
3	Morphology	Chamberlin	500	3	*sy234, sy238, sy240*
4	Morphology	Chamberlin	5800	12	*sy278, sy279, sy281, sy282, sy285, sy287, sy294, sy295, sy307, sy316, K.33.3, Q.29.3*
5	Gift (Morph)	Emmons	?	1	*bx31*

Table 3. B lineage defects observed in male mutants. abn-*n* indicates the lineage was abnormal, producing *n* progeny. n.d. = not determined (lineage not followed to completion for that cell). Other notation as in Chapter 2, Table 1. Animals #602, #603, and #612 are from non-backcrossed strains.

genotype	animal #	**aa** ant.	**aa** post.	**pp** ant.	**pp** post.	**ap** left	**ap** right	**pa** left	**pa** right	B.p(a/p)
wild type		α	β	γ	δ	ε	ε	ζ	ζ	wt
A. late defect										
1. *sy29*	73	α	β	γ	δ	ε	ε	ζ	ζ	wt
	116	α	β	γ	δ	ε	ε	ζ	ζ	wt
	163	abn-5	β	γ	δ	abn-5	ε	ζ	ζ	wt
2. *sy32*	67	n.d.	n.d.	n.d.	abn-3	(abn	n.d.	n.d.	n.d.)	wt
	96	α	β	γ	δ	n.d.	n.d.	n.d.	n.d.	wt
	484	abn-5	β	γ	δ	ε*	ε*	ζ	ζ	wt
	639	α	β	γ	abn-3	ε	ε*	ζ	ζ	wt
3. *sy202*	90	n.d.	n.d.	n.d.	abn-3	n.d.	n.d.	n.d.	n.d.	n.d.
	483	α	β	γ	δ	ε	ε	ζ	ζ	wt
4. *sy234*	482	ατ	abn-6	abn-7	δτ	ε	ε	ζ	ζ	wt
	485	ατ	α	γτ	δτ	ε	ε	ζ	ζ	wt
	486	abn-5(l)	abn-5(r)	γτ	δτ	ε	ε	ζ	ζ	wt
	487	abn-5(l)	ατ(r)	γτ	δτ	ε	ε	ζ	ζ	wt
	633	α	abn-5	abn-7	δτ	ε	ε	ζ	ζ	wt
5. *sy258*	196	α(abn)	β	γ	δ	ε	ε	ζ	ζ	wt

genotype	animal	**aa** ant.	**aa** post.	**pp** ant.	**pp** post.	**ap** left	**ap** right	**pa** left	**pa** right	B.p(a/p)
wild type		α	β	γ	δ	ε	ε	ζ	ζ	wt
6. *sy278*	602	β	β	γ*	δ	abn-3	ε*	ζ	ζ	wt
	613	β	abn-5	γ	δ	ε*	ε*	ζ	ζ	wt
	614	βτ	βτ	γ*	δ	ζ	ε*	ζ	ζ	wt
	615	βτ	βτ	γ	δ	ε*	ε*	ζ	ζ	wt
7. *sy287*	643	α	β	δτ	δτ	ε	ε	ζ	ζ	wt
	644	α	β	δτ	δτ	ε	ε	ζ	ζ	wt
8. *sy295*	603	abn-5	β	γ	δτ	ε	ε	ζ	ζ	wt
9. *sy316*	612	β	β	γ	δ	ε	ε	ζ	ζ	wt
10. *bx31*	63	α	β	γ	δ	ε	ε	ζ	ζ	wt
11. *n301*	74	abn-5	abn-5	γ	δ	ε	ε	ζ	ζ	wt
B. mid defect										
1. *sy238*	464	α(l)	β(r)	γ*	δ	ε	ε	ζ	ζ	wt
2. *sy285*	641	α	β	γ	δ	ε	ε	ζ	abn-4	wt
	642	α	β	γ	δ	ε	ε	ζ	ζ	wt

Table 4. Phenotypes associated with *let-23* mutations. *sy97/sy97* data are from Aroian and Sternberg (1991). Numbers indicate the number of wild type/number of animals screened. wt = wild type.

	male	hermaphrodite			
	tail	egglaying	vulva ind	fertility	P11/P12
wild type	wt	wt	wt	wt	wt
sy278/sy278	0/20 wt	wt	wt	wt	5/5 wt
sy97/sy97	0/19 wt		0/21 wt	21/22 wt	19/20 wt
sy278/sy97	0/11wt	11/11 wt			
sy278/mnDf61	0/21wt	20/20 wt	20/20 wt	20/20 wt	9/9 wt

Fig. 1. Genetic maps showing approximate positions of mutations and markers used in this study. Left is up, right is down. Mutations identified in this study or the genes they represent are indicated in bold.

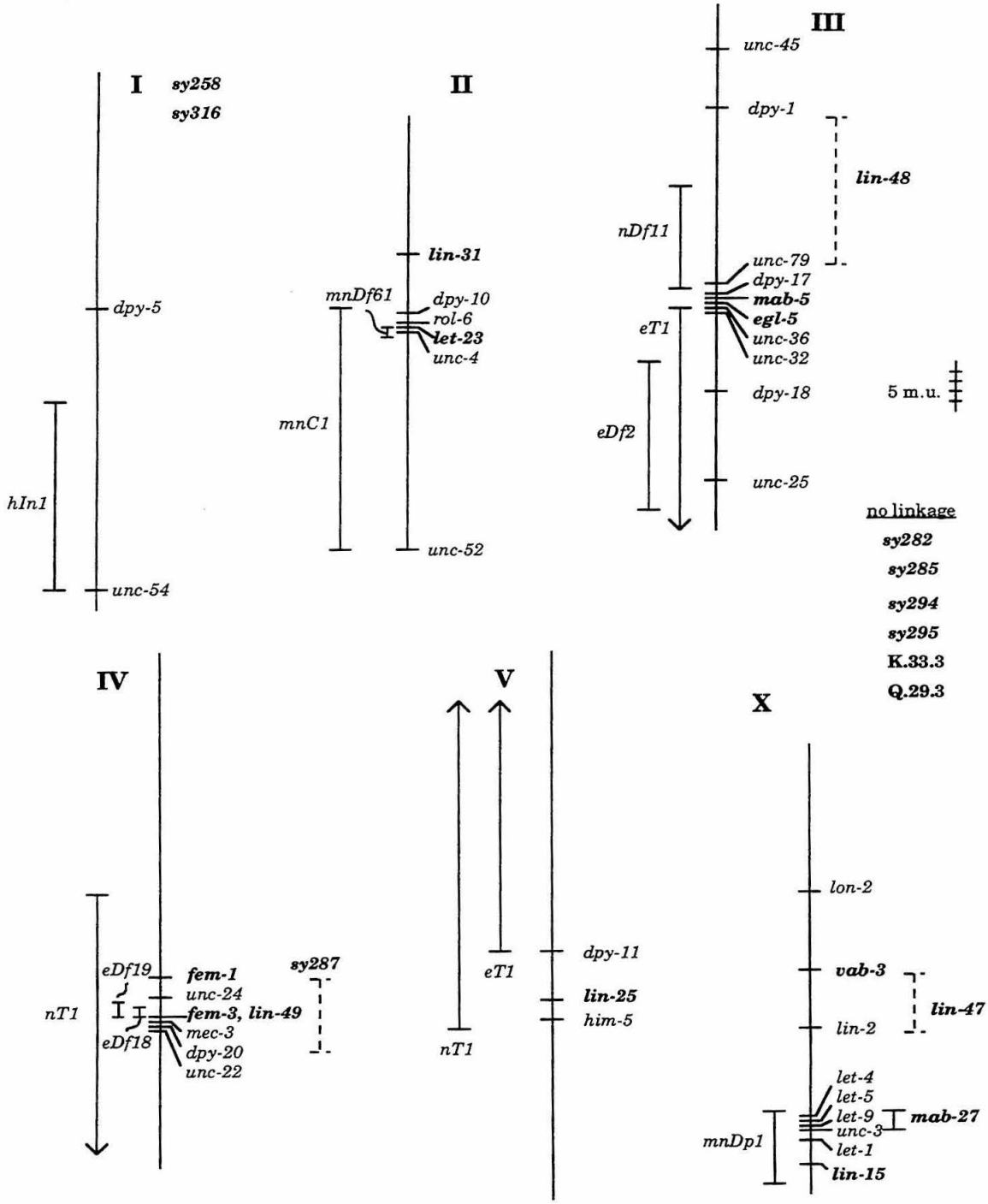
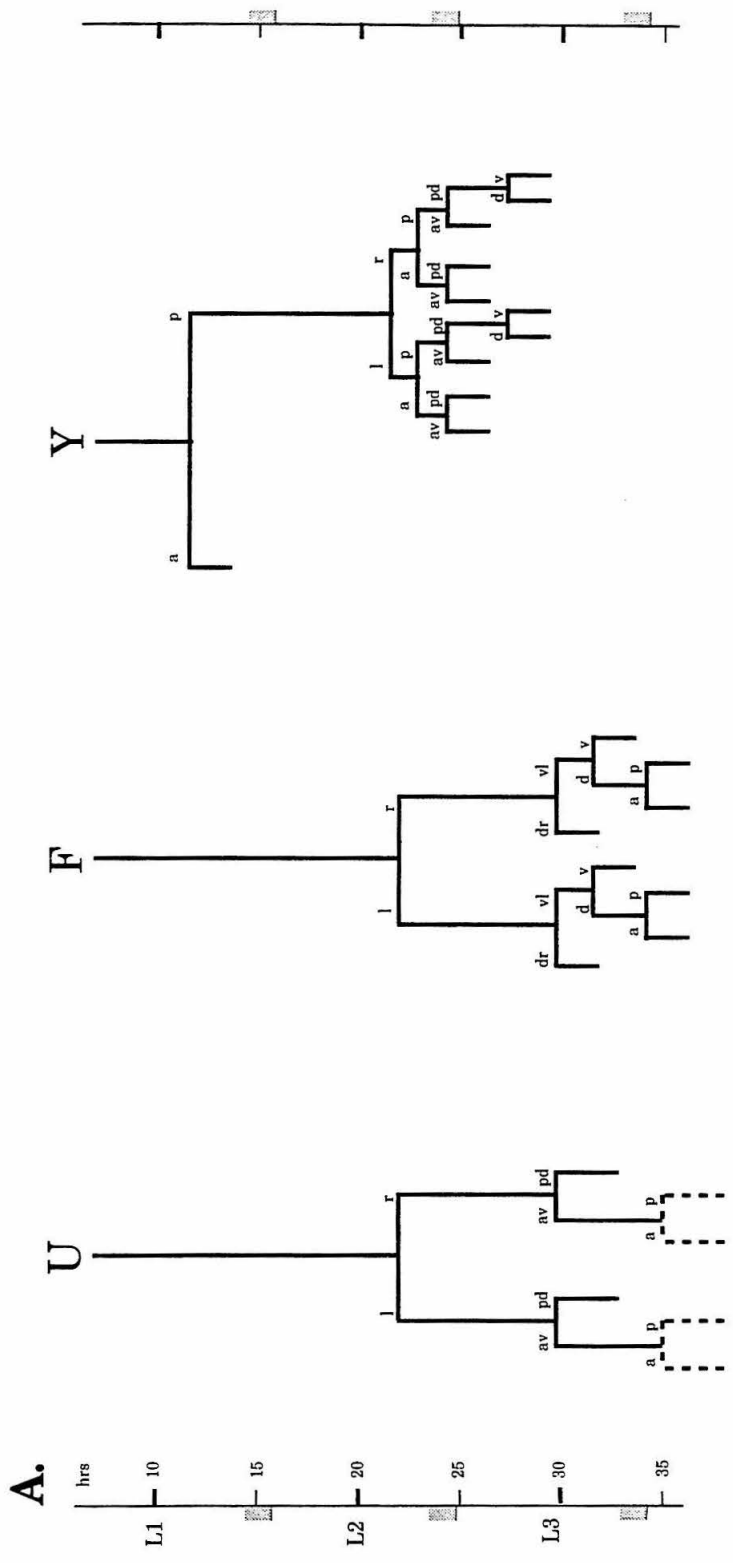
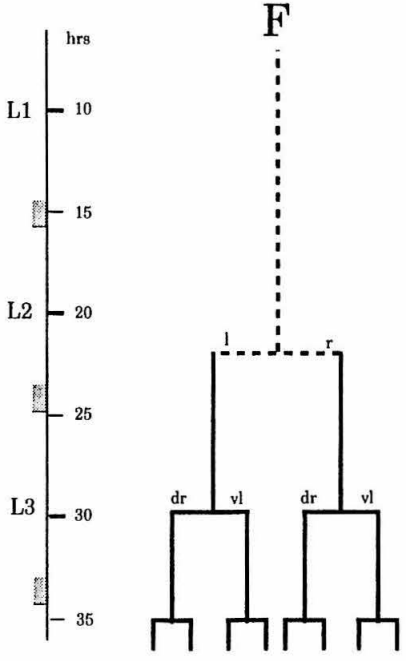

I
sy258
sy316
dpy-5
hIn1
unc-54
II
lin-31
mnDf61
dpy-10
rol-6
let-23
unc-4
mnC1
unc-52
III
unc-45
dpy-1
lin-48
nDf11
unc-79
dpy-17
mab-5
egl-5
eT1
unc-36
unc-32
dpy-18
5 m.u.
eDf2
unc-25
no linkage
sy282
sy285
sy294
sy295
K.33.3
Q.29.3
IV
eDf19
fem-1
sy287
unc-24
nT1
fem-3, lin-49
eDf18
mec-3
dpy-20
unc-22
V
dpy-11
eT1
lin-25
him-5
nT1
X
lon-2
vab-3
lin-47
lin-2
let-4
let-5
let-9
mab-27
unc-3
mnDp1
let-1
lin-15

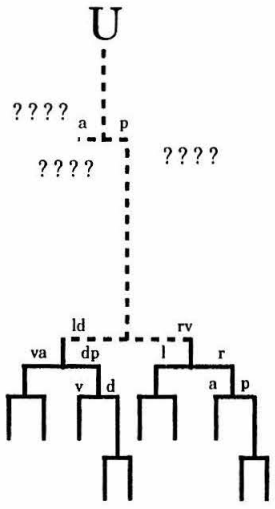
Fig. 2. (A) Wild type U, F, and Y lineages. (B) Abnormal F lineage observed in a *lin-49(sy238)* mutant. The U cell was not present in this animal. (C) U lineage in a *sy294* mutant. Later divisions of U are like a normal Y.p cell. It has not yet been established if U divides early, and thus if U is misspecified as Y, or as Y.p. (D) F lineage in *sy287* animal. The U lineage was normal in this animal. (E) F and U lineages from two *lin-48(sy234)* mutants. Dotted lines in (A) indicate variable lineage. Dotted lines in (B) - (E) indicate inferred (not followed) lineage. The division axes are indicated above each cell.


A.
hrs
10
15
20
25
30
35
L1
L2
L3
U
F
Y
r
l
a
p
av
pd
vl
dr
d
v

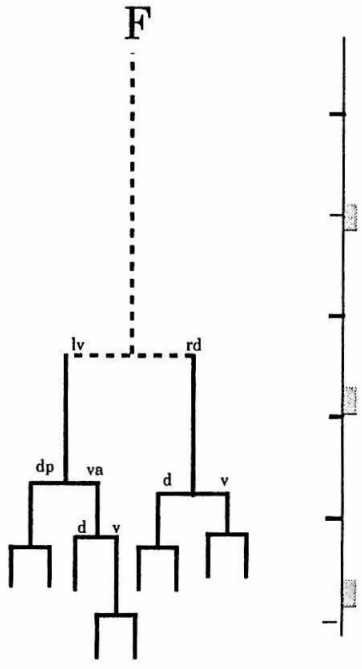
B. *lin-47(sy238)* #646

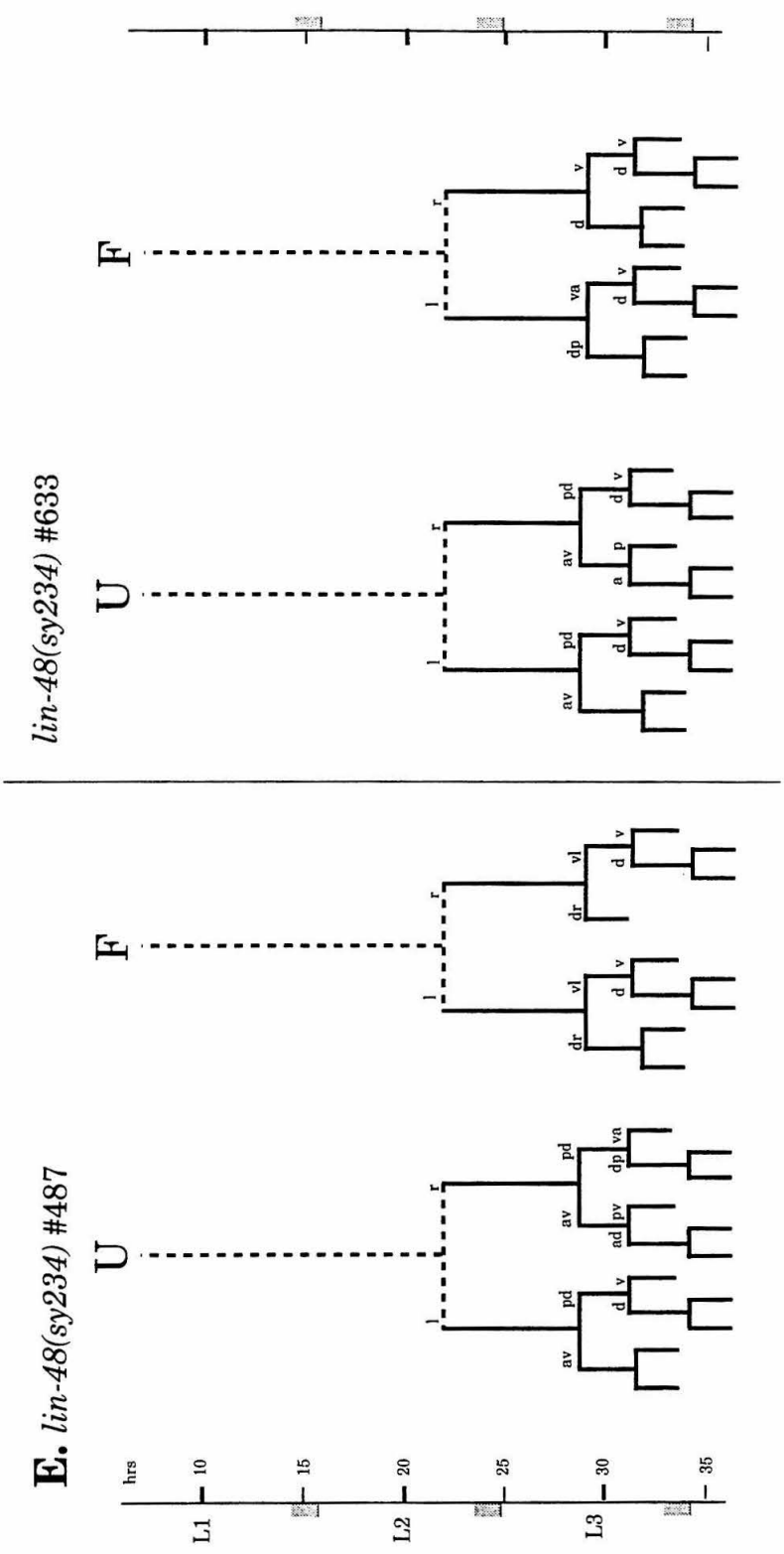


C. *sy294* #645



D. *sy287* #644




E. lin-48(sy234) #487
lin-48(sy234) #633
U
F
hrs
10
15
20
25
30
35
L1
L2
L3

References

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Chapter 5

Asymmetric cell divisions and the segregation of fate potential: The role of *lin-17* and *vab-3* in the *Caenorhabditis elegans* male B lineage

INTRODUCTION

Fate specification in a cell division that results in two daughters with two distinct fates can be mediated by cell interactions or by the asymmetric distribution of factors. However, visibly unequal cell divisions marked by asymmetric cytokinesis suggest that asymmetry exists in the mother cell, and fate potential may be asymmetrically distributed between the two daughters. Asymmetric cytokinesis can result in an unequal volume of cytoplasm and other cellular components distributed between daughter cells. In addition, it can be associated with the asymmetric distribution of specific factors that can play a direct role in the specification of one of the daughter fates.

During the development of the *C. elegans* male tail, the initial division of the B cell is asymmetric. The anterior daughter of B, B.a, is larger, it divides earlier, and it produces more progeny that the posterior daughter, B.p. The differentiated fates of the progeny from each cell are also different. B.a is the precursor to all of the cells of the copulatory spicules, whereas B.p produces no spicule fates (Sulston and Horvitz, 1977; Sulston, et al., 1980). The gene *lin-17* is required to mediate the asymmetric division of B. In *lin-17* mutants cytokinesis of the B cell is symmetric, and the two daughter cells behave like the normal B.a cell: they both divide early and produce more progeny than a normal B.p cell (Sternberg and Horvitz, 1988). Thus *lin-17* is necessary for the asymmetric division of B and for the production of normal B.p fates. *lin-17* is also required to mediate a variety of other postembryonic asymmetric cell divisions.

In this Chapter I describe one function of *vab-3*, a gene that is necessary for normal fates of the anterior daughter of the B cell, and its

interaction with *lin-17*. I also describe additional functions of *lin-17* in the B lineage subsequent to the first division.

MATERIALS AND METHODS

Nematode strains and handling

Nematode strains were cultured according to standard techniques (Brenner, 1974; Sulston and Hodgkin, 1988). In general, mutant genotypes include *him-5(e1490)* in the background. The following mutations were used in this study: *lin-17(n671, n677)* I (Ferguson and Horvitz, 1985). *him-5(e1490)* V (Hodgkin, et al., 1979). *vab-3(e648, e1176, sy66)* X.

Cell lineage and ablation

Cell nuclei divisions in living animals were directly observed using Nomarski differential interference contrast optics as described by Sulston and Horvitz (1977). Nomenclature follows the standard of Sulston and Horvitz (1977), with modifications of Chamberlin and Sternberg (1993). Cell nuclei were destroyed by a laser microbeam as described by Avery and Horvitz (1987).

RESULTS

vab-3 is required for normal B.a fates

Candidate mutations in *vab-3* were recovered in the screen for male tail mutants (*sy66*, *sy281*; Chapter 4). These mutations result in a variable

notched-head phenotype, and a characteristic pattern of six B progeny in males (rather than the normal ten) at the L3 stage, phenotypes also associated with mutations in the gene *vab-3* (A. Chisholm, pers. comm.).

Lineage analysis of *vab-3* mutants indicates that *vab-3* is necessary for the normal B.a lineage. The initial division of B is asymmetric as in wild type, and the B.a cell divides in early L2 larval stage along a left/right axis. However, B.al and B.ar fail to divide until about 5-6 hours later, often when the B.p cell divides. These cell divisions produce the six progeny observed at the L3 stage. The observed abnormal lineages of these cells are consistent with both B.al and B.ar behaving like a normal B.p cell (Figs 1, 2). Although the more anterior daughters of B.a(l/r) may divide more than once (in contrast to B.pa), overall the cells divide with the normal timing and appropriate axes of the B.p cell. In addition, these cells produce at least one of the differentiated fates of the B.p lineage. The B.ppaa cell normally undergoes a programmed cell death, and in *vab-3* mutants the corresponding cells from B.a(l/r) lineages can likewise die. Although cell death is a fate also observed in the normal B.a lineage, the specific pattern of which cell dies is distinctly B.p-like.

The *vab-3* and *lin-17* mutant defects are additive

To establish the relationship between *vab-3* and *lin-17* in specification of B.a and B.p fates, I constructed *lin-17; vab-3* double mutants and compared them to *lin-17* and *vab-3* single mutants (Table 1). At the L3 stage, the B cell can divide to produce sixteen progeny in *lin-17* mutants, whereas it produces six progeny in *vab-3* mutants. In the *lin-17; vab-3* double mutants

the B cell can produce eight progeny. Thus *lin-17* is necessary for the asymmetric division of B and mutations make B.a and B.p similar. However, *vab-3* is necessary for B.a fates. Both B.a and B.p behave like B.a in *vab-3* single mutants, and produce only four progeny at this stage.

I have followed the B lineage in two *lin-17; vab-3* animals to determine if both B.a and B.p behave like the B.a cell in *vab-3* mutants and produce two B.p lineages (Fig. 3). Although the lineages bear some similarity to normal B.p lineages, in general they were abnormal. The asymmetric timing of different cell divisions (e.g., B.ppp normally divides about 2 hours prior to B.ppa) was generally lost, with most of the cells of a given generation dividing within 10-15 minutes of each other. This defect was observed both in the double mutant with eight early L3 B progeny and the one with six. Since mutations in *lin-17* can disrupt the B.p-like lineages in a *vab-3* mutant *lin-17* may play additional roles in the B lineage, specifically in mediating normal asymmetric cell divisions in the B.p lineage.

lin-17 has additional functions in both the B.a and B.p lineages

Although in many *lin-17* mutant animals both the B.a and B.p cells behave like B.a, some animals escape the lineage defect at the first division and produce the normal ten B progeny at the L3 stage. To corroborate the B.p lineage defect observed in *lin-17; vab-3* double mutants, I have followed the B cell lineage in these escaper animals. In these animals, mutations in *lin-17* disrupt the normal B.p lineage, as both daughters of B.pp are of equal size and divide at the same time and produce only two progeny (Fig. 4).

However, the B.pa cell divides later than the B.pp cell, and produces only two progeny as it does in wild type.

A side effect of the abnormal duplication of the B.a lineage in *lin-17* mutants is that the eight progeny of each B daughter end up in abnormal positions with abnormal neighbors. Since proper fate specification for these cells requires a variety of cell interactions (see Chapter 2), it is difficult in intact animals with sixteen L3 B progeny to identify if a lineage defect results from the abnormal environment of a cell, or is a direct result of mutant *lin-17*. I have used two methods to identify additional requirements for *lin-17* in the B.a lineage. First, I also followed the B.a lineage in the escaper animals with ten L3 B progeny. Second, I ablated the B.p cell in two *lin-17* mutants, and followed the lineage of the B.a cell. In wild type animals, this ablation results in a normal lineage from the B.a cell (Chamberlin and Sternberg, 1993). Thus any defects observed following ablation in *lin-17* mutants reflect a requirement for *lin-17*.

Lineage analysis of both escapers and B.p ablated animals suggest that *lin-17* mediates additional asymmetric cell divisions in the B.a lineage (Table 2). 7/12 ε lineages were disrupted, with 4/4 disrupted in the animals in which B.p was ablated. The ε lineage is normally produced by the anterior cell of the lateral pairs (B.a(l/r)**ap**). The first division of an ε lineage is asymmetric: the anteroventral daughter is larger, divides earlier, and produces more progeny than the posterodorsal daughter. In *lin-17* mutants the cell division is symmetric, and both cells behave like the normal anteroventral daughter. Other lineages with asymmetric divisions such as the ζ lineage and the γ lineage are also occasionally disrupted. Thus *lin-17* is required for both the

initial division of the B cell and specific asymmetric divisions later in the B lineage.

DISCUSSION

lin-17 and *vab-3* act to specify fate in the asymmetric division of the B cell

The gene *lin-17* is necessary for the asymmetric division of the B cell, and in *lin-17* mutants both B.a and B.p behave like B.a cells. Although the B.p cells are not normal in *lin-17* mutants, *lin-17* is not specifically necessary for B.p fate, but rather the asymmetric distribution of B.a and B.p fate potential. The gene *vab-3* is necessary for B.a fate; in *vab-3* mutants both daughters of B.a behave like B.p cells. Unlike *lin-17,* mutations in *vab-3* uncouple fate specification from the asymmetric cell division. *vab-3* may represent a gene thats product is asymmetrically distributed between B.a and B.p, or a gene that is activated by an asymmetrically distributed factor in B.a. The gene *lin-44* is also required for the normal division of the B cell. Mutations in *lin-44* result in a reversal of the polarity of the B cell: B.a behaves like a normal B.p, and B.p behaves like a normal B.a (Herman and Horvitz, 1994). *lin-17* is epistatic to *lin-44*, as *lin-17 lin-44* double mutants have the *lin-17* phenotype. *lin-17*, *lin-44*, and *vab-3* thus represent three major functions in an asymmetric cell division. *lin-17* is necessary to establish the asymmetric division, *lin-44* is necessary to establish the proper orientation of the division, and *vab-3* activity is asymmetrically distributed by the division (Fig. 5).

lin-17 acts at several distinct steps in the B lineage to mediate specific asymmetric cell divisions

Lineage analysis of *lin-17; vab-3* double mutants, *lin-17* animals with ten L3 B progeny, and *lin-17* animals with B.p ablated suggest that *lin-17* functions both at the initial division of the B cell as well as specific asymmetric cell divisions later in the lineage. In the B.p lineage *lin-17* is required for the asymmetric division of the B.pp cell and possibly the B.p cell itself. The B.p lineage in *lin-17* escaper animals retains the asymmetry in division timing between B.pa and B.pp, whereas both anterior and posterior cells in the *lin-17; vab-3* double mutants generally divide at the same time. This discrepancy may be a side effect of selecting animals with ten L3 B progeny, as it is possible that polarity in the division of B.p is not independent from polarity in the division of B.

There are many asymmetric cell divisions -- divisions that result in two daughters that are different from each other -- in the B lineage. Lineage analysis suggest that *lin-17* acts at some, but notably not all of these cell divisions. *lin-17* appears to play a role in visibly unequal cell divisions. For instance, the division of B and many of the cells in the B.p lineage result in daughters that are morphologically distinct. In contrast, the B.a(l/r) cells each divide asymmetrically to produce four cells with distinct potentials that respond differently to the same extracellular cues (see Chapters 2 and 3). Although there is a subtle timing difference in the division of B.a(l/r) daughters (Sulston and Horvitz, 1977; Chamberlin and Sternberg, 1993), the four cells are morphologically similar. Interestingly, mutations in *lin-17* do not disrupt either of the two asymmetric cell divisions that would be required to generate these four progeny types. For instance, the **aa** cells produce

generally normal **aa** fates in the *lin-17* mutants. They do not produce the fates normally associated with their sisters (**ap** cells and ε fate) or their lineal homologs (**pa** cells and ζ fate) that would be expected if these divisions were disrupted. The late lineage defects in *lin-17* mutants do not represent a transformation from one intermediate blast fate to another. The disrupted ε lineages are not like any other normal B.a progeny fate. Although the rare disrupted ζ lineages superficially produce six progeny in a 3+3 pattern like a normal β lineage, the timing of divisions and appearance of the progeny cells are more consistent with both cells producing a lineage like the normal **pap** cell. Cytokinesis is unequal in both the ε and the ζ lineages, and the anteroventral and posterodorsal daughters are distinct from each other in the timing of division and in the number of progeny. Thus in the B lineage *lin-17* appears to mediate a subset of asymmetric cell divisions in which unequal division correlates with the fates of the daughter cells.

Do *lin-17*, *lin-44*, and *vab-3* represent a genetic pathway that functions generally as a unit at unequal cell divisions? This does not appear to be the case. Although in the B lineage *lin-17* is required for specific unequal divisions, it also functions in other postembryonic cell divisions that are not visibly asymmetric (Sternberg and Horvitz, 1988). Other unequal divisions, such as the first division of the Pn cells or the male Y cell, are not disrupted in *lin-17* mutants. Thus, although *lin-17* is required for unequal cell division in certain cells, its function is not one required generally for asymmetric cytokinesis.

The three genes also do not always act together as a genetic "module." Without the use of mosaics, we cannot test whether *vab-3* acts in the later B lineage cell divisions that require *lin-17*. However, although *lin-17*, *lin-44*,

and *vab-3* are required in several other postembryonic lineages, they are not required in the *same* lineages. For example, *vab-3* is required for a normal Y lineage but not male Pn.p lineages (data not shown). Mutations in *lin-17*. in contrast, disrupt the Pn.p lineages but not Y (Sternberg and Horvitz, 1988). *lin-44* is required in a subset of *lin-17*-mediated cell divisions, but not all (Herman and Horvitz, 1994). Thus these genes function together in mediating the division of B and the specification of its progeny, but they can also function independently.

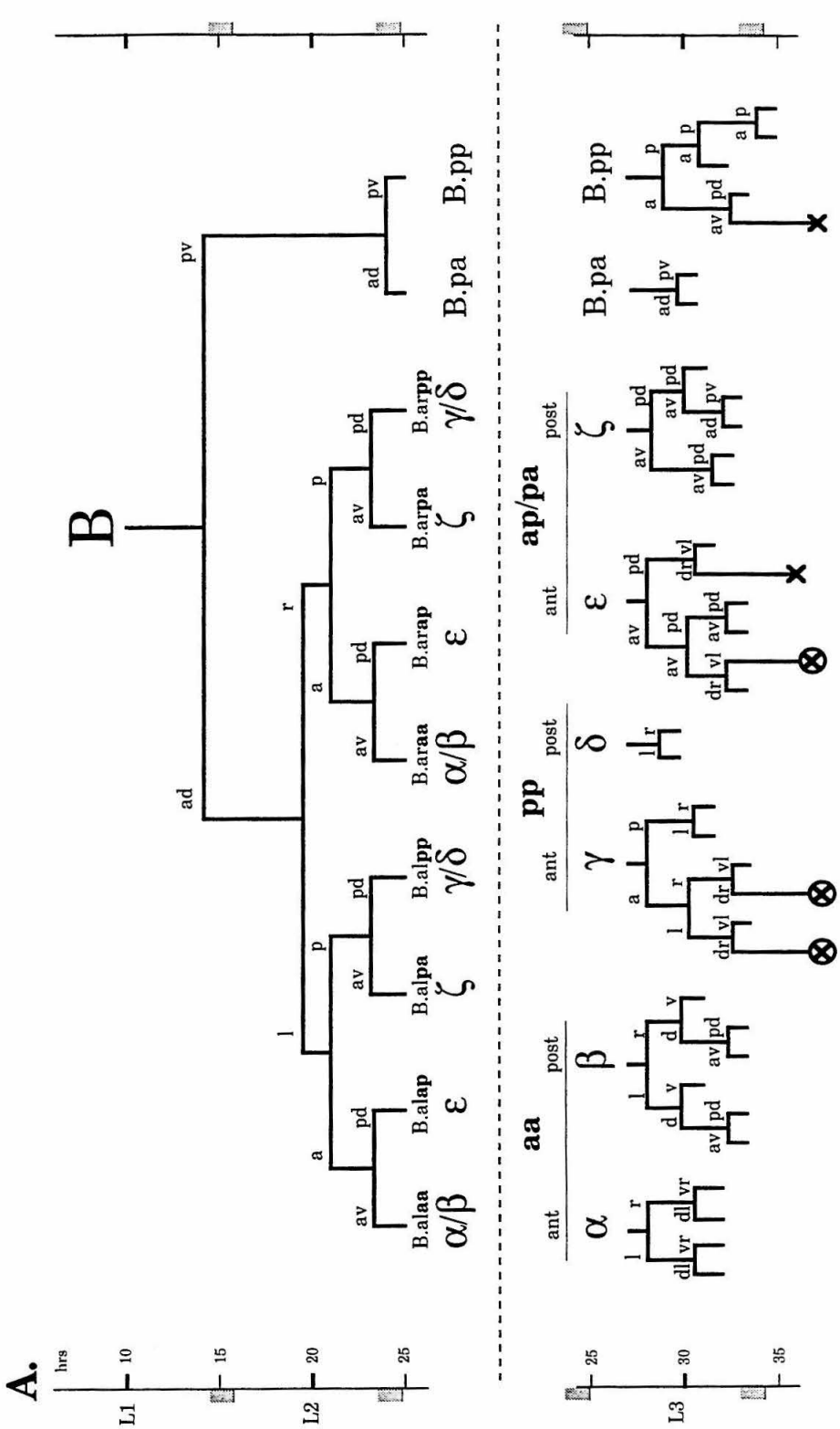
Table 1. Number of B progeny in early L3 stage males. The Table indicates the number of animals with the indicated number of B progeny at the early L3 larval stage. Wild type animals always produce ten progeny: eight from B.a and two from B.p. If both B progeny behave like B.a, there are sixteen progeny (*lin-17* phenotype). If B.a divides once and then all three cells behave like B.p, there are six B progeny (*vab-3* phenotype).

	Number of B progeny at early L3 stage					
genotype	**6**	7	**8**	**10**	11 to 15	**16**
wild type	.	.	.	**all**	.	.
lin-17(n671)	.	.	.	**8**	**2**	**9**
lin-17(n677)	.	.	.	**6**	**1**	**13**
vab-3(e648)	**17**	.	.	.	.	.
vab-3(e1178)	**9**	.	.	.	.	.
n677; e648	**15**	**2**	**5**	.	.	.

Table 2. Late B lineages in *lin-17* mutants. The Table indicates the lineages observed in *lin-17* ablated (B.p-) and intact, escaper animals (B=10). Wild type, B.p- data are from Chapter 2. Although the *lin-17* defect is variable, B.a and B.p were approximately the same size in animal #648, and in animal #647 B.a and B.p were initiating cell division at the same time when B.p was ablated. In the B.p lineages, 2+4 indicates a lineage like that illustrated in Fig. 4. In animal #651, the B.p cell produced only four progeny. However, cytokinesis was not complete in the division of B.p, so B.pa and B.pp shared a common cytoplasm and divided synchronously. This defect is occasionally observed in many genotypes, and likely represents genetic background or damage to the animal rather than a specific effect of *lin-17(n677)*.

genotype		animal	**aa** ant.	**aa** post.	**pp** ant.	**pp** post.	**ap** left	**ap** right	**pa** left	**pa** right	B.p(a/p)
wild type			α	β	γ	δ	ε	ε	ζ	ζ	2+5
wild type	B.p-		α	β	γ	δ	ε	ε	ζ	ζ	x
n671	B.p-	647	β	α	abn-8	δ	abn-8	abn-8	ζ	ζ	x
	B.p-	648	α	β	γτ	δ	abn-7	abn-8	ζ	ζτ	x
n671	B=10	649	α	β	γ	δ	ε	abn-7	abn-6	ζ	2+4
	B=10	650	α	β	abn-5	δ	ε	ε	ζ	ζ	2+4
	B=10	652	abn-5	β	γ	δ	abn-7	ε	abn-6	ζ	2+4
n677	B=10	651	α	βτ	γ	δ	abn-8	ε	ζ	ζ	2+2

Figure 1. B lineage abnormalities in *vab-3* mutants. A. The wild type B lineage. B. Composite B lineage of *vab-3* mutants. This composite was constructed from the eight L3 lineages of C and additional lineages of L1 and L2 animals. C. B lineage charts from two *sy66* and six *e648* animals. Normal B lineage after Sulston et al. (1977). Larval stage and approximate developmental time are indicated on the left axis. Dotted lines in B indicate variable lineage. Dotted lines in C indicate inferred lineage. The cell division axes are indicated above each cell division: a = anterior, p = posterior, d = dorsal, v = ventral, l= left, r= right. X indicates cell death. X in a circle indicates a conditional cell death where either one or the other, but not both, of the pair dies. (X) indicates a presumptive death marked by unequal division of the mother, compact nucleus, and other hallmarks of cell death. Cytokinesis was incomplete in the division of B.p in animal #560, so the nuclei shared a common cytoplasm as in animal #651 in Table 2.


A.
hrs
L1
L2
L3
B
B.alaa
B.alap
B.alpa
B.alpp
B.araa
B.arap
B.arpa
B.arpp
B.pa
B.pp
α/β
ε
ζ
γ/δ
α/β
ε
ζ
γ/δ
aa
pp
ap/pa
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δ
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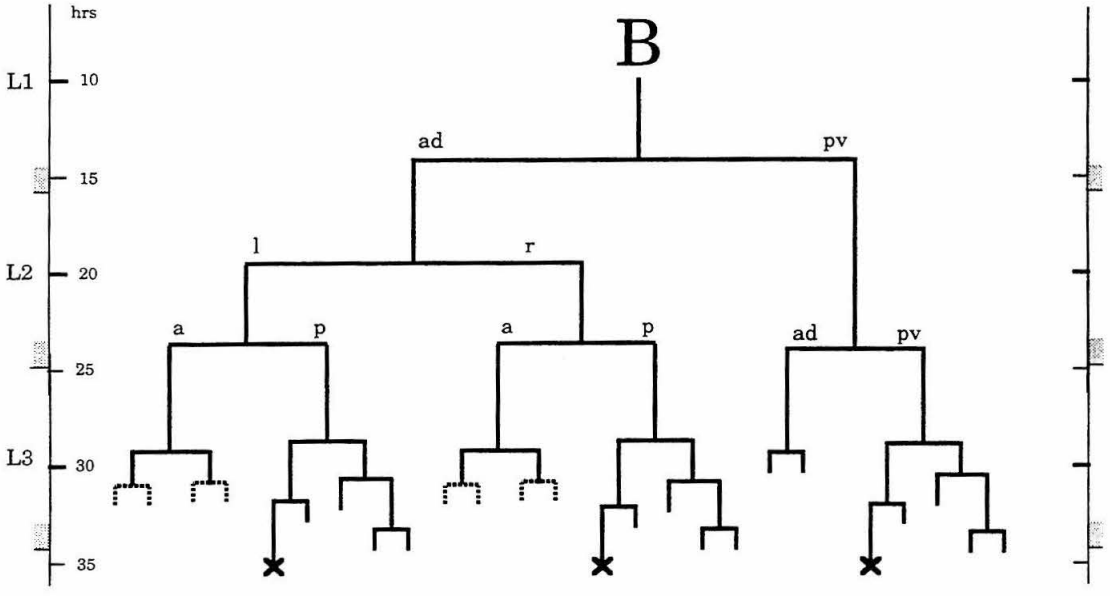
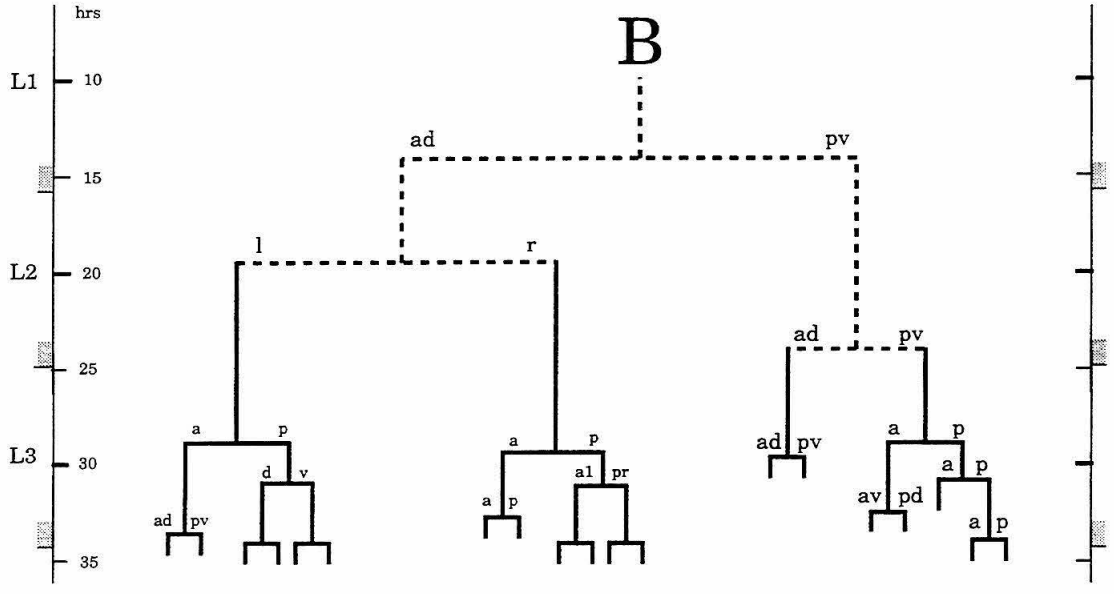
ζ

B.

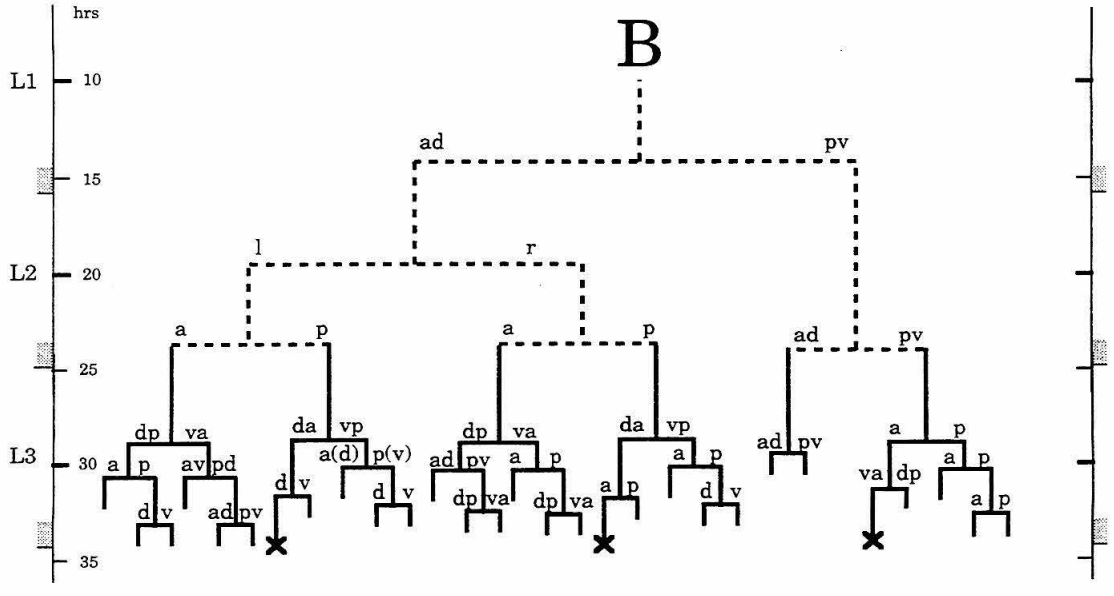


C.

e1490; sy66 #68

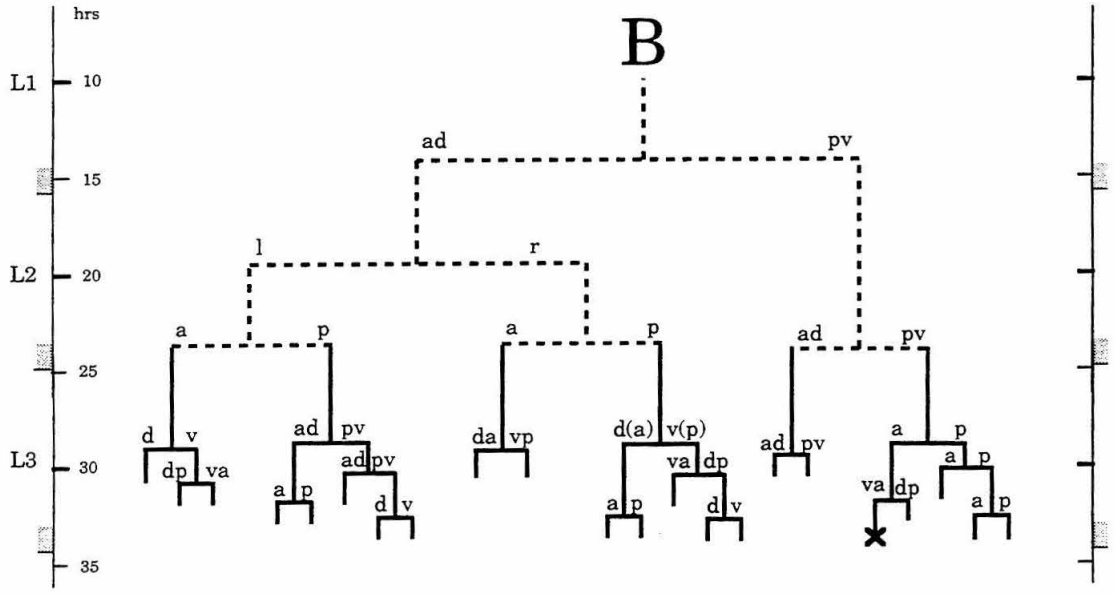


e1490; sy66 #79

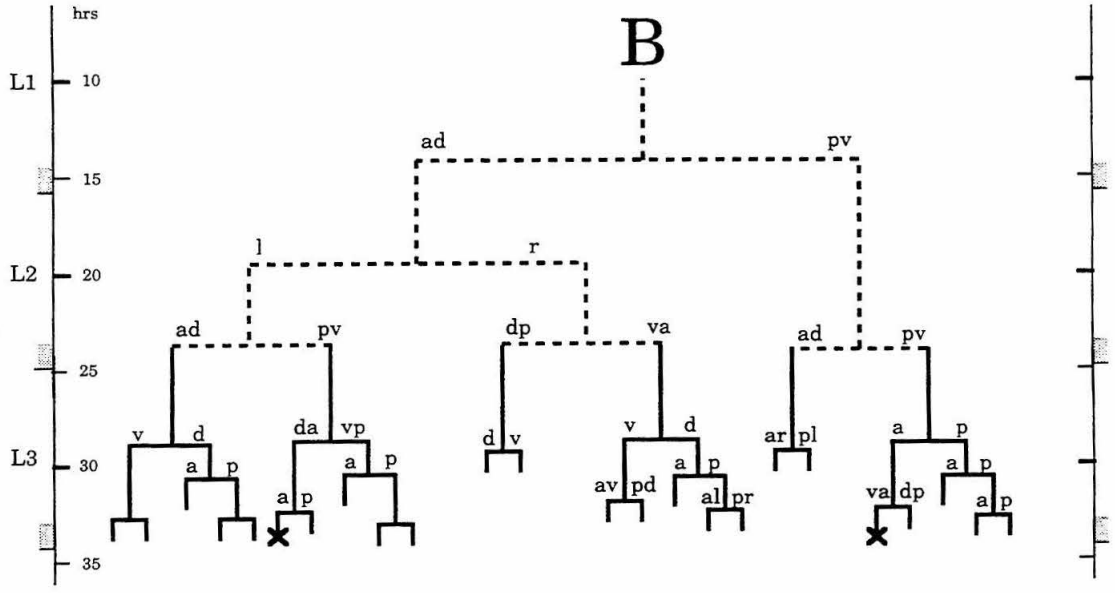


C. cont.

e1490; e648 #77

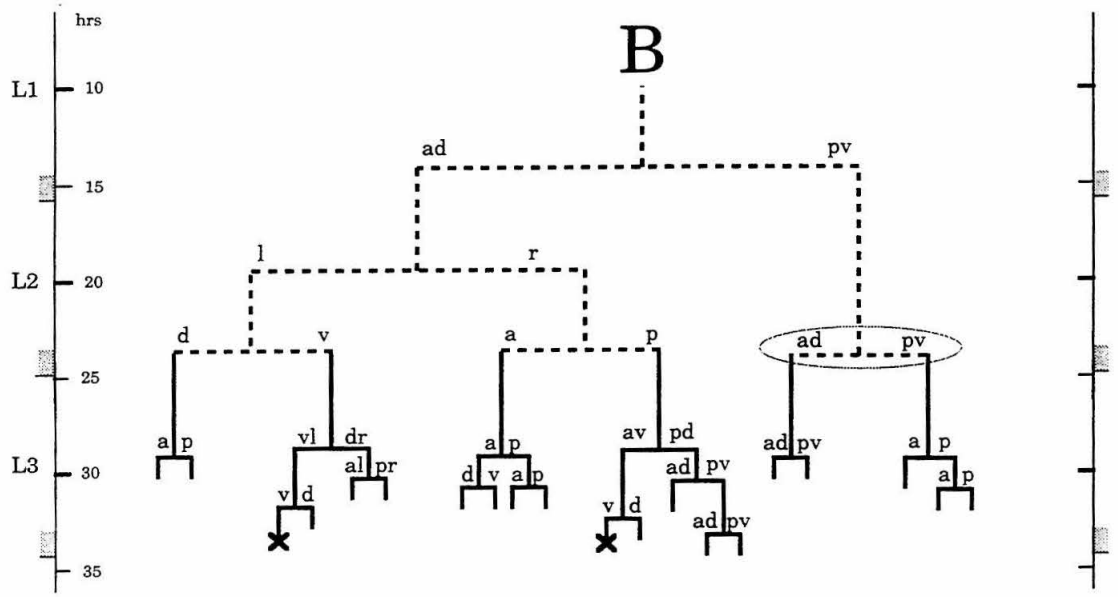


e1490; e648 #559

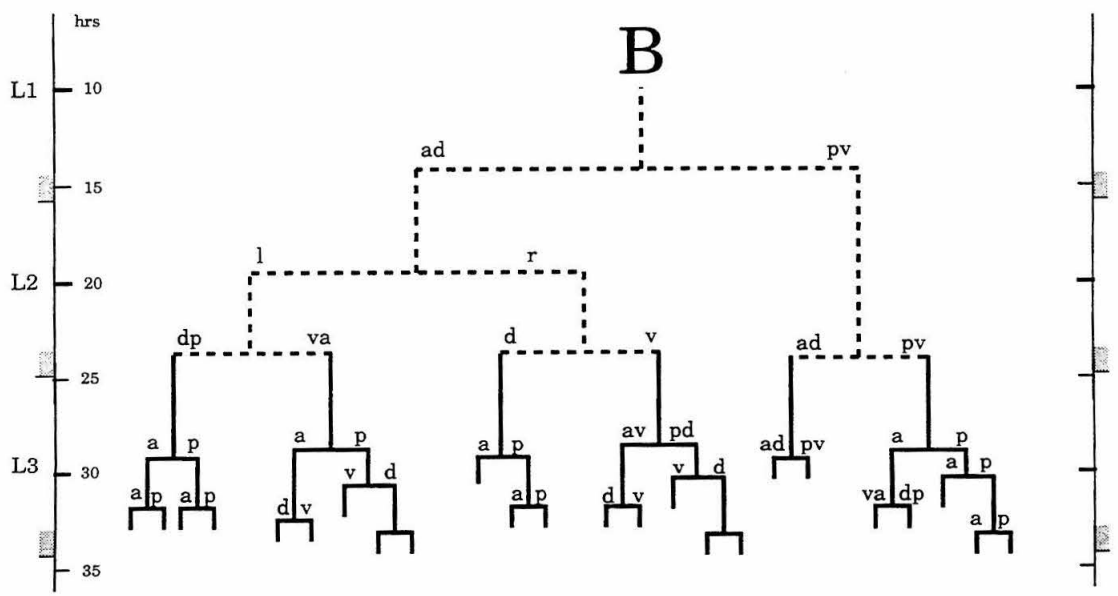


C. cont.

e1490; e648 #560



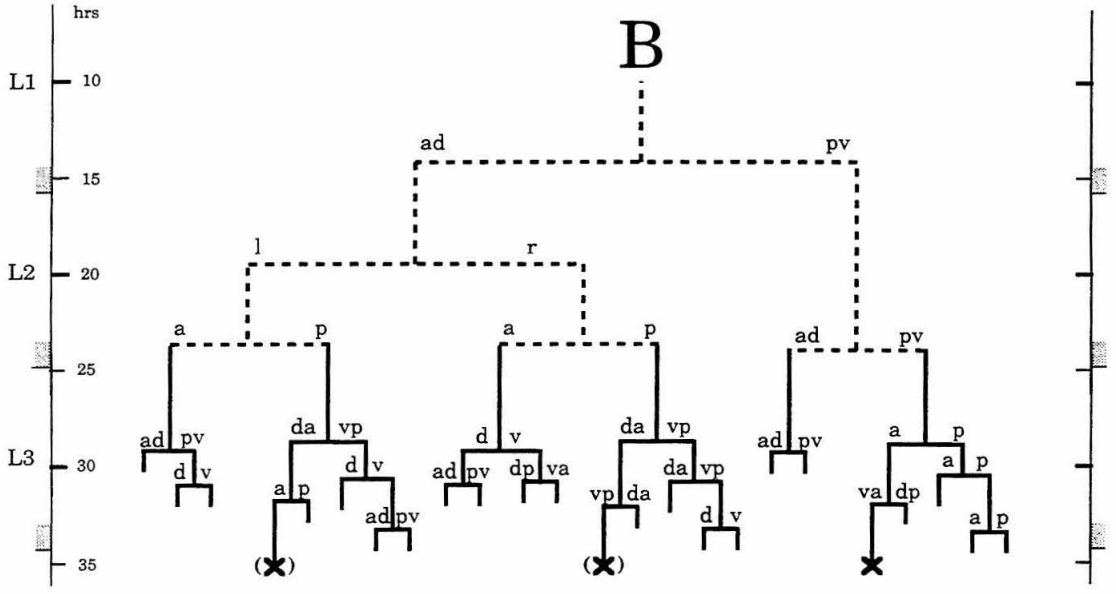
e1490; e648 #581



not followed to cell deaths

C. cont.

e1490; e648 #616



e1490; e648 #617

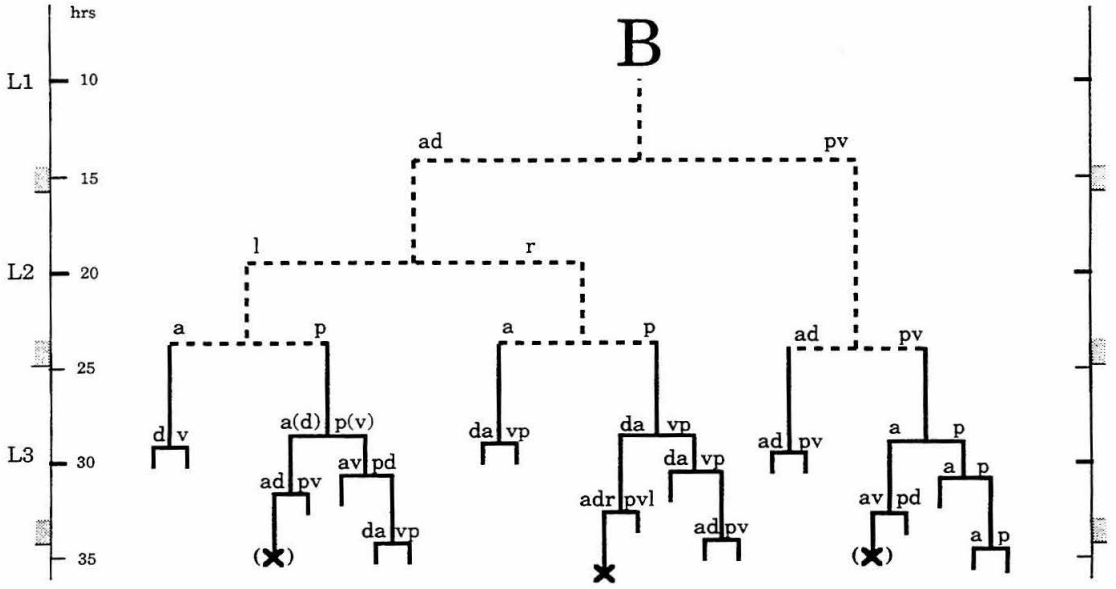


Figure 2. Nomarski photomicrograph showing the lineage defect in *vab-3* mutants. In *vab-3* mutants B.a(l/r) behave like a normal B.p cell. This includes dividing once in late L2. The two daughters don't divide until mid-L3, after the first division of the Pn.p cells. B.pp, B.pa, and B.arp are initiating cell division (arrows) at the same time, whereas B.pa (line) and B.ara (out of the plane) are intact. The P11.p and P10.p cells have already divided. Genotype: *him-5(e1490); vab-3(sy66)*

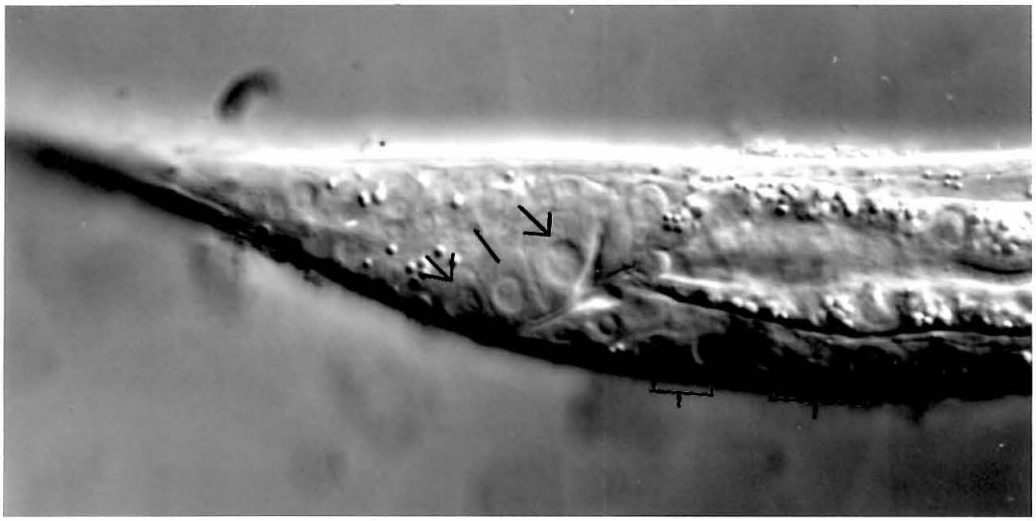
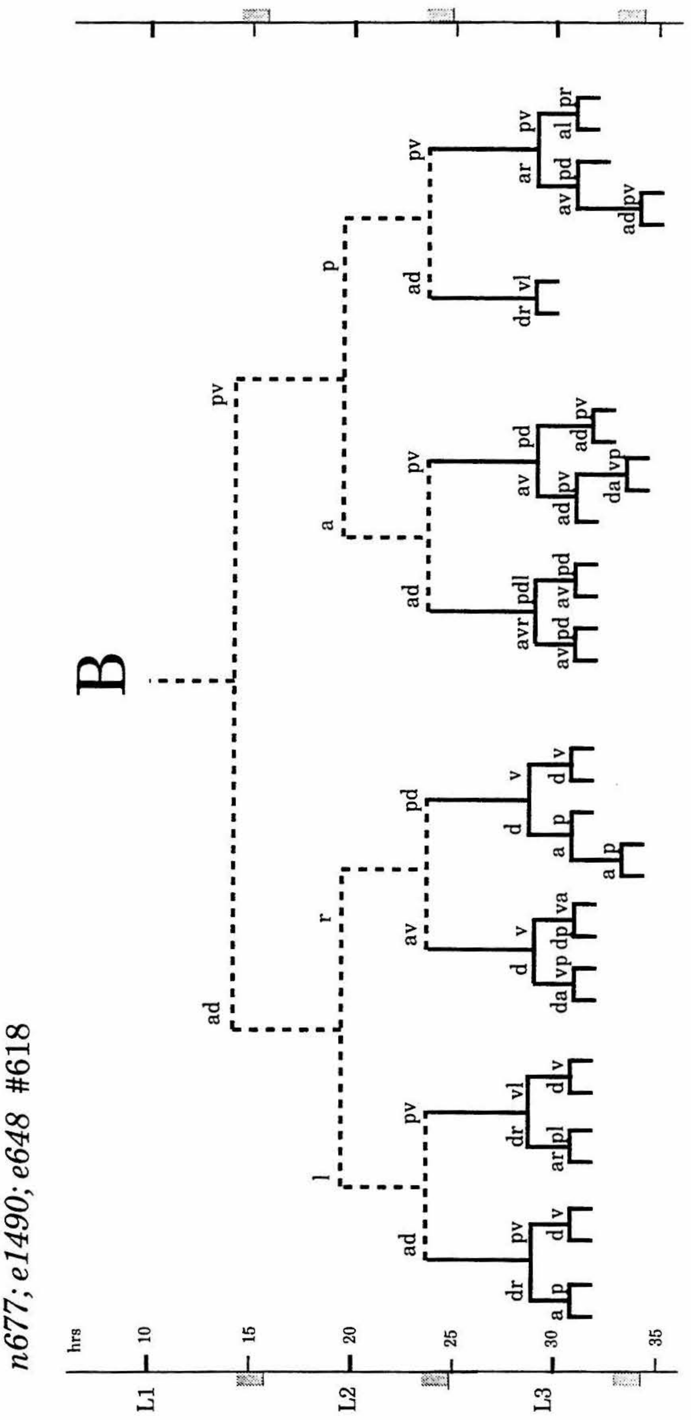


Figure 3. B lineage in *lin-17; vab-3* double mutants. Notation as in Fig. 1.


n677; e1490; e648 #618
B
hrs
10
15
20
25
30
35
L1
L2
L3

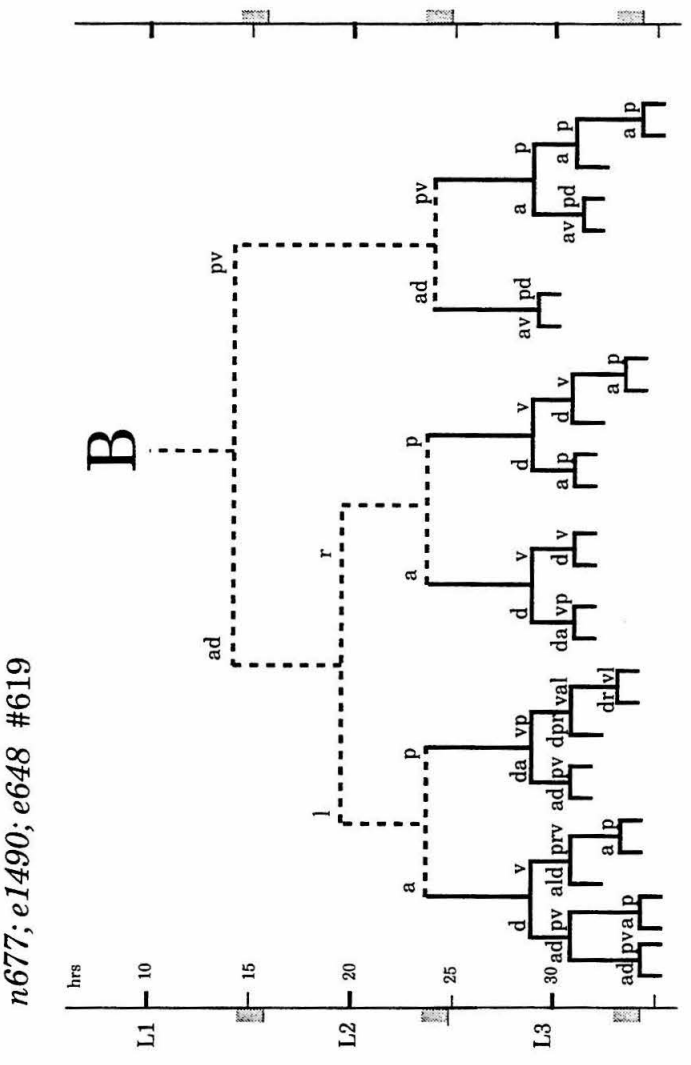

n677; e1490; e648 #619
B
hrs
10
15
20
25
30
L1
L2
L3
ad
l
r
pv
a
p
d
v
da
vp
dpn
val
dr
vl
ald
prv
pva
av
pd

Figure 4. B lineage defect in B.p of *lin-17* mutants that escape the lineage defect of the initial B division. A. Normally the B.pp cell division is asymmetric, and the posterior daughter is larger, divides earlier, and produces more progeny than the anterior daughter. B. In *lin-17* mutants the B.pp cell division is equal, and both B.ppa and B.ppp divide only once. Notation as in Fig. 1.

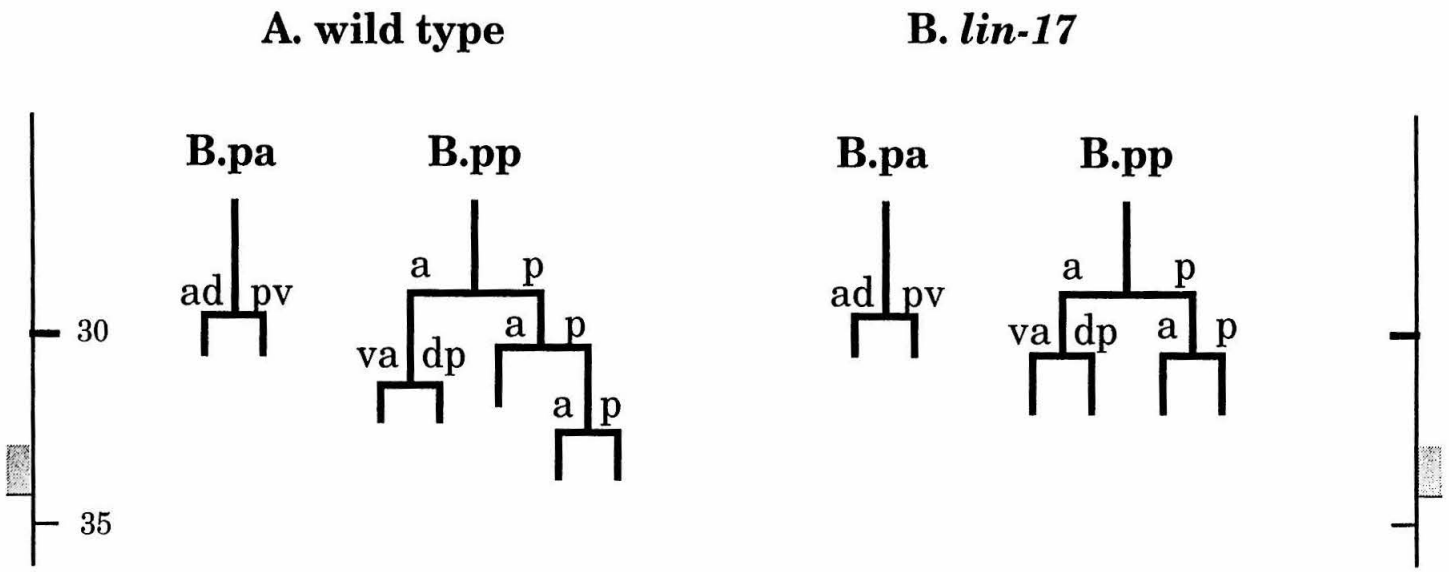
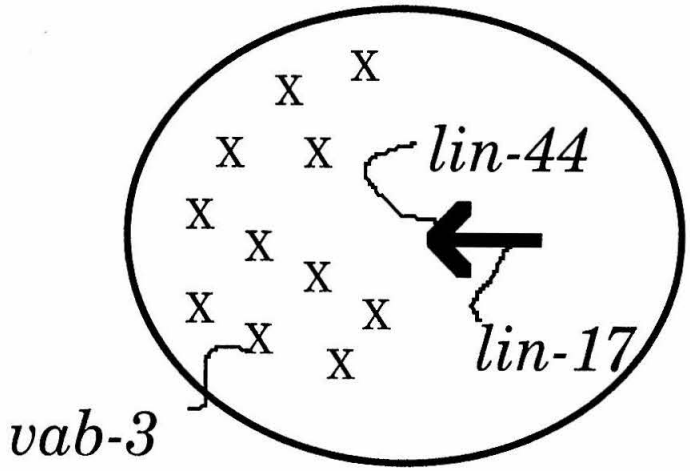

A. wild type
B. lin-17
B.pa
B.pp
ad
pv
a
p
va
dp
30
35

Figure 5. Model for the action of *lin-17*, *lin-44*, and *vab-3*. *lin-17* is necessary to establish the asymmetric division of B, *lin-44* is necessary to establish the proper orientation of the division, and *vab-3* activity is asymmetrically distributed by the division.

B cell



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Chapter 6

Summary

Several related observations contributed to the initiation of the experiments described in this thesis. Three results hinted at the cell interactions that play a role in fate specification in the B lineage: (1) the correlation of α/β and γ/δ fates with cell position but not cell lineage (Sulston and Horvitz, 1977; Sulston, et al., 1980; Sulston and White, 1980), (2) the lineage defect in the γ/δ pair in *lin-12(0)* mutants (Greenwald, et al., 1983), and (3) the pleiotropic defects in mutants of other genes required for vulval induction (Ferguson and Horvitz, 1985; Aroian and Sternberg, 1991). An early model was that *lin-12* might mediate fate specification in the γ/δ pair whereas the *lin-3/let-23* pathway might act in the α/β pair. In addition to the question of cell interactions, the role of *lin-17* in the first division of the B cell (Sternberg and Horvitz, 1988) suggested that mutations that disrupt different steps in the development of the B cell could be recovered, and the function(s) of the corresponding gene could be interpreted using cell lineage analysis. Here, I review the results in the context of stepwise fate specification and multiple signal integration.

The integral relationship between autonomous and conditional fate specification

The initial division of the B cell is asymmetric and unequal. The genes *lin-17*, *lin-44*, and *vab-3* act at this division to mediate the asymmetric distribution of fate potential and the specification of fate (Chapter 5). In both *lin-17* and *lin-44* mutants the defect is observed in the cytokinesis of the B cell itself, suggesting a mechanism of autonomous fate specification. The *vab-3* phenotype is consistent with this model. In *vab-3* mutants the initial

division is normal, but B.a fates are misspecified, and B.al and B.ar behave like B.p. One difference between B.a progeny and B.p progeny is that B.a progeny are responsive to a set of specific cell interactions, and require the genes of the *lin-3/let-23* signalling pathway for normal fate specification (Chapters 2 and 3). All eight intermediate B.a progeny utilize these genes, and thus inducing the expression of the *let-23* receptor, for instance, may be a function (direct or indirect) of *vab-3*. Cell interactions are likely not absent from the B.p progeny, as mutations in *lin-12* can disrupt the fates of B.pa and B.pp (Greenwald, et al., 1983). However, none of the cell ablations or *lin-3/let-23* class of mutations that disrupt B.a progeny fates disrupt B.p progeny fates. Furthermore, ectopically placed cells with B.p fates (as in the *vab-3* mutants) can be essentially normal. Thus the progeny of B.a and B.p are different in their ability to respond to positional cues, and this difference is specified, at least indirectly, by the asymmetric distribution of *vab-3* activity.

Following the cell interactions that establish anterior and posterior fates among the eight progeny of B.a, the cells must execute the fate appropriate to their position. Both cell interactions and cell divisions that asymmetrically distribute fate potential may play a role at this later step. In the **ap/pa** pairs, *lin-17* is required for the asymmetric division associated with ε and ζ fates (although both defects are variable; Chapter 5). In contrast, cell ablation experiments (Chapter 2) and ectopic expression of *lin-3* (Chapter 3) suggest that additional cell interactions may play a role in the execution of the γ lineage. Normally a γ lineage comprises an initial anterior/posterior division, with γ.p producing two progeny and γ.a producing four. However, ectopic expression of *lin-3*, or a failure to localize the F/U

positional cue, can result in both daughters behaving like γ.a, such that there are a total of eight progeny (the abn-8 lineage) rather than the normal six.

Although these experiments at least begin to identify the genes and processes involved at different steps of fate specification throughout the B lineage, some key steps are still poorly understood. Specifically, what mechanisms are involved in establishing the differences between the cells of the different pairs of intermediate B.a progeny? The **aa** cells, for instance, respond to ectopic LIN-3 differently from the **ap/pa** cells or the **pp** cells. Cell ablation experiments did not identify any examples where cells in one pair adopted the fates normally associated with cells in another pair (Chapter 2). Although the negative result cannot completely rule out the possibility of cell interactions, there is no evidence that they play a role. Analysis of the defects associated with one gene required for a variety of asymmetric cell divisions, *lin-17*, suggests that the fates of the different precursors are specified by a *lin-17*-independent mechanism (Chapter 5). It is possible that a mutant that will be useful to understand these steps in B lineage development exists among the mutants with a mid-stage class of defect recovered in the genetic screen (Chapter 4). In a mutant where intermediate B.a progeny fates are abnormal, the cells may not migrate to their normal dorsal/ventral positions, but to the position associated with their misspecified fate. Thus, *sy285*, possibly *sy294*, or mutations like them represent candidate mutations that disrupt specification of intermediate B.a progeny fate. Further lineage analysis and cell ablation experiments may be able to verify if this is the case.

It is interesting to note the parallels between the development of the male B lineage and early *C. elegans* embryogenesis. Both involve an initial

asymmetric division that produces a larger anterior and a smaller posterior daughter. The posterior daughter divides with a modified stem-cell-like lineage, where one progeny retains the function of the mother cell. The first division of the anterior daughter is equivalent, and a complex set of cell interactions specify different fates among the progeny. One difference is that in the embryo, all mechanisms of fate specification must come from "within" the lineage; maternal factors are localized in the ooplasm, and cell interactions are restricted to occurring among the progeny of P0. In contrast, the B cell develops within the context of a multicellular organism, and positional cues can arise from neighboring cells. In terms of fate specification the two processes may share some mechanisms, whereas others may be unique. Presently, however, several genes have been identified that are required for early embryogenesis and others required for B cell development, but no gene has been demonstrated to function in both processes. This may reflect the differences in selection protocols for mutations that disrupt each process, as well as the likelihood that all of the functions may not be understood for a given gene.

Fate specification and the integration of multiple signals

Cell ablation experiments have identified four active cell interactions that establish anterior/posterior fates in each pair of intermediate B.a progeny. How do the responding cells integrate these positional cues? The *lin-3 / let-23* signalling pathway mediates the F/U positional cue that promotes anterior fates. Double mutant analysis indicates that *lin-15* likely acts antagonistic and in parallel to *lin-3* as a negative regulator of *let-23*

activity as it does in vulval development (Chapter 3). Thus both *lin-3* and *lin-15* are integrated at the *let-23* receptor.

The disruption of posterior **pp** fate following ablation of Y.p is not blocked by any mutation in genes of the *lin-3/let-23* pathway, so the Y.p cue is integrated at some point after *lin-45*. The Y.p cue does not represent the activity mediated by *lin-15*, and it may represent an independent signalling pathway that acts in parallel to, or after, the *lin-3/let-23* pathway. The **pa** cells are required for normal anterior fates in the **pp** cells (Chapter 2). However, this function is likely to localize (modulate) the Y.p positional cue so that it does not act inappropriately on the anterior **pp** cells. Thus the **pa** and Y.p information is integrated at the level of the Y.p signal. There are presently no candidate mutations that disrupt the Y.p signalling pathway.

The lateral interaction, at least in the **pp** cells, is mediated by *lin-12*, and the integration of *lin-12* information may parallel the relationship between *lin-3/let-23* and *lin-12* in vulval development. As in vulval development, the *lin-12(d)* defect can be overridden by *lin-3* (or at least the presence of F and U). In relation to the Y.p cue, *lin-12(d)* is sufficient to produce δ fates even in the absence of Y.p, whereas Y.p is necessary when *lin-12* is not mutant.

The information from the other B.a progeny likely represents two functions: a posterior positional cue and an insulating function that localizes or modulates the positional cues from F/U and Y.p (Chapter 2). Because both functions are eliminated when the cells are ablated, it is difficult to predict what the lineage defect would be if just the positional cue is eliminated, as it would be in a genetic mutant. It is possible that this positional cue would be redundant, or partially redundant, with the modulatory function and the

other positional cues from F/U and Y.p. If this is the case, then we cannot rule out the possibility that *lin-15* represents a component of this signal. Alternatively, this interaction may be disrupted in *lin-47(sy234)* animals.

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