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The evolution of cell lineage in nematodes

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SUMMARY

The invariant development of free-living nematodes combined with the extensive knowledge of *Caenorhabditis elegans* developmental biology provides an experimental system for an analysis of the evolution of developmental mechanisms. We have collected a number of new nematode species from soil samples. Most are easily cultured and their development can be analyzed at the level of individual cells using techniques standard to *Caenorhabditis*. So far, we have focused on differences in the development of the vulva among species of the families Rhabditidae and Panagrolaimidae. Preceding vulval development, twelve Pn cells migrate into the ventral cord and divide to produce posterior daughters [Pn.p cells] whose fates vary in a position specific manner [from P1.p anterior to P12.p posterior]. In *C. elegans* hermaphrodites, P(3-8).p are tripotent and form an equivalence group. These cells can express either of two vulval fates (1° or 2°) in response to a signal from the anchor cell of the somatic gonad, or a non-vulval fate (3°), resulting in a 3°-3°-2°-1°-2°-3° pattern of cell fates. Evolutionary differences in vulval development include the number of cells in the vulval equivalence group, the number of 1° cells, the number of progeny generated by each vulval precursor cell, and the position of VPCs before morphogenesis. Examples of three Rhabditidae genera have a posterior vulva in the position of P9-P11 ectoblasts. In *Cruznema tripartitum*, P(5-7).p form the vulva as in *Caenorhabditis*, but they migrate posteriorly before dividing. Induction occurs after the gonad grows posteriorly to the position of P(5-7).p cells. In two other species, *Mesorhabditis* sp. PS 1179 and *Teratorhabditis palmarum*, we have found changes in induction and competence with respect to their presumably more *C. elegans*-like ancestor. In *Mesorhabditis*, P(5-7).p form the vulva after migrating to a posterior position. However, the gonad is not required to specify the pattern of cell fates 3°-2°-1°-2°-3°. Moreover, the Pn.p cells are not equivalent in their potentials to form the vulva. A regulatory constraint in this family thus forces the same set of precursors to generate the vulva, rather than more appropriately positioned Pn.p cells.

Key words: nematodes, evolution, cell lineage, induction, cell migration, cell death

INTRODUCTION

Most nematodes display invariant cell lineages, that is, a similar pattern of cell divisions in all individuals of a species. One hundred years ago, Boveri and zur Strassen used the invariance of *Ascaris* embryology in their morphological and developmental studies (Boveri 1899; zur Strassen, 1896). They described the early germline - soma differentiation in the embryo of *Ascaris*, visualized by the elimination of chromatin material only in the somatic cells. Together with centrifugation and polyspermy experiments of early *Ascaris* embryos these studies indicated for the first time the necessity of interactions between the cytoplasm and the nucleus in the generation of different cell fates during ontogeny (Boveri, 1910). As an extrapolation of the early germline - soma differentiation, pioneer developmental biologists considered nematodes as a very extreme example of preformistic development with autonomous cell specification.

Over the last twenty years a resurgence of interest in nematode development has been led by the establishment of the free-living nematode *Caenorhabditis elegans* as a model system for genetics, developmental biology, neurobiology and genome analysis (e.g., Brenner, 1974; Wilson et al. 1994). The invariant development of this species, combined with the small cell number, allowed description of the complete cell lineage (Sulston et al., 1983; Sulston and Horvitz, 1977; Kimble and Hirsh, 1979). The combination of genetics, cell lineage and experimental analysis by cell ablation gave insight into a variety of developmental processes. We now know that the invariant development in *Caenorhabditis* results from both autonomous and conditional cell specification, with highly reproducible cell-cell interactions occurring because homologous cells in different individuals have homologous neighbors (reviewed by Lambie and Kimble, 1991; Wood and Edgar, 1994).

The striking invariance of nematode development also made it possible to use this group of organisms for evolutionary developmental analysis. Comparative studies have been initiated in both embryonic and postembryonic development (Sternberg and Horvitz, 1981, 1982; Sulston et al., 1983; Ambros and Fixsen, 1987; Skiba and Schierenberg, 1992). Here we first describe the types of results obtained from genetic analysis in one species, and from comparison of the complete postembryonic cell lineages of two species representing different nematode families. We then focus on the results of our recent studies of vulval development in one

family of nematodes (Sommer and Sternberg, 1994; R. Sommer and P. Sternberg, unpublished data).

POSSIBLE EVOLUTIONARY CHANGES INFERRED FROM GENETIC STUDIES

Variation in ontogeny ultimately arises from mutation. What types of changes in development can be caused by single or a few mutations? Cell lineage mutants in *Caenorhabditis* have defined some of the types of changes that can affect a cell lineage in one step. The best example is provided by the "heterochronic" genes, which control the relative timing of specific developmental events in several tissues (Ambros and Horvitz, 1984; reviewed by Ambros and Moss, 1994). Mutations in the genes *lin-4, lin-14, lin-28* and *lin-29* alter the timing of particular events in relation to events in other tissues. These temporal transformations lead to "heterochrony," considered as a possible major source of evolutionary novelty (De Beer, 1958; Gould, 1977). Heterochronic mutations can cause precocious or retarded development (Ambros and Moss, 1994). For example, recessive mutations in *lin-14* cause particular developmental events to occur earlier than normal. Vulva formation normally begins in the third larval stage (L3), whereas precocious *lin-14* alleles it cause to start during the second larval stage (L2). However, semidominant alleles of *lin-14* cause retarded development, resulting in supernumerary larval molts beyond the four wild-type molts.

Genetic and phenotypic analyses of the heterochronic genes revealed a phenotypic hierarchy of the genes *lin-4, lin-14, lin-28* and *lin-29* (Ambros and Moss, 1994). *lin-28* and *lin-29* affect only a subset of the developmental events controlled by *lin-4* and *lin-14*. Consistent with the phenotype described above, molecular analysis has shown that *lin-14* is highly active in the L1 stage and regulates a L1-specific program (Ruvkun and Giusto, 1989). *lin-4* encodes a small RNA that post-transcriptionally regulates *lin-14* by an antisense mechanism (Lee et al., 1993; Wightman et al., 1993). Thus molecular analysis of heterochronic genes in *Caenorhabditis* gives plausible mechanisms for how temporal aspects of developmental events are controlled and how they could be altered during evolution.

SPECIES COLLECTION AND THE COMPARATIVE APPROACH

One approach for the investigation of the evolution of development is comparison between species. Ideally, this approach requires a species collection that spans several different level of taxa. The nematode phylum fulfills this requirement. Together with insects, nematodes show the highest level of radiation in the animal kingdom. Average estimates calculate one million nematode species, many of which are free-living. We have collected nematodes species from soil samples, by placing soil samples on standard *C. elegans* Petri plates and picking the nematodes that crawl out. This approach allows for easy laboratory culture, but biases the worms we extract. So far, we have collected 33 new isolates of Rhabditidae, defining 18 species. Thirteen of these species are new (L. Carta, K. Thomas and P.W.S., unpublished data; L. Carta and P.W.S., unpublished observations). Some nematodes can survive years in dry soil after collection (e.g., Aroian et al., 1993). Most of these nematodes are easily cultured, and moreover, most strains can be frozen at −70°C for long term storage (L. Carta, Y. Hajdu and P.W.S., unpublished observations; M. Edgley, D. Riddle, T. Stiernagle and R. Herman, personal communication). Most of these species belong to the family Rhabditidae, like *Caenorhabditis elegans*, but species of the families Panagrolaimidae and Neodiplogasteridae were also found (see Table 1 for classification of species described in this review). These three families belong to the Order *Rhabditida*, one of approximately 20 nematode orders. Within the Rhabditidae, the collected species span several distinct branchpoints, according to the phylogeny of Sudhaus (1976).

The development of these free-living species can be analyzed at the level of individual cells using techniques standard to *Caenorhabditis* (cell lineage, cell ablation, genetic analysis, and potentially transgenic technology), because most nematodes have a similar bauplan.

POSTEMBRYONIC CELL LINEAGE COMPARISON

Comparison of the cell lineages of two related but morphologically distinct species reveals ways in which cell lineages can change. The complete postembryonic lineage of the free-living nematode *Panagrellus redivivus* of the familiy Panagrolaimidae was compared to *Caenorhabditis elegans* of the family Rhabditidae (Sternberg and Horvitz, 1981, 1982). The changes in the cell lineage between these two species define five classes of modification (Fig. 1). Each of these classes could involve either changes in cell-cell interactions or intrinsic programming.

(1) Switches in the fate of a cell to a fate associated with another cell (Fig. 1 part 1)

One example of a fate switch is in theV5.ppp lineage: in *C. elegans* males the cell V5.ppp generates a sensory ray as well as seam and hyp7 epidermal cells; in *P. redivivus* males the V5.ppp cell generates only seam and hyp7 epidermal cells. A striking example of a fate switch is that of the gonadal cell

Table 1. A very simplified classification of some genera of the phylum Nematoda, class Secernentea

Classification	Vulva position* (%)	Gonad type†	AC-dependence‡
Order Rhabditida			
Rhabditidae			
Mesorhabditis	80	M	–
Cruznema	80	M	+
Teratorhabditis	95	M	–
Pelodera	50	D	+
Caenorhabditis	50	D	+
Oscheius	50	D	+
Panagrolaimidae			
Panagrellus	60	M	+
Order Ascarida			
Ascaris§	35	D	?

*Position of vulva from anterior end (0%) to rectum (100%).
†M, monodelphic; D, didelphic.
‡+, dependent on anchor cell signal; –, independent of anchor cell signal.
§According to Schmidt et al. (1985).

Z4.pp, that becomes a distal tip cell (DTC) in *Caenorhabditis* hermaphrodites, but undergoes programmed cell death in *Panagrellus* females. This cell death would in principle be sufficient to cause the anatomical transformation of a two-armed gonad into a one-armed gonad (see below; Sternberg and Horvitz, 1981); ablation of the posterior distal tip cell in *Caenorhabditis* causes an essentially one-armed gonad to form (Kimble and White, 1981). There is a surprisingly wide range of interspecific differences in lineages in which programmed cell death occur (Sulston and Horvitz, 1977; Sulston et al., 1983; Sternberg and Horvitz, 1981, 1982; R. J. S. and P. W. S., in preparation). Raff's hypothesis for cell death as a default (Raff, 1992) provides an explanation for these promiscuous changes in programmed cell death: it is in principle relatively easy to program cell death merely by blocking response to a survival factor. Ellis and Horvitz (1991) have identified single gene mutations (defining the genes *ces-1* and *ces-2*) that prevent the programmed death of specific cells, as opposed to all programmed cell death; changes in such genes might underlie behavioral and morphological evolution.

(2) Reversal in the polarity of a sublineage (Fig. 1 part 2)

The fates of two sisters are interchanged; the consequence is a change in the spatial distribution of cells without change in cell number or type. Lineage reversals have been described in the male tail lineages between *Caenorhabditis* and *Panagrellus*, but also exist between the sexes of *Panagrellus* in parts of their gonadal lineage (Sternberg and Horvitz, 1981, 1982), and the early embryonic founder lineage, in the division of P2 between *Caenorhabditis* and *Cephalobus* (Skiba and Schierenberg, 1992). Genes have been identified that alter polarity of a lineage, for example, *lin-18* and *lin-44* (Ferguson et al., 1987; Herman and Horvitz, 1994; W. Katz and P. W. S., unpublished data).

(3) Alterations in the number of rounds of cell divisions (Fig. 1 part 3)

Addition (Fig. 1-3A) or suppression (Fig. 1-3B) of a cell division is the most common type of cell lineage alteration seen between *Caenorhabditis* and *Panagrellus*. Additional cell divisions can either be symmetric or asymmetric. Symmetric additional divisions form a duplication of sublineages (e.g. Z1.ppp lineage in *Panagrellus* female), whereas asymmetric additional divisions can produce a pattern reiteration (Fig. 1-3C; e.g., AR.pp gonadal lineage in *Panagrellus*). *unc-86* mutations lead to reiterations in particular lineages (Chalfie et al., 1981).

The generation of additional cells is likely to be of evolutionary importance. After duplication, the additional cell will be functionally equivalent to its sister. But as with the duplication of genes or gene-clusters, this cell might be able to adopt novel characters and functions because of different extrinsic (different neighbors) or different intrinsic (different gene expression) properties. Thus, cell duplication might create cell diversity over evolutionary time scales, as proposed by Goodman (1977) and Chalfie et al. (1981).

(4) Changes in the relative timing of cell divisions (Fig. 1 part 4)

Interesting examples include the early embryonic founder lineage, in which there is no known consequence (Skiba and Schierenberg, 1992) and Z1 in *P. redivivus* females, in which the delayed division is the first difference in the Z1 and Z4 lineages, ultimately involving the death of Z4.pp but not the homologous Z1.aa (Sternberg and Horvitz, 1981). This is a subtle example of heterochrony.

(5) Altered segregation of lineage potential (Fig. 1 part 5)

The developmental potential to generate particular cell types might be transferred from one cell to its sister. One examples is in the male gonadal lineage between *Caenorhabditis* and *Panagrellus* (Sternberg and Horvitz, 1981). Since fates might be specified by cell-cell interactions between sisters and cousins (e.g., Posakony, 1994), this type of change might involve a reversal in the polarity of division potential, with the same cell interactions occurring, such that the posterior daughter rather than the anterior daughter divides, but the middle cell is nonetheless of type B. At a molecular level, *mex-1* mutants are defective in localization of the SKN-1 determinant (Bowerman et al., 1993).

The finding that similar types of lineage transformations have also been observed in *Caenorhabditis* after cell ablation and in mutant animals suggests that relatively minor genetic changes might cause the observed changes in species characters. Thus, alterations at the level of cells or genes can cause the evolution of cell lineages.

COMPARISON OF VULVA DEVELOPMENT

The induction of the hermaphrodite vulva is one of the best

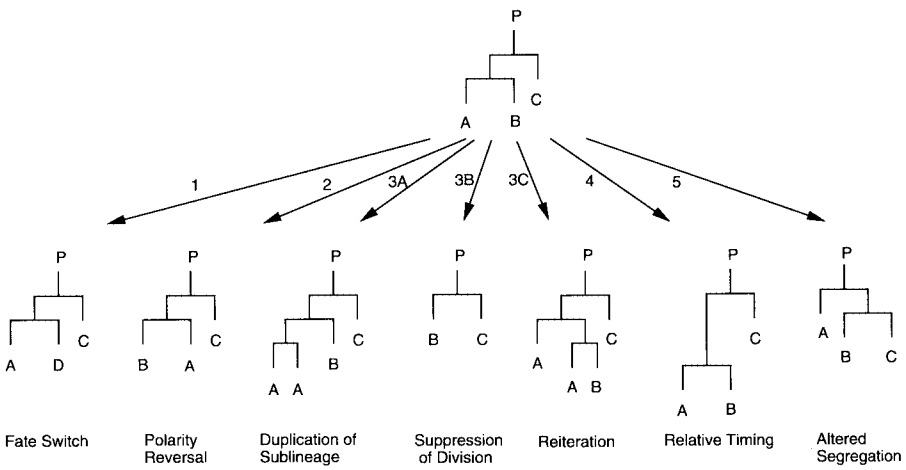

Fig. 1. Different types of cell lineage transformations observed between nematode species. P, a precursor cell. A, B and C, different cell types. 1. Fate Switch, cell with fate B instead has fate D. 2. Polarity Reversal, A and B are produced in different positions. 3. Changes in number of cell divisions: 3A. Duplication of A cell. 3B. Suppression of division; might change the fate of parent of A and B to A, B (as shown) or other fate. 3C. Reiteration, B has fate of its parent, i. e., and extra asymmetric cell division. 4. Change in the relative timing of divisions; such a change can affect subsequenct cell interactions. 5. Altered segregation (see text).

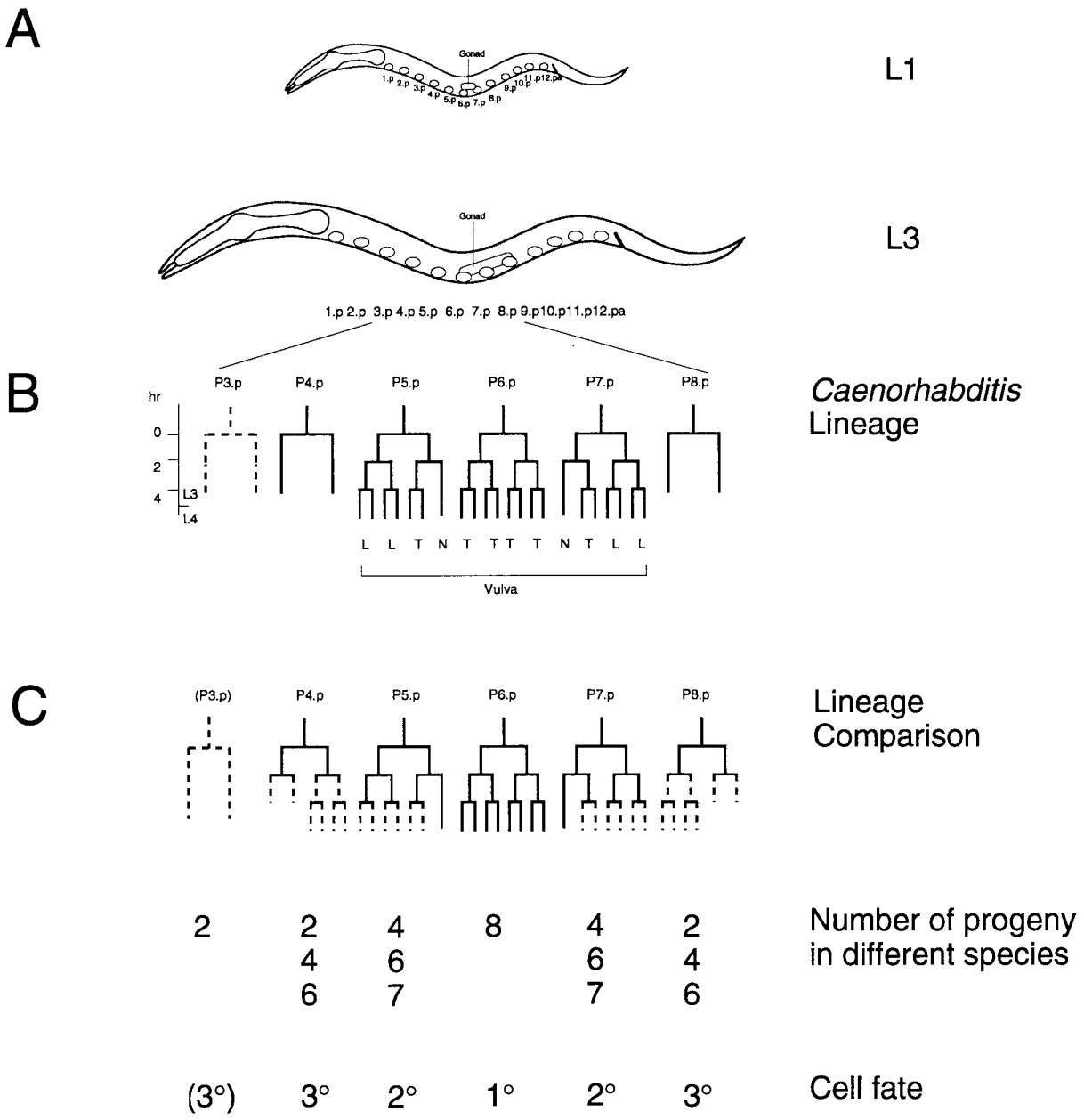

Fig. 2. Schematic summary of vulva development in *Caenorhabditis elegans* and nematodes with vulva development in the central body region. (A) Position of the Pn.p ectoblasts in the L1 stage and the early L3 stage. Cells are homogenously distributed between pharynx and anus. (B) Vulva cell lineage in *Caenorhabditis elegans*. L, longitudinal division; T, transverse division; N, non-dividing cell; according to Sternberg and Horvitz (1986). (C) Schematic comparison of the vulva cell lineage in other species. The plain lines refer to cell divisions occurring in all species. Dashed lines represent the cell divisions that occur only in one or some species. P6.p generates 8 progeny in all analyzed species, whereas P(5,7).p generate 4, 6 or 7 progeny and P(4,8).p generate 2, 4 or 6 progeny. Examples of the different number of progeny are: 2°, 4 in *Oscheius*, 6 in *Pelodera*, 7 in *Caenorhabditis*; 3°, 2 in *Caenorhabditis*; 4 in *Oscheius* and 6 in *Pelodera*. P3.p is 3° in 50% of *Caenorhabditis* hermaphrodites, but is not a VPC in the other species.

understood examples of postembryonic development in *Caenorhabditis* (reviewed by Hill and Sternberg, 1993). Twelve Pn ectoblast cells migrate into the ventral cord and divide to produce posterior daughters (Pn.p cells) whose fate varies in a position-specific manner (Sulston and Horvitz, 1977; Fig. 2A). Some Pn.p cells are competent to generate vulval cells; others are non-specialized epidermal cells. In *Caenorhabditis*, the Pn.p cells located in the central body region (P3.p-P8.p) are tripotent vulva precursor cells (VPCs) and form an equivalence group (Fig.2B). In intact animals, P(5-7).p respond to a signal from the gonadal anchor cell (AC) (Kimble, 1981). P6.p has the 1° cell fate generating 8 progeny; P(5,7).p have the 2° cell fate and generate 7 progeny; together these progeny form the vulva. The remaining cells, P(3,4,8).p have a non-vulval fate, called 3°, resulting in a 3°-3°-2°-1°-2°-3° pattern of cell fates. After ablation of individual Pn.p cells,

more AC-proximal cells, within the equivalence group have the potential to replace more AC-distal cells and thereby regenerate the vulval pattern (Sulston and Horvitz, 1977; Sternberg and Horvitz, 1986).

Over the last few years molecular analysis has shown that one of the *Hom-C* genes, *lin-39*, contributes positional information to establish the vulva equivalence group within the linear array of the 12 Pn ectoblast cells (Wang et al., 1993; Clark et al., 1993) (Fig. 3A). Further patterning within the equivalence group is initiated by an EGF-like growth factor inductive signal (LIN-3) that stimulates a receptor tyrosine-kinase mediated signal transduction pathway (Hill and Sternberg, 1992, 1993; R. Hill, W. Katz, T. Clandinin and P. Sternberg, unpublished observations; Fig. 3B). Additional lateral signaling between the VPCs acts via a distinct receptor and signal transduction pathway (Greenwald et al., 1983; Sternberg, 1988; Yochem et al., 1988; Sternberg and Horvitz, 1989). A negative signal, presumably from the hyp7 epidermis, and requiring the action of *lin-15* as well as other genes, may block basal activity of the LET-23 receptor (Herman and Hedgecock, 1990; Huang et al., 1994). While *lin-15*-controlled signaling appears to convey no patterning information in *Caenorhabditis*, it is conceivable that it might do so in another taxa.

Caenorhabditis and many other species form the vulva in the central body region. Analysis of vulva development in the species *Oscheius sp.* PS1131, *Oscheius tipulae*, *Rhabditella axei*, *Rhabditoides regina*, *Pelodera strongyloides* and *Protorhabditis* sp. PS1010 by cell lineage observation and cell ablation experiments revealed that in all these species the vulva is formed by the progeny of P(5-7).p as in *Caenorhabditis* (R.J.S. and P.W.S., unpublished data). Nonetheless, evolutionary alterations are present at several levels.

(1) Number of VPCs constituting the equivalence group (Fig. 2C)

So far, only in *Protorhabditis* does P(3-8).p form the equivalence group as in *Caenorhabditis*. In all other analyzed species P(4-8).p are VPCs; the final pattern within the equivalence group is 3°-2°-1°-2°-3°. This is an example of a fate change with respect to P3.p (Fig. 1-1).

(2) The cell lineage generated by the 1°, 2° and 3° VPCs (Fig. 2C)

P6.p, which has the 1° cell fate, generates eight progeny in all analyzed Rhabditidae species with a central vulva. The cells with the 2° cell fate, P(5,7).p, generate between four and seven progeny in a species-specific manner. The 3° cell lineage of P(4,8).p varies between two and six progeny in different species. In the *Rhabditoides* and *Pelodera* 3° lineage the AC-proximal two cells of the four-cell stage undergo a third round of cell division and produce an asymmetric lineage. These are examples of changes in the number of rounds of cell division (Fig. 1, part 3).

How is this mirror image difference between P4.p and P8.p regulated? After ablation of the gonad, all VPCs express a 3° lineage with the asymmetry normally found in P4.p, suggesting this is the ground state. Thus, vulva induction by the gonad is directly, or indirectly involved in reversing the polarity of the asymmetric lineage of P8.p. A similar polarity reversal occurs in the 2° cell lineage in *Caenorhabditis*; genetic and cell ablation experiments indicate that a signal from the gonad reverses the polarity of the P7.p lineage (W. Katz and P.W.S., unpublished data).

(3) Variability of cell lineages

Pelodera strongyloides displays striking variability in its 3° vulva lineages. P3.p and P9.p divide in nearly 50% of the females, expressing a partial or complete 3° lineage, generating two, four or six progeny. Increasing the temperature changes the frequency of this variability, a phenomenon also known to occur in, for example, the occasional division of P5.ppp and P7.paa in the *Caenorhabditis* hermaphrodite at 25°C (Sternberg and Horvitz, 1986).

An important question is how invariant cell lineages change during evolution into other cell lineages. Variability might play a role in the evolutionary transition process from one invariant cell lineage to another. More detailed comparative studies based on a solid phylogeny might help test this hypothesis. A taxon that includes members with two distinct invariant cell lineages, and other members with variable lineages would support this hypothesis and provide the experimental material to analyze the genetic and molecular basis for the transition. Another hypothesis is that variability results from a failure to select precision in the underlying genetic program, for example in a lineage undergoing extensive proliferation (e.g., Sternberg and Horvitz, 1981, 1982).

FORMATION OF A POSTERIOR VULVA

One major difference between species exists in the body position of the developing vulva (central vs. posterior vulva formation) (Figs 4, 5). This difference coincides with different gonad morphology. Central vulva species form two ovaries symmetrically about the vulva (didelphic); posterior vulva

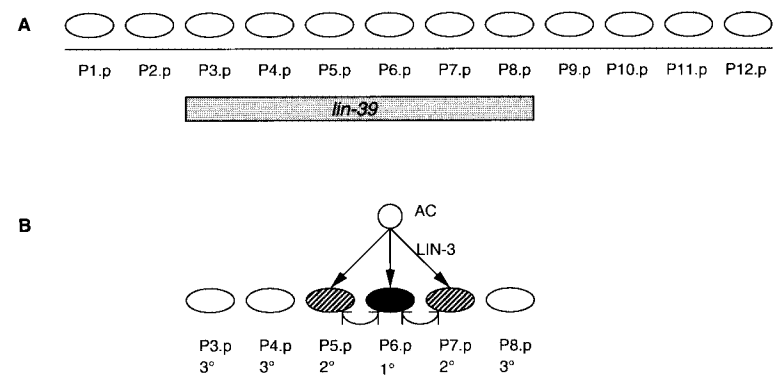

Fig. 3. Schematic summary of the early steps in vulva pattern formation in *Caenorhabditis elegans*. (A) The twelve Pn.p cells have different developmental potentials because of positional information generated by the Hom-C genes. Only the two Hom-C genes influencing the central body region are shown. *lin-39* is involved in the establishment of the vulva equivalence group. (B) After P(3-8).p have been specified as the vulval equivalence group, further pattern formation is initiated by an inductive signal from the gonadal anchor cell (AC) (indicated by bold arrows). Lateral signaling is one of the additional interactions necessary for proper vulva development (curved arrows).

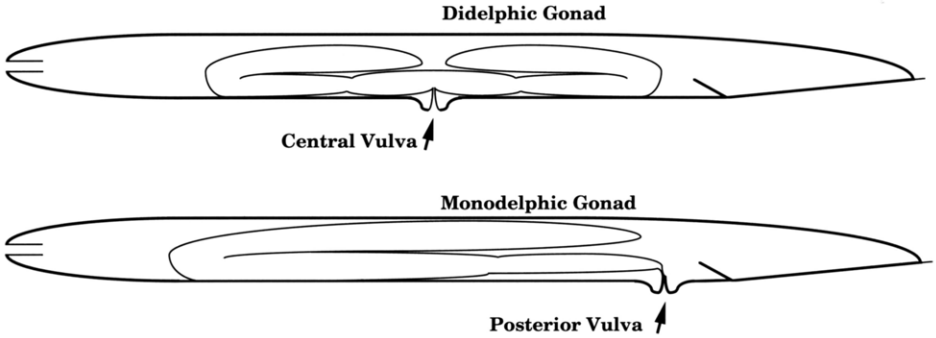

Fig. 4. Correlation of gonad type and vulval position. Central vulva species have two-armed (didelphic) gonads with two ovaries. Posterior vulva species have one-armed (mondelphic) gonads with a single ovary directd anterior from the vulva.

species form a single ovary, directed anterior from the vulva (monodelphic). A didelphic gonad with a vulva in the central body region is considered to be the ancestral character in the taxa we have considered so far (Chitwood and Chitwood, 1950).

Which set of precursor cells make the vulva in species with posterior vulva formation? In *Panagrellus redivivus*, which has a vulva at 60% body length (head = 0%; tail = 100%), the equivalence group and the gonad primordium are shifted posteriorly. In principle, a more extreme shift in the equivalence group, for example to P9.p-P11.p, and gonad primordium would allow posterior vulva formation. In three genera, *Mesorhabditis, Teratorhabditis* and *Cruznema*, cell lineage analysis revealed that the central P-ectoblasts still form the vulva equivalence group (Sommer and Sternberg, 1994). In *Cruznema tripartitum*, P(3-8).p, and in *Mesorhabditis* sp. PS 1179 and *Teratorhabditis palmarum* P(4-8).p migrate posteriorly during the L2 stage. As in *Caenorhabditis*, P(5-7).p form the vulva in intact animals of these species. An example of P-cell location before and after migration and of the vulva cell lineage is shown for *Mesorhabditis* (Fig. 6).

Cruznema

In *Cruznema,* induction of the vulva by the AC occurs after the gonad grows posteriorly to the position of the VPCs (Sommer and Sternberg, 1994). In the intact animal the AC forms a specific contact with P6.p, the cell adopting the 1° cell fate. After ablation of P(5,6).p, their neighbors P4.p and P7.p can assume the 1° cell fate. In these experiments, the AC stops migration in the region of P4.p or P7.p respectively, indicating that cell-cell interactions occur between the AC and the VPCs prior or simultaneously to induction. In central vulva species, the AC is born close to P6.p and thus it is more difficult to assess the role of such interactions. However, in *Caenorhabditis*, the anchor cell will extend a process towards the cluster of cells generated by a misplaced 1° VPC, presumably in response to a signal from the VPC grandprogeny (K. Tietze and P. S., unpublished data). Posterior vulva formation in *Cruznema* is thus gonad-dependent and occurs within a set of VPCs that seems to form an equivalence group as in *Caenorhabditis*.

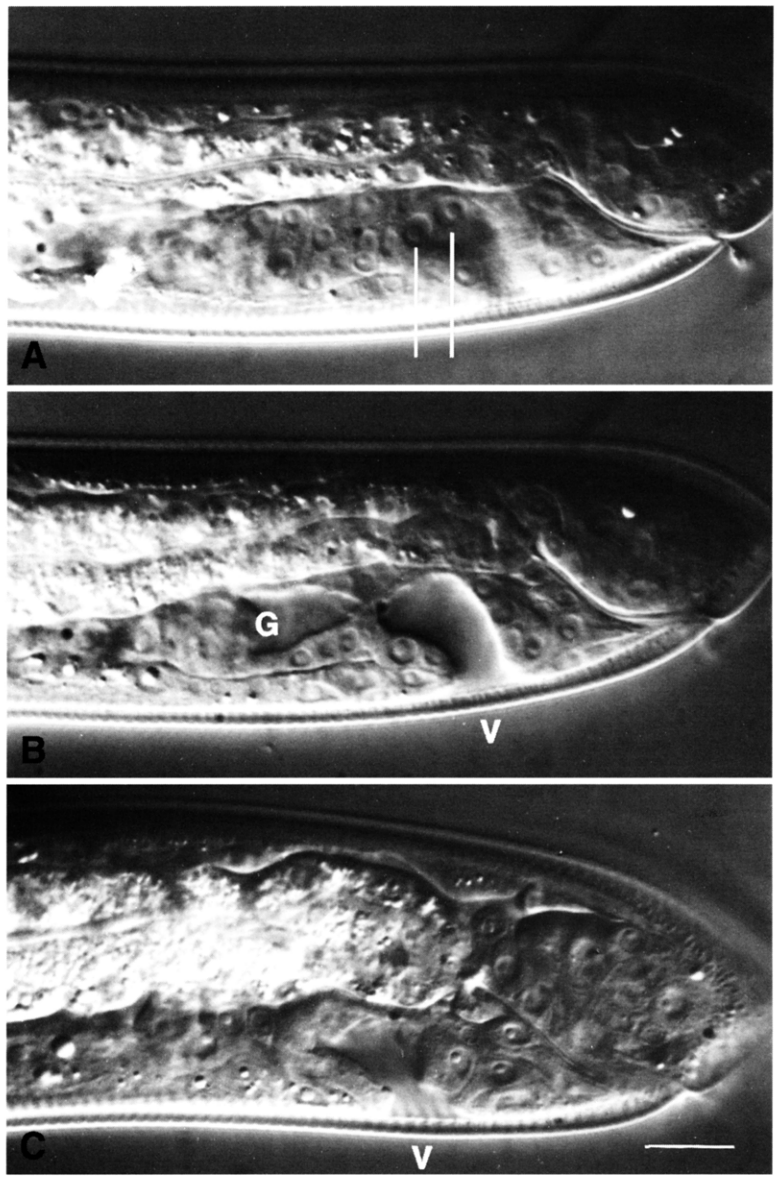

Fig. 5. Nomarski photomicrographs of lateral views of *Teratorhabditis* females at different stages of vulva development in intact (A,B) and cell-ablated (C) animals. (A) Late L3 stage of vulva development in an intact animal with two progeny of P6.p visible in this plane of focus. (B) Mid L4 stage showing a medial focal plane with the gonad (G), vulva (V) and the rectum. (C) Mid L4 stage of a gonad-ablated worm. No uterus is formed, the vulva is still present. Scale bar, 20 μm.

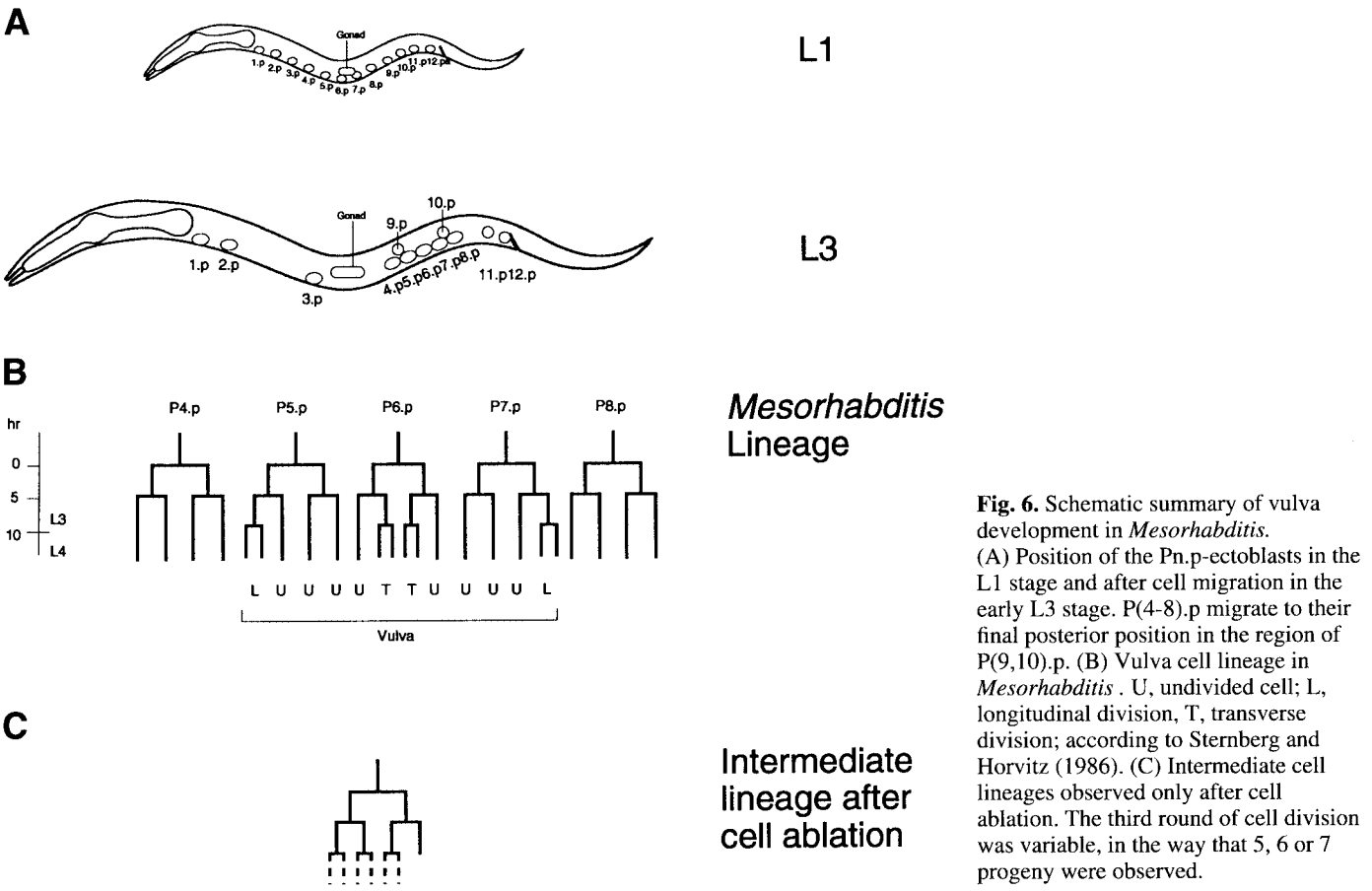

Fig. 6. Schematic summary of vulva development in *Mesorhabditis*. (A) Position of the Pn.p-ectoblasts in the L1 stage and after cell migration in the early L3 stage. P(4-8).p migrate to their final posterior position in the region of P(9,10).p. (B) Vulva cell lineage in *Mesorhabditis* . U, undivided cell; L, longitudinal division, T, transverse division; according to Sternberg and Horvitz (1986). (C) Intermediate cell lineages observed only after cell ablation. The third round of cell division was variable, in the way that 5, 6 or 7 progeny were observed.

Mesorhabditis/Teratorhabditis

In *Mesorhabditis* and *Teratorhabditis* vulva development is not induced by the AC (Sommer and Sternberg, 1994). The VPCs undergo two rounds of cell division before the AC contacts the forming vulva and ablation of the AC precursor does not affect vulva formation. Is vulva development still induced by other cells or do the VPCs self organize (Fig. 7)? We were unable to find other cells that induce the vulva (Sommer and Sternberg, 1994). Therefore, a simple hypothesis is that the VPCs form a correct pattern solely through interactions among themselves. The VPCs might secrete a diffusible inductive signal. The concentration of signal could be highest in the center of the 5-cell region, allowing P6.p to assume the 1° cell fate and promoting P5.p and P7.p towards a 2° cell fate. This hypothesis is testable by ablating all but two VPCs (Fig. 7). According to the model, both remaining cells should secrete and receive equal amounts of signal, resulting in random patterning (1°-2° or 2°-1°). However, ablation experiments revealed that only one specific cell in a pair adopts

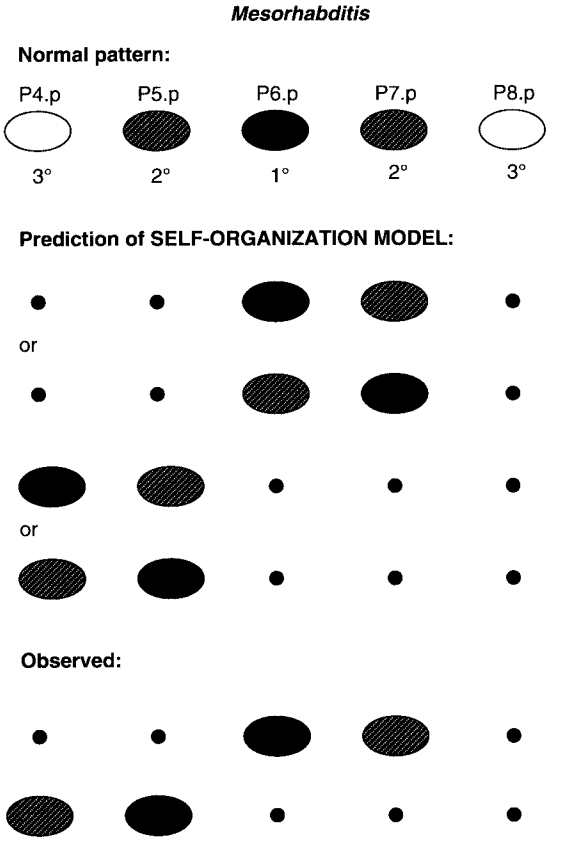

Fig. 7. Self organization of the VPCs: model, prediction and results. (See text for further details). If self organization is responsible for pattern formation within this group of cells, one would expect random specification after ablation of three of the five VPCs. The observed pattern after corresponding ablation experiments in *Mesorhabditis* show that only one cell, P6.p of P(6,7).p, and P5.p of P(4,5).p have the 1° cell fate, arguing against a simple self organization.

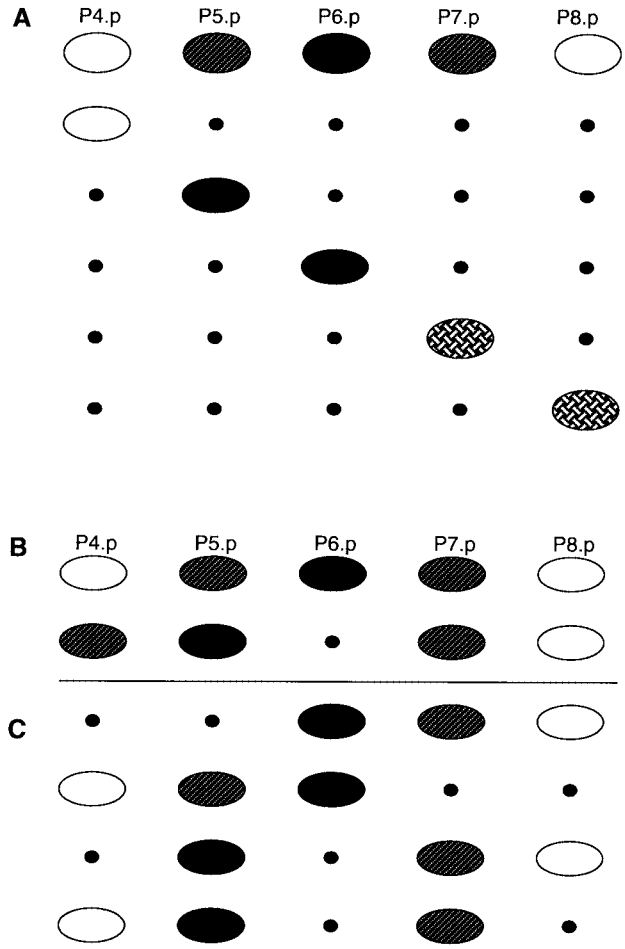

Fig. 8. Schematic summary of cell ablation experiments in *Mesorhabditis*. (A) After ablation of four of the five cells, the remaining cell has a different developmental potential depending on which cell is remaining. P4.p has only the 3° cell fate as an isolated cell. P(5,6).p can adopt the 1° cell fate as an isolated cell, whereas P(7,8).p have a fate, intermediate between that of a 1° and a 2° cell fate. (B) After ablation of P6.p, always P5.p has the 1° cell fate. In the corresponding experiment in *Caenorhabditis* P5.p or P7.p have the 1° cell fate. (C) Additional experiments where two or three VPCs were ablated. Only P(5,6).p can adopt the 1° cell fate. 1°, black oval; 2°, hatched oval; 3°, white oval; intermediate lineage, cross-hatched oval.

the 1° cell fate (Fig. 7). Therefore it is unlikely that vulva formation is due to simple self organization of equivalent VPCs.

Further analysis of *Mesorhabditis* revealed that the VPCs are not equivalent (Fig. 8). After ablation of VPCs only P5.p or P6.p can assume a 1° cell fate . In contrast, P7.p and P8.p assume 2°, 3° or an intermediate cell fate with characteristics of both 1° and 2° lineages. P4.p has the 3° cell fate as an isolated VPC. Thus, changes in induction and competence occur during the evolution of posterior vulva development in *Mesorhabditis* and *Teratorhabditis*. Vulval pattern formation does not require an inductive signal from the gonad, and the VPCs are not equivalent in their competence to form vulval tissue.

Two models can explain how the pattern of VPC fates is established in *Mesorhabditis*. After an initial specification process making some VPCs distinct from one another, final vulva patterning might result from either an inductive signal (most likely from the posterior body region) or solely by lateral interactions among the already different VPCs (Fig. 9). For example, P6.p, once specified, could prevent P5.p from becoming 1° (lateral inhibition) and signal P7.p to be 2° (induction).

A regulatory constraint

All the three posterior vulva genera, *Cruznema, Mesorhabditis* and *Teratorhabditis*, form the vulva with the progeny of P(5-7).p rather than the more appropriately positioned posterior Pn.p cells (Fig. 5). Early morphogenetic processes that regulate anterior-posterior pattern formation by the Hom-C genes (Wang et al., 1993; Clark et al., 1993) might create a regulatory constraint at the cellular level in the Rhabditidae that forces the same set of precursors to generate the vulva. In other families, such as the Panagrolaimidae, there is posterior shifting of the set of precursors, as discussed above.

The newly acquired character of the VPCs is the migration to a more posterior position. While it is unknown how this migration is regulated, in *Caenorhabditis*, there are several examples of genetic control over specific cell migrations. The migratory behaviour of the neuroblast QL is controlled by the *Antennapedia*-like gene *mab-5* acting in the neuroblasts (Salser and Kenyon, 1992). The circumferential migration of a variety of cell types is controlled by the UNC-6 system, with expression of the UNC-5 integrin determining dorsalward response to a global guidance cue (Hamelin et al., 1993).

The difference in induction and competence between *Cruznema* and *Mesorhabditis/Teratorhabditis* might give important evolutionary cues to their phylogenetic relationship. Central vulva development is considered ancestral in this family (Chitwood and Chitwood, 1950), but it is not known if posterior vulva development within the Rhabditidae evolved once or several times independently. This question is of evolutionary importance because it might imply different relationships and origins of the derived characters "posterior vulva" and "gonad independent vulva development." According to the phylogram in Fig. 10B (based on Sudhaus, 1976), posterior vulva development in *Cruznema* and *Mesorhabditis* evolved independently. If so, we cannot determine whether posterior vulva formation and gonad independence evolved simultaneously or subsequently in *Mesorhabditis*.

Based on our results, the most parsimonious phylogram is as depicted in Fig. 10A, in which posterior vulva development evolved just once within the *Rhabditidae*, generating a *Cruznema*-like intermediate ancestor. This phylogram implies subsequent acquisition of gonad-independent vulva development. If this is true, the evolution of a posterior vulva in a *Cruznema*-like intermediate ancestor might have helped reset developmental conditions. In this context one can consider posterior vulva development as a heterotopic change that allows further changes of the developmental process. Changing boundary conditions by destroying the proximity of VPCs and AC, might thus be an important requirement for the acquistion of subsequent evolutionary novelty. Similar theoretical suggestions have been made concerning the importance of heterochronic changes during development (Buss, 1987).

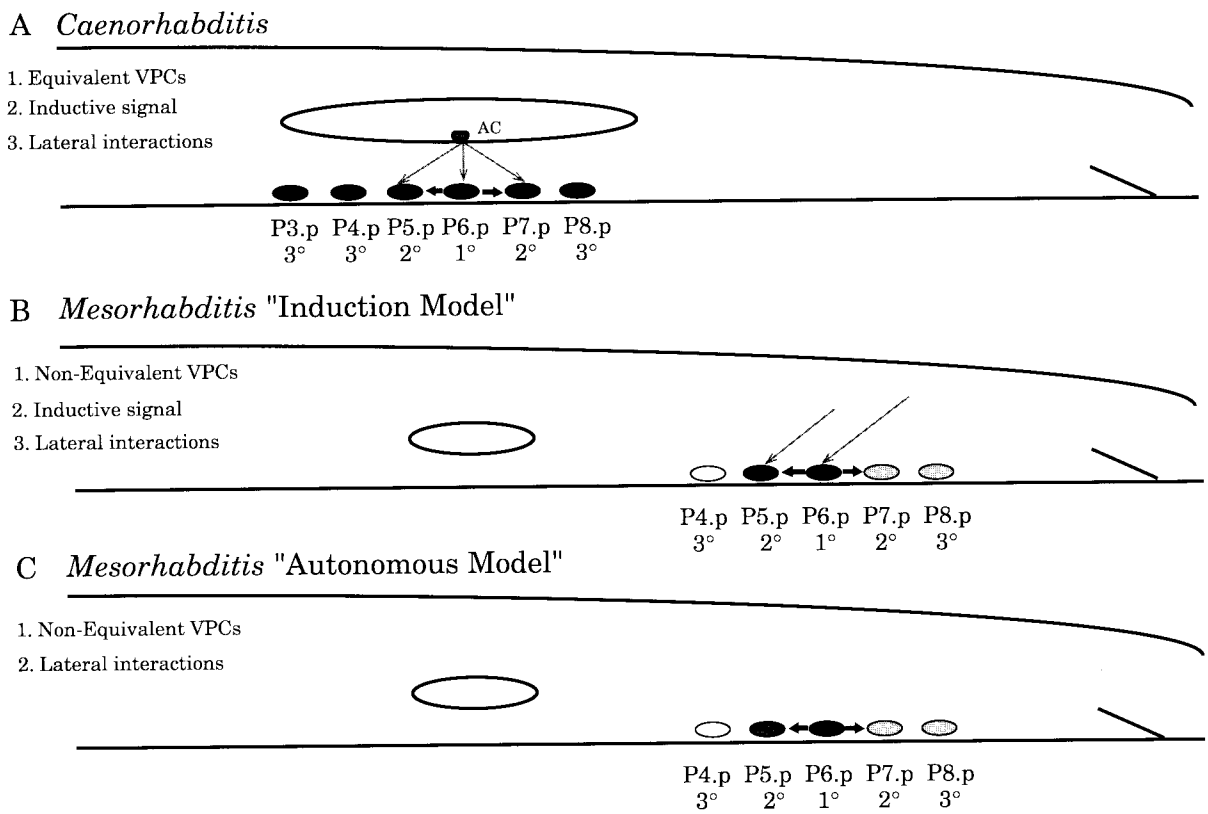

Fig. 9. Two models for the initiation of vulva pattern formation in *Mesorhabditis*. Two alternative models for the early steps of vulva pattern formation in *Mesorhabditis* (B, C) compared to the existing model in *Caenorhabditis* (A). According to the "Induction Model" a pre-bias between P4.p-P(5,6).p-P(7,8).p exists. An inductive signal, perhaps from the posterior body region initiates further cell specification. Lateral signaling ensures final cell fate of the VPCs. According to the "Autonomous Model" a pre-bias establishes four different types of VPCs; P4.p-P5.p-P6.p-P(7,8).p. P6.p is proposed to be intrinsically different from P5.p allowing it to adopt the 1° cell fate. After ablation of P6.p only P5.p has the ability to replace P6.p. Lateral signaling is involved in the determination of the final cell fates (plain arrows). Positional information is indicated by the different shading of the VPCs. The inductive signal is indicated by the grey arrows. The arrows between the VPCs (black) refer to the lateral inhibitory signal which exists in *Caenorhabditis*, and which we suggest exists in *Mesorhabditis*.

EQUIVALENCE GROUPS AND INVARIANT CELL LINEAGES

Cell fate specification in nematodes, as in most animals, proceeds by progressive restrictions in potential. Equivalence groups represent a transition state, and might be an intermediate between specification of cell type using oligopotent cells and using cells whose fates are tightly constrained by lineage. From comparative studies, there are two examples in which loss of multipotentiality of cells within an equivalence group appears to be a derived character: AC specification in *Panagrellus* and VPC fate specification in *Mesorhabditis*.

The anchor cell versus ventral uterine precursor equivalence group in *Caenorhabditis* hermaphrodites arises from the generation of two cells (Z1.ppp and Z4.aaa) with the same bipotentiality by two homologous lineages. The difference between the cells results solely from interactions among them via a lateral signaling mechanism (Kimble and Hirsh, 1979; Seydoux and Greenwald, 1989). In *Panagrellus* females, only the posterior homolog, Z4.aaa becomes the anchor cell; Z1.ppp is a ventral uterine precursor (Sternberg and Horvitz, 1981). Since *Panagrellus* monodelphic gonad development is likely an evolutionarily derived character, it is probable that the fixed lineage in *Panagrellus* evolved from an equivalence group. By contrast, the analogous equivalence group in the male, the linker cell/vas deferens precursor cell group, exists in both species (Kimble and Hirsh, 1979; Sternberg and Horvitz, 1981), further supporting the derived character of apparently autonomous anchor cell specification.

Vulva development in *Caenorhabditis*, *Panagrellus* and other nematode species starts with a set of equipotent precursors, the vulval equivalence group. Although the cell fate and cell lineage of each individual precursor is invariant in intact animals, these cells have the potential to replace each other after cell ablation. By contrast, in *Mesorhabditis*, vulval precursor cells are not equivalent. It might be possible during the course of evolution to transform initially equivalent blast cells into non-equivalent blast cells. The possibility that a *Cruznema*-like species was indeed the ancestor to *Mesorhabditis* would support this view. One could also imagine that in other derived evolutionary lines, all vulval precursors might already be committed, creating an autonomous mode of specification as is thought to exist in many cell lineages in the animal kingdom (see Davidson, 1991).

PROSPECTS

Many different developmental mechanisms are used to

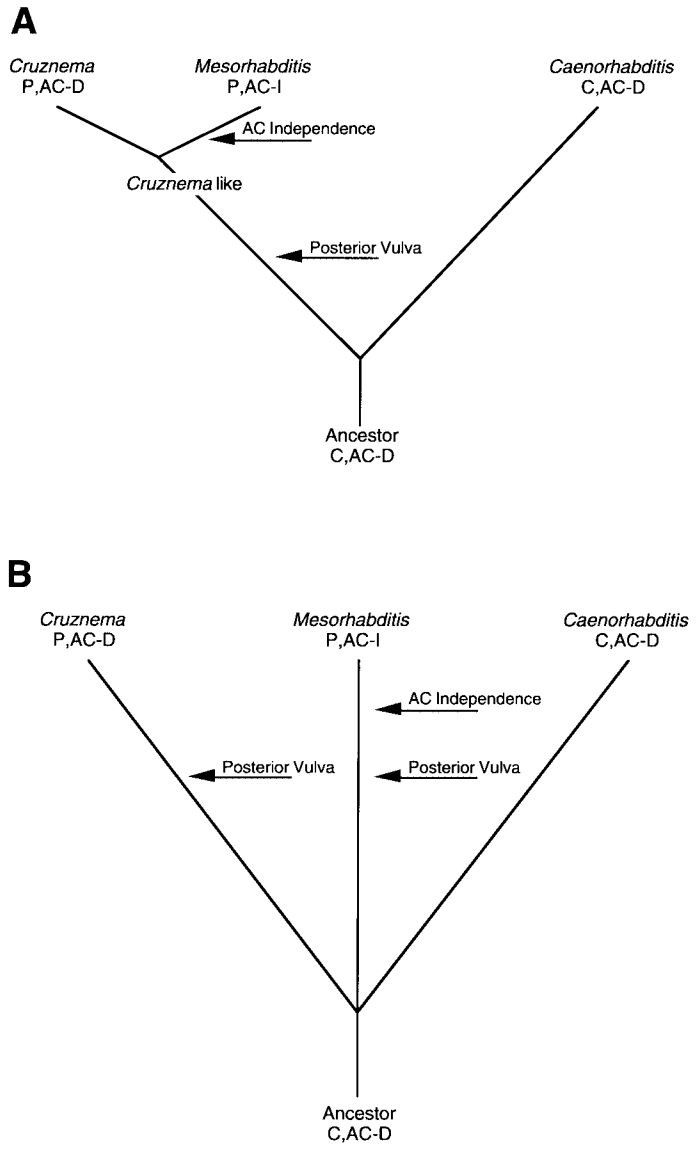

Fig. 10. Two phylogenetic trees indicating the different possible relationships between the posterior vulva species. (A) Posterior vulva development evolved just once, generating a *Cruznema*-like ancestor. AC-independence evolved later in the sublineage guiding to *Mesorhabditis*-like forms. (B) Posterior vulva development evolved several times independently in different lineage. According to this tree *Cruznema* and *Mesorhabditis* are no closer relatives to each other. Developmental evolutionary implications are discussed in the text.

generate cell identity during nematode development. Autonomous cell specification, conditional cell specification by induction and lateral signaling as well as the generation of positional information by Hom-C genes are present in *Caenorhabditis*. The analysis of these mechanisms and of the genes involved is becoming increasingly more detailed. Comparative developmental studies involving cell lineage and cell ablation experiments, are beginning to reveal the types of changes in development that have occurred during evolution of nematodes. By comparing what is known of the genetic control of development in *Caenorhabditis* to the inferred changes in phylogeny, we can formulate hypotheses as to the mechanistic differences in the development of the various nematodes. These hypotheses will be testable by genetics, molecular biology and transgenic nematode technology. Genetic analysis of other free-living nematodes will define the types of cell lineage changes that can occur in species other than *Caenorhabditis*. Molecular cloning, examination of the expression and gene transfer experiments with homologous genes from related species can test specific hypotheses concerning genes that might have mutated to cause the observed changes in development.

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