

Cell Lineage

A D Chisholm

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doi: 10.1006/rwgn.2001.0172

Chisholm, A D

Department of Biology, University of California,
Santa Cruz, CA 95060, USA

The cell lineage of an organism is the pattern of cell divisions during its development. Cell lineages are described by following cell divisions in living individuals, or by marking cells and examining their progeny. Some organisms or precursor cells display invariant patterns of cell division, in which specification of cell fates is correlated with cell division patterns; in other organisms, lineage patterns are variable and not correlated with cell fates. Invariant cell lineages reflect both cell-autonomous mechanisms of fate determination and highly reproducible cell–cell interactions. Genetic analysis of cell lineages has focused on systems where cell lineage and cell fates are correlated, such as *Caenorhabditis elegans* or the nervous system of *Drosophila*. Mutations affecting cell lineages in these animals have been informative in understanding both the mechanisms of cell fate specification and the control of cell proliferation.

Overview of Biology of Cell Lineages

History of Cell Lineage Studies

Cell lineage studies began with Whitman's description of cleavage patterns in leech embryos in the 1870s, and continued with descriptions of lineages in many invertebrate animals, including nematodes, sea urchins, and ascidians. It was found that in some animal groups, such as nematodes and ascidians, the pattern of cell divisions was almost identical from individual to individual. Such 'invariant' cell lineages allowed the reconstruction of extensive lineage trees. In other animals, such as leeches and insects, stereotyped patterns of cell division ('sublineages') were seen in the progeny of particular precursor cells. Because of the correlation between cell lineage and cell fate in such invariant lineages, it was assumed that cell fates were determined by factors segregating within the dividing cells (termed 'determinate' cleavage). This mode of development was contrasted with the 'indeterminate' cleavages observed in other animals, in which cell lineages are variable and cell fates are determined by a cell's interaction with its environment. However, as discussed below, invariant cell lineages do not

necessarily mean that cell fates are determined by the cell lineage pattern (see Moody, 1999 for examples). Over time, the term 'cell lineage' has acquired multiple meanings (Slack, 1991; Price, 1993). Here, cell lineage is defined as the pattern of cell divisions in the development of an organism, whether invariant or not.

How Cell Lineages Are Followed

Direct observation

In the nineteenth century, lineages were followed either by direct observation, or by reconstruction from fixed specimens. Such studies required embryos that were small, transparent, and rapidly developing, but were necessarily limited to early embryogenesis where the cells were large and few in number. More extensive observations of cell lineages have been made possible by the development in the 1960s of Nomarski differential interference contrast microscopy, which allows the imaging of transparent specimens. The complete cell lineage of the nematode *C. elegans* was followed using Nomarski microscopy; cell lineages in the *Drosophila* central nervous system have also been described by direct observation. More recently, time lapse microscopy in multiple focal planes ('four-dimensional' microscopy) has allowed entire cell lineages of individual animals to be recorded digitally.

Clonal analysis

In large, opaque, or slowly developing embryos, direct observation of cell divisions is not feasible. To analyze cell lineages in such cases, it is necessary to mark individual cells by physical or genetic means, and later to identify their progeny by expression of the marker. Such techniques are known as clonal analysis, because the progeny of a single cell forms a clone. In many animals cells can be labeled by injection with a nondiffusing dye such as fluorescein-conjugated dextran. A problem with this technique in growing tissues is that the dye can become progressively diluted with each round of cell division. In vertebrates, cells can be marked by infection of an embryo with a replication-defective retrovirus that expresses a reporter gene such as β -galactosidase or green fluorescent protein (GFP). At low virus concentrations single cells can be infected and their progeny recognized by reporter gene expression; there is no dilution of the marker because each cell in the clone expresses the reporter gene. This technique has been used to analyze cell lineages in chick and mammalian neural development.

In *Drosophila*, individual cells can be marked genetically for clonal analysis by mitotic recombination (**Figure 1A**). This technique is based on the observation that X-irradiation of mitotically dividing

cells causes homologous chromatids to recombine. Thus, if a parent cell that is heterozygous for a mutation ($m/+$) undergoes recombination between the mutation and the centromere in the G_2 phase of the cell cycle, it will divide to produce one homozygous mutant cell (m/m) and one homozygous wild-type cell ($+/+$). Recessive mutations that cause cell-autonomous phenotypes will be expressed only in the clone of mutant cells derived from the m/m daughter, allowing this clone to be visualized. The size of the clone depends on the number of cell divisions between irradiation and the time of analysis. Inducible expression of recombinases such as the yeast FLP enzyme causes mitotic recombination between chromatids bearing the FLP recognition sequence (FRT sites), allowing clones to be made at specific times and in specific tissues. Clones of genetically marked cells can also be generated in plants by induced excision of a transposon from within a transgenic reporter gene.

Chimeric embryos are a different form of genetic mosaic and have also been useful in defining lineage relationships. Chimeras are embryos formed from cells of two different genotypes. Most chimeras involve multiple cells of each type and thus these approaches involve the analysis of multiple rather than single clones. Mammalian chimeras are made by combining blastomeres from two early embryos; if the cells are genetically or physically distinct their progeny can be identified later. Chimeras can be made between chick and quail embryos; the quail cells can be distinguished by nucleolar morphology, allowing lineage relationships to be traced. Interspecific chimeras have also been used to examine lineages in plant development.

Types of Cell Division Pattern

Cell division patterns are typically represented as a branching tree (**Figure 1B**). Three basic types of division can be distinguished (Stent, 1998). In a ‘proliferative’ cell division, a cell divides symmetrically to give rise to two daughters, each of which behaves like its parent (cell type A divides to give two cells of type A). The other two types of division are asymmetric, in that the fates of the daughter cells are different. In a ‘stem-cell’ division, the parent cell gives rise to one daughter that resembles the parent and one daughter of a different type (A divides to make A + B). Finally, in a ‘diversifying’ lineage the two daughters are different in fate from each other and from their parent (A divides to make B + C). Some bacteria, such as *Bacillus subtilis* and *Caulobacter crescentus*, and single-celled eukaryotes such as the budding yeast *Saccharomyces cerevisiae* develop by stem-cell-like cell divisions and provide models for understanding asymmetric cell division in multicellular animals. Because asymmetric

cell divisions give rise to daughters with different fates they are important in understanding how different cell types arise, and have been the focus of intense genetic analysis (see below; reviewed by Horvitz and Herskowitz, 1992; Jan and Jan, 1998).

Intrinsic and Extrinsic Mechanisms in Cell Fate Determination

In animals displaying invariant cell lineages, the ancestry, environment, and fate of a cell are correlated. It was often assumed that invariant cell lineages reflected intrinsic (cell-autonomous) mechanisms of cell fate determination (also known as the ‘mosaic’ mode of development), in which the fate of a cell is determined only by its inheritance of factors segregated in ancestral cell divisions. However, lineage invariance is not sufficient evidence for a lineage-intrinsic mechanism. It is important to note that in an invariant cell lineage both a cell’s *environment* and its *ancestry* are correlated with its fate. Thus, cell fates could be specified by reproducible cell–cell interactions rather than reproducible inheritance of intrinsic factors. To prove that fates are specified autonomously, experiments in which a cell is isolated or transplanted must be performed. Although nematodes and ascidians both display invariant lineages, modern experiments have shown that many aspects of development in these animals are not cell-autonomously programmed, but instead rely on invariant cell–cell interactions.

Genetics of Cell Lineage in Nematode *Caenorhabditis elegans*

Cell Lineage

Our understanding of cell lineages in *Caenorhabditis elegans* is uniquely privileged in that the complete cell lineage from zygote to adult has been determined (**Figure 2**), a heroic work of direct observation of living specimens (reviewed by Sulston, 1988). In conjunction with maps of cell nuclei, the cell lineage provides a complete fate map, and makes it possible to analyze the results of experimental manipulations and mutants with single-cell resolution.

The *C. elegans* zygote undergoes a series of asymmetric cell divisions to generate six blastomeres (AB, MS, E, C, D, and P₄), known as embryonic founder cells (**Figure 3A**). Each founder cell is distinctive in terms of its cell lineage pattern and the cell fates it generates. For example, the zygote divides asymmetrically to form a larger anterior daughter denoted the AB founder cell, which undergoes a set of initially symmetrical divisions to generate neurons, muscle cells, and some epidermal cells. Most cell proliferation occurs during the first half of embryogenesis; a small number of postembryonic blast cells divide in larval

development to generate neuronal and epidermal cells, the gonad, and sexually dimorphic structures. During the development of a *C. elegans* hermaphrodite 1090 somatic cells are generated, of which 131 undergo programmed cell death, to yield an adult containing 959 somatic cell nuclei (the number of cells is lower because some cells fuse to form multinucleate syncytia).

In *C. elegans* lineage studies each cell is given a unique name reflecting its lineage history. Certain key embryonic and postembryonic precursors are given arbitrary names (e.g., AB, Z1). Their progeny are named by adding letters denoting the axis of the cell division relative to the body axes (a/p for anterior/posterior, etc.). Thus, Z1.ppp is the posterior daughter of the posterior daughter of the posterior daughter of Z1.

The somatic cell lineage of *C. elegans* is largely invariant, with limited exceptions. Within some pairs of cells there is variation in terms of which member of the pair adopts one fate and which adopts the other fate. For example, two adjacent gonadal precursor cells, Z1.ppp and Z4.aaa, generate two cells known as an anchor cell (ac) and a ventral uterine precursor (VU) cell. In an individual animal, either Z1.ppp or Z4.aaa becomes an anchor cell, and the other cell becomes a VU cell. Since in normal development the Z1.ppp/Z4.aaa pair never generates two anchor cells or two VU cells, the two cells must communicate to ensure the normal pattern of fates. The Z1.ppp/Z4.aaa pair of cells is an example of an ‘equivalence group’: a group of cells equivalent in developmental potential. In the case of the Z1.ppp/Z4.aaa pair, the choice of fates appears to be entirely stochastic; in other equivalence groups, the choice of fates is biased.

The vast majority of cell divisions in *C. elegans* are asymmetric, in that the fates of the daughters are different. Most cell types (neurons, muscles, epidermis) are generated in patterns that, while not random, do not show simple lineage relationships. The germline and intestine are exceptional in that they develop as clones from the precursors E and P₄, respectively. Furthermore the germline develops in a proliferative lineage that is variable from animal to animal.

A striking feature of the lineage is that repeated ‘sublineages’ are evident, in which homologous precursors divide in identical ways to make homologous sets of cells. Such sublineages, in which cell fate and lineage correlate in multiple instances, suggest the existence of lineage-intrinsic mechanisms specifying fates. For example, along the length of the ventral side of the first stage larva are 12 postembryonic blast cells denoted P1 through P12 (these are different from the embryonic blast cells P₁–P₄). Each P cell divides to generate an anterior daughter with neuroblast fate and a posterior daughter with epidermal fate;

the anterior daughters all divide in similar patterns to generate five motor neuron types at identical positions in each lineage tree (**Figure 3B**). P cells in different body regions divide in the same basic pattern with slight modifications. Because isolation or transplantation of P cells is not technically feasible, it is not known to what extent cell fates are determined intrinsically within each sublineage.

An example of extrinsic control of cell fates was provided by Priess and Thomson, 1987. Normally the anterior and posterior daughters of AB have different fates. If the division axis of AB is reversed by micro-manipulation, such that the anterior daughter now lies posteriorly, the AB daughters display regulation and a normal embryo is formed. Thus, differences between AB daughters cannot result from cell-autonomous mechanisms but must involve interactions with each cell’s environment.

Isolation of Cell Lineage Mutants

Mutations affecting *C. elegans* cell lineages have been isolated in many genetic screens. The most common approach has been to isolate mutants with morphological or behavioral defects, and subsequently to identify cell lineage defects. Because *C. elegans* can propagate as a self-fertilizing hermaphrodite, many mutants with severe defects in morphology or behavior can be recovered. Alternative approaches have been to screen directly for alterations in the pattern or number of cells generated, visualizing cells by Nomarski microscopy or by staining with DNA-binding dyes. Early screens focused on mutants affecting postembryonic cell divisions; more recently, screens for maternal-effect and zygotic embryonic lethal mutants have identified genes required for embryonic cell lineages. The genes defined by such cell lineage mutants form a diverse set, with roles ranging from general requirements in cell division to roles in certain types of cell division or specific cell fates (reviewed by Horvitz, 1988).

Genes Identified by Cell Lineage Mutations

Genes required for cell–cell interactions that specify fates

Many mutations result in ‘homeotic’ cell fate transformations, that is, a particular cell is not simply abnormal but takes on the fate (as evidenced by a cell lineage transformation or other markers) of another cell normally found in a different body region, in a different developmental stage, or in the other sex.

An example of a homeotic transformation of cell lineage is provided by mutations in the *lin-12* gene (*lin* stands for cell lineage abnormal). *lin-12* mutants display a variety of homeotic transformations, often

involving the members of equivalence groups. For example, in the ac/VU (Z1.ppp/Z4.aaa) equivalence group, a reduction of *lin-12* function causes both cells to become anchor cells (**Figure 4A**). Elevation of *lin-12* activity causes both cells to become VU cells. Because opposite changes in *lin-12* activity cause opposite effects on cell fates, *lin-12* is an example of a binary switch gene, whose activity controls which of two alternative fates a cell can adopt. The LIN-12 protein is a transmembrane receptor of the Notch family, and functions in cell–cell communication between members of an equivalence group. Thus, in normal development, LIN-12 is likely initially expressed in both Z1.ppp and Z4.aaa. By chance, LIN-12 becomes more active in one cell than the other; elevated activity of LIN-12 feeds back positively to keep LIN-12 on in that cell, and negatively to turn LIN-12 off in the other cell. As a result, LIN-12 activity increases in the cell that becomes the VU cell, and decreases in the cell that becomes the anchor cell.

Genes required for timing of cell lineage patterns

C. elegans normally develops through four larval stages (L1–L4). Postembryonic precursor cells undergo stage-specific patterns of cell division within each larval stage. A fascinating class of mutants known as heterochronic mutants display either precocious or retarded expression of these cell lineage patterns. Genes defined by heterochronic mutations thus function in controlling the temporal pattern of cell fates during larval development.

Mutations in the *lin-14* gene affect stage-specific patterns of cell division (**Figure 4B**). Reduction of LIN-14 function results in a precocious phenotype (early stages express the patterns of later larval stages), while abnormally high LIN-14 function causes retarded cell lineage patterns (all stages express early patterns). Thus, the level of LIN-14 activity determines whether a precursor undergoes early or late division patterns. The *lin-14* locus encodes nuclear proteins of unknown biochemical function that are present at high levels in early larvae and low levels in late larvae.

Genes involved in asymmetric cell divisions

Many cell lineage mutants display defects in the normal asymmetry of cell divisions, and have provided insights into the mechanisms by which determinants of cell fates are segregated in such asymmetric divisions. The first division of the zygote is asymmetric along the anteroposterior axis, which is determined by the point of fertilization. Because the oocyte appears to be symmetrical, and after fertilization is isolated within an eggshell, the asymmetry of the first division

is likely to be set up cell-autonomously rather than by environmental cues. Maternal-effect mutations affecting the asymmetry of the first division define several *par* (defective partitioning) genes, the products of which are asymmetrically distributed in the zygote. The asymmetry of subsequent cell divisions may involve both intrinsic mechanisms that provide a cellular memory of this initial asymmetry, and cell–cell interactions. All asymmetric divisions in *C. elegans* involve cell division along the anteroposterior axis, and in many of these divisions the protein POP-1 is asymmetrically distributed with higher POP-1 levels in the anterior daughter. In many cells this asymmetry of POP-1 levels requires cell signaling via the Wnt pathway.

Several genes have been identified that function in asymmetric cell divisions in later development. One gene, *unc-86*, is required in diversifying neuroblast lineages. In *unc-86* mutants, the diversifying character of such divisions is lost, revealing an underlying stem-cell type of division (**Figure 4C**). The UNC-86 protein is a POU-domain transcription factor that is asymmetrically activated in the daughter cell that requires its function.

Cell Lineages in Insects

Insects mostly display cell lineages that are variable at the level of individual cell divisions. However, in the central and peripheral nervous systems (CNS and PNS), precursor cells undergo stereotyped sublineages giving rise to neurons and neuronal support cells. Analysis of such lineages has involved a combination of direct observation, dye labeling, and examination of lineage-specific molecular markers.

Genetic analysis of cell lineages in insects has focused on *Drosophila* CNS and PNS neuroblast lineages. In the development of a peripheral sensillum such as a bristle, a precursor cell generates one neuron and three support cells (**Figure 5A**). If activity of the Notch signaling pathway is reduced, all cells become neuronal, indicating that Notch signaling normally promotes the non-neuronal fate. Notch signaling appears to operate between sister cells in the lineage (**Figure 5B**). Thus, although fates are specified autonomously within the lineage, they require local interactions between cells in the same lineage.

Other mutations disrupting neuroblast lineages define several genes required for the normal asymmetry of cell division and cell fates. Such genes may be involved in determining the polarity of the asymmetry itself, or may be segregated in response to the polarity, such as *numb*. Mutations in the *numb* gene cause sister cell transformations in peripheral neuroblasts, leading to a total absence of sensilla (**Figure 5C**). The *numb*

protein is asymmetrically localized in the dividing precursor cell, is segregated to the one daughter cell that will make a neuron, and thus can be considered a localized determinant. The function of the numb protein is to antagonize the effects of Notch signaling, and thus promote neuronal development. Several other genes have been found that regulate the asymmetric cell division itself. Some of these genes may be involved in setting up or responding to the apical/basal asymmetry of the neuroepithelium from which the neuroblasts arise.

Cell Lineage in Vertebrates

The size and cell number of most vertebrate embryos make direct observation of cell division patterns difficult, and thus lineage relationships have been largely defined using clonal analysis. Cell marking and transplantation experiments in amphibians and the zebra fish *Danio rerio* have shown that the early cleavages are not determinative, and that cells do not become committed to specific fates until the blastula stage.

Cell lineage studies in the vertebrate CNS and retina showed that individual cells can generate a wide variety of cell fates, even in very small clones. Thus, cell fates in these situations appear to be specified by a cell's environment and not by its lineal ancestry. Evidence suggestive of lineage-autonomous mechanisms of fate determination has come from the analysis of vertebrate homologs of proteins such as numb and Notch, both of which are asymmetrically localized in dividing neuroblasts in the mammalian cerebral cortex. However, the role of these proteins in cell fate specification in vertebrates has not yet been determined.

Cell Lineage in Plant Development

Stereotyped cell lineages have been observed in the development of many plants. Asymmetric cell divisions occur in the development of colonial algae such as *Volvox*, in which they segregate somatic versus germline fates. Early cell divisions of flowering plants such as *Arabidopsis* are highly stereotyped. However, cell interactions appear to be more important than ancestry in specifying fates. Stereotyped cell lineages are also observed during development of *Arabidopsis* root and floral meristems, and in stomatal development, but again the pattern of cell fates may be determined by interactions rather than ancestry.

Evolution of Cell Lineages

Once a cell lineage has been described for one species, one can examine equivalent lineages in related species

to understand how cell lineages have been modified in evolution – in effect, comparative anatomy with single-cell resolution. Comparative cell lineage analysis has been performed in nematodes, molluscs, insects, and ascidians. Studies of cell lineages in nematodes have begun to yield insights into how morphological change occurs in evolution (reviewed by Félix and Sternberg, 1997). For example, in *C. elegans* the choice of fates in the the ac/VU (Z1.ppp/Z4.aaa) equivalence group is stochastic, with each precursor equally capable of becoming an ac or a VU. In some nematode species the allocation of fates is variable but biased, while in other species the allocation of fates is invariant. Cell killing experiments show that in such species cell fates are no longer dependent on cell–cell interactions. An emerging theme is that alterations in the behavior of single cells can result in dramatic morphological changes.

Conclusions

Studies of cell lineages have been critical in our understanding of how cell fates are specified in development and how fates are correlated with cell division patterns. Invariant lineages or sublineages, although initially considered to imply 'lineage-intrinsic' mechanisms of fate determination, are now thought to reflect both intrinsic and extrinsic mechanisms. Thus, animals with invariant cell lineages may not develop in fundamentally different ways from larger animals in which cell lineages are variable. In insects and vertebrates, cells mostly function in groups, within which cell communication specifies fate. In such animals development may be described as a lineage of cell groups. Selection for rapid development and small size might have led to the reduction of such cell groups to individual cells, and thus the appearance of animals with defined cell lineages.

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See also: 0152, 0170, 0171, 0801

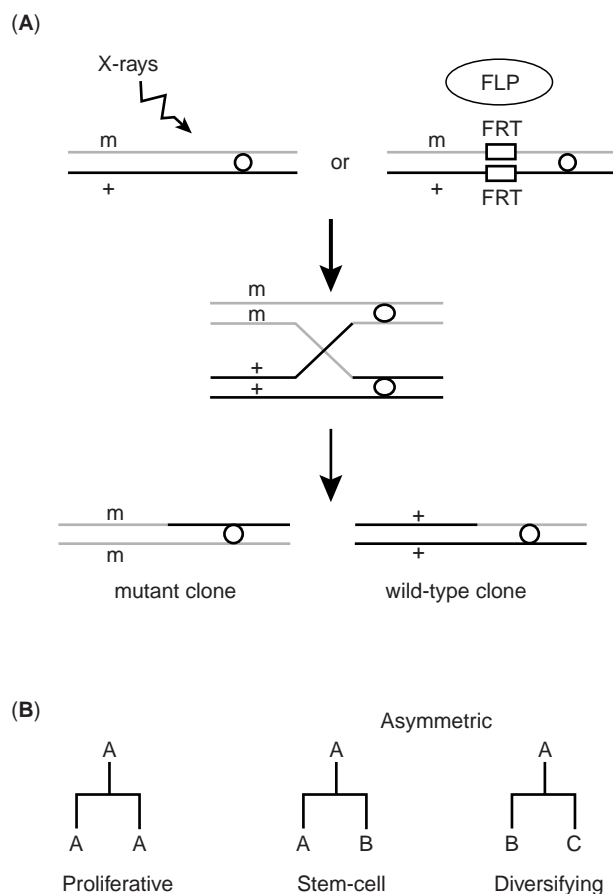


Figure 1 (A) Clonal analysis techniques. Generation of homozygous mutant clones in *Drosophila* by X-ray irradiation or by FLP recombinase-catalyzed recombination between FRT sites. The centromere is denoted by the circle between chromatids. *m*, cell-autonomous recessive marker mutation. (B) Types of cell division: proliferative, stem-cell, and diversifying.

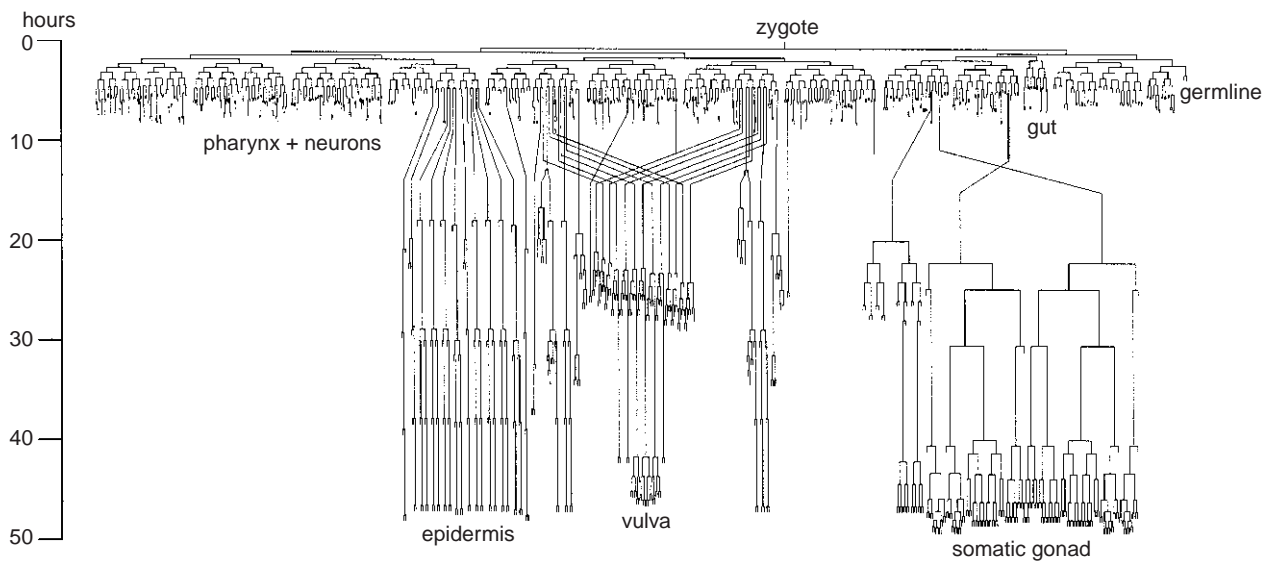
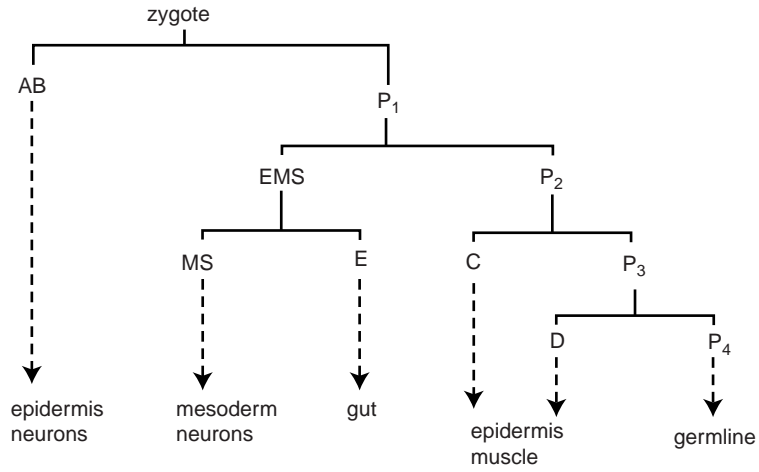


Figure 2 The *Caenorhabditis elegans* cell lineage. Time axis is vertical; each cell division is a horizontal line. The origin of some cell types is indicated.

(A) *C. elegans* embryonic lineage



(B) P cell lineage

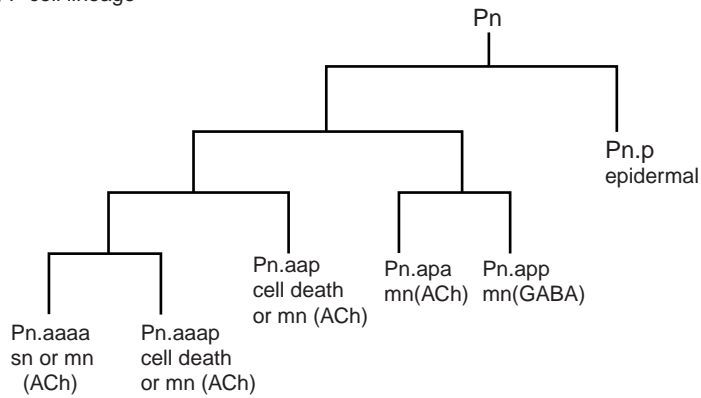


Figure 3 (A) Abbreviated embryonic lineage of *Caenorhabditis elegans*, showing the relationships of the major embryonic founder cells. (B) P cell sublineage, showing the classes of cell generated. mn, motor neuron, with neurotransmitter indicated (ACh, cholinergic; GABA, GABAergic); sn, sensory neuron.

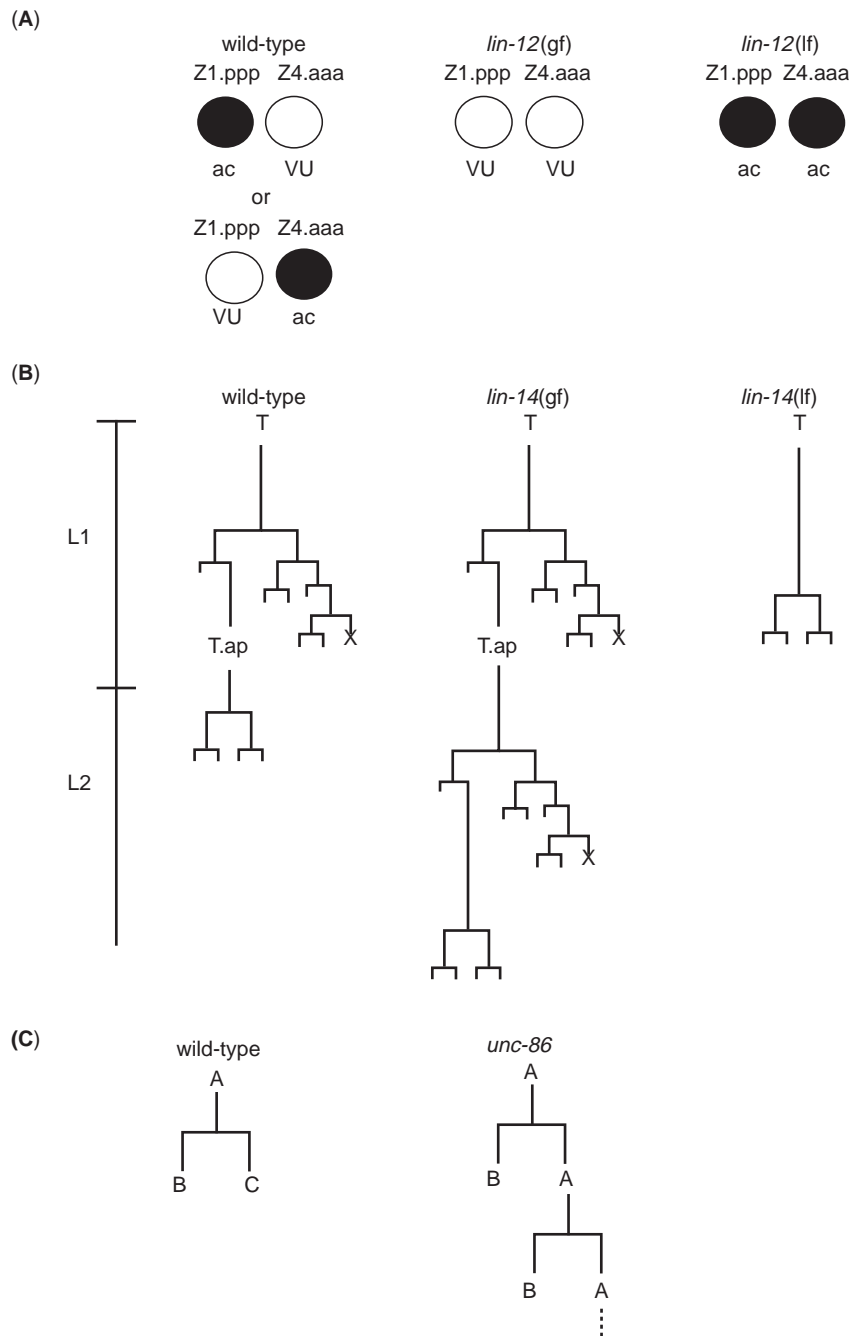


Figure 4 Effects of cell lineage mutations. (A) Effects of *lin-12* mutations on cell fates in the anchor cell/ventral uterine (ac/VU) equivalence group. Filled circle = anchor cell; open circle = ventral uterine precursor. *lin-12(gf)* = gain-of-function *lin-12* mutation causing overactivity of the LIN-12 protein; *lin-12(lf)* = loss or reduction of LIN-12 function. (B) Effects of *lin-14* mutations on the temporal control of the lineage of the postembryonic blast cell T. In normal development T generates the lineage shown in the L1 and L2 larval stages. In *lin-14(gf)* mutants the LIN-14 protein is overactive and early (L1-specific) lineages are reiterated (retarded phenotype); in mutants that cause loss of LIN-14 function (*lin-14(lf)*), L1-specific patterns are bypassed and T undergoes an L2-specific lineage. (C) Effect of *unc-86* mutations on diversifying lineages in the nervous system. In *unc-86* loss-of-function mutants, diversifying lineages are transformed to a reiterating stem-cell-like pattern. UNC-86 protein is expressed within the daughter affected in the mutants (C in the figure).

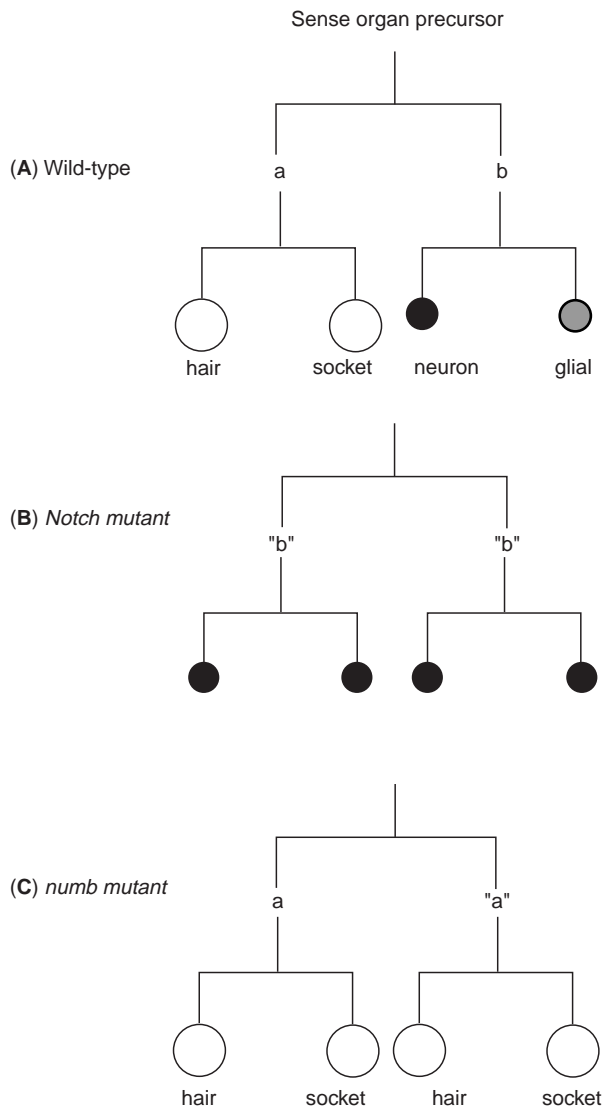


Figure 5 Cell lineage of a *Drosophila* peripheral sensory organ precursor. (A) A single precursor undergoes two rounds of asymmetric divisions to generate four cells: a sensory neuron, a hair cell, a socket cell, and a glial cell. (B) In a *Notch* mutant, all four cells are converted into neurons; (C) in a *numb* mutant, the opposite effect is seen, in which all cells adopt non-neuronal fates.