Investigating *C. elegans* development through mosaic analysis

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Summary

The analysis of genetically mosaic worms, in which some cells carry a wild-type gene and others are homozygous mutant, can reveal where in the animal a gene acts to prevent the appearance of a mutant phenotype. In this primer article, we describe how *Caenorhabditis elegans* genetic mosaics are generated, identified and analyzed, and we discuss examples in which the analysis of mosaic worms has provided important information about the development of this organism.

Introduction

C. elegans derives its power for elucidating animal development from its suitability for mutant analysis (Brenner, 1974). A gene reveals itself as important for development when its loss of function causes a developmental phenotype. Genetic mosaics allow one to ask what the phenotypic consequences are when some cells in an animal carry a wild-type gene and the other cells are homozygous mutant. By analyzing a collection of genetic mosaics, one can ask which cell or cells in an animal must carry the wild-type gene to produce a wild-type phenotype. The responsible cell or cells are referred to as the anatomical focus of the gene's action or function with respect to the phenotype under study.

As the anatomical focus of a gene's function may well be in only a subset of cells in which the gene is expressed, knowing its complete expression pattern does not necessarily tell one where the gene normally functions with respect to a particular phenotypic effect. Furthermore, the anatomical focus of a gene's action may or may not be in cells that exhibit a mutant phenotype. A gene is said to act cell autonomously when the phenotype of a given cell is affected by the gene's mutation and when that phenotype in mosaic animals depends solely on whether or not the cell has the wild-type gene and is unaffected by the genotypes of other cells. A gene is said to act cell non-autonomously when a cell carrying the wild-type gene in a mosaic animal exhibits a mutant phenotype or when a homozygous mutant cell exhibits a wild-type phenotype. Cell non-autonomy implicates cell-cell interactions, and mosaic analysis can be used to identify the responsible interacting cells. Some genes are used repeatedly during development, and the loss of an early essential role of such a gene in a mutant can lead to developmental arrest that precludes analyzing the gene's role in later developmental events. This problem can be overcome through mosaic analysis, as mosaic animals may be able to complete the early stages of development to reveal a gene's later role.

How to generate *C. elegans* genetic mosaics

In nearly all mosaic analyses that have been carried out in *C. elegans*, mosaic animals have been generated by the spontaneous mitotic loss of an extrachromosomal genetic element that carries the wild-type allele of a gene in an otherwise homozygous mutant background. When the extrachromosomal element – and the wild-type gene carried by it – is present in all cells, the worm exhibits a completely wild-type phenotype. But when, as occurs at low frequency, the extrachromosomal element fails to be transmitted to one of the daughters of a cell division, all the descendants of that cell, a clone, will be homozygous mutant. Cells within a mosaic animal that lack the wild-type gene can be independently identified as mutant if the extrachromosomal element also carries a marker gene whose absence from a cell affects the cell's appearance (i. e. acts cell autonomously). Because the *C. elegans* cell lineage is invariant (Sulston et al., 1983), a cell autonomous marker can allow one to determine precisely where in a worm's lineage the extrachromosomal element was lost, which helps to verify, by reference to the known lineage, which cells in the mosaic animal are homozygous mutant and which are not. The frequency of loss of an extrachromosomal element per cell division is approximately the same throughout development (Hedgecock and Herman, 1995; Yochem et al., 1998). However, extrachromosomal elements are occasionally lost at two or more consecutive cell divisions to give a pattern of mosaicism that is somewhat more complicated than that corresponding to a single clone of mutant cells (Hedgecock and Herman, 1995; Yochem et al., 1998). Possible misinterpretations caused by this effect can be avoided by scoring for the presence or absence of the extrachromosomal element in more than a few cells in the lineage of interest.

Two kinds of extrachromosomal element have been used for mosaic analysis: free chromosome fragments (Herman, 1984) and extrachromosomal arrays (Lackner et al., 1994; Miller et al., 1996). *C. elegans* chromosomes do not have localized centromeres (Albertson and Thomson, 1982) – they are said to be holocentric – which means that a suitably large fragment of any part of a chromosome, referred to as a free duplication, can retain some centromeric function, and can behave as a fairly stable mini-chromosome and be maintained in genetic stocks.

Extrachromosomal arrays, which are routinely generated to demonstrate transformation rescue (or complementation) of a mutant phenotype (Mello and Fire, 1995), are now often used in preference to free duplications for mosaic analysis. When a

mixture of DNA that contains a wild-type gene and a gene that encodes a cell autonomous marker is microinjected into the syncytial germline of an adult hermaphrodite, a mini-chromosome composed of many copies of the DNAs tends to form spontaneously (Stinchcomb et al., 1985) (Fig. 1). One can then select animals that carry an array that shows good expression of both the gene to be analyzed, as judged by the rescue of the mutant phenotype, and the marker gene. Arrays are generally present in one copy per cell. Mitotic loss leads to mosaic animals in which a clone of cells lacks the array and therefore lacks both the marker gene and the wild-type gene under study (Fig. 2). Mitotic losses of free duplications or extrachromosomal arrays frequently involve non-disjunction, in which one daughter cell receives no duplication and the other daughter receives two copies (Hedgecock and Herman, 1995; Yochem et al., 1998). Some arrays are lost at a frequency that may be inconveniently high for mosaic analysis, say >1/50 per cell division, which means every animal will contain many independent mutant clones. Preliminary work in selecting a suitable array is therefore a good idea. A crucial assumption when using extrachromosomal arrays for mosaic analysis is

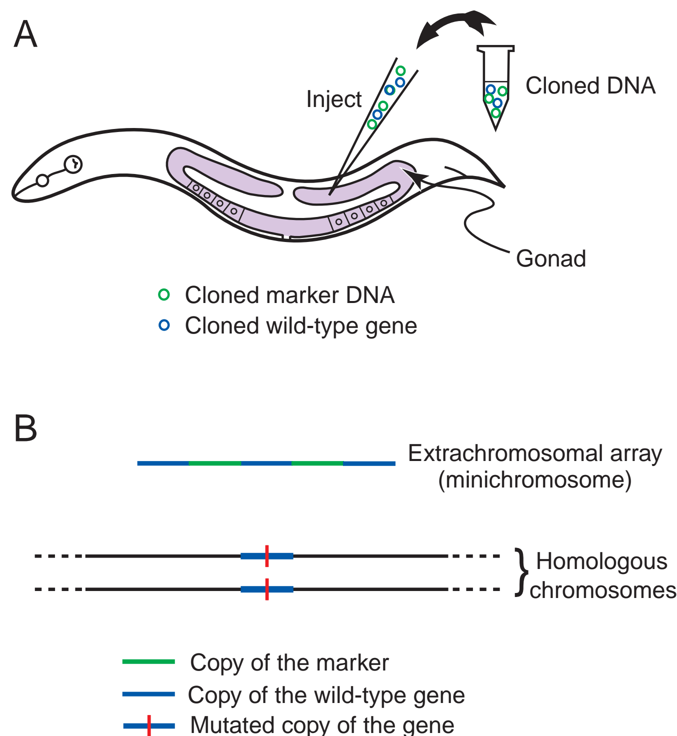


Fig. 1. Extrachromosomal arrays for mosaic analysis in *C. elegans*. (A) Mini-chromosomes, called extrachromosomal arrays, form in vivo from DNA that has been injected into the syncytial part of the gonad of hermaphrodites. Green circles represent plasmids that contain a marker gene that cell-autonomously expresses green fluorescent protein (GFP) in nuclei of transgenic worms; blue circles represent plasmids that contain a wild-type copy of a gene under study. (B) An array can contain multiple copies of each type of injected DNA. Shown in green is the marker gene that expresses GFP in nuclei. In blue are wild-type copies of the gene under study. Endogenous copies of the blue gene have a loss-of-function mutation, as indicated by a red line. The wild-type copies on the array fully complement the mutant copies on the homologous chromosomes.

that the expression of the wild-type gene on the array mimics its normal pattern of expression. [For more information on this and other issues that are important in *C. elegans* mosaic analysis, see also previous reviews (Herman, 1995; Yochem et al., 2000).]

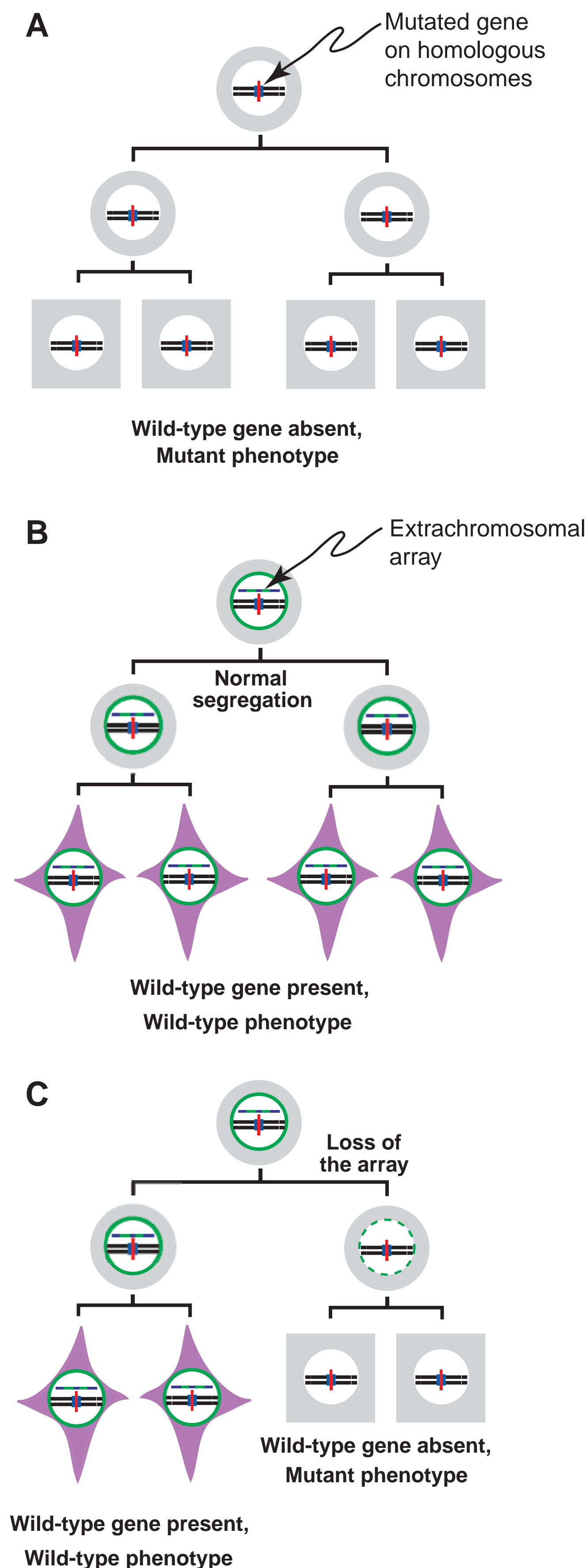
How to identify mosaic animals

To track the mitotic loss of an extrachromosomal element, one wants a cell autonomous marker that: (1) can be readily scored in virtually all cells – preferably in nuclei – of living animals, even when losses occur very late in the lineage; and (2) has no other phenotypic effect that could confound mosaic analysis. One excellent marker is a wild-type *ncl-1* gene in a mutant *ncl-1* background; nearly all homozygous mutant *ncl-1* cells exhibit enlarged nucleoli (Fig. 3) with no other apparent effects (Hedgecock and Herman, 1995; Frank and Roth, 1998). Both free duplications and extrachromosomal arrays carrying *ncl-1(+)* have been used for mosaic analysis. Alternatively, the gene that encodes GFP (green fluorescent protein) when expressed under the control of a strong promoter, as in *sur-5::gfp*, can be used to track the inheritance of extrachromosomal arrays in living animals (Fig. 4) (Yochem et al., 1998).

Additional genetic markers that can be scored in mutant animals at low magnification are sometimes used for rapidly identifying potential mosaics, which are then scored for their cellular phenotypes at high magnification. For example, consider zygotes of genotype *unc-36(–); Ex100[unc-36(+) sur-5::gfp]*. This nomenclature means that the zygotes are homozygous mutant for *unc-36* and harbor an extrachromosomal array, *Ex100*, that carries both *unc-36(+)* and the marker *sur-5::gfp*. A low magnification stereomicroscope equipped with a lamp for exciting green fluorescence can be used to screen rapidly for uncoordinated animals that are at least partly green, and these animals can then be scored at single-cell resolution using a compound microscope at high magnification. The fully uncoordinated (Unc-36) phenotype in mosaics is caused by array loss at AB or ABp in the *C. elegans* cell lineage (Fig. 5) because the anatomical focus of *unc-36* action is among the descendants of both daughters of ABp: ABpl and Abpr. Array loss by either ABpl or ABpr leads to a weaker uncoordinated phenotype (Kenyon, 1986). Fig. 6 illustrates how the Unc-36 phenotype can be used to pick out rapidly classes of animals that are mosaic for essential genes; in these examples, the extrachromosomal array carries the wild-type allele of an essential gene under study in addition to *unc-36(+)* and *sur-5::gfp*.

How to analyze mosaic worms

If one wants to delimit the focus of action of a gene that affects the whole animal when mutated, such as by causing uncoordinated movement, lengthened lifespan or inviability, then as many different types of mosaics should be collected as possible to see whether one can correlate the mutant phenotype with the absence of gene expression in a particular cell or set of cells. A good start in such cases is to look first for worms that contain large mutant clones, which have been generated by loss of the extrachromosomal element very early in the embryonic lineage. Loss at P_1 (Fig. 7), for example, would give rise to an animal, which we denote as a P_1(–) mosaic, in which 94 of its 95 adult body wall muscle cells would lack the wild-type gene, whereas almost all of its neurons would have it. Thus, P_1(–) mosaics and their complement, AB(–) mosaics,



can quickly distinguish whether a gene acts primarily in the development of muscles or neurons.

Certain mosaics can also exhibit novel phenotypes, which may be informative. For example, if the focus of a gene's action is broadly distributed among several cells, then mosaics in which only a subset of the cells are mutant might exhibit a mildly mutant phenotype, as in the *unc-36* example noted above. Mosaics of a recessive lethal gene might be viable but show novel cellular abnormalities. Homozygous germline clones in an array-bearing hermaphrodite parent may give rise to a more severe homozygous mutant phenotype than that produced by a heterozygous parent germline, thus revealing a maternal effect. Conversely, germline mosaics can be used to indicate that a gene is fully zygotic in its action (Chen et al., 1994).

When a loss-of-function mutation causes a specific cellular abnormality, one can ask whether or not the gene behaves cell autonomously (Fig. 2). In this case, one wants to identify mosaics in which the cell of interest is genotypically different from its neighbors and other potentially interacting cells. If the gene of interest behaves cell non-autonomously, a careful mosaic analysis should be able to identify the interacting cells responsible for the effect.

Limitations of mosaic analysis in *C. elegans*

Two potential problems or complications associated with the analysis of mosaic worms should be noted. The first is called perdurance, which refers to the persistence of a gene product in a cell that lacks the gene. Thus, a wild-type product synthesized in an ancestral cell prior to gene loss might persist and be transmitted to descendant cells even though they did not inherit the gene. Because the effect of perdurance would be to weaken the expected mutant phenotype, mosaics that exhibit a fully expressed mutant phenotype seem to be free of perdurance. Perdurance is also unlikely to be a problem when the loss of a duplication or an array occurs early in the embryonic cell lineage or when the gene product is involved in terminal cellular differentiation and is synthesized only late

Fig. 2. An example of mosaic analysis and cell autonomy. (A) In worms that are homozygous for the mutation of the blue gene mentioned in Fig. 1, four hypothetical cells develop an abnormal shape and color. (B) Worms are identified in which the four cells have green fluorescent nuclei, a consequence of inheriting the extrachromosomal array, mentioned in Fig. 1, that expresses GFP cell autonomously. The cells have therefore inherited wild-type copies of the blue gene, because they are also present on the array, and the array is known from preliminary work to complement the mutant phenotype of the blue gene in transgenic worms that are not mosaic. The cells are observed to undergo wild-type development, which involves a change in cell shape and color soon after birth. This pattern of inheritance, however, does not prove that the blue gene must function within the four cells (cell autonomously). Proper development of the four cells may instead depend on the expression of the gene in another cell or cells, which signal to the four cells to change their shape and color. This would be an example of cell non-autonomy. Mosaic worms must therefore be examined carefully for their overall patterns of mosaicism. (C) Loss of the array when the grandmother of the cells divides produces mosaicism within the four cells. The left clone has a wild-type phenotype, and the right clone is mutant. Note that the phenotype correlates with inheritance of the array, as would be expected for the cell autonomous action of the blue gene.

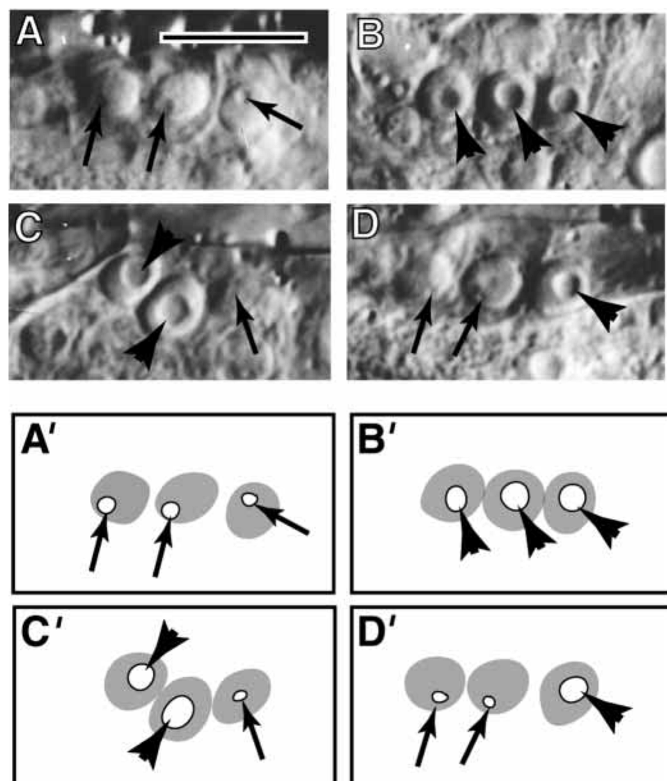


Fig. 3. The Ncl phenotype. (A) Nomarski image of normal nucleoli (arrows) in the nuclei of three neurons that have the genotype *ncl-1(e1865)/ncl-1(e1865); sDp3[ncl-1(+)]*. Although the endogenous copies of the *ncl-1* gene carry *e1865*, a loss-of-function mutation that is completely recessive to the wild-type gene, each neuron inherited a free duplication (*sDp3*) that has a wild-type (+) copy of *ncl-1*, which fully complements the *e1865* mutation. (B) Neurons that fail to inherit *sDp3* have the genotype *ncl-1(e1865)/ncl-1(e1865)*. They show the Ncl phenotype, a cell-autonomous enlargement of nucleoli (arrowheads). (C) The three nuclei are mosaic for the duplication. The two nuclei on the left have enlarged nucleoli, indicating that they failed to inherit *sDp3*; the nucleus on the right has a normal nucleolus, indicating its inheritance of the duplication. (D) The opposite pattern of mosaicism results when the two nuclei on the left, but not the nucleus on the right, inherit the duplication. (A′-D′) The boundaries of the nuclei (gray) and of the nucleoli (white circles) are indicated for each of the upper panels. Scale bar: 10 μm.

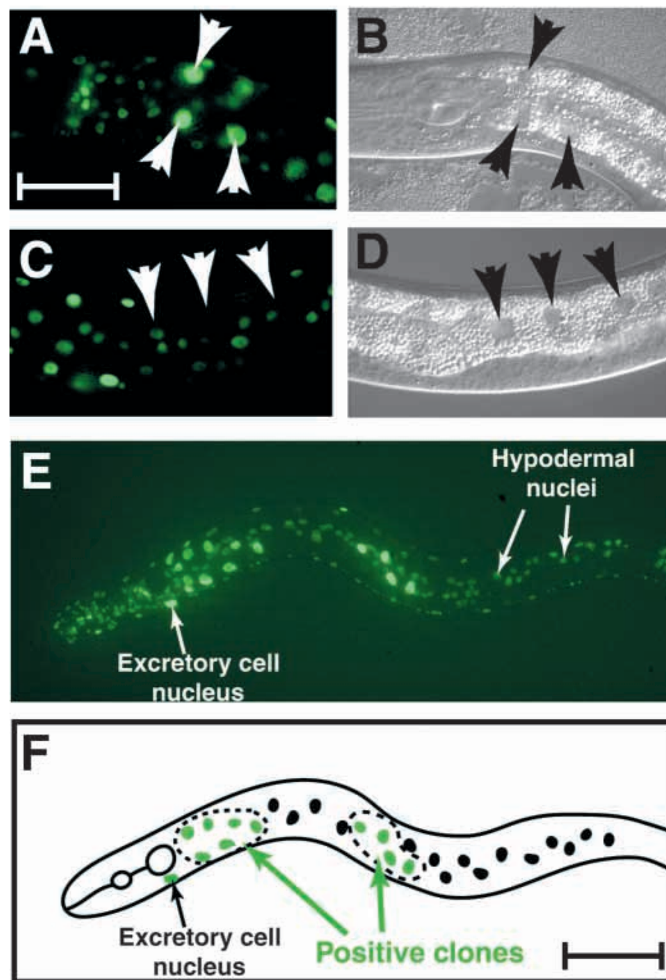


Fig. 4. Expression of *sur-5::gfp* in nuclei. (A) Fluorescent image of a non-mosaic worm. Arrowheads indicate three intestinal nuclei that have inherited an extrachromosomal array that expresses *sur-5::gfp*. (B) The corresponding Nomarski image. (C,D) Lack of fluorescence of three intestinal nuclei is indicated with arrowheads in a mosaic worm that lost the array in the embryonic cell E, the progenitor of the gut. (E,F) Patchy mosaicism in the gut. Two positive clones can be seen within an otherwise dark intestine, indicating consecutive losses of the array within the gut cell lineage. Consecutive losses occur more frequently for extrachromosomal arrays than for free duplications. Scale bars: in A, 50 μm for A-D; in F, 150 μm for E,F.

in development. Very late losses affecting either *ncl-1* or *sur-5::gfp* give clear mosaic phenotypes, indicating that there is very little perdurance of the wild-type gene product for either of these marker genes.

The second complication concerns limitations that occur as a consequence of the nature of the worm cell lineage. This is generally not a problem when investigating a cellular phenotype because mosaics can usually be identified in which the cell under investigation is genotypically different from cells that are candidates for interaction. However, the worm cell lineage can be limiting when a gene's focus of action is diffuse and distributed among cells of disparate lineage. The fully mutant phenotype may then only be apparent in mosaic animals when the duplication or array is lost by a progenitor of all or nearly all of the responsible cells, in which case it may be difficult to pinpoint the responsible cell types. A particularly difficult tissue to investigate in mosaic worms is the hypodermis, called hyp7, which forms the skin for the main body of the animal. Hyp7 is a single syncytial cell that is formed by the fusion of many mononucleate cells that descend from both AB and P_1, the daughters of the very first embryonic cleavage (Sulston et al., 1983); therefore, no mosaic animal can contain a single, completely mutant hyp7 clone (Fig. 7). Mosaic analyses have, nonetheless, been used to implicate hyp7 as the focus of action of several genes, as we illustrate below.

Insights into worm development from mosaic analyses

More than 70 genes affecting worm development have been studied in genetic mosaics. Rather than attempt a

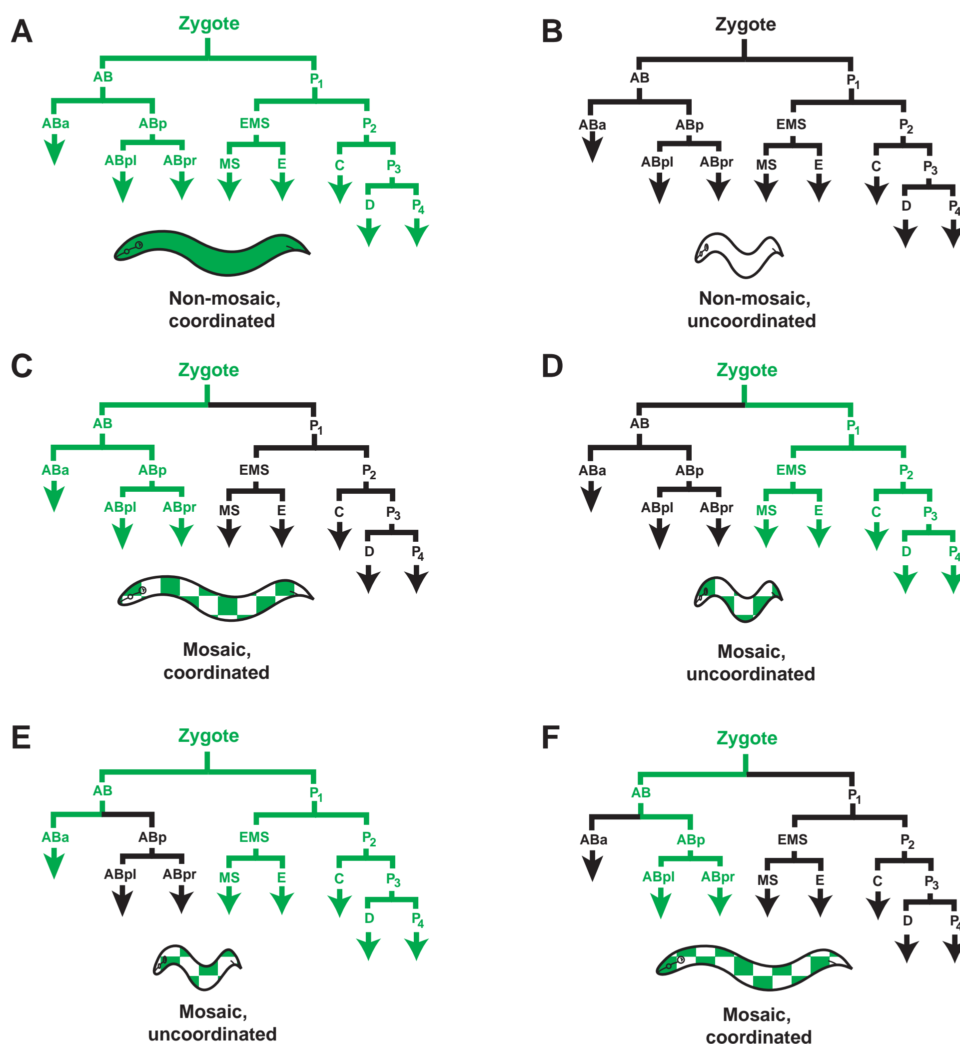


Fig. 5. Correlation of phenotype with mosaic cell lineages. The phenotypes of certain progeny of a worm of genotype *unc-36(–); Ex100[unc-36(+) sur-5::gfp]* are indicated below diagrams of the early divisions of the invariant cell lineage of *C. elegans*. *Ex100* is an extrachromosomal array that has wild-type (+) copies of the *unc-36* gene, which rescue a loss-of-function mutation in the endogenous copies of *unc-36*. The array also expresses GFP from a cell-autonomous marker gene. Based on which cells are green, the marker allows one to deduce the cell division at which the array was lost in a mosaic animal. (A) Inheritance of the array by all cells, indicated in green in the diagram, produces a non-mosaic worm whose movement is completely coordinated. (B) Failure to inherit the array (indicated in black), owing to meiotic segregation in the mother, results in an uncoordinated (Unc) animal. (C) Loss of the array (indicated in black) in P_1 produces a mosaic worm that has normal coordination. The focus of action of the *unc-36* gene is therefore not in P_1 or its descendants. (D) By contrast, loss of the array in AB, the sister of P_1, produces a mosaic worm that is fully uncoordinated. (E) Loss of the array in ABp also produces the uncoordinated phenotype. (F) Loss of the array in ABa and P_1 – an example of consecutive losses of the array – gives a coordinated worm. The focus of action of *unc-36* is therefore among the descendants of ABp (Kenyon, 1986).

comprehensive review of those genes here, we will discuss a few examples to illustrate how mosaic analysis has been used to elucidate certain mechanisms of development.

The foci of action of several genes implicated in cell-to-cell signaling during development have been determined by mosaic analysis. Two classic examples are *glp-1* and *lin-12*, which encode members of the Notch family. The proteins encoded by these genes contain copies of a motif that resembles epidermal growth factor (EGF). Because EGF is an extracellular factor that affects other cells, and because it is cleaved from a membrane-bound precursor, it was natural to consider LIN-12 (the protein encoded by *lin-12*) and GLP-1 as possible sources of extracellular signal. However, mosaic analysis indicated that both *glp-1* and *lin-12* act cell autonomously in the cells whose fates require their function (Austin and Kimble, 1987; Seydoux and Greenwald, 1989), as would be expected if the genes encode receptors and not precursors for secreted signals.

By contrast, *lin-44* encodes a Wnt signal that affects the polarity of a cell in the worm's tail, called T, and that was shown by mosaic analysis to act cell non-autonomously – not in T but most likely in tail hypodermal cells (Herman et al., 1995), the cells believed to secrete the Wnt signal. Another gene that has been shown by mosaic analysis to act cell non-autonomously is *her-1* (Hunter and Wood, 1992), a gene that is essential for determining male sexual fate (Hodgkin 1980). This result was consistent with the later molecular characterization of *her-1* (Perry et al., 1993), which suggested that HER-1 protein is secreted and acts as a signaling molecule in the worm sex determination pathway.

Loss-of-function mutations in *unc-5* lead to defects in the dorsal-ward guidance of pioneering axons and migrating cells during worm development. Mosaic analysis has shown that *unc-5* acts cell autonomously (Leung-Hagesteijn et al., 1992); for example, a migrating cell was found to be defective in its dorsal-ward migration if, and only if, it lacked *unc-5(+)*. This result, together with the molecular characterization of *unc-5* (Leung-Hagesteijn et al., 1992), led to the proposal that UNC-5 is a transmembrane receptor that promotes the movement of migrating axons and cells away from a high ventral concentration of the extracellular matrix protein UNC-6 (Wadsworth et al., 1996). UNC-6 is a member of the netrin family of proteins (Ishii et al., 1992), which have been shown to affect axon guidance in vertebrate embryos (Serafini et al., 1994). The *vab-8* gene has also been shown by mosaic analysis to affect axon guidance cell autonomously; in this case, Wolf et al. were able to conclude that *vab-8* must be expressed in certain neurons despite the fact that

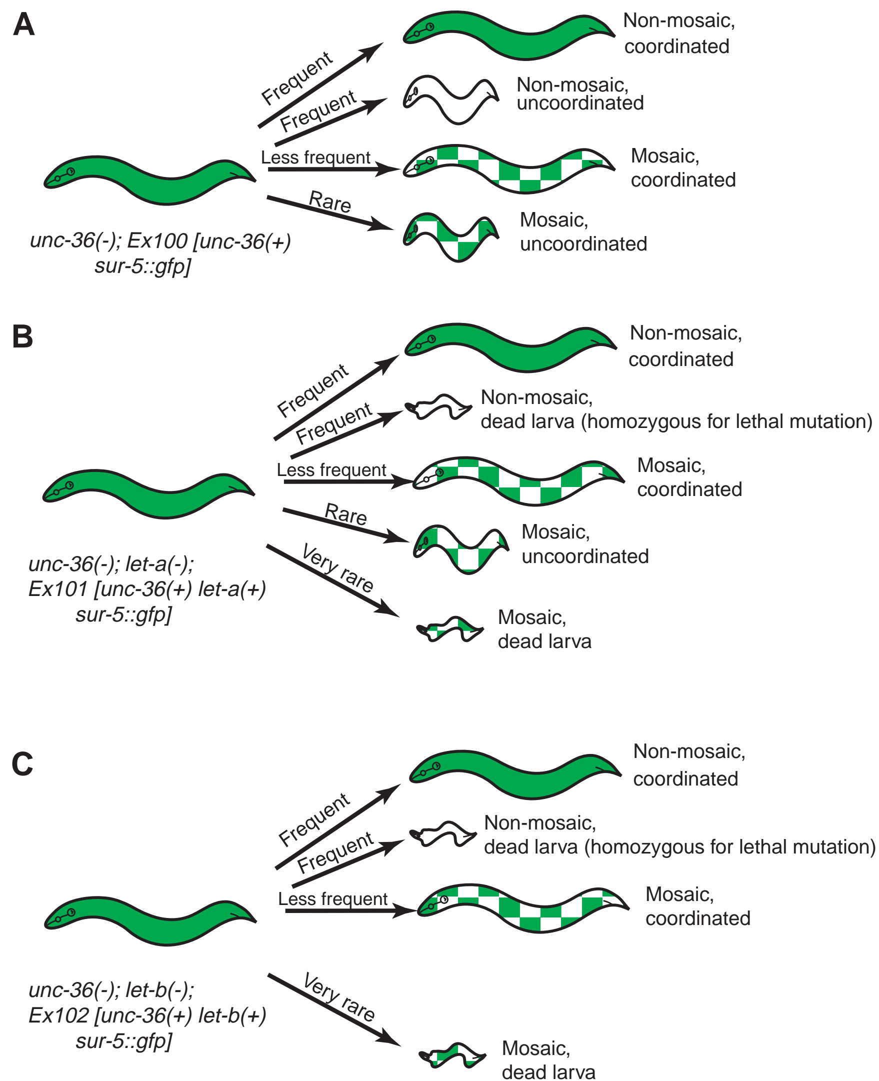


Fig. 6. Using *unc-36* to identify specific classes of mosaics. (A) The phenotypes of the progeny of *unc-36(–); Ex100[unc-36(+) sur-5::gfp]* hermaphrodites, as shown in Fig. 5. The upper two classes of progeny are the most frequent. The non-mutant (fully coordinated), non-mosaic worms have the same genotype as the mother and can be used to propagate the strain. The uncoordinated, non-mosaic progeny derive from zygotes that failed to inherit the array owing to meiotic segregation. (B) The segregation pattern for *let-a*, a hypothetical gene that is essential for viability (*let* – lethal when mutant). Homozygosity for a recessive mutation in the gene, designated as *let-a(–)*, results in death soon after hatching. The segregants are from mothers with the genotype *unc-36(–); let-a(–); Ex101[unc-36(+) let-a(+) sur-5::gfp]*. *Ex101* is an extrachromosomal array that has wild-type copies of the *unc-36* gene, wild-type copies of the *let-a* gene and a marker gene that expresses GFP. The segregation of mosaic worms that are fully viable but uncoordinated indicates that the focus of the lethal mutation is not in ABp, because loss of the array in ABp, which affects coordination, has no effect on viability. (C) The segregation pattern for a different lethal gene, *let-b*. Mutation of this gene also causes death soon after hatching. The segregants derive from mothers with the genotype *unc-36(–); let-b(–); Ex102[unc-36(+) let-b(+) sur-5::gfp]*. The failure to see older larvae and adults that are uncoordinated indicates that the focus of *let-b* includes the same part of the cell lineage as the focus of *unc-36*.

immunofluorescent staining was only able to detect VAB-8 expression in body muscle (Wolf et al., 1998).

The example of *let-23*, which encodes an EGF tyrosine kinase receptor (Aroian et al., 1990), is also instructive. Activation of LET-23 by an EGF-like ligand occurs repeatedly during *C. elegans* development to trigger diverse developmental events. One of these events is the induction of vulval development, in which three vulval precursor cells generate 22 cellular descendants that form the vulva. In the absence of an EGF signal from the gonadal anchor cell, none of the vulval precursor cells contributes to vulval development, and no vulva is formed. Because vulval development is not essential to a worm's survival, it can be analyzed in *let-23* mosaics, even though *let-23* is an essential gene. Such an analysis has shown that normal vulval development can occur when only one vulval precursor cell contains *let-23(+)* (Simske and Kim, 1995; Koga and Ohshima, 1995). According to the picture that emerges from these studies, the vulval precursor cell closest to the signaling anchor cell is activated by the reception of the EGF signal and then induces its neighboring vulval precursor cells on each side (through LIN-12-mediated signaling) to embark on vulval development.

Downstream of LET-23 in the vulval signal-transduction pathway is the small G protein RAS, which is encoded by *let-60* (Beitel et al., 1990; Han and Sternberg, 1990). The lethality of a *let-60* loss-of-function mutation has been traced by mosaic analysis to a single cell, the excretory duct cell (Yochem et al., 1997). An unexpected mosaic phenotype was encountered in this study: when descendants of ABpl (Fig. 7), which normally includes the excretory duct cell, were mutant for *let-60*, a *let-60(+)* cell assumed the duct cell fate.

Another example in which mosaicism has given an unexpected phenotype involves a natural ambiguity in the cell lineage between two cell fates. Either one of two cells defined by the lineage randomly assumes the anchor cell (AC) fate, with the other cell becoming VU (Kimble, 1981). The VU fate is specified by an AC-to-VU signal and requires *lin-12* function: in a *lin-12* loss-of-function mutant, both cells become AC, and in a *lin-12* gain-of-function mutant, both become VU (Greenwald et al., 1983; Seydoux and Greenwald, 1989). Mosaic analysis has shown, as expected, that *lin-12* function is required in VU and not in AC (Seydoux and Greenwald, 1989). But the collection of mosaics in which one of the two AC/VU cells was homozygous for a *lin-12* loss-of-function mutation

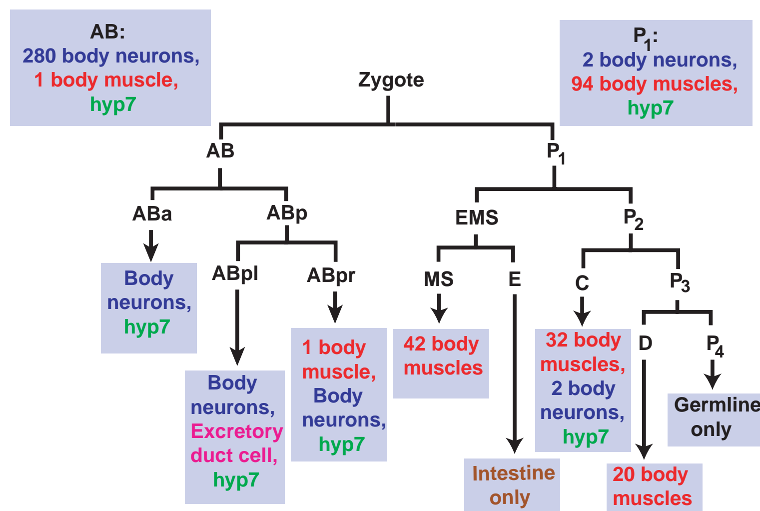


Fig. 7. The embryonic progenitors of several cell types in a hermaphrodite. D, E and P_4 each gives rise to only one cell type, and C gives rise only to two neurons, 32 body muscles and nuclei that form part of hyp7, a large syncytium that forms most of the hypodermis, or skin, of the animal. Additional cell types derive from ABa, ABpl, ABpr and MS, but only those cell types discussed in the text are indicated. For a complete picture of the embryonic cell lineage of *C. elegans*, please go to http://www.wormatlas.org/Sulstonemblin_1983/results.html

and the other was *lin-12(+)* gave an unexpected result: in every animal, the mutant cell became AC and the wild-type cell became VU. Thus, loss of *lin-12* in one cell had a non-autonomous effect that forced the other cell to become VU. This result is explained by a model in which the two AC/VU cells signal to each other until the greater activation of LIN-12 receptor in one (VU) leads to the loss of LIN-12 activity in the other (AC) (Seydoux and Greenwald, 1989).

Mosaic analysis also revealed an unexpected anatomical focus for the action of *unc-52*, which encodes the worm homolog of mammalian perlecan (Rogalski et al., 1993). Null *unc-52* mutants are unable to assemble a myofilament lattice and undergo embryonic arrest typical of that caused by defective body muscle (Williams and Waterston, 1994). UNC-52 localizes between muscle and hypodermis, and it had been concluded that the protein is synthesized by muscle. But mosaic analysis showed that *unc-52* does not function in muscle, because P_1(–) mosaics (Fig. 7) were viable and fertile (Spike et al., 2002). It was therefore concluded that UNC-52 is synthesized and secreted by hypodermis.

The anatomical foci of other whole-animal, developmental phenotypes have been deduced from mosaic analysis. For example, analysis of mosaics of *sma-3*, which encodes a component of a TGFβ signaling pathway that regulates body size, lent weight to the conclusion that *sma-3* acts in the hypodermis to control body size (Wang et al., 2002). The *daf-2* gene, which encodes a homolog of insulin-like growth factor I receptors (Kimura et al., 1997), affects two whole-animal phenotypes: lifespan and the decision of young larvae to enter a state of diapause (the dauer stage) rather than progressing to adulthood. A partial loss-of-function mutation in *daf-2* leads to extended lifespan (Kenyon et al., 1993), whereas a stronger *daf-2* mutation leads to the inappropriate formation of dauer larvae (Riddle et al., 1981). The characterization of many *daf-2* mosaics with different mutant clones has led to the conclusion that the wild-type DAF-2 receptor acts diffusely in multiple cell lineages to regulate the production or activity of a secondary signal, which then affects lifespan and dauer formation by affecting the tissues of the whole animal (Apfeld and Kenyon, 1998).

Final considerations

Mosaic analysis in *C. elegans* requires a knowledge of worm anatomy and development, both to pinpoint where in the cell lineage a genetically-marked extrachromosomal element has been lost and to analyze the cellular phenotypes of mosaic animals. This can seem formidable at first, but knowledge of this organism's anatomy can be one of the joys of working with *C. elegans*. (To begin acquiring such knowledge, we recommend you go to http://www.wormatlas.org/index.htm) One prospect for the future is additional computer-assisted tutorials on worm anatomy. Another future development that would assist the field would be the creation of improved markers to allow for the even more rapid examination of thousands of worms at low magnification for specific mosaics.

Another method for determining the anatomical focus of a gene's action is to introduce transgenic copies of the wild-type gene under the control of cell- or tissue-specific promoters into otherwise homozygous mutant animals. The rescue of a mutant defect by a particular transgene indicates that the gene's expression in the indicated cell type can provide the required function. However, one cannot be certain that such promoters are absolutely specific in their effects, and one must make a new construct when testing each promoter with a gene of interest. In addition, rescue by expression from the transgene in this case (generally overexpression because of the multiple gene copies) does not prove that the wild-type gene normally acts in the same cell type or tissue. But this approach, especially in combination with mosaic analysis, can help to build a strong case (e.g. Zhen and Jin, 1999; Inoue and Thomas, 2000; Zhen et al., 2000; Wang et al., 2002). The inclusion of mosaic analysis provides greater confidence that one has identified the cell or tissue that requires the activity of a gene, and we expect future mosaic analyses to yield more unexpected insights into the mechanisms of *C. elegans* development.

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References

Albertson, D. G. and Thomson, J. N. (1982). The kinetochores of *Caenorhabditis elegans*. *Chromosoma* **86**, 409-428.

Apfeld, J. and Kenyon, C. (1998). Cell nonautonomy of *C. elegans* DAF-2 function in the regulation of diapause and life span. *Cell* **95**, 199-210.

Aroian, R. V., Koga, M., Mendel, J. E., Ohshima, Y. and Sternberg, P. W. (1990). The *let-23* gene necessary for *Caenorhabditis elegans* vulval induction encodes a tyrosine kinase of the EGF receptor subfamily. *Nature* **348**, 693-699.

Austin, J. and Kimble, J. (1987). *glp-1* is required in the germ line for regulation of the decision between mitosis and meiosis in *Caenorhabditis elegans*. *Cell* **51**, 589-599.

Beitel, G. J., Clark, S. G. and Horvitz, H. R. (1990). *Caenorhabditis elegans ras* gene *let-60* acts as a switch in the pathway of vulval induction. *Nature* **348**, 503-509.

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

Chen, L., Krause, M., Sepanski, M. and Fire, A. (1994). The *Caenorhabditis elegans* MYOD homologue HLH-1 is essential for proper muscle function and complete morphogenesis. *Development* **120**, 1631-1641.

Frank, D. J. and Roth, M. B. (1998). *ncl-1* is required for the regulation of cell size and ribosomal RNA synthesis in *C. elegans*. *J. Cell Biol.* **140**, 1321-1329.

Greenwald, I. S., Sternberg, P. W. and Horvitz, H. R. (1983). The *lin-12* locus specifies cell fates in *Caenorhabditis elegans. Cell* **34**, 435-444.

Han, M. and Sternberg, P. W. (1990). *let-60*, a gene that specifies cell fates during *C. elegans* vulval induction, encodes a ras protein. *Cell* **63**, 921-931.

Hedgecock, E. M. and Herman, R. K. (1995). The *ncl-1* gene and genetic mosaics of *Caenorhabditis elegans. Genetics* **141**, 989-1006.

Herman, M. A., Vassilieva, L. L., Horvitz, H. R., Shaw, J. E. and Herman, R. K. (1995). The C. elegans gene *lin-44*, which controls the polarity of certain asymmetric cell divisions, encodes a Wnt protein and acts cell nonautonomously. *Cell* **83**, 101-110.

Herman, R. K. (1984). Analysis of genetic mosaics of the nematode *Caenorhabditis elegans. Genetics* **106**, 165-180.

Herman, R. K. (1995). Mosaic analysis. *Methods Cell Biol.* **48**, 123-146.

Hodgkin, J. (1980). More sex-determination mutants of *Caenorhabditis elegans*. *Genetics* **96**, 649-664.

Hunter, C. P. and Wood, W. B. (1992). Evidence from mosaic analysis of the masculinizing gene *her-1* for cell interactions in *C. elegans* sex determination. *Nature* **355**, 551-555.

Inoue, T. and Thomas, J. H. (2000). Targets of TGF-β signaling in *Caenorhabditis elegans* dauer formation. *Dev. Biol.* **217**, 192-204.

Ishii, N., Wadsworth, W. G., Stern, B. D., Culotti, J. G. and Hedgecock, E. M. (1992). UNC-6, a laminin-related protein, guides cell and pioneer axon migrations in *C. elegans*. *Neuron* **9**, 873-881.

Kenyon, C. (1986). A gene involved in the development of the posterior body region of *C. elegans*. *Cell* **46**, 477-487.

Kenyon, C., Chang, J., Gensch, E., Rudner, A. and Tabtiang, R. (1993). A *C. elegans* mutant that lives twice as long as wild type. *Nature* **366**, 461-464.

Kimble, J. (1981). Alteration in cell lineage following laser ablation of cells in the somatic gonad of *Caenorhabditis elegans*. *Dev. Biol.* **87**, 286-300.

Kimura, K. D., Tissenbaum, H. A., Liu, Y. and Ruvkun, G. (1997). *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans. Science* **277**, 942-946.

Koga, M. and Ohshima, Y. (1995). Mosaic analysis of the *let-23* gene function in vulval induction of *Caenorhabditis elegans*. *Development* **121**, 2655-2666.

Lackner, M. R., Kornfeld, K., Miller, L. M., Horvitz, H. R. and Kim, S. K. (1994). A MAP kinase homologue, *mpk-1*, is involved in ras mediated induction of vulval cell fates in *Caenorhabditis elegans*. *Genes Dev.* **8**, 160-173.

Leung-Hagesteijn, C., Spence, A. M., Stern, B. D., Zhou, Y., Su, M.-W., Hedgecock, E. M. and Culotti, J. G. (1992). UNC-5, a transmembrane protein with immunoglobulin and thrombospondin type 1 domains, guides cell and pioneer axon migrations in *C. elegans*. *Cell* **71**, 289-299.

Mello, C. C. and Fire, A. (1995). DNA transformation. *Methods Cell Biol.* **48**, 452-482.

Miller, L. M., Waring, D. A. and Kim, S. K. (1996). Mosaic analysis using a *ncl-1(+)* extrachromosomal array reveals that *lin-31* acts in the Pn.p cells during *Caenorhabditis elegans* vulval development. *Genetics* **143**, 1181-1191.

Perry, M. D., Li, W., Trent, C., Robertson, A., Fire, A., Hageman, J. M. and Wood, W. B. (1993). Molecular characterization of the *her-1* gene suggests a direct role in cell signaling during *Caenorhabditis elegans* sex determination. *Genes Dev.* **7**, 216-228.

Riddle, D. L., Swanson, M. M. and Albert, P. S. (1981). Interacting genes in nematode dauer larva formation. *Nature* **290**, 668-671.

Rogalski, T. M., Williams, B. D., Mullen, G. P. and Moerman, D. G. (1993). Products of the *unc-52* gene in *Caenorhabditis elegans* are homologous to the core protein of the mammalian basement membrane heparan sulfate proteoglycan. *Genes Dev.* **7**, 1471-1484.

Serafini, T., Kennedy, T. E., Galko, M. J., Mirzayan, C., Jessell, T. M. and Tessier-Lavigne, M. (1994). The netrins define a family of axon outgrowth-promoting proteins homologous to C. elegans UNC-6. *Cell* **78**, 409-424.

Seydoux, G. and Greenwald, I. (1989). Cell autonomy of *lin-12* function in a cell fate decision in *C. elegans. Cell* **57**, 1237-1245.

Simske, J. S. and Kim, S. K. (1995). Sequential signalling during *Caenorhabditis elegans* vulval induction. *Nature* **375**, 142-146.

Spike, C. A., Davies, A. G., Shaw, J. E. and Herman, R. K. (2002). MEC-8 regulates alternative splicing of *unc-52* transcripts in *C. elegans* hypodermal cells. *Development* **129**, 4999-5008.

Stinchcomb, D. T., Shaw, J. E., Carr, S. H. and Hirsh, D. (1985). Extrachromosomal DNA transformation of *Caenorhabditis elegans. Mol. Cell. Biol.* **5**, 3484-3496.

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

Wadsworth, W. G., Bhatt, H. and Hedgecock, E. M. (1996). Neuroglia and pioneer neurons express UNC-6 to provide global and local netrin cues for guiding migrations in C. elegans. *Neuron* **16**, 35-46.

Wang, J., Tokarz, R. and Savage-Dunn, C. (2002). The expression of TGFβ signal transducers in the hypodermis regulates body size in *C. elegans*. *Development* **129**, 4989-4998.

Williams, B. D. and Waterston, R. H. (1994). Genes critical for muscle development and function in *Caenorhabditis elegans* identified through lethal mutations. *J. Cell Biol.* **124**, 475-490.

Wolf, F. W., Hung, M.-S., Wightman, B., Way, J. and Garriga, G. (1998). *vab-8* is a key regulator of posteriorly directed migrations in *C. elegans* and encodes a novel protein with kinesin motor similarity. *Neuron* **20**, 655-666.

Yochem, J., Sundaram, M. and Han, M. (1997). Ras is required for a limited number of cell fates and not for general proliferation in *Caenorhabditis elegans. Mol. Cell. Biol.* **17**, 2716-2722.

Yochem, J., Gu, T. and Han, M. (1998). A new marker for mosaic analysis in *Caenorhabditis elegans* indicates a fusion between hyp6 and hyp7, two major components of the hypodermis. *Genetics* **149**, 1323-1334.

Yochem, J., Sundaram, M. and Bucher, E. A. (2000). Mosaic analysis in *Caenorhabditis elegans. Methods Mol. Biol.* **135**, 447-462.

Zhen, M. and Jin, Y. (1999). The liprin protein SYD-2 regulates the differentiation of presynaptic termini in *C. elegans. Nature* **401**, 371-375.

Zhen, M., Huang, X., Bamber, B. and Jin, Y. (2000). Regulation of presynaptic terminal organization by *C. elegans* RPM-1, a putative guanine nucleotide exchanger with a RING-H2 finger domain. *Neuron* **26**, 331-343.