Caenorhabditis elegans as an Experimental Organism

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Genetic analysis in the small nematode worm *Caenorhabditis elegans* has elucidated the mechanisms of many basic biological processes.

Secondary article

Article Contents

Introduction

The nematode worm *Caenorhabditis elegans* has been the subject of intensive molecular and genetic analysis. This simple metazoan animal was selected for two practical reasons: first, it is very easy to do *C. elegans* genetics, and second, *C. elegans* is well suited for electron microscopic analysis. Since the pioneering work of Sydney Brenner in the late 1960s, several hundred laboratories around the world now study diverse fields of biology using *C. elegans*. *C. elegans* is the only animal for which the entire cell lineage and synaptic connectivity have been determined, and was the first metazoan genome to be completely sequenced. Studies in *C. elegans* have contributed to our understanding of many biological processes such as signal transduction, embryogenesis, neural function, programmed cell death and ageing. (***see*** Brenner, Sydney.) (***see*** Genome, proteome, and the quest for a full structure–function description of an organism.)

Life Cycle

C. elegans is a nonparasitic terrestrial nematode that normally inhabits soil and feeds on bacteria from decaying plant matter. *C. elegans* is found worldwide; the standard laboratory strain, known as N2, was isolated in Bristol, England in the 1950s. In the laboratory, *C. elegans* is grown on agar plates with *Escherichia coli* as a food source. The anatomy of *C. elegans* is typical of nematodes (**Figure 1**), and has been completely reconstructed from electron micrographs of serial sections. Briefly, the animal is enclosed by a single epidermal layer ('hypodermis') that secretes a collagenous cuticle, providing the hydrostatic skeleton of the body. Neurons are closely associated with the epidermis. Muscles, digestive tract and gonads lie in the internal body cavity or pseudocoelom. A separate excretory system composed of four cells may function in osmoregulation. (***see*** Nematoda (roundworms).) (***see*** Invertebrate body plans.)

Fertilization and embryogenesis

C. elegans has two sexes, a self-fertilizing hermaphrodite (XX karyotype) and male (XO). The hermaphrodite germline makes sperm then oocytes. Hermaphrodite oocytes can be self-fertilized by the hermaphrodite's own sperm or by cross-fertilization by male sperm, which outcompete the hermaphrodite sperm. Fertilized eggs develop within the hermaphrodite for 2–3 hours after fertilization and then are laid via the vulva. (***see*** Reproduction and life cycles in invertebrates.)

The fertilized egg undergoes cleavage to generate a small number of embryonic founder cells that undergo distinctive cleavage patterns. By 300 minutes postfertilization, cell division is largely complete, and differentiation and organogenesis occurs. The gut and the germline are generated from the founder cells E and P_4, respectively. All other founder cells generate a mixture of epidermal, neuronal and mesodermal fates; thus, the classical divisions of ectoderm, mesoderm and endoderm do not correspond to particular cell lineages.

The cell lineage of *C. elegans* is almost completely invariant and has been followed in its entirety from the fertilized egg to the adult using microscopy of living animals (Sulston *et al.*, 1983) (**Figure 2**). This monumental work has led to the most complete description of development available for any metazoan animal. For example, during embryogenesis, precisely 671 cells are generated, of which 113 die via programmed cell

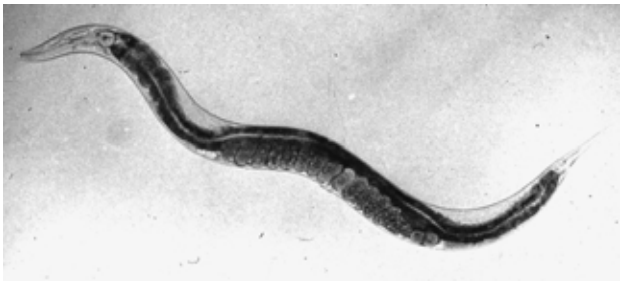

Figure 1 *Caenorhabditis elegans* adult hermaphrodite, bright-field view; anterior to the left and dorsal up; the animal is about 1 mm long.

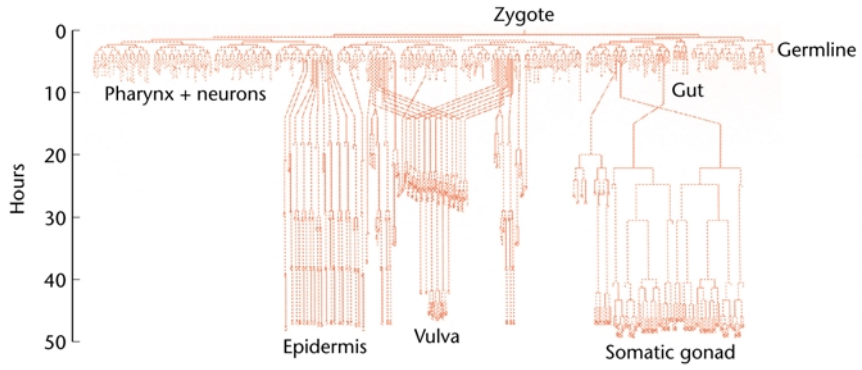

Figure 2 Cell lineage of a *C. elegans* hermaphrodite. The vertical axis represents time postfertilization; each cell is represented as a vertical line, cell divisions by horizontal lines. The lineage origin of some tissues has been indicated. The germline lineage is variable and has not been determined. Many somatic cells later fuse to form multinucleate syncytia (not shown).

death (apoptosis), leaving 558 cells in the L1 larva. (***see*** *Caenorhabditis elegans* embryo: genetic analysis of cell specification.)

Postembryonic development

After 13 hours the embryo hatches to become the first stage (L1) larva. *C. elegans*, like most nematodes, has four larval stages (L1–L4). During postembryonic development the animal increases from 250 μm to 1 mm in length. Cell proliferation occurs in the epidermis throughout larval development, and in the peripheral nervous system (ventral cord) in the L1 and L2 stages. Sexual maturation occurs during the L3 and L4 stages, and involves the growth of the somatic gonad, proliferation of the germline, and formation of the hermaphrodite vulva and male copulatory apparatus (tail). Somatic cell lineages are largely invariant, and result in an hermaphrodite adult containing 959 somatic cell nuclei (as some cells fuse to form syncytia, the number of cells is less than the number of nuclei; **Figure 3a**). (***see*** *Caenorhabditis elegans* embryo: determination of somatic cell fate.) (***see*** *Caenorhabditis elegans*: determination of germ cell fate.)

The four larval stages are separated by moults, in which the previous cuticle is shed and a new cuticle secreted. The L4 larva moults to become an adult. Adult hermaphrodites produce about 300 progeny over the first 4 days of adulthood. The number of progeny is limited by the number of hermaphrodite sperm; males can sire over 2000 cross progeny from a single hermaphrodite.

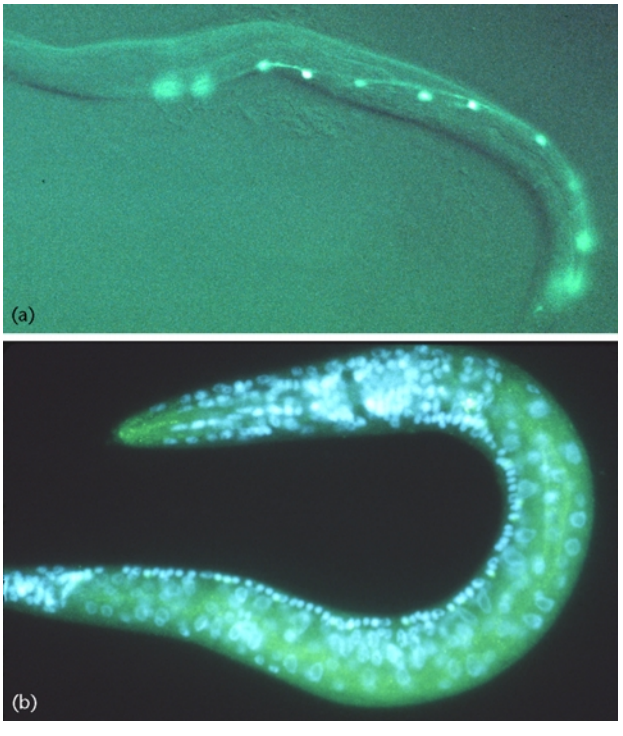

Figure 3 (a) *C. elegans* adult hermaphrodite stained with DAPI to show nuclei; the line of small nuclei along the ventral side of the animal represents ventral cord neurons. (b) GABAergic neurons in a living first larval stage animal, visualized using a transgene in which green fluorescent protein is expressed under the control of the *unc-30* gene. The bright spots are cell bodies in the ventral cord; some fluorescence in cell processes can also be seen.

Heterochronic genes, dauer larvae and ageing

The timing of the different larval stages and the stage-specific cell division patterns within them are controlled by a set of 'heterochronic' genes; mutations in these genes can cause either precocious or retarded developmental changes in many tissues. Genetic analysis showed that two genes, *lin-4* (cell lineage abnormal) and *lin-14*, play key regulatory roles in determining the sequence of early larval stages. The gene *lin-4* encodes a small RNA that represses the gene *lin-14* via complementary base pairing to the *lin-14* 3′ untranslated region. (***see*** Heterochrony.)

C. elegans can enter a diapause state known as the 'dauer larva' in response to environmental cues early in larval development. Low levels of food or crowding (as sensed by

a pheromone) can induce this alternative developmental pathway. The dauer larva form is an alternative L3 stage that displays thicker cuticle and low metabolism. Dauer larvae have a lifespan of over 3 months, and thus may be considered nonageing relative to the 2 week lifespan of continuously developing animals. Dauers can re-enter the normal life cycle in response to food; following re-entry, their lifespan is that of normal worms. (***see*** *Caenorhabditis elegans* culture.)

Genetic analysis has identified many genes required for normal dauer development. The signals that trigger dauer development are sensed by sensory cells in the head. Reception of the signal triggers neurosecretory pathways that involve both transforming growth factor β (TGFβ)-like signals and the insulin receptor-like protein DAF-2 (Kimura *et al*., 1997). These pathways in turn cause dauer-specific alterations in cuticle structure and behaviour. Mutations in some dauer pathway genes can cause longevity without invoking other aspects of the dauer state, suggesting that ageing is under specific genetic control. (***see*** Signal transduction: overview.) (***see*** Ageing genes: gerontogenes.)

Genetics and genome

C. elegans was chosen for study primarily for its simple genetics, and genetic analysis has been the main tool used in studying *C. elegans* biology. *C. elegans* is well suited for genetic analysis for several reasons. First, it is easy to propagate in the laboratory. Because hermaphrodites reproduce by selfing, stocks can be maintained without setting up crosses. Second, it reproduces rapidly (3.5-day life cycle) and prolifically. A third unique advantage is that the animals can be preserved cryogenically, allowing stable long-term storage of thousands of mutant stocks. A final advantage is that all *C. elegans* genetic studies use a single defined wild-type stock, derived from that used by Brenner. Mutants can be induced by chemical mutagens such as ethyl methane sulfonate (EMS), by irradiation or by transposon insertion. (***see*** Experimental organisms used in genetics.) (***see*** Delete 98.)

C. elegans genes defined by mutation are named according to their mutant phenotype, for example, mutations in *unc* genes cause uncoordinated phenotypes. Within such phenotypic classes genes are numbered in order of discovery, thus *unc-1*, *unc-2*, etc. Mutations are named using a prefix for the laboratory in which they were isolated and a number, thus *e1* is the first mutation isolated in the laboratory in Cambridge, England. Gene products are designated in uppercase, e.g. the UNC-1 protein. (***see*** Mutagenesis.)

The nuclear genome of *C. elegans* comprises about 100 million base pairs of DNA, arranged in five autosomes (I–V) and one sex chromosome (X). This is one of the smallest genomes known for any animal. The complete sequencing of the *C. elegans* genome (*C. elegans* Sequencing Consortium, 1998) allowed the first glimpse of the entire set of genes required by a metazoan animal. A major finding is that the number of genes (about 19 000) is much higher than that expected from genetic analysis, suggesting that many genes play redundant or subtle roles, at least under laboratory conditions. About 20% of *C. elegans* genes are shared with those of the yeast *Saccharomyces cerevisiae* and form a common set of 'core' genes, required for basic metabolic and cellular functions (Chervitz *et al*., 1998). Of the other 80% of genes, many are conserved between all multicellular animals, while others are specific to nematodes. (***see*** The promise of whole genome sequencing.) (***see*** Genome catalogue.)

Gene structure and processing in *C. elegans* are similar to those of other animals, with two exceptions. First, many *C. elegans* messenger RNAs (mRNAs) undergo '*trans*-splicing', in which a short 'leader' RNA derived from an unlinked locus is spliced to the 5′ end of nascent messages. *Trans*-splicing occurs in other nematodes, yet its function remains obscure. Second, some *C. elegans* genes are organized into operons, that is, clusters of two to three genes transcribed from a single promoter region; the transcripts from such clusters are processed into separate mRNAs by cleavage. Some operons comprise functionally related genes, suggesting that the operon organization allows coordinate regulation. (***see*** Messenger RNA in eukaryotes.)

The analysis of the enormous amount of *C. elegans* genomic data has been made feasible by the development of two reverse genetic techniques. The first, RNA interference (RNAi) is a recent discovery, and exploits the ability of injected double-stranded RNA encoding the gene of choice to transiently inhibit the endogenous gene, and thus induce a phenocopy (Fire *et al*., 1998). The mechanism by which RNAi operates is under investigation, but at present it provides a rapid means of testing function of genes defined by sequence. The second approach is to make deletion mutations in genes using standard mutagenesis treatments. Large libraries of mutagenized worms can be made and screened using polymerase chain reactions (PCRs) with primers flanking the gene of interest to identify deletions in that gene. Individual mutants can be recovered by subdividing the library and re-screening, potentially allowing recovery of knockout mutations in any gene. (***see*** Antisense RNA.) (***see*** Mutagenesis: site-specific.)

Sex Determination

The sex of *C. elegans* is determined by the ratio of the number of X-chromosomes to the number of autosomes. Experiments in which the number of X-chromosomes or autosomes was altered showed that it is not the absolute

number of X-chromosomes that is assessed, but rather the ratio of X-chromosomes to autosome sets (the 'X:A ratio'). The activities of a small number of X-linked genes form the 'numerator' of the ratio; the nature of the autosomal 'denominator' is unknown, and may not involve discrete genetic loci. The readout of the X:A ratio is the activity of a single gene, *xol-1*, which acts as a binary switch gene controlling the choice of both sexual fate and dosage compensation mode. X-linked numerator genes are thought to repress *xol-1* function in a dose-dependent manner. The activity of *xol-1* is set by the X:A ratio early in zygotic development. *xol-1* in turn regulates three *sdc* genes. The *sdc* genes function as coordinate regulators of two distinct sets of genes: those involved in sex determination and those involved in dosage compensation. (***see*** Sex determination.) (***see*** *Caenorhabditis elegans*: determination of germ cell fate.)

Dosage compensation is the process by which the expression levels of X-linked genes are equalized between XX and XO animals. In *C. elegans* this is achieved by repression of X-linked gene transcription in hermaphrodites. Repression requires a large multiprotein complex that in XX animals is targeted to the X-chromosome. The sex-specificity of the X-chromosomal association results from the SDC-2 protein, which is only expressed in XX animals and which is sufficient to assemble a dosage compensation complex on the X. The *cis* elements that distinguish the X-chromosome from autosomes have not been defined. (***see*** Repression mechanism.)

Sex determination involves an intercellular signalling pathway, the function of which may be to ensure that all cells in the animal adopt the same sexual fate. The terminal global regulator of sexual fate is the TRA-1 transcriptional regulator, which acts cell-autonomously to activate hermaphrodite fates and repress male fates. TRA-1 probably functions via a number of downstream genes, one of which (*mab-3*) is related to a *Drosophila* sex determination gene, doublesex (Raymond *et al*., 1998). Although both *Drosophila* and *C. elegans* determine fate by formally analogous means (the X:A ratio regulates a hierarchy of global regulatory genes), the only similarity at the molecular level between the two species is at the level of these terminal effector genes. This may imply that the hierarchy of regulatory genes in sex determination is evolving rapidly. (***see*** Transcriptional gene regulation in eukaryotes.) (***see*** *Drosophila* gametogenesis.) (***see*** Chromosome function: sex differences.)

Specification of Embryonic Cell Fate

Although early embryologists viewed nematode embryos as 'mosaic' embryos, in which fates were assigned without cell–cell interactions, current work has shown that most embryonic cell fates are assigned by an interplay of cell-autonomous mechanisms and cell–cell interactions.

The first difference in cell fate occurs at the very first mitosis, in which the zygote divides asymmetrically to give rise to a large anterior daughter cell, AB and a smaller posterior cell, P_1. The asymmetry of the first division is foreshadowed by the asymmetric organization of the zygote, which in turn is triggered by the entry of sperm at fertilization. The unfertilized oocyte does not appear to be polarized; the site of sperm entry defines the future posterior pole of the embryo. (***see*** *Caenorhabditis elegans* embryo: establishment of asymmetry.)

The transcription of the zygotic genome does not begin until at least the 8–10-cell stage, and thus the fates of the early embryonic blastomeres must be specified by maternally provided gene products. Extensive screens for maternal-effect mutations have identified many genes required for early embryogenesis. The *par* genes function to create the asymmetry of the zygote. The fates of anterior blastomeres require signalling from posterior blastomeres via the GLP-1 receptor. Other proteins, such as PIE-1 and SKN-1, play cell-autonomous roles in specifying specific blastomere fates.

Little is known of how the fates of these early blastomeres are translated into the final pattern of the embryo. *C. elegans*, like other animals, possesses a cluster of homeobox genes that play roles in patterning the anteroposterior axis; however these genes play only a small role in embryogenesis. (***see*** Evolutionary developmental biology: homologous regulatory genes and processes.) (***see*** Evolutionary developmental biology: *Hox* gene evolution.)

Nervous System

General organization

The nervous system of the *C. elegans* hermaphrodite is composed of 302 neurons, classified into 118 classes based on morphology. The entire synaptic connectivity has been reconstructed from electron micrographs of serial sections (White *et al*., 1986). (***see*** *Caenorhabditis elegans* nervous system.)

Neuronal cell bodies form ganglia located in the head and tail, and nerve processes run as bundles adjacent to the epidermis. The major neuropils are the circumpharyngeal nerve ring and the longitudinal ventral and dorsal nerve cords. Twenty neurons in the pharynx control pharyngeal muscle movements, and are linked to the rest of the nervous system through two neurons.

The pattern of synaptic connectivity is remarkably similar between wild-type animals. Nerve processes are stereotypically arranged within bundles. Chemical synapses occur *en passant* between adjacent nerve processes. As in other nematodes, neurons do not send processes onto muscles, but rather muscles send processes, known as

muscle arms, that contact neural processes. The nervous system has about 5000 chemical synapses, 2000 neuromuscular junctions and 700 gap junctions. (***see*** Synapses.) (***see*** Cell junctions.)

General features of neurons and support cells

Most *C. elegans* neurons are monopolar or dipolar and have simple unbranched processes. In many neurons a single neuronal process can have both presynaptic and postsynaptic zones, and thus does not fit the classical distinction between axon and dendrite. Within such processes the components for presynaptic and postsynaptic specializations are spatially segregated. (***see*** Neurons.)

Electrophysiological properties of *C. elegans* neurons have not been studied until recently because of their small size. A classical Na^+ action potential has not been detected in *C. elegans* neurons, although they exhibit voltage-dependent membrane currents that can be accounted for by K^+ and Ca^{2+} ion flows (Goodman *et al*., 1998). *C. elegans* neurons are not myelinated, and most do not have support cells. The exceptions are some specialized sensory neurons that are supported by two types of glial-like cell: sheath cells that enclose the sensory neuron endings, and socket cells that form an interface with the epidermis.

Development of the nervous system

Neuronal precursor cells derive from ectodermal precursor cells that also generate the epidermis. The fates of individual neurons are primarily determined by cell lineage and final position; a few neurons undergo long-range migrations. (***see*** *Caenorhabditis elegans* neural development.) (***see*** Neuronal migration.)

C. elegans is an ideal organism to study neural growth cone migration because of its transparent body and the small number of neurons. Individual neurons can be seen in living animals with the use of green fluorescent protein (**Figure 3b**). Global guidance systems for growth cones have been identified that act along the longitudinal and dorsoventral axes of the animal. Dorsoventral axon guidance is controlled by the secreted protein UNC-6 and its receptors UNC-5 and UNC-40. These proteins are highly conserved in evolution, and their homologues, known as netrins and netrin receptors, control axon guidance in the vertebrate spinal cord. (***see*** Axon growth.) (***see*** Axon guidance.)

Neural function

C. elegans neurons use the same neurotransmitters as other animals, and the machinery of neurotransmitter release and reception is also similar to those of vertebrates. The functions of individual neurons have been studied by laser microsurgery and genetic analysis. Because *C. elegans* can reproduce by self-fertilization, most of the nervous system is dispensible under laboratory conditions, allowing genetic analysis of many neural functions. (***see*** Neurotransmitters.)

C. elegans can detect a wide range of sensory stimuli. Chemosensory neurons can discriminate a variety of water-soluble and volatile chemicals, including the signals that regulate dauer formation (Bargmann *et al*., 1993). *C. elegans* avoids high osmolarity, and can detect differences in temperature as small as 0.1°C (Hedgecock and Russell, 1975). (***see*** Chemosensory systems.)

Muscle movements are controlled by motor neurons. The sinusoidal locomotion pattern is generated as the result of a balance between excitatory and inhibitory inputs from the ventral cord neurons. Other motor behaviours such as pharyngeal pumping, defecation and egg-laying may involve neural modulation of intrinsic myogenic rhythms. (***see*** Locomotion in invertebrates.)

Analysis of behaviour in *C. elegans* has elucidated many molecular mechanisms of neural function. One example is the choice between social or solitary behaviour, which can be regulated by a single gene (de Bono and Bargmann, 1998). Another elegant example is provided by the mechanosensory or touch response. Five mechanosensory cells mediate the responses to gentle touch: animals move backwards when gentle touch is applied to anterior mechanoreceptors, and forward when posterior receptors are stimulated. Seventeen genes are specifically required for touch sensation. MEC-4 and MEC-10 encode stretch-sensitive membrane receptors. The stomatin-like protein MEC-2 may link the membrane receptors to intracellular specialized microtubules encoded by *mec-7* and *mec-12*, which transmit the stretch signals to the rest of the neuron. The expression of all these genes may be regulated at the transcriptional level by the MEC-3 and UNC-86 proteins. (***see*** Touch.) (***see*** Sensory transduction mechanisms.) (***see*** Sensory systems in invertebrates.)

Morphogenesis

Morphogenesis of the embryo

The early embryo is a mass of morphologically similar cells. The first morphogenetic movements are in gastrulation, in which germline, gut and pharyngeal precursors ingress into the interior, leaving a prominent ventral cleft that is filled in by the short-range movements of many neuroblasts. Gut and pharynx precursors arrange themselves into a central cylinder.

Epidermal cells are born in the dorsal surface of the embryo, and differentiate into epithelial cells that form a dorsal sheet. This sheet undergoes epiboly, driven by changes in the shapes of the epithelial cells, and expands to enclose the embryo. Four cells at the anterior end of the

sheet extend ventrally to meet up with their contralateral counterparts at the ventral midline. Following this the posterior epidermal cells close up by a purse-string mechanism. The epidermis of the extreme anterior appears to develop by a different mechanism. After the epidermis has completely enclosed the embryo, epidermal cells contract around their circumference, putting pressure on internal cells. This circumferential contraction is actin-mediated and drives the process of elongation, in which the long axis of the embryo expands 4-fold. (***see*** Secondary induction: overview.)

Morphogenesis of the cuticle

The nematode cuticle is a complex and highly organized extracellular matrix that is secreted by the epidermis and which determines the overall morphology of the animal. Mutations perturbing cuticle development cause gross phenotypic defects, known as 'dumpy', 'roller', 'long' and 'blistered'. Some genes affected by such mutations encode cuticular collagens, while others encode proteases required for the assembly of cuticular components. The morphogenesis of the cuticle remains very poorly understood. (***see*** Extracellular matrix.)

Vulval Development

The development of the hermaphrodite vulva is a classic example of an inductive cell–cell interaction, and has been subject to intense genetic and molecular analysis. This analysis has shown that vulval induction requires the integration of three different cell–cell interactions (**Figure 4**): (***see*** *Caenorhabditis elegans* vulval induction.)

1. A signal from the gonadal anchor cell that induces vulval precursor cells to adopt vulval fates, mediated by a receptor tyrosine kinase/ras/MAP kinase cascade.
2. Lateral inhibitory signals between prospective vulval precursor cells, mediated by the LIN-12 receptor.
3. A repressive signal from surrounding epidermal cells, requiring the LIN-15 proteins.

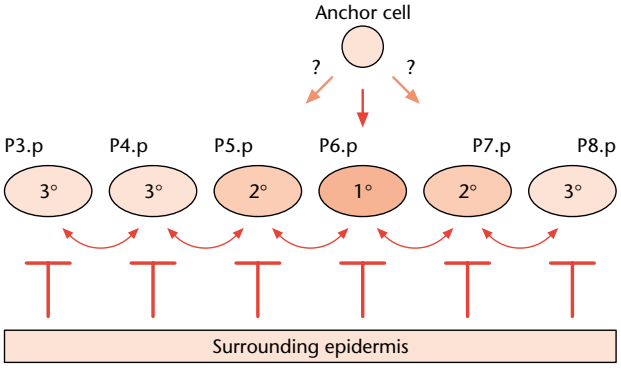

Figure 4 Overview of the cell signals occurring during vulval development.

The vulva develops from six epidermal vulval precursor cells (VPCs) (**Figure 4**). During the L3 and L4 stages the three central VPCs (P5.p, P6.p and P7.p) adopt fates known as 2°, 1° and 2° respectively and divide to generate the 22 cells of the vulva; three outer VPCs adopt a nonvulval (3°) fate. Laser killing experiments have shown that the six VPCs are initially equivalent in fate – that is, any VPC can potentially adopt the 1°, 2° or 3° fate. The six VPCs thus form an 'equivalence group' or developmental field, in which cell fate is assigned by position rather than ancestry. During the L2 stage the gonadal anchor cell induces the three closest VPCs to adopt vulval fates. The P6.p cell is normally closest to the anchor cell, and becomes the 1° fate; its neighbours adopt 2° fates.

Genetic analysis of mutants in which vulval induction was defective or constitutive identified the pathway responsible for the anchor cell signal. The anchor cell produces a TGFα-like protein, LIN-3, that acts as a ligand for the LET-23 receptor tyrosine kinase, expressed on all VPCs. Activation of the LET-23 receptor triggers a cytoplasmic ras/MAP kinase signalling pathway in the VPCs that results in their adopting vulval fates. The ras/MAP kinase cascade is a highly conserved signalling module used throughout evolution. (***see*** Transforming growth factor beta (TGF beta).) (***see*** Signal transduction: overview.)

Cell–cell interactions between VPCs are also required for the normal pattern of cell fates, in which a single 1° cell is flanked by two 2° cells. Isolated VPCs always adopt the 1° fate, yet if two VPCs are in contact, only one will adopt the 1° fate. The LIN-12 protein, similar to *Drosophila* Notch, mediates this lateral inhibitory signal. (***see*** *Drosophila* patterning: Delta-Notch interactions.)

A third pathway normally functions to inhibit vulval induction. *lin-15* mutants display excessive vulval induction. Genetic mosaic analysis suggested that *lin-15* may be required outside of the VPCs, possibly in the surrounding epidermis. The LIN-3/LET-23 signal is thus necessary to overcome this repressive effect of surrounding epidermis.

How is the normal highly reproducible pattern of vulval fates generated? Two models have been proposed: the 'graded signal' model, and the 'sequential induction' model. In the first model, the pattern of 2°–1°–2° fates arises as the result of different levels of a graded signal from the anchor cell; high levels of LIN-3 induce VPCs to adopt the 1° fate, intermediate levels of LIN-3 induce 2° fates; low levels permit the default 3° fate to be adopted. In the sequential induction model, LIN-3 only induces the 1° fate; the alternating pattern of 1° and 2° fates results from the LIN-12-dependent lateral inhibition of flanking cells. In normal development both mechanisms may operate to promote normal vulval development. (***see*** *Caenorhabditis elegans* embryo: genetic analysis of cell specification.)

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