Genetics of Egg-Laying in Worms

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Annu. Rev. Genet. 2006. 40:487–509

The *Annual Review of Genetics* is online at http://genet.annualreviews.org

This article's doi: 10.1146/annurev.genet.40.110405.090527

0066-4197/06/1215-0487$20.00

Abstract

Genetic studies of behavior in the nematode *Caenorhabditis elegans* have provided an effective approach to investigate the molecular and cellular basis of nervous system function and development. Among the best studied behaviors is egg-laying, the process by which hermaphrodites deposit developing embryos into the environment. Egg-laying involves a simple motor program involving a small network of motorneurons and specialized smooth muscle cells, which is regulated by a variety of sensory stimuli. Analysis of egg-laying–defective mutants has provided insight into a number of conserved processes in nervous system development, including neurogenesis, cell migration, and synaptic patterning, as well as aspects of excitable cell signal transduction and neuromodulation.

*Erratum (16 Nov. 2006): See online log at http://arjournals.annualreviews.org/errata/genet

Contents

EGG-LAYING AND THE EGG-LAYING CIRCUIT

Along with body locomotion, feeding, defecation, and (in males) copulation, egg-laying represents one of the basic motor outputs of the *Caenorhabditis elegans* nervous system. In part because of the anatomical simplicity of neural circuits hypothesized to control egg-laying, this behavior has been extensively used for the genetic analysis of nervous system function and development. This section provides a brief overview of egg-laying in *C. elegans* and of the neurons and muscle cells that mediate oviposition. Genes affecting the development and function of these cells, and the biological insights gained from their analysis, are discussed in the following sections.

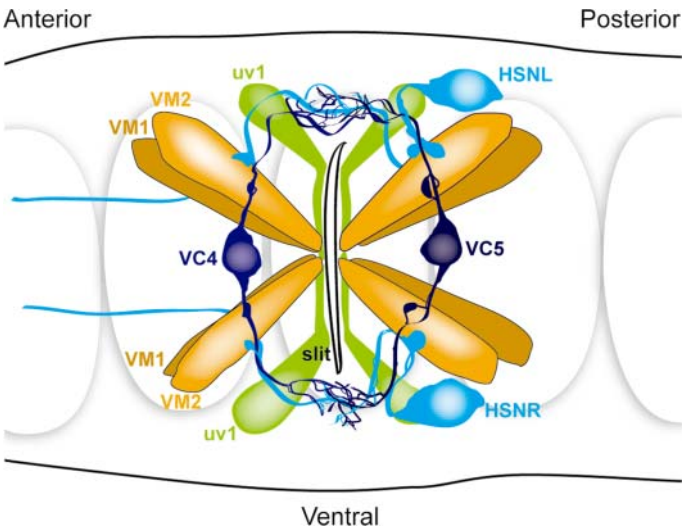

Figure 1

Anatomy of the egg-laying neuromusculature. Shown is a ventral view of the hermaphrodite vulva and cells associated with egg-laying. Inferred functions of individual cells are described in the text.

Reproduction in *C. elegans*

C. elegans hermaphrodites are self-fertile, producing first sperm, which are stored in the spermatheca, and then oocytes. Within the first day of the L4/adult molt, hermaphrodites accumulate fertilized eggs in the uterus; a young adult hermaphrodite will generally have a store of 10–15 eggs in its uterus at any given time. Egg-laying occurs when specialized sex-specific muscles contract, opening the vulva and allowing eggs to be expelled. Egg-laying behavior can thus be reduced to the question of how the animal controls the timing and spatial positioning of these egg-laying events in response to environmental and/or homeostatic cues.

The Egg-Laying Neuromusculature

The structure of the egg-laying circuit, defined in the anatomical studies of White and colleagues (124), is relatively simple, with three critical cell types: the vm2 vulval muscles, the HSN motorneurons, and the VC motorneurons. The functions of these cells are described briefly below **(Figure 1)**.

Egg-laying muscles. Egg-laying occurs through contraction of specialized smooth muscle cells that open the vulva and compress the uterus so that eggs can be deposited into the environment. A total of 16 muscle cells are likely to be involved in the mechanics of egg-laying. Of these, the four vm2 vulval muscles have been shown to be particularly critical. The vm2s are the only egg-laying

muscles receiving significant synaptic input from neurons (124). They are arranged in a cross shape, with their apical ends attached to the vulva, and are electrically coupled to one another through gap junctions. Ablation of the vm2s completely abolishes egg-laying, indicating that contraction of the vm2s is essential for opening the vulva (M. Stern, personal communication). Although vm2 vulval muscle cells can contract individually, egg-laying events appear to involve simultaneous contraction of several if not all the vulval muscles.

Two additional classes of hermaphrodite-specific smooth muscles are thought to be involved in egg-laying. The four vm1 vulval muscles are arranged in an X-shaped pattern similar to the vm2s, and are also thought to be involved in opening the vulva. The vm1s are electrically coupled to the vm2s (124), but receive no significant synaptic input and do not markedly alter egg-laying when ablated (M. Stern, personal communication). There are also eight uterine muscles, which form bands surrounding the anterior and posterior arms of the uterus. Contraction of these muscles might be expected to promote egg-laying by constricting the uterus and thus pushing eggs out of the vulva. However, ablation of all eight uterine muscles does not cause a gross egg-laying defect (M. Stern, personal communication).

HSN motorneurons. The egg-laying muscles receive synaptic input from two classes of motorneurons, the two HSNs and the six VCs (**Figure 2**). The HSNs are hermaphrodite-specific motorneurons [in the male they undergo programmed cell death (105)] that play a central role in egg-laying behavior. The HSN cell bodies are located lateral and slightly posterior to the vulval, and extend a long process ventrally into the ventral nerve cord and then anteriorly into the nerve ring. In the vulval area, the HSNs make extensive neuromuscular junctions with the vm2 vulval muscles, and also direct synaptic output to the VC5 motorneurons. The HSNs also receive

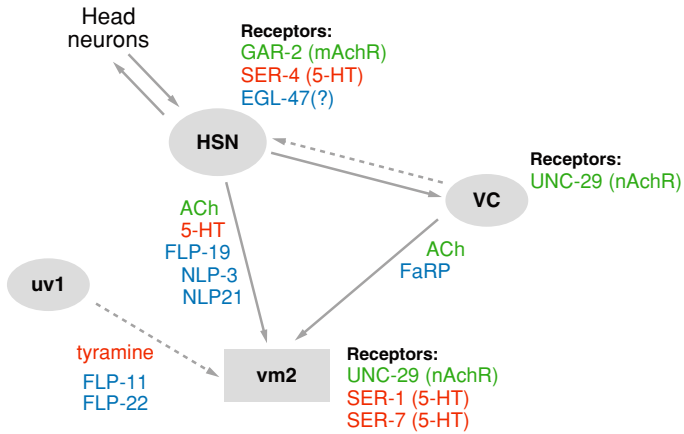

Figure 2

Connectivity and neurochemistry of the egg-laying circuit. Muscle cells are indicated by rectangles, neurons and putative neurosecretory cells by ovals. Synaptic connections are indicated by solid arrows, and putative extrasynaptic connections are indicated by dashed arrows. Neurotransmitters released by individual cells are indicated next to arrows; receptors expressed in individual neurons are listed beside the cell. Classical neurotransmitters are indicated in green, biogenic amines in red, and peptides in blue. For references, see text.

synaptic input near the cell body from the PLM mechanosensory neurons (124). In the nerve cord the HSNs are primarily presynaptic. The HSNs use at least three neurotransmitters: serotonin (24, 43), acetylcholine (28), and at least three neuropeptides, encoded by *flp-19*, *nlp-3*, and *nlp-15* (35, 57, 79, 99).

VC motorneurons. The VCs are six hermaphrodite-specific neurons in the ventral nerve cord; their male homologues are the serotonergic CP motorneurons involved in tail curling (70, 104). The VCs can be further subdivided into two classes: the vulva-proximal VC4 and VC5 neurons, and vulva-distal VC1-3 and VC6. VC4 and VC5 direct their synaptic output exclusively to the vulval muscles and to the other VCs. These neurons are cholinergic (28) and express one or more RF-amide neuropeptides (99). In addition, they express the vesicular monoamine transporter (VMAT) CAT-1 (27) and stain weakly for serotonin (28), though they are not reported to express the serotonin biosynthetic enzyme tryptophan hydroxylase (108).

RF-amide: the C-terminal amino acids are arginine (R) and amidated phenlyalanine (F-amide)

Thus, these neurons probably also release a monoamine, possibly serotonin, which might enter the cells by reuptake from vulval muscle neuromuscular junctions. VC5 in particular receives significant synaptic input from the HSNs. The other VCs (VC1-3 and VC6) make fewer neuromuscular junctions with the vulval muscles than VC4 and VC5. Additionally, unlike the proximal VCs, which extend only short processes that innervate the vulval muscles, the distal VCs also extend a longer process into the ventral nerve cord that directs synaptic output to both the body muscles and the D-class GABAergic motorneurons. These neurons also do not express CAT-1/VMAT, and are therefore not thought to be aminergic (28).

Other cells. The direct involvement of neurons other than the HSNs and VCs in the egg-laying motor circuit has not been clearly established. There are single synapses between a vm1 muscle arm and three ventral cord motorneurons, two cholinergic and one GABAergic. Because of the small number and size of these synapses, it is not clear how significant these connections are to the control of egg-laying. Another group of cells that may be important for egg-laying behavior are the uv1 cells, which are located at the junction between the uterus and the vulval epithelium. These cells appear to function as gland cells, as they express at least two FMRFamide-related neuropeptide genes [*flp-11* and *flp-22*; (57)]. They also express tyramine, a neuromodulator that appears to affect egg-laying behavior (2). The vulD vulval epithelial cells also express neuropeptides, and thus might participate in the hormonal regulation of egg-laying (57).

Egg-Laying Behavior

Temporal pattern of egg-laying. Under conditions favorable to egg-laying, egg-laying events (i.e., openings of the vulva leading to the expulsion of one or sometimes two eggs) occur in a specific temporal pattern. Specifically, egg-laying mostly occurs in short bursts lasting approximately 1–2 min, which are separated by longer quiescent periods averaging about 20 min in duration (119). Both the duration of the inactive periods between bursts and the intervals between events within a burst model as independent, exponentially distributed random variables with different characteristic rate constants (127). Thus, the timing of egg-laying events can be modeled as a stochastic process in which animals fluctuate distinct behavioral states—an active phase, during which egg-laying events are frequent, and an inactive phase, during which eggs are retained. This pattern implies that the likelihood of an egg-laying event is not strongly dependent on the precise number of eggs in the uterus. If it were, the duration of an interval following the end of an active phase (during which the number of eggs in the uterus can be reduced by as many as 10) would tend to be unusually long, whereas the intervals following an inactive phase (during which the number of eggs in the uterus has increased) would tend to be unusually short. Since the duration of an interval appears to be independent of the previous interval, it is unlikely that a mechanism exists to maintain a precise number of eggs in the uterus. More generally, the fact that the egg-laying pattern can be broken down into these specific temporal parameters may imply specific roles for particular neurons or neurotransmitters in egg-laying modulation.

Control by sensory cues. Egg-laying is regulated by a number of environmental conditions. For example, mechanical stimulation such as vibration of the culture medium inhibits egg-laying, an effect that requires the body touch receptors ALM and PLM (94). Hypertonic salt solutions such as M9 salts also strongly inhibit egg-laying (47). Finally, the egg-laying rate in the presence of abundant food is significantly higher than in the absence of food (47). This effect has been demonstrated using several experimental paradigms (20, 26a, 46, 94, 118), and may in fact be mediated by multiple regulatory mechanisms.

Coordination with other behaviors. In addition to receiving modulatory input from sensory cells, egg-laying is also coordinated with other motor programs. Specifically, egg-laying is temporally correlated with locomotion. Immediately prior to an egg-laying event, there is a transient increase in locomotor velocity; in addition, reversals are inhibited during egg-laying (45). In *Prionchulus punctatus*, a free-living predatory species, egg-laying and locomotion are correlated in the converse manner: Egg-laying occurs only during periods of inactivity (72).

GENES AFFECTING EGG-LAYING BEHAVIOR

Because egg-laying is dispensable for viability and reproduction, it has been relatively straightforward to isolate and characterize *C. elegans* mutants with abnormal egg-laying behavior. Wild-type animals typically begin laying eggs shortly after the L4/adult molt, and typically retain about 10–15 eggs in the uterus, which are laid at the 30–100-cell blastula stage. Egg-laying–defective (Egl or Egl-d) mutants, which lay eggs more slowly than wild-type animals, can most easily be identified by the accumulation of an excessive number of eggs in the uterus (i.e., bloating) and/or by the retention of late-stage (comma, twofold, or pretzel stage) embryos (113). Egl mutants typically differ from vulvaless (Vul) mutants, which fail to produce a functional vulva, in that they often eventually release some or all of their eggs, and frequently do not form "bags of worms" (in which unreleased progeny hatch within and eventually consume the mother), Conversely, mutants with hyperactive egg-laying (egg-laying–constitutive or Egl-c mutants) either retain fewer than normal numbers of eggs, lay abnormally early stage embryos, or lay eggs under normally inhibitory conditions (i.e., hypertonic or food-free media) (**Table 1**).

Both Egl and Egl-c phenotypes were initially observed in mutants with other visible phenotypes, especially uncoordinated (Unc) and dauer-constitutive (Daf-c) mutants. Subsequently, directed screens for Egl mutants were conducted by Trent et al. in the Horvitz lab (113). In addition to identifying additional alleles of several previously known *unc* and *daf* genes, these screens defined 40 new *egl* genes. These genes were subsequently classified using a simple pharmacological test: whether the egg-laying responded to the HSN neurotransmitter serotonin or to the serotonin reuptake blocker imipramine. Mutants that failed to respond to either drug (termed class A mutants) were inferred to most likely affect vulval muscle function. Mutants in class B responded to serotonin but not imipramine; because this phenotype mimicked the behavior of animals with ablated HSNs, these mutants were inferred to have defects in HSN function or development. Subsequent screens were carried out to specifically identify such class B mutants (25). Mutants in class C responded to both serotonin and imipramine; because the Daf-c Egl mutants also showed this phenotype, they were hypothesized to mediate modulation of egg-laying by sensory cues, perhaps functioning upstream of or in parallel to the HSNs. One class D mutant, carrying a dominant allele of the *egl-2* gene, showed resistance to serotonin but sensitivity to imipramine (the nature of this puzzling phenotype is discussed in the next section). The remaining mutants, grouped in class E, showed variable responses to both drugs, and thus were inferred to have partial or incompletely penetrant defects in some component of the circuitry.

Most of the genes identified on the basis of egg-laying phenotypes have since been cloned and characterized at the molecular level. In addition, a number of genes involved in egg-laying have been identified through reverse genetic or candidate screening approaches. In the following sections, genes involved in the development or function of specific components of the egg-laying circuitry are discussed. Although it has not been possible to describe every gene known to affect these processes, an attempt has been made to focus on those

Table 1 Genes affecting egg-laying behavior

Process affected		Gene	Phenotype	Encodes	Reference
Vulval muscle development	SM migration	*egl-15*	Egl (A), Mig	FGF receptor	(28)
		egl-17	Egl (A), Mig	FGF	(12)
		egl-20	Egl (E), Mig	Wnt	(128)
	M lineage	*sem-4*	Egl	Zn finger txn factor	(6)
Vulval muscle function	Ion channels	*egl-2*	dom Egl (D)	eag K^+ channel	(127)
		egl-23	dom Egl ()	twk K^+ channel	(97)
		egl-36	dom Egl ()	shaw K^+ channel	(32, 54)
		unc-93	dom Egl (A)	twk accessory protein	(70)
		sup-9	dom Egl (A)	twk K^+ channel	(23)
		sup-10	dom Egl (A)	twk accessory protein	(23)
		unc-103	dom Egl ()	erg K^+ channel	(41, 91)
		egl-19	Egl (E)	L-type Ca^+ channel	(68)
	Signal transduction	*egl-30*	Egl (E)	$G_q\alpha$ subunit	(10)
		unc-43	dom Egl	CaM kinase II	(89)
		ser-1	5-HT resistant	Serotonin receptor	(24, 49)
		ser-7	5-HT resistant	Serotonin receptor	(49)
	Nicotinic receptors	*unc-29*	Lev resistant	nAChR	(59)
		unc-38	Lev resistant	nAChR	(59)
		lev-1	Lev resistant	nAChR	(59)
HSN function	Serotonin production	*tph-1*	Egl (B)	Tryptophan hydroxylase	(113)
		cat-4	Synthetic Egl	Pterin cofactor	(73, 121)
		cat-1	Weak Egl	VMAT	(29)
	Signal transduction	*egl-10*	Egl (B)	RGS	(61)
		egl-30	Egl (E)	$G_q\alpha$	(10)
		goa-1	Egl-c	$G_o\alpha$	(77, 104)
		eat-16	Egl-c	RGS	(47)
		egl-47	Egl (B)	GPCR	(81)
		ser-4	5-HT hypersensitive	Serotonin receptor	(49)
		gar-2	Egl-c	mAChR	(4)
HSN development	Neurogenesis	*hlh-14*	Egl	HLH txn factor	(38)
	Cell death	*egl-1*	dom Egl (B)	Cell death activator	(19)
	Migration	*egl-5*	Egl (B)	Antp txn factor	(14)
		egl-43	Egl (B)	Zn finger txn factor	(43)
		ham-2	Egl (B)	Zn finger txn factor	(8)
		egl-44	Egl (B)	Zn finger txn factor	(130)
		egl-46	Egl (B)	Zn finger txn factor	(130)
	Differentiation	*unc-86*	Egl (B)	Lim txn factor	(35)
	Synapse placement	*syg-1*	Egl^+	Ig superfamily	(105)
		syg-2	Egl^+	Ig superfamily	(106)
VC development	Axon outgrowth/ guidance	*unc-34*	Egl-c	txn factor	(4)
		unc-76	Egl-c	PKC binding protein	(4)
		unc-4	Egl-c	txn factor	(4)
		unc-5	Egl-c	Netrin receptor	(4)
		unc-75	Egl-c	RNA binding protein	(4)
		unc-115	Egl-c	Actin binding protein	(4)
		unc-42	Egl-c	txn factor	(4)
	Cell death	*lin-39*	Vul	Antp txn factor	(13)
	Axon branching	*bam-2*	Egl^+	Neurexin-related	(18)

(*Continued*)

Table 1 (*Continued*)

Process affected		Gene	Phenotype	Encodes	Reference
Sensory control	Neuropeptides	*egl-3*	Egl (C)	Proprotein convertase	(55)
		egl-21	Egl (C)	Carboxypeptidase E	(52)
		flp-1	Egl	FaRP	(83, 123)
	TGF-β	*daf-1*	Egl, Daf-c	TGF-β receptor	(99)
		daf-4	Egl, Daf-c	TGF-β receptor	(99)
		daf-7	Egl, Daf-c	TGF-β	(99)
		daf-8	Egl, Daf-c	SMAD	(99)
		daf-14	Egl, Daf-c	SMAD	(99)
	Other	*egl-4*	Egl (E)	PKG	(22, 39, 64)
		tdc-1	Egl-c	Tyrosine decarboxylase	(2)

whose initial identification was based on an egg-laying mutant phenotype.

Vulval and Uterine Muscles

Because the vulval muscles are absolutely required for egg-laying, many egg-laying mutants are defective in genes that are required for the development or function of the vulval muscles. For example, some of the structural components of vulval muscles are shared with the body wall muscles that generate locomotion, including the myosin encoded by the *unc-54* gene (71) and the filament protein twitchin encoded by *unc-22* (77). Loss-of-function mutations in these genes therefore confer defective egg-laying in addition to uncoordinated movement. Furthermore, there are also a number of genes whose products are required more specifically for the development or function of the egg-laying muscles; these are discussed in more detail below.

Genes affecting vulval muscle development. The vulval and uterine muscles are derived from an embryonic mesoblast called M. In hermaphrodites, M undergoes a series of postembryonic divisions that give rise to 16 body muscle cells, 2 coelomocyte scavenger cells, and 2 sex myoblasts (104). The sex myoblasts then migrate anteriorly from the tail, where they are born, toward the gonad, where they eventually undergo a series of divisions to generate all the vulval and uterine muscles. Using a mutant strain in which the gonad is misplaced, it has been demonstrated that gonadal cells serve as guideposts to direct the final positioning of the sex myoblasts and, consequently, the vulval and uterine muscles (111).

Several egg-laying–defective mutants disrupt one or more stages of sex myoblast development, and thereby prevent the proper generation of the egg-laying muscles. For example, loss-of-function mutations in the gene *sem-4* result in an abnormal M lineage in which the sex myoblasts are not generated (6). *sem-4* encodes a zinc-finger transcription factor related to Drosophila *spalt* that is involved in cell fate decisions in a variety of neuronal, endodermal, epidermal, and muscle cell lineages (11, 29, 42, 112). *sem-4* appears to play a conserved role in the M lineage of the relatively distantly related nematode *Pristionchus pacificus* (85).

Several other genes are involved in guiding sex myoblast migrations per se. In mutants with loss-of-function in these genes, the egg-laying muscles are misplaced, and therefore do not function properly in opening the vulva. An example of such a gene is *egl-20*, which encodes a Wnt molecule also required for the migrations of a number of neurons and neuroblasts in *C. elegans* (35, 73, 123). Although the sex myoblast migration defect is almost certainly responsible for the Egl phenotype, the mechanism by which Wnt signaling controls sex myoblast migration is not well

understood. Another set of genes affecting sex myoblast migration includes *egl-15*, *egl-17*, and *sem-5*. *egl-15* encodes a homologue of the fibroblast growth factor receptor (FGFR), a receptor tyrosine kinase that plays a conserved role in signal transduction in a wide range of organisms (26). In *C. elegans*, EGL-15 functions cell-autonomously in the migrating sex myoblasts to direct proper cell movement. The ligand for this EGL-15 is encoded by the *egl-17* gene, which encodes a homologue of fibroblast growth factor (FGF) that is produced by the gonad and functions as an instructive cue to direct the sex myoblast migrations (10). *sem-5* encodes a multisubstrate adaptor protein that appears to function as an effector of the EGL-15 receptor in the migrating sex myoblasts (14). Ultimately, EGL-15 signaling is mediated by the Ras/MAP kinase pathway, which is involved in many aspects of *C. elegans* development including vulval differentiation (106). Ras signaling in *C. elegans* is a rich topic that has been reviewed in detail elsewhere (53, 59, 107).

Genes affecting vulval muscle function. In addition to genes affecting vulval muscle development, many of the class A Egl genes encode molecules that are important for excitation of the vulval muscles. A major group of such genes are defined by dominant mutations that cause defective egg-laying as well as defects in excitation of one or more additional excitable cell types (82, 87). The majority of these so-called muscle-activation genes encode potassium channels that are constitutively activated by the Egl mutation, which therefore leads to constitutive hyperpolarization of cells expressing the mutant channel. The *C. elegans* genome contains a large number (>70) of potassium channel genes, including members of all the gene families known from vertebrates (5, 93, 120). Thus far, all loss-of-function mutations in *C. elegans* potassium channel genes have essentially wild-type behavior, indicating a substantial degree of redundancy in K^+ channel function even within individual excitable cells. K^+ channel genes defined by dominant Egl mutations include *egl-36*, which encodes a homologue of the *shaw* voltage-gated K^+ channel (30, 51); *egl-2*, the *C. elegans* homolog of *eag* (122); and *unc-103*, which encodes a homologues of the *erg* channel (38). Members of the TWK family of two-domain K^+ channels are also defined by dominant Egl mutations. One of these, *sup-9*, interacts genetically with two other dominant Egl genes, *unc-93* and *sup-10*, which appear to encode accessory subunits that facilitate K^+ channel function (21, 67, 68). Dominant mutations in another twk channel gene, *egl-23*, also lead to an egg-laying–defective phenotype, though *egl-23* does not interact genetically with the *sup-9/unc-93/sup-10* group and therefore may participate in a functionally distinct K^+ channel complex (93).

The dominant Egl mutation in *egl-2* is of particular interest because the constitutively active mutant channel is specifically blocked by the antidepressant imipramine (122). This explains the previously puzzling observation that *egl-2* mutants are resistant to serotonin but sensitive to a serotonin reuptake blocker; in these mutant animals, imipramine stimulates egg-laying not by potentiating serotonin neurotransmission but by directly blocking a hyperpolarizing channel in the vulval muscles. (Other serotonin reuptake blockers, such as fluoxetine, do not block the EGL-2 channel, nor do they stimulate egg-laying in *egl-2* dominant mutants). The unusual properties of the EGL-2 channel have provided a novel means of conditionally silencing neurons by heterologously expressing the mutant *egl-2* allele in cells of interest and comparing behavior in the presence or absence of exogenous imipramine (15).

Another ion channel that can mutate to give an egg-laying–defective phenotype is *egl-19*, which encodes the *C. elegans* homolog of L-type voltage calcium channels (65). Null alleles of *egl-19* are lethal, but reduction-of-function alleles affect the excitation of multiple muscle groups in *C. elegans*, including vulval, pharyngeal, and body wall muscles. Gain-of-function alleles of *egl-19* have also

been identified, which result in hyperactive egg-laying as well as abnormal locomotion and pharyngeal pumping. Even weak *egl-19* alleles with only slightly reduced egg-laying rate are strongly serotonin resistant, which has led to the suggestion that EGL-19 calcium channels may be a target for serotonin modulation of vulval muscle activity (102, 113, 119).

A number of genes have also been identified that encode components of signal transduction pathways that modulate vulval muscle activity. Serotonin stimulates vulval muscle activity by increasing the frequency of spontaneous calcium transients (102). Two serotonin receptors, SER-1 and SER-7, appear to be involved in activating vulval muscle activity; both *ser-1* and *ser*-7 loss-of-function single mutants are resistant to serotonin stimulation of egg-laying, and *ser-1; ser*-7 double mutant animals show a visible Egl phenotype (22, 46). In vitro, SER-1 appears to couple to G_q while SER-7 couples to G_s. Although the G_s α-subunit is expressed in the vulval muscles, its role has been difficult to establish owing to the lethal phenotype of *gsa-1* null mutants (60, 83). However, the G_q α-subunit subunit, encoded by the *egl-30* gene (9), is clearly important for serotonin stimulation of vulval muscle activity. Loss-of-function mutations in *egl-30* confer serotonin resistance with respect to egg-laying stimulation, and gain-of-function mutations partially suppress the egg-laying defect resulting from HSN ablation (7, 102). While *egl-8*, which encodes the only *C. elegans* homolog of phospholipase Cβ (the canonical effector of G_q), is also required for normal egg-laying and serotonin response, double mutant analyses suggest that it primarily functions independently from *egl-30*, and probably represents at most a minor effector of EGL-30/G_q signaling (7). The primary effector of G_q signaling the vulval muscles thus remains to be identified.

Another set of signal transduction molecules that may function in the vulval muscles includes calcium/calmodulin-dependent protein kinase II (CaMKII), encoded by *unc-43* (86), and calcineurin, encoded by the *tax-6* (α-subunit) and *cnb-1* (β-subunit) genes (3). Loss-of-function mutations in *cnb-1* and gain-of-function mutations in *unc-43* cause a serotonin-resistant and levamisole-resistant Egl-phenotype, whereas loss-of-function *unc-43* mutations and gain-of-function *tax-6* mutations (64) confer an egg-laying constitutive phenotype. Since loss-of-function mutations in the G_o α-subunit gene *goa-1* also have an Egl-c phenotype (74), and since *goa-1* and *unc-43* appear to interact genetically in motorneurons (90), it is possible that calciuneurin and CaM kinase II participate in some sort of G_o-mediated modulation of vulval muscle activity. However, although *cnb-1*, *unc-43*, and *goa-1* are expressed in vulval muscles, they are also expressed in motorneurons, and at least a major component of the GOA-1 effect on egg-laying behavior appears to involve modulation of HSN activity (78).

Finally, the stimulatory effects on egg-laying of acetylcholine and cholinergic agonists such as levamisole appear to be mediated by nicotinic receptors in the vulval muscles. Loss-of-function mutations in the canonical levamisole receptor subunit genes *unc-29*, *unc-38*, and *lev-1* (34) confer resistance to levamisole-induced egg-laying (56), a phenotype that can be rescued (at least in the case of *unc-29*) by expression of a wild-type allele specifically in the vulval muscles (117). However, even an *unc-29; unc-38; lev-1* triple mutant has only a subtle abnormality in egg-laying behavior in the absence of drug (56). Since the more general nAChR agonist nicotine can still weakly stimulate egg-laying in a LevR mutant background, other nAChRs that are functionally redundant with the levamisole receptor may also play a role in the excitation of the vulval muscles by acetylcholine.

HSN Motorneurons

Another important cellular focus for many egg-laying genes is the HSN motorneurons. Ablation experiments have demonstrated that hemaphrodites lacking HSN neurons have

significant defects in egg-laying behavior leading to clear accumulation of embryos in the uterus, but are otherwise viable, healthy, and behaviorally normal (23, 113). Thus, any gene that is specifically required for the development or function of the HSNs should be a clear target for Egl mutant screens. Moreover, because the egg-laying defect of HSN-ablated animals can be completely rescued by exogenous application of the HSN neurotransmitter serotonin but not by serotonin reuptake blockers (which potentiate endogenous serotonin signaling), mutants with HSN-related egg-laying defects can usually be identified based on their serotonin sensitivity and resistance to serotonin reuptake blockers. These mutants, which correspond roughly to the class B Egl mutants of Trent et al., have provided significant insight into the functional importance of the HSNs in the egg-laying circuit. In addition, many of these mutant genes play critical roles in the development of the HSNs or in signal transduction pathways that regulate their activity; analysis of these genes has therefore provided important insights into these processes.

Genetic analysis of the functional importance of the HSNs. Genetic analysis has proven effective in assessing the functions of the HSN neurons in the egg-laying circuit. Particularly useful for such studies are semidominant alleles of the gene *egl-1*, which lead specifically to the loss of HSNs in hermaphrodites through programmed cell death. *egl-1* encodes an upstream activator of the apoptotic cell death pathway, and null alleles of *egl-1* (isolated as revertants of the semidominant Egl alleles) are defective in apoptosis (17). In males, EGL-1 protein is synthesized in the HSN cells, and they consequently undergo cell death during development. The semidominant Egl alleles affect a *cis*-acting regulatory sequence that normally mediates hermaphrodite-specific repression of *egl-1* expression in HSN. Since the HSNs are the only neurons that normally undergo cell death in males but not in hermaphrodites, the *egl-1* semidominant alleles result in a highly specific genetic ablation of the HSNs in hermaphrodite animals. Thus, by comparing the behavior of *egl-1(sd)* animals to wild-type, it is possible to infer precisely the roles of HSN neurons in egg-laying and other behaviors.

As noted previously, *egl-1* animals, like animals in which the HSNs have been ablated with a laser, are strongly egg-laying defective and accumulate many unlaid embryos in the uterus. However, egg-laying does occur in HSN-deficient animals, and most embryos are laid before hatching. Closer analysis of the timing of egg-laying events in HSN-ablated animals indicates that the HSNs are specifically important for inducing the onset of egg-laying active phases; clusters of egg-laying events are about threefold less frequent than in normal animals, though egg-laying within these bursts occurs normally (119). Since exogenous serotonin rescues these egg-laying defects (113), the HSNs likely promote egg-laying at least in part by releasing serotonin as a neuromodulator. Calcium imaging studies have shown that serotonin acts directly on the vulval muscles to increase the frequency of spontaneous calcium transients (102), an effect most likely mediated by the G_q homolog EGL-30 (7). However, because serotonin-deficient mutants (e.g., *tph-1*) have less severe egg-laying defects than HSN-ablated animals (56, 121), the HSNs may also release another neuromodulator (perhaps a peptide) that functions semiredundantly with serotonin to promote egg-laying.

Because the HSNs are the major synaptic link between the egg-laying circuit and the rest of the nervous system, it is likely to function as an important conduit for regulatory feedback to and from the egg-laying motor program. The coordination of egg-laying and locomotion in *C. elegans* appears to be mediated by feedback from the HSN motorneurons to interneurons in the head that promote forward movement. The direct postsynaptic output of HSN is to AVF, which in turn synapses onto the forward command

interneuron AVB. Ablation of the HSNs or the AVFs or interference with serotonin neurotransmission leads to the absence of the velocity burst prior to egg-laying events (45). These results imply that the activity of the HSNs during periods of active egg-laying also modulates interneurons controlling locomotion. This observation raises the possibility that the behavioral states observed for egg-laying may in fact represent general states of neural circuit function that could affect diverse, seemingly unrelated, motor outputs.

The evidence that HSN is involved in communication between head neurons and the egg-laying circuit is less clear. For example, *egl-1* mutant animals lacking the HSNs are still capable of modulating their egg-laying rate in response to food abundance, suggesting that humoral or other extrasynaptic modulation of the vulval muscles and/or VCs may mediate this regulation (117). Exactly how the activity of the HSNs is controlled, and how this regulation relates to egg-laying behavior, is one of the key unanswered questions about *C. elegans* egg-laying.

Genes affecting HSN development. Among the remaining members of the B class of egg-laying mutants are several with obvious abnormalities in the proper development of the HSN neurons. In wild-type animals, the HSNs are generated from asymmetric divisions of a bilateral pair of neuroblasts, each of which generates an HSN precursor as well as a PHB chemosensory neuron (105). During embryogenesis, the HSN precursors migrate anteriorly from the tail, where they are born, to a position in the middle of the animal adjacent to the germline primordium. The HSN neuroblasts remain in this position until the end of the third larval stage, at which point they begin to extend their process toward the nerve ring. During the fourth larval stage, axonal branches form off the HSN process in the vicinity of the developing vulva, which ultimately form neuromuscular junctions with the vm2 vulval muscles and synapses with the VC motorneurons (39). At this stage, the HSNs also begin to express characteristic markers such as the neurotransmitter serotonin.

A number of genes with Egl mutant phenotypes appear to function in various steps of HSN development. Among the earliest-acting is *hlh-14*, which encodes a basic helix-loop-helix transcription factor of the achaete-scute family (36). In *hlh-14* loss-of-function mutants, the asymmetric division that normally generates the HSN and PHB neuroblasts does not occur, leading to an absence of these neurons in mutant animals. HLH-14 appears to be required for neurogenesis in a number of other neuroblast lineages as well, a function that appears to involve dimerization with the more general bHLH protein HLH-2. Another early acting transcriptional regulator of HSN development is the Hox gene *egl-5*, which encodes the *C. elegans* homolog of Abd-B and is required for the specification of cell fates in the most posterior region of the embryo (12). *egl-5* mutants generate HSN neuroblasts, but they neither migrate nor express characteristic HSN markers such as serotonin. Several other transcription factors have more specific effects on HSN development. For example, the POU transcription factor gene *unc-86* affects serotonin expression and other late events in HSN development, but not HSN migration (33). Conversely, two genes encoding zinc-finger transcription factors, *egl-43* and *ham-2*, affect HSN migration but not serotonin expression (8, 40). While *egl-43* appears to function independently from *egl-5*, *ham-2* and *unc-86* appear to be downstream of *egl-5* in a cascade of transcriptional activation, and these two genes themselves appear to act to inhibit *egl-43* expression late in HSN development (8).

Another group of genes involved in HSN migration and development includes *egl-44* and *egl-46*. Loss-of-function mutations in these genes result in a characteristic defect in which the HSN cell bodies migrate along their normal trajectories but stop at abnormally anterior positions (24). Mutants HSNs also have abnormally directed axons and fail

GPCR: G-protein coupled receptor

to express serotonin. *egl-44* and *egl-46* both encode zinc-finger transcription factors that are expressed in the HSNs and promote HSN cell fate (125). This pair of transcription factors functions in other neurons to control cell fate decisions; specifically, they function in the FLP neurons to repress body touch neuron fates and to promote cell-specific gene expression in the male HOB neurons (76, 126).

Genes have also been identified that affect later steps in HSN development, including axonal branching and synapse formation and placement. Using fluorescent protein tags that specifically label HSN synapses, it has been demonstrated that specialized vulval epithelial cells, termed guidepost cells, provide an inductive signal that causes axonal branching and synaptogenesis to occur in the vicinity of the vulval muscles. In animals lacking vulval cells (e.g., in vulvaless mutants such as *lin-3*), synapses are anteriorly displaced; in contrast, when the vulval muscles are absent, synapses form in the correct location, though their postsynaptic targets are not present. Two genes, *syg-1* and *syg-2*, have been identified that encode molecules that direct proper positioning of HSN neuromuscular synapses (100, 101). SYG-1 and SYG-2 are both immunoglobulin superfamily members; SYG-2 is expressed in the guidepost cells, whereas SYG-1 is expressed in the HSN process and appears to physically interact with SYG-2 to identify the correct site for synaptogenesis. Both *syg-1* and *syg-2* mutants lay eggs efficiently, indicating that serotonin and/or other neuromodulators can still activate egg-laying muscle contraction when released from misplaced synapses.

Genes affecting HSN function. Among the class B Egl mutants (which respond to serotonin but not to serotonin reuptake blockers) are several in which the HSNs are morphologically normal and still contain serotonin. These mutant genes are therefore inferred to affect HSN function rather than HSN development.

The genes in this category that have been cloned and characterized in detail primarily affect signal transduction networks in the HSNs that modulate their activity. In particular, these genes affect a signal transduction network involving the *C. elegans* homologs of the G_o and G_q heterotrimeric G proteins. The G_o and G_q pathways appear to act antagonistically in the HSNs to regulate their activity; *egl-30*, which encodes the G_q α-subunit, is required for HSN activity (7) and has an egg-laying–defective phenotype, whereas *goa-1*, which encodes the G_o α-subunit, negatively regulates HSN activity and has an egg-laying-constitutive loss-of-function phenotype (78, 102). Mutations in the RGS proteins, which negatively regulate G-protein signaling, have phenotypes converse to those of their G-protein counterparts; loss-of-function mutations in the *goa-1* RGS *egl-10* are egg-laying defective (58), whereas loss-of-function of the *egl-30* RGS *eat-16* are egg-laying constitutive (44). Although *egl-30*, and perhaps *goa-1* as well, also function in vulval muscle signal transduction (see previous section), the principal focus of action of all these genes appears to be the HSNs. The G_o/G_q network appears to modulate the activity of ventral cord motorneurons that control locomotion in a way very similar to its role in the HSNs (63, 75, 81, 89).

Analysis of egg-laying mutants has provided some insight into how this network acts within the HSNs to modulate egg-laying behavior. In particular, several receptors have been identified that putatively couple to G_o/GOA-1 to negatively regulate HSN activity. The *egl-47* gene encodes two GPCRs (G-protein coupled receptor) that function in the HSN neurons; *egl-47* mutations with an egg-laying–defective phenotype are dominant, gain-of-function alleles that appear to constitutively activate one of these receptors (78). Although the ligand(s) for the EGL-47 proteins are not known, the EGL-47 appears to act through GOA-1/G_o, since *goa-1* loss-of-function mutations are completely epistatic to *egl-47*. Loss-of-function

mutations in *egl-47* have no detectable egg-laying phenotype, indicating that other GPCRs in HSN are capable of activating inhibitory signaling through GOA-1 in these neurons. One candidate for such a receptor is encoded by the *gar-2* gene (4). *gar-2* encodes a muscarinic acetytylcholine receptor that is expressed in the HSNs (66). Loss-of-function mutations in *gar-2* result in a slightly hyperactive egg-laying phenotype and confer resistance to the inhibitory effects on egg-laying of the acetylcholinesterase blocker aldicarb (4). The GAR-2 receptor might act in an autoinhibitory capacity or could be responsible for inhibitory feedback from the VCs to the HSNs. Another GPCR that may inhibit HSN activity is the neuronal serotonin receptor SER-4. Serotonin suppresses spontaneous neural activity in the HSNs, an effect requiring the G_o homolog GOA-1 (102) and which may account for the inhibition of egg-laying caused by chronic exposure to exogenous serotonin (97). In mutants defective in the stimulatory vulval muscle serotonin receptor SER-7, even acute serotonin treatment strongly inhibits egg-laying (46). *ser-4* mutations suppress this inhibition, suggesting that serotonin might act through SER-4 receptors on the HSNs to inhibity their activity. However, neither SER-4 nor another candidate mediator of serotonin inhibition of egg-laying, the serotonin-gated chloride channel MOD-1 (10b), has been shown to be expressed in the HSNs, VCs or vulval muscles (114). Thus, it is possible that SER-4 and/or MOD-1 might function in other neurons to indirectly affect the activity of the egg-laying circuit.

VC Motorneurons

Functional importance. The importance of the VC motorneurons to egg-laying behavior has been more difficult to evaluate than in the case of the HSNs. No mutations that cause a specific genetic ablation of the VCs (analogous to *egl-1* for the HSNs) have been identified; although mutations in the Antp homologue *lin-39* result in inappropriate cell death of the VCs (13), in completely penetrant alleles they also result in a vulvaless phenotype that makes egg-laying impossible to assay. The effects of the VC neurons on egg-laying behavior have been assessed by laser ablation in wild-type animals, but these observed effects are far less striking than those seen for HSN ablation.

However, several lines of evidence suggest that at least the vulva-proximal VCs (VC4 and VC5) stimulate vulval muscle activity. Ablation of VC4 and VC5 in an *egl-1* mutant background significantly enhances the egg-laying–defective phenotype caused by loss of the HSNs (116). Moreover, in vivo calcium imaging studies have shown that VC activity is temporally correlated with vulval muscle movement (102; M. Zhang & W. Schafer, unpublished). Seemingly paradoxically, there is also compelling evidence for an inhibitory role for the VCs in egg-laying. In particular, a number of egg-laying-constitutive mutants have defects in VC morphology or function (4) or have general neurotransmission defects with an apparent focus of action in the VCs (96, 97). In addition, VC4/VC5-ablated animals accumulute fewer unlaid eggs than intact animals, suggesting that their overall rate of egg-laying is elevated (4).

The question of the VCs' role in egg-laying is closely related to the role of acetylcholine, a key VC neurotransmitter. Nicotinic agonists strongly stimulate egg-laying (113, 121), an effect requiring the function of specific nicotinic receptors in the vulval muscles (117, 121). In contrast, chronic increases in cholinergic neurotransmission (e.g., in cholinesterase-deficient mutants) inhibit egg-laying in a manner dependent on the HSN-expressed muscarinic receptor GAR-2 (4). As for serotonin, these inhibitory effects of acetylcholine on the HSNs may involve the G_o signal transduction pathway (78). A model consistent with all existing data is that the release of acetylcholine from the VCs stimulates vulval muscle contraction through nicotinic receptors while feedback inhibits the HSNs through muscarinic receptors. In

addition, other neurotransmitters (such as neuropeptides) expressed in the VCs may play stimulatory or inhibitory roles in egg-laying behavior.

Genes affecting development of the VC motorneurons. The VCs are derived from the same postembryonic lineages that generate the adult ventral cord motorneurons that control locomotion. At the end of the first larval stage, the 12 P cell ectoblasts, distributed anteroposteriorly in rows of 6 on the left and right sides of the animal, migrate circumferentially into the ventral nerve cord. Each of the P cells first divides asymmetrically to give rise to an anterior daughter, which becomes a neuroblast, and a posterior daughter, which ultimately gives rise to epidermal cells. The Pn.a neuroblast then divides several times to yield one neuron each of the VA, VB, and AS cholinergic motorneurons and one VD GABAergic motorneuron. In addition, the 6 *lin-39*-expressing Pn.a cells (P3.a-P8.a) each give rise to a single VC neuron (in the other Pn.a cells, the corresponding cell undergoes apoptosis). Though these neurons are generated in the first larval stage, the VCs do not extend processes until the late third larval stage, and innervate the developing vulval muscles in the fourth larval stage. The VC neurons closest to the vulva (VC4 and VC5) also undergo a short cell migration around the time axon outgrowth ensues, by which their cell bodies move longitudinally to positions immediately anterior and posterior to the vulval opening (103, 104).

Although the VCs appear to play both stimulatory (by exciting the vulval muscles) and inhibitory (by inhibiting the HSNs) roles in egg-laying, the stimulatory function appears to be largely redundant with the HSNs. Thus, mutants with abnormalities in VC development have generally been identified on the basis of an Egl-c hyperactive egg-laying phenotype rather than an egg-laying defect (4). Because these mutants also have defects in the development of other postembryonic ventral cord motorneurons, they also often exhibit a coiling uncoordinated (Unc) phenotype. Defects seen in these mutants include missing neurons (possibly due to failed P-cell migration or inappropriate cell death), gaps in the VC processes in the ventral nerve cord (probably due to axon extension defects), and grossly misguided axons. Although many of these Unc Egl-c mutant genes have been cloned, little systematic effort has been made to determine their specific roles in the development of the VCs.

Using fluorescent markers, other genes with more specific defects in VC development have been identified. For example, the migrations of the VC4 and VC5 cell bodies, like several other short-range neuronal migrations, are at least partially dependent on the voltage-gated calcium channel gene *unc-2* (109). Genes affecting VC axonal branching have also been identified. As with the HSNs, branching of the VC processes in the vicinity of the vulval muscles requires an inductive signal from guidepost cells in the vulval epithelium; thus, vulvaless mutants (but not mutants lacking vulval muscles) are specifically defective in VC axonal branching (69). Loss-of-function mutations in the *bam-2* gene also result in an abnormal VC branching phenotype; axons often extend too far medially and frequently cross the ventral midline near the vulval opening (16). *bam-2* encodes a neurexin-related transmembrane protein that is functionally expressed in the VulF epithelial guidepost cells. Thus, the BAM-2 protein is likely to mediate cell-cell signaling between the guidepost cells and the developing VC axons to direct proper branching, similar in many ways to the role of SYG-2 in directing proper placement of HSN synapses (101).

Genes Affecting Egg-Laying Sensory Inputs

While the cellular focus of action of many egg-laying genes is within the egg-laying muscles or motorneurons, a number of these genes are likely to act outside the egg-laying motor circuit. In particular, many of these genes are likely to function in processes mediating

the control of egg-laying behavior by sensory stimuli. The first group of such mutants to be genetically identified were the Daf-c Egls, which in addition to being egg-laying defective constitutively form dauer larvae at 25°C. The dauer larva is an alternative third larval stage that is induced by starvation and crowding; because starvation also inhibits egg-laying, it has been hypothesized that these mutants are defective in a pathway mediating behavioral responses to food (1, 115). Loss-of-function mutations in 5 genes (*daf-1*, *daf-4*, *daf-7*, *daf-8*, and *daf-14*) show egg-laying–defective as well as dauer-constitutive phenotypes, which are both suppressed by loss-of-function dauer-defective mutations in the *daf-3* and *daf-5* genes (110, 113). All of these genes have been cloned, and have been shown to encode components of a TGF-β signal transduction pathway. *daf-7* encodes a TGF-β homologue that is expressed in the amphid chemosensory neuron ASI (88, 95). *daf-1* and *daf-4* encode type II and type I, respectively, TGF-b receptor proteins (31, 41), while *daf-8*, *daf-14*, and *daf-3* encode SMAD proteins that function as TGF-β effectors in both vertebrates and invertebrates (48, 84). *daf-5* encodes a novel proline-rich protein that may be specific to nematodes; DAF-5 may play a key role in linking TGF-β signaling to the control of egg-laying behavior (19). There are no direct connections between the amphids and the egg-laying motor circuit, raising the possibility that the TGF-β pathway genes influence egg-laying through hormonal signaling.

Another molecule that appears to be involved in the control of egg-laying by food is the cyclic GMP-dependent protein kinase EGL-4 (37, 62). Mutations in *egl-4* slow the rate of egg-laying on food and render animals insensitive to food modulation of egg-laying rate (20). These phenotypes are suppressed by mutations in *daf-3* and *daf-5* (113), which suggests that PKG and the TGF-β pathway may function in a common pathway to mediate the sensory control of egg-laying. *egl-4* affects a number of other aspects of *C. elegans* behaviors, and at least for some of these (62) it appears to function in chemosensory neurons. Thus, EGL-4 may function in or downstream of sensory neurons that influence the egg-laying circuitry; however, the identities of these sensory neurons, and neural mechanisms by which they might influence egg-laying behavior, are not known.

The modulation of egg-laying rate by food abundance also appears to involve neuropeptides. Two class C egg-laying genes, *egl-3* and *egl-21*, encode enzymes involved in neuropeptide processing (49, 52). Since loss-of-function *egl-3* and *egl-21* mutants respond to serotonin reuptake blockers, they do not appear to be strongly defective in HSN function, suggesting that EGL-3 and EGL-21 may be required to produce peptides that act upstream of, or parallel to, the HSNs to control egg-laying. A candidate gene for encoding such a peptide is *flp-1* (92). Deletion mutants of the *flp-1* gene, which encodes a number of FMRFamide-related neuropeptides, are egg-laying defective, show diminished responses to serotonin, and are defective in the modulation of egg-laying by food (80, 119). *flp-1* is expressed in a number of sensory neurons and head interneurons including ASK, AIA, and AIY, which have synaptic connections to HSN (57). However, at least some of the effects of *flp-1* on egg-laying appear to be HSN-independent, as a *flp-1* deletion mutation still inhibits serotonin-induced egg-laying in an *egl-1* mutant background (118) Potentially, *flp-1*-encoded peptides could act humorally on the vulval muscles, the VCs, or other cells that directly or indirectly influence egg-laying behavior. A peptide identical to one encoded by *flp-1* has an inhibitory effect on the female sex muscles of the parasitic nematode *Ascaris suum* (32), and in *C. elegans* *flp-1* peptides are inhibitory in the pharyngeal muscle (91). If inhibition is a general property of *flp-1* peptides in *C. elegans*, this may imply an indirect mode of action for the stimulation of egg-laying by *flp-1*.

Another neuromodulator that may mediate sensory control of the egg-laying motor circuit is tyramine (2). Mutant animals

specifically defective in the production of tyramine are egg-laying constitutive and fail to decrease their egg-laying rate in the absence of food. Based on expression patterns of enzymes required for tyramine and octopamine biosynthesis, tyramine (but not octopamine) is released from the uv1s, endocrine cells found at the junction between the uterus and the vulva, as well as the RMD head motorneurons. It is reasonable to suppose that the uv1s are the source of tyramine responsible for the modulation of egg-laying behavior, though how these cells might be connected to the sensation of food is not clear. One speculation is that tyramine release from the uv1s might be negatively regulated by *flp-1* peptides. Further work is necessary to address these possibilities.

PERSPECTIVES

The genetic analayis of egg-laying behavior has provided many useful insights into fundamental aspects of nervous system development and function. For example, the mechanisms of directed cell migration have been elucidated in part through analysis of mutants defective in the migrations of the egg-laying muscle precursors, the sex myoblasts, as well as the HSN egg-laying motorneurons. Mutants defective in HSN development have also provided insight into the transcriptional control of neurogenesis and neuronal cell fate selection. Likewise, studies of egg-laying defective and egg-laying constitutive mutants have led to a number of key discoveries related to the the G_o/G_q signaling network and its function in neuronal signal transduction, particularly regarding the roles of RGS proteins. Given the relative simplicity of egg-laying assays and the dispensability of egg-laying for hermaphrodite viability and fertility, egg-laying should continue to offer many advantages to genetic studies of the *C. elegans* nervous system.

Despite the long history of egg-laying genetics and the simplicity of the egg-laying neuromusculature, many questions remain about how the egg-circuit works at the molecular and neural levels. However, recent technical advances should accelerate progress on these questions. Of particular importance has been the completion of the *C. elegans* genome sequence and the systematic generation of deletion mutants in identified open reading frames (10a, 50). The advantages of this approach are well-illustrated by the example of neurotransmitter signal transduction in the egg-laying circuit. While forward screens for egg-laying–defective mutants failed to identify receptors for serotonin or other neurotransmitters known to control egg-laying, analysis of the genomic sequence made it possible to identify candidate serotonin receptors, assess their expression in the egg-laying circuitry using reporter transgenes, and analyze the loss-of-function phenotypes of deletion mutants (which as a result of genetic redundancy were not visibly Egl as single mutants). A second critical technical advance has been the development of methods for in vivo imaging of neuronal activity in individual cells during behavior (54). This has made it possible not only to determine how a particular gene affects behavior, but also to explain these effects mechanistically in terms of how genes affect particular cells in neural circuits, and how the activities of those neurons correlate with behavioral events.

The apparent anatomical simplicity of the *C. elegans* egg-laying circuit belies a surprising complexity at the molecular and neurochemical levels. The use of multiple neurotransmitters by the egg-laying motorneurons allows a simple circuit to generate complex temporal patterns of behavior. Among the central questions for future investigation is how sensory information modulates the rate of egg-laying. The structure of the *C. elegans* nervous system provides few clues as to how information could be conveyed from sensory neurons such as the amphid chemoreceptors to the egg-laying motorneurons. Potentially, neurohormonal or other extrasynaptic signaling may play an important role in these processes, raising the possibility that the cellular network involved in egg-laying control is considerably more complex than previously suspected.

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Annual Review of Genetics

Volume 40, 2006

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