Neurogenetics, in *Caenorhabditis elegans*

K J Yook and E M Jorgensen

doi: 10.1006/rwgn.2001.0892

Yook, K J
Department of Biology, University of Utah, Salt Lake City, UT 84112, USA

Jorgensen, E M
Department of Biology, University of Utah, Salt Lake City, UT 84112, USA

Overview

The human brain is an extraordinarily complex and beautiful organ. It has one hundred billion neurons and an inestimable number of synaptic connections. Since there are very few opportunities to ethically test experimental hypotheses on the human brain, we rely on model organisms to understand how neurons work in humans. Such an organism, the nematode *Caenorhabditis elegans*, with a total of only 302 neurons, has contributed substantially to our understanding of the development and function of the human nervous system.

Using a Soil Nematode to Study Neurobiology

In 1974 Sydney Brenner introduced *C. elegans* as a genetic model organism that could be used to elucidate the molecular nature of the nervous system. The main advantages of *C. elegans* as a model organism for the study of genetic pathways in general include the simplicity of worm maintenance, the ease of isolating mutants, and the availability of molecular reagents for gene analysis. In addition, the nematode possesses a number of features that make it particularly well suited for the study of its nervous system. First, the number and positions of the neurons are invariant between individuals (**Figure 1**). This feature allowed researchers to reconstruct the connectivity of the nervous system from serial electron micrographs. Second, the worm is transparent, so individual cells can be identified using a light microscope and can be killed by firing pulses from a laser microbeam into the cell. By using cell-ablation studies, researchers can infer the role that each cell plays in the behavior of the worm. Third, the nervous system is largely nonessential under laboratory conditions. The worm does not need a functional nervous system to eat. This nematode ingests bacteria using a muscular pump called the pharynx, and the pharynx will pump even in the absence of neuronal input. In addition, *C. elegans* is a self-fertilizing hermaphrodite, so it does not need a nervous system to search for mates and to reproduce. However, laser ablation studies have demonstrated that there are two neurons, M4 and CAN, which when ablated will cause the animal to die. The M4 motor neuron regulates the peristaltic movements of the pharynx and the CAN neuron is required for osmoregulation.

Genetic Dissection of Neuronal Development

The development of the nervous system can be divided into four steps, the determination of neuronal cell fates, the specification of cell identity, the outgrowth of axons, and the differentiation of synaptic connectivity. In *C. elegans* the cell lineage is invariant, that is, every cell division generates two daughter cells and the fates of the daughter cells of each division are largely fixed. Early divisions generate six founder cells (**Figure 2**); individual founder cells generally give rise to one type of tissue. For example, all daughter cells of the P4 founder cell are germ cells, all daughter cells of the E founder cell are intestinal cells, and the D founder cell only gives rise to muscle cells. Thus, tissue-type determinants are likely to be expressed very early in these lineages. On the other hand, neuronal tissues are derived from AB, MS, and C founder cells. Thus, there is no founder cell that gives rise to only neuronal tissue. In fact, neuronal cell fates are frequently segregated in the terminal cell division of lineages that also generate other ectodermal derivatives such as epidermal or glial cells (**Figure 2**). Thus, neuronal determinants are likely to be expressed late during embryonic cell divisions.

How then does a cell adopt a neuronal cell fate? The mechanisms that determine neuronal cell fate seem to be conserved among *C. elegans*, *Drosophila*, and mice. Specifically, proneural genes related to members of the basic helix-loop-helix (bHLH) family of transcription factors function in neurogenesis in *C. elegans*. For example, *lin-32*, an ortholog of the *Drosophila atonal* gene, is required for the generation of many sensory cells. In *lin-32* mutants, these cells become epidermal cells instead. Sensory cell lineages expressing LIN-32 are then modified by the *unc-86* gene. UNC-86 is a member of the POU homeodomain family of transcription factors. UNC-86 expression prevents a daughter cell from adopting its mother's fate. For example, the Q neuroblast divides to generate an anterior daughter which will eventually

produce a sensory cell called AQR. The *unc-86* gene is expressed in the posterior daughter, and this lineage will eventually generate a mechanosensory neuron called AVM. In *unc-86* mutants, the posterior daughter retains the Q neuroblast identity and continues to generate an anterior AQR progenitor cell and a posterior Q neuroblast. In the wild-type, the UNC-86 protein remains expressed in a subset of these cells, after cell divisions are complete, and acquires a new function. In particular, it is required to specify the correct cell identity in conjunction with other transcription factors. One such partner, MEC-3, a LIM homeodomain transcription factor, is required for the specification of six mechanosensory neurons. MEC-3 expression in these cells activates genes required for mechanosensation cell function, such as the gene encoding a specific tubulin required for mechanosensory neurites. In the absence of *unc-86* expression, there are no mechanosensory precursors, and also no *mec-3* expression. In the absence of *mec-3* expression, mechanosensory cells are produced but cannot function as mechanosensory neurons. These POU and LIM homeodomain transcription factors were originally identified in *C. elegans* and have since defined new families of transcription factors found in other invertebrates and vertebrates.

After acquiring a cell fate, a neuron must send out axons to form connections with other neurons. The direction of axonal outgrowth is determined by chemoattractants and chemorepellents. In some cases, this can be the same molecule. For example, responses to the secreted netrin/UNC-6 protein is mediated by the UNC-40 and UNC-5 receptors. The UNC-40 receptor causes axons to be attracted to secreted UNC-6 molecules, whereas axons expressing both the UNC-40 and the UNC-5 receptors are repelled by UNC-6. Neurons of the head ganglia appear to rely on partially redundant gradients produced by three signal transduction pathways, which play similar roles in *Drosophila* and vertebrates: the UNC-6 pathway, the Robo/Slit pathway and the Eph pathway. For example, axon migrations of the amphid sensory neurons require the parallel action of all three of these guidance systems. Worms simultaneously mutated in any combination of two of the processes result in stronger mutant phenotypes than worms mutated in only one process.

The growth cone converts these guidance cues into changes in the cytoskeleton which will redirect its trajectory. The conversion of these cues is mediated through signaling pathways made up of Rho-family guanosine triphosphatase (GTPase) proteins. These proteins exist in an inactive guanosine diphosphate (GDP)-bound state or an active guanosine triphosphate (GTP)-bound state. Switching from one state to the other requires a guanine nucleotide exchange factor (GEF) which exchanges GTP for GDP. Mutations in GEFs such as UNC-73, a homolog of the human Trio, result in abnormal axonal guidance and premature termination of axons. There are several Rho-family GTPases which are possible targets of UNC-73. Mutations in any one of these produces weak defects in migration. For example, mutations in the MIG-2 GTPase result in migration defects in only the Q neuroblasts. However, mutations in multiple GTPases produce severe phenotypes, reminiscent of *unc-73* defects. It is likely that these GTPases regulate growth cone activity via the WASP actin nucleation and polymerization proteins. UNC-34, a homolog of the *Drosophila*-enabled and mammalian Mena proteins appears to act in parallel with these pathways. Mutations in *unc-34* result in partially disrupted cell migrations and axon outgrowth. However, loss of WASP expression in an *unc-34* null background results in synthetic lethality. Thus, multiple pathways converge on the regulation of actin dynamics during cell and axon migrations.

After outgrowth, neurons need to activate genes specific to neuronal classes and to form the proper connections with their target cells. For example, UNC-30 and UNC-4, members of the orthodenticle class of homeodomain transcription factors, are required to specify a subset of GABA and acetylcholine neurons, respectively. *unc-30* is required to specify D-type GABA neurons of the ventral nerve cord. UNC-30 controls the expression of GABA-specific genes such as *unc-25* and *unc-47* in these D neurons. *unc-25* encodes glutamic acid decarboxylase (GAD), the biosynthetic enzyme required to synthesize the neurotransmitter GABA, and *unc-47* encodes the GABA vesicular transporter, required to load GABA into synaptic vesicles. In addition, UNC-30 is required to specify the correct synaptic connections for the GABA neurons. Similar to *unc-30*, *unc-4* is required to specify the fate of the A-type neurons, another class of ventral cord neurons. Specifically, *unc-4* is required for VA motor neurons to form synaptic connections with the correct set of interneurons. In worms mutant for *unc-4*, the A-type VA neuron is functionally transformed into the B-type VB neuron. Since VA motor neurons control backward movement, the loss of UNC-4 activity results in an animal that is unable to move backward.

Genetic Dissection of Neurotransmission

Communication between neurons is mediated by neurotransmitters. When a neuron is depolarized, calcium enters the neuron via voltage-sensitive calcium

channels; calcium then causes the synaptic vesicles to fuse with the plasma membrane and release neurotransmitter into the synaptic cleft. Once the vesicle has released its contents, the vesicle and its associated proteins are retrieved from the membrane and prepared for another cycle of fusion. Biochemical studies of yeast and mammalian cells have identified many of the proteins required for vesicle dynamics. Genetic studies in *C. elegans* have identified additional components and have also elucidated the functions of these proteins at the synapse. The proteins uncovered in these genetic studies can be divided into two categories: those required for a specific neurotransmitter type; and those required for the functions of all synapses, for example, proteins required for synaptic vesicle kinetics. We will emphasize those components which were discovered in *C. elegans*.

The genes required for the two neurotransmitters which function at neuromuscular junctions, GABA and acetylcholine, are the most well studied. The behavioral phenotype associated with the loss of GABA neurons was determined by laser ablation. Screens for mutants which mimicked the loss of GABA identified six genes required for GABA function. Three of the genes identified in these screens were *unc-25*, the biosynthetic enzyme for synthesizing GABA, *unc-47*, the vesicular GABA transporter, and *unc-49*, the GABA receptor. Screens assaying for altered levels of acetylcholine led to the identification of *cha-1*, the biosynthetic enzyme required for acetylcholine synthesis and *unc-17*, the vesicular acetylcholine transporter. The discovery of the vesicular transporters for GABA and acetylcholine led to their subsequent identification in vertebrates.

Proteins required for the functioning of all synapses include proteins required to transport materials from the cell body to the synapse and proteins required to dock, fuse, and recycle synaptic vesicles at the active zone. The cell body of the neuron is often far from the synapse. To transport synaptic vesicle precursors to the synapse, neurons use a kinesin-like motor protein encoded by *unc-104*. Worms with reduced function of *unc-104* accumulate vesicle precursors in the cell body. Vertebrate homologs have been discovered and comprise the KIF1 family of kinesins.

The fusion of synaptic vesicles with the plasma membrane requires the formation of the SNARE complex. The SNARE complex is comprised of three proteins: synta-xin/UNC-64, SNAP-25/RIC-4, and synapto-brevin/SNB-1. Both syntaxin and SNAP-25 are associated with the plasma membrane, whereas synaptobrevin is an integral membrane protein of the synaptic vesicle. These three proteins form a helical bundle that pulls the vesicle close to the plasma membrane at the active zone, which is thought to induce membrane fusion. The SNARE complex is required unquestionably for vesicle fusion. Null mutations in syntaxin and synaptobrevin abolish synaptic vesicle release. Two proteins implicated in regulating the formation of the fusion complex are UNC-18 and UNC-13. The discovery of *unc-18* and *unc-13* in *C. elegans* has led to the identification of homologs in vertebrates. Null mutations in either one of these proteins result in a severe decrease in neurotransmission. Both proteins have been demonstrated to bind to syntaxin/UNC-64. UNC-18 plays both a facilitory and inhibitory role in vesicle fusion. In the absence of UNC-18, there is a severe decrease in the release of synaptic vesicles; thus it must be playing a facilitory role in neurotransmission. In addition, UNC-18 stabilizes syntaxin in a conformation which prevents binding to synaptobrevin, thus UNC-18 inhibits the formation of the SNARE complex and subsequent fusion events. Unlike *unc-18*, *unc-13* only plays a facilitory role in synaptic release. In animals with reduced UNC-13 function, synaptic release is abolished. Since vesicles dock normally to the plasma membrane in these mutants, *unc-13* is not required for vesicle docking. Rather, *unc-13* is required for the priming step that makes synaptic vesicles competent for fusion. Moreover, this protein is the target of modulatory cascades that increase neurotransmission. Specifically, UNC-13 acts in a G-protein signaling pathway downstream of Gq alpha/EGL-30 and phospholipase C/EGL-8. Activation of these modulatory pathways stimulates the association of UNC-13 with the plasma membrane and a concomitant increase in vesicle priming. Once the vesicle is primed, the actual exocytosis fusion event is triggered by calcium influx. The Ca^{2+} sensor is likely to be synapto-tag-min/SNT-1. Synaptotagmin is an integral membrane protein of the synaptic vesicle, contains two C-2 Ca^{2+}-binding domains, and has been demonstrated to bind to the SNARE complex. Absence of synaptotagmin causes a loss of calcium-dependent release in mice.

Surprisingly, synaptotagmin plays a dual role in the synaptic vesicle cycle. Once exocytosis is complete, the synaptic vesicle and its associated proteins are retrieved from the plasma membrane through endocytosis. Endocytosis is mediated by the formation of a clathrin cage, which buds membrane into the cell. Synaptotagmin appears to be required to recruit clathrin adapter proteins to the plasma membrane. In *snt-1* mutants, there is a striking loss of vesicle endocytosis. Another gene, *unc-11*, which encodes an adapter protein called AP180, recruits the synaptic vesicle protein synaptobrevin, in addition to clathrin, to the membrane targeted for endocytosis. When the clathrin coat is assembled around the invaginating vesicle, dynamin, encoded by *dyn-1*, cleaves the

vesicle from the plasma membrane. To complete vesicle recycling, the clathrin coat must be removed. One protein implicated in this process is synaptojanin/UNC-26 a polyphosphoinositide phosphatase which converts phosphatidylinositol-4, 5-bisphosphate (PIP_2) to phosphatidylinositol (PI). Mutations in *unc-26* result in an accumulation of coated vesicles, presumably because the adapter proteins which bind PIP_2 remain attached to synaptic vesicle lipids. In addition, budded but uncleaved vesicles accumulate. Thus, the lipid composition of synaptic membranes plays an important role in regulating progress through endocytosis.

Genetic Dissection of Behavior

Nematodes monitor environmental signals such as odorants, salt concentrations, temperature, and hormones to migrate to favorable conditions and to retreat from unfavorable ones. Sensory neurons mediate either an attractive response or an aversive response to the compounds which they sense. For example, the AWA chemosensory neuron mediates attractive responses. AWA expresses ODR-10, a seven-transmembrane G-protein-coupled chemoreceptor, which detects the volatile compound diacetyl. When a worm senses diacetyl, it will move up a gradient of the compound. However, when *odr-10* was misexpressed in the AWB chemosensory neuron, a neuron which normally mediates aversive responses, the diacetyl became a repellent. Therefore the attractive or aversive nature of the odorant was controlled by the sensory cell rather than by the molecular nature of the output of the receptor.

Worms can change their response to odorants or chemicals in a process called sensory adaptation. Through adaptation, worms become less responsive to particular odorants or tastes when exposed to a stimulus for long periods of time. Adaptation is likely to be a change in the transduction pathway of the receptor rather than the cell, since adaptation to a compound does not affect the responses to other compounds sensed by that same cell. Worms can exhibit long-term changes in their responses to temperature and chemicals also. Plasticity in thermotactic and chemotactic responses has been demonstrated by classical conditioning paradigms built to test associative learning ability. For example, *C. elegans* can associate a specific temperature or ion with the presence of food.

Genetic Dissection of Brain Diseases

In certain circumstances, *C. elegans* can act as a model system for human disorders of the brain. Homologs of genes implicated in human brain diseases have been identified in *C. elegans*. The analysis of these genes will identify the molecular pathways underlying these diseases. For example, *sel-12* was identified in a screen for suppressors of *lin-12* mutants; *lin-12* encodes a signaling molecule involved in cell fate determination. SEL-12 is a transmembrane protein that functions as part of the LIN-12/Notch signaling pathway. SEL-12 is the *C. elegans* homolog of presenilin, a protein implicated in Alzheimer's disease. Since human presenilin can substitute for SEL-12 in the worm, it is probable that presenilin also functions in a Notch signaling pathway in the human brain.

Future

The *C. elegans* genome contains over 19 000 genes; however, only about 2000 have been identified by mutations. The absence of known mutations in these other 17 000 loci might be due to either redundancy or ignorance. First, redundancy has been observed for a number of loci; specifically, a phenotype is only observed when multiple genes are mutated. Second, we are still very naive about the many biochemical processes which are required by an organism and therefore have not yet designed screens capable of revealing them. For example, the *C. elegans* genome contains up to 1000 G-protein-coupled chemoreceptors for which specific functions remain unknown. In the future, clever screens may begin to identify genes for which functions have not yet been assigned.

The *C. elegans* genome, of course, is the most important resource for further study. However, another largely unexplored resource is the completely determined neuronal connectivity of the *C. elegans* nervous system. Electrophysiological techniques have been developed which allow one to record from identified cells in the central nervous system of *C. elegans*. These methods will allow researchers to explore how neural circuits with known connectivities, and with defined molecular components, such as voltage-sensitive ion channels and ligand-gated receptors, function together to generate an electrical and behavioral output.

References

Bargmann CI and Kaplan JM (1998) *Annual Review of Neuroscience* 21: 279–308.

Bargmann CI (1998) *Science* 282: 2028–2033.

Brenner S (1974) *Genetics* 77: 71–94.

Chalfie M and Jorgensen EM (1998) *Trends in Genetics* 14: 506–512.

Mori I (1999) *Annual Review of Genetics* 33: 399– 422.

Riddle DL, Blumenthal T, Meyer BJ and Priess JR (eds) (1997) *C. elegans* vol. II. Plainview, NY: Cold Spring Harbor Laboratory Press

White JG, Southgate E, Thomson JN and Brenner S (1986) *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 314: 1–340.

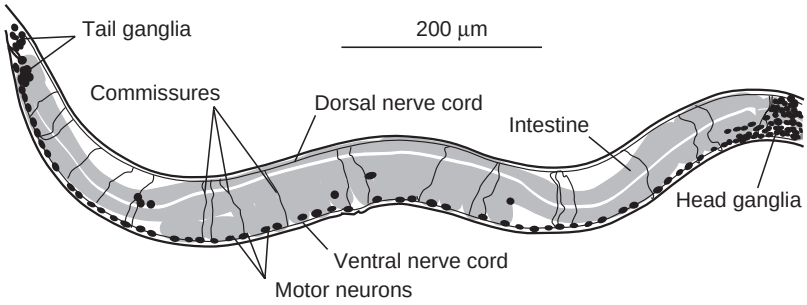

Figure 1 The adult nervous system of *C. elegans*. There are 302 neurons in an adult nematode. Most of the cell bodies of the neurons are found in the head ganglia, in the ventral nerve cord, or in the tail ganglia. Not all axon bundles are shown.

See also: **0152, 0172, 0149**

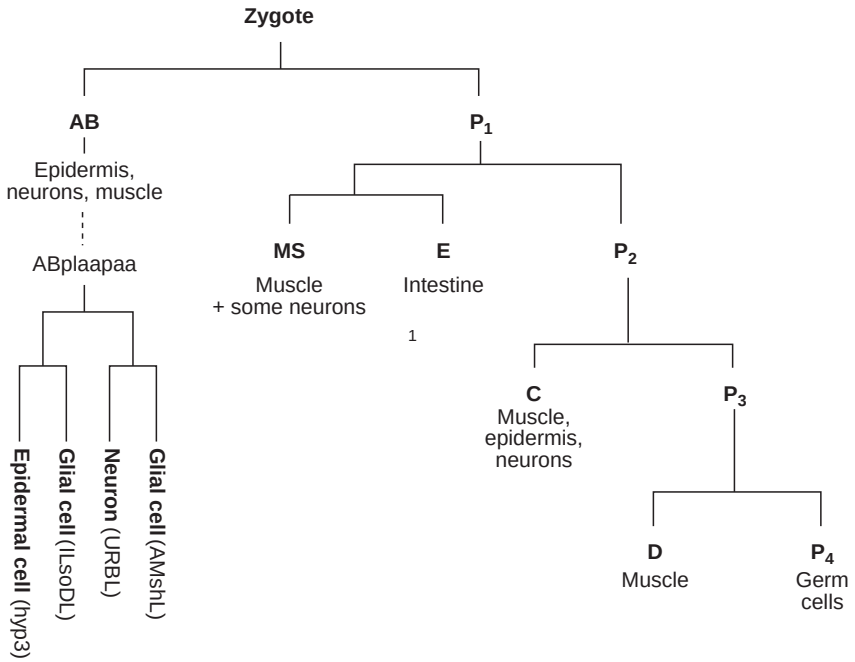

Figure 2 The early cell lineage of *C. elegans*. The founder cells and the tissues which are derived from their descendents are shown (the names of individual cells are noted in parentheses). Horizontal lines represent cell divisions. A very small sublineage from the AB founder cell is shown. The 'a,' 'p,' and 'l' suffixes attached to AB refers to the anterior, posterior, and left daughters, respectively, of the previous cell in the lineage.