# Perspectives

## Anecdotal, Historical and Critical Commentaries on Genetics

*Edited by James F. Crow and William F. Dove*

## Pathway to RAS

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WHEN I started my laboratory at Caltech in 1987, I hung on my door a wall chart from Oncogene Sciences with all the known proto-oncogenes and their cellular locations and regulatory relationships. One of these, *ras*, was identified by viral and cancer genetics as a dominant oncogene in many tumors and in transforming retroviruses. The RAS protein is a small GTPase, which undergoes a cycle of guanine nucleotide exchange and hydrolysis. RAS was known to be active when bound to GTP, but its normal role in the cell was not known, nor was how it became activated and what it did once it was. RAS and epidermal growth factor receptor (EGFR) resided on opposite sides of the wall chart. The molecular genetics of *Caenorhabditis elegans* vulval development allowed us to draw a big arrow between RAS and EGFR in 1990 and other arrows in 1992. The intellectual pathway to RAS is a genetic story, the highlight being 15 years ago when we described the initial genetic pathway from a cell surface receptor to the *ras* proto-oncogene *let-60* in *C. elegans* (Han *et al.* 1990). This article places this genetic analysis in perspective.

The genetics of this pathway were worked out in *C. elegans* in the context of vulval development (see *Perspectives* by Horvitz and Sulston 1990). The anchor cell of the gonad induces three vulval precursor cells to generate the vulva (Kimble 1981). In the absence of the anchor cell, hermaphrodites lack a vulva and cannot lay eggs because the vulval precursor cells generate only epidermis. Horvitz and Sulston (1980) had found vulvaless mutants that mimicked the ablation of the anchor cell, as well as multivulva mutants in which the precursor cells generate vulval tissue even in the absence of the anchor cell (Ferguson *et al.* 1987). These observations implied that there must be a signaling pathway from the anchor cell to the precursor cells. Among the vulvaless mutants would be those that lacked this signaling pathway; among the multivulva mutants would be those in which the signaling pathway might be constitutively activated.

My own scientific interests evolved from trying to understand the logic of cell lineage to trying to understand the molecular basis for inductive signaling during development. This goal led directly to the discovery that RAS has a crucial role in normal development and acts downstream of growth factor receptors such as the EGFR. I started working on *C. elegans* because in February 1978 Bob Horvitz showed me the just-published cell lineage diagrams from his article with John Sulston (Sulston and Horvitz 1977); it looked as if worm development had some logical structure, and I thought to myself, "I can figure that out." I did not get a chance to actually work on cell lineage until June 1979, when I started comparing the essentially invariant lineages of another free-living nematode, *Panagrellus redivivus*, with those of *C. elegans* to infer how the cell lineages might be genetically programmed (Sternberg and Horvitz 1984). In Horvitz's lab I learned to watch *C. elegans* nuclei and cells divide. I had earlier learned to watch *Physarum polycephalum* nuclei divide as a visiting undergraduate in Stuart Kauffman's laboratory at the University of Pennsylvania. Mainly, it involved lots of attention, quick note taking, and not much sleep. My undergraduate advisor at Hampshire College, Lynn Miller, had told me that to get a position in a genetics laboratory I should stress my experience working long hours at tedious jobs under less-than-optimal conditions.

In Horvitz's lab I collaborated with and learned from two talented geneticists with drastically different styles. Iva Greenwald's style was intensive genetic analysis of one locus, which she applied to *unc-93* (Greenwald and Horvitz 1980) and later to *lin-12* (Greenwald *et al.* 1983). Chip Ferguson's style was to work with tens of loci at once (Ferguson and Horvitz 1985, 1989). It would take him 2 weeks to transfer and clean up his

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~600 strains, spreading over both our benches. One year we puzzled over the vulval pathway working two shifts, talking each night at 11:30 when Ferguson came in and the next morning at around 8:30 when I came in. Eventually I broke down and switched to the all-night schedule; Ferguson and I would go out for breakfast at 6:00 AM and move our cars out of the forbidden Massachusetts of Technology (MIT) parking lot to prime on-the-street locations. From this 2-year conversation we derived a first pathway for vulval development (Ferguson *et al.* 1987). The only viable allele of *let-23* was *n1045*, a strange hypomorph that we do not yet fully understand (see Aroian and Sternberg 1991; Aroian *et al.* 1994). Ferguson had found that *let-23(n1045)* and *lin-15(n309)* suppressed each other's phenotypes. During this time I realized that *let-23* and *lin-15* had opposite effects not only on the vulva precursor cells but also on a two-celled group, P11/P12, and I was convinced that *let-23* was a key to vulval induction and might encode the receptor for the inductive signal from the anchor cell.

After an enjoyable postdoc in yeast with Ira Herskowitz, I visited Horvitz at MIT in early 1987. I explained what I wanted to work on in my laboratory. I was excited about two genes with interpretable pleiotropies, *let-23* and *lin-17* (Sternberg and Horvitz 1988). Horvitz said they were already trying to clone *lin-17*, but *let-23* was about third on his handwritten list of potential projects for new students. He quietly crossed *let-23* off his list. *lin-17* turned out to encode a WNT receptor, a receptor for a different type of developmental signal (Sawa *et al.* 1996).

After writing my first National Institutes of Health grant application in January 1987, I set out to obtain additional alleles of *let-23* and of other loci that worked with it, using suppression of *lin-15* to focus attention on the desired mutations. This approach worked. We eventually screened ~100,000 gametes and recovered only 10 alleles: 2 of *let-23*, 7 of *let-60*, and 1 of *lin-45*. I carried out a pilot genetic screen while still at the University of California at San Francisco, when Cynthia Kenyon kindly let me use her new *C. elegans* lab. I got the first suppressor, *sy1* (inaugurating my *C. elegans* strain collection), and set up the complementation test with a *let-23* allele before going to the Sixth International *C. elegans* Meeting at the Cold Spring Harbor Laboratory. I scored the cross at Greenwald's new lab in Princeton en route. Raffi Aroian joined the lab in September 1987 and screened for *lin-15* suppressors of which he found two. L28S, isolated on September 28, carried the third viable *let-23* allele, *sy97*, while L19O (October 19) became *sy90* and defined a locus that we temporarily called *dov-1* for "*do*minant *v*ulvaless." Ultimately *sy90* was found to be allelic to *let-60*. The next summer (1988), Caltech sophomore Ron Rogge obtained seven more suppressors but had trouble mapping them in the 10 weeks allotted (most were dominant suppressors but recessive lethal). Rogge actually wanted to be a script writer, so he moved across town to the University of California at Los Angeles and Utpal Banerjee's lab where he isolated Drosophila *Sos* (more about that later) and appeared in the movie "Jurassic Park," pipetteman in hand. When Min Han joined my lab in August 1988, Rogge gave him the strains and showed him how to set up worm crosses. A few months of mapping later, Han and Aroian realized that they both were working on *let-60* (originally *dov-1*) and set out on a blackboard a joint series of experiments that needed to be done to solve this interesting locus. During this same time, Scott Clark and Greg Beitel in Horvitz's laboratory found a variety of *let-60* alleles by screening for suppressors of a temperature-sensitive allele of *lin-15*.

During the 1980s developmental geneticists defined genes as interesting if they appeared to act as if they were binary switches (Hodgkin 1984). We called them, tongue-in-cheek, IDCGs for *i*mportant *d*evelopmental *c*ontrol *g*enes or more seriously, "switch genes." [As mentioned above, I had worked with Stuart Kauffman while an undergraduate and was thus on the lookout for binary switches controlling development (Kauffman 1973; Thomas 1973).] The paradigmatic switch genes in *C. elegans* were *lin-12* (Greenwald *et al.* 1983), *lin-14* (Ambros and Horvitz 1984), and *tra-1* (Hodgkin 1983), controlling space, time, and sex, respectively. Their characteristic was that loss-of-function (amorphic) alleles had an effect on a developmental decision opposite to that of the effect of gain-of-function (hypermorphic or neomorphic) alleles. As described below, *let-60* has gain-of-function alleles that cause a multivulva phenotype (previously known as *lin-34*; Ferguson and Horvitz 1985) and loss-of function alleles that cause a vulvaless phenotype. Thus, we were primed to realize that *let-60* was a new switch gene (Beitel *et al.* 1990; Han *et al.* 1990). While loss-of-function alleles are crucial for inferring gene function, gain-of-function mutations can indicate the sufficiency of gene action and can be used in reversion screens to generate null alleles (*e.g.*, Greenwald and Horvitz 1980). As Hodgkin (1984) describes, this concept of switch genes was based on the work of Ed Lewis (1994) who had shown that opposite *gf* and *lf* alleles of the Bithorax complex demonstrated its controlling rather than its permissive role in developmental decisions.

We eventually realized that *let-60* was defined by four classes of alleles. Loss-of-function alleles are recessive lethal and were isolated by screens for recessive-lethal mutations near *unc-22* (Rogalski *et al.* 1982; Clark *et al.* 1988). Gain-of-function mutations were first isolated on the basis of their semidominant multivulva phenotype. The dominant, vulvaless alleles are antimorphs and were isolated as suppressors of the *lin-15* multivulva phenotype. Recessive hypomorphs that are vulvaless and semiviable were also isolated as *lin-15* suppressors (Beitel *et al.* 1990).

To understand the dominant negative (*dn*) mutations, Han reverted a *dn* allele as a dominant, going

from a vulvaless heterozygote (*dn/+*) to a wild-type heterozygote (*dn lf/+ +*), by obtaining a suppressor mutation linked to the *dn* allele. A complementation test with a recessive-lethal *let-60* allele (*dn +/+ lf*) suggested that the *dn* mutations were *let-60* alleles, provided that they were only *cis*-dominant. The *cis–trans* test could then be done: animals carrying the *trans* configuration (*dn +/+ lf*) were dead, while those with the *cis* configuration were wild type (*dn lf/+ +*). The *dn* alleles were thus *let-60* alleles. A deletion of the region does not cause a dominant vulvaless phenotype, so the *dn* alleles involve some type of gain of function. Beitel *et al.* (1990) have a nice series of hypomorphic alleles, helping to establish the null phenotype of *let-60* as vulvaless (and lethal). Thus, *let-60* is necessary for vulval induction.

The *gf* alleles of *let-60* were isolated in five separate screens. In addition to the original "*lin-34*" allele (Ferguson and Horvitz 1985), four more were found as suppressors of vulvaless mutants: by Gregg Jongeward as a dominant suppressor of *let-23* in the same screen that identified *sli-1* (Jongeward *et al.* 1995), by Dianne Parry and Stuart Kim as a suppressor of *lin-10* in the same screen that identified *gap-1* (Hajnal *et al.* 1997), by Scott Clark as a suppressor of *let-341*, and by Min Han as a *trans* dominant suppressor of a *let-60(dn)*. Beitel *et al.* (1990) screened for suppressors of the multivulva phenotype of one of the *gf* alleles and obtained a recessive, *cis*-dominant (and thus intragenic) revertant: *gf/+* and *gf +/ + lf* are multivulva but *gf lf/+ +* is wild type. The intragenic revertants failed to complement a *let-60* recessive-lethal allele, and thus the dominant multivulva mutations are also *let-60* alleles. This and other gene dosage studies suggested that increased function of *let-60* is sufficient to cause vulval development in the absence of the anchor cell. Taken together, we then knew that *let-60* acts as a switch to control whether or not vulval precursor cells generate epidermis or vulval tissue. Indeed, Beitel *et al.* (1990) later found that the sequences of all five dominant multivulva alleles involved the identical amino acid substitution in codon 13, causing a weak activation of *let-60* by decreasing intrinsic GTPase activity, the mechanism by which RAS proteins become inactive.

Han also screened for mutations that reverted the ability of a heterozygous *dn* mutation (*dn/+*) to suppress the multivulva phenotype of *lin-15* by going from *dn/+* to *dn lf/+ +*. One of these second-site suppressors was linked to the balancer chromosome (*dpy-20*); it was a new *gf* allele (*dn +/+ gf*)! Having a *gf* mutation induced on a *dpy-20* chromosome allowed us to map *let-60* to the left of *dpy-20*, setting up the positional cloning described below. The observation that the dominant-negative alleles were suppressed by a gain of function indicated that the *dn* form of RAS interfered with RAS activation rather than blocking interaction with an effector (Han and Sternberg 1991).

The pathway genetics continued to be satisfying. We showed that *let-60(gf)* multivulva mutations suppressed *let-23* recessive vulvaless mutations. We inferred that *let-23* is necessary to activate *let-60*. The anchor cell induces the vulva via *let-23*, which acts via *let-60* to promote vulval differentiation. The next step was to find out what these two genes encoded.

Clark *et al.* (1995) had just cloned *dpy-20*, so we were looking to its left on the physical map. With a rotation student, Hiroyuki Mori, Han tried to identify by microinjection experiments the cosmid in the region that included *let-60* but soon realized that the physical map was incorrect, owing to the insertion of a piece of *Escherichia coli* sequence into one of the cosmids. When we told Alan Coulson by fax that the cosmid had *E. coli* DNA, he removed it, recalculated the physical map, and faxed back a very small interval a day later. Han then identified the cosmid by transformation rescue on New Year's Eve 1989 around 9 pm. In that pregenomic era, going from cosmid to the sequence of a gene was an involved process. Indeed, it took until May to get subclones and good sequence. Han wasted a month or so manually sequencing to get a long open reading frame. When he was ready to use BLAST, the server was down and it took 3 days to get the result. One night, he left an excited message on my home answering machine that *let-60* was what sounded like "RAF," which was clarified as RAS when I called him. We then knew that RAS in *C. elegans* plays critical roles in development and acts in an intercellular signaling pathway (Han and Sternberg 1990). What controls RAS? And what does it actually do?

Meanwhile in 1989, Aroian and Jane Mendel were trying to clone *let-23*, which lay in an ill-defined megabase-sized region of chromosome II. Aroian mapped *let-23* with *Tc1* polymorphisms (using the method of Ruvkun *et al.* 1989), determining an approximate location as well as a left boundary. Mendel identified the left breakpoint of *mnDf67* (left of *rol-6*) and made a genomic library from the *mnDf67* strain to clone the deletion breakpoint. She found the right breakpoint, and this gave a right boundary. Smack in the middle of this region was a clone called *kin-7*, which was homologous to the EGFR tyrosine kinase. In 1989 we faxed Makoto Koga and Yasumi Ohshima about collaborating to determine if we both had the same gene; most of the information on the physical map was unpublished. Aroian used Koga's genomic clone to rescue *let-23*. Koga obtained the genomic and cDNA sequence of *let-23*. The whole collaboration was carried on by fax and mail; we did not meet until the International *C. elegans* Meeting at Madison, Wisconsin, in 1991, well after our joint article was published (Aroian *et al.* 1990). LET-23 was the likely receptor for anchor cell signal, and activation of LET-23 led to RAS activation.

During this same period, Russell Hill was trying to clone *lin-3*, which was still a candidate for the inductive

signal. This region of the genome was not covered by cosmids, and one night he had a nightmare that the contig turned into overlapping hotdogs. The next day he glued plastic hotdogs to the right side of a paper contig map as surrogate cosmids. Hill screened for *lin-3* alleles that failed to complement existing vulvaless alleles, using a mutator background that had high levels of transposon Tc1 activity. He eventually obtained a Tc1 allele that tagged *lin-3* (Hill and Sternberg 1992). There were three especially pleasing aspects to this study: *lin-3* encodes an EGF family growth factor, overexpression of *lin-3* causes a multivulva phenotype suppressed by mutations in *let-23* or *let-60*, and *lin-3* is expressed in the anchor cell at the right time to be the inductive signal.

Andy Golden arrived at Caltech in May 1990 and quickly cloned a *C. elegans raf* homolog (*lin-45*) since (according to that week's literature) it interacted physically with EGFR (LET-23). Golden and Han figured out that *lin-45* encoded RAF and acted downstream of RAS; *lin-45* was the 10th of our *lin-15* suppressors (Han *et al.* 1993). By this time, RAF was known to be an upstream kinase in the MAP kinase cascade (formally MAP kinase kinase kinase), and a plausible connection from surface receptor to nucleus was thus established. However, we still did not know how LET-23 activates LET-60 RAS.

Our *lin-15* screen had been amazingly specific, recovering alleles of only three genes. By contrast, Clark, while a student with Horvitz, screened for suppressors of a temperature-sensitive mutation of *lin-15* and found many more loci (Clark *et al.* 1992b). He was swamped with interesting mutations, many of which are still in the freezer. He did, fortunately, focus on *sem-5*, which became one of the two keys to understanding the receptor-to-RAS pathway (Clark *et al.* 1992a). The other key came from studies of R7 photoreceptor specification in the Drosophila eye. During the same period, Mike Simon and Gerry Rubin worked out their sensitized R7 screen (Simon *et al.* 1991) and found alleles defective in RAS as well as in SOS. SOS was also found by Rogge in his Drosophila work with Banerjee. SOS is a guanine nucleotide exchange factor for RAS, controlling RAS activation. The SEM-5-SOS complex couples receptor activation to RAS activation. Whereas we were able to obtain viable or semiviable strains, the Drosophila geneticists could get even recessive lethal alleles by dominant enhancement. Having SOS allowed biochemists to confirm the regulatory relationships inferred from the genetic epistasis experiments. We know in retrospect that Clark had identified the locus *let-341*, which encodes *C. elegans* SOS early on, and showed that it acted upstream of RAS, but it proved very difficult to clone. Its sequence was not even in the 1998 *C. elegans* genome article. Chieh Chang, after failing to clone a SOS homolog during a laboratory rotation, eventually found the sequence in December 1998. Chang figured out it was *let-341* but only in 2000, by which time the game was over (Chang *et al.* 2000).

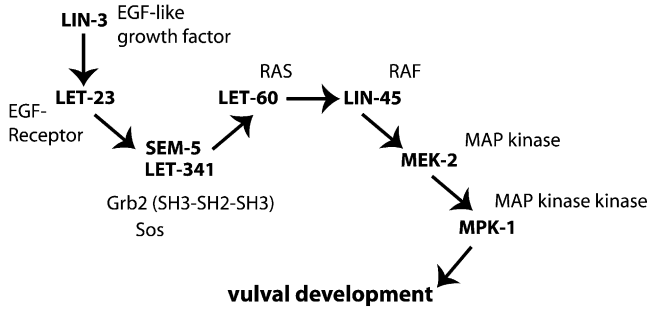


Figure 1.—The genetic pathway to RAS. *C. elegans* proteins are indicated in boldface type; the mammalian proteins are in regular type. Arrows indicate positive regulation. The pathway was inferred from tests of epistasis between loss-of-function mutations with a vulvaless phenotype and gain-of-function mutations or transgenes with a multivulva phenotype.

The initial pathway of *let-23* controlling *let-60* activity was discovered in 1990. By 1993, we in *C. elegans* had put together a linear pathway with *lin-3*, *let-23*, *sem-5*, *let-60*, and *lin-45* (see Figure 1). Studies in Drosophila and mammalian cell lines were also hot on the trail of the RAS pathway, and progress was rapid during these 3 years. In 1993, a physical interaction of RAS and RAF was demonstrated, assembling the complete pathway. The genetic analysis came to fruition at just the right time to inform the definitive biochemical experiments. There still is too large a gap between our ability to study, by molecular genetics, proteins acting in a multicellular organism *in vivo* and our ability to do single-cell biochemistry. Fortunately, the conservation of signaling pathways has allowed their elucidatation by the combination of whole-organism genetics coupled with biochemistry and cell biology applied *in vitro* or to heterologous systems. The gap has been narrowed by mouse molecular genetics on the mammalian side and by *in vivo* imaging techniques on the model organism side.

The similarities between the RAS signaling pathways of *C. elegans*, *Drosophila melanogaster*, and mammalian cells served as a key example of conserved core animal signal transduction. Earlier studies of yeast, for example, the complementation of a *Schizosaccharomyces pombe* cell-cycle mutant with the human cyclin-dependent kinase *cdc2* (Lee and Nurse 1987), had demonstrated deep conservation at the level of individual proteins and of core eukaryotic pathways such as the cell cycle. These studies and many others that followed led to our current expectation that regulatory pathways will be conserved. This conservation has made molecular genetics of model organisms a standard tool in understanding pathways of interest to human biology. The pathway to RAS was also a pathway to the age of model organisms.

I thank Bob Horvitz for showing me my first *C. elegans* cell division and for his generosity when I returned as a "ghost" to work on the vulva. I especially thank my many colleagues for making the pathway to RAS so exhilarating, productive, and fun.

## LITERATURE CITED


Ambros, V., and H. R. Horvitz, 1984 Heterochronic mutants of the nematode *Caenorhabditis elegans*. Science **226:** 409–416.

Aroian, R. V., and P. W. Sternberg, 1991 Multiple functions of *let-23*, a *Caenorhabditis elegans* receptor tyrosine kinase gene required for vulval induction. Genetics **128:** 251–267.

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

Aroian, R. V., G. M. Lesa and P. W. Sternberg, 1994 Mutations in the *Caenorhabditis elegans let-23* EGF receptor-like gene define elements important for cell-type specificity and function. EMBO J. **13:** 360–366.

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

Chang, C., N. A. Hopper and P. W. Sternberg, 2000 *C. elegans* SOS-1 is required for multiple RAS-dependent events and cooperates with a SOS-1-independent pathway during vulval induction. EMBO J. **19:** 3283–3293.

Clark, D. V., T. M. Rogalski, L. M. Donati and D. L. Baillie, 1988 The *unc-22 (IV)* region of *Caenorhabditis elegans*: genetic analysis of lethal mutations. Genetics **119:** 345–353.

Clark, S. G., M. J. Stern and H. R. Horvitz, 1992a *C. elegans* cell-signalling gene *sem-5* encodes a protein with SH2 and SH3 domains. Nature **356:** 340–344.

Clark, S. G., M. J. Stern and H. R. Horvitz, 1992b Genes involved in two *Caenorhabditis elegans* cell-signaling pathways. Cold Spring Harbor Symp. Quant. Biol. **57:** 363–373.

Clark, D. V., D. S. Suleman, K. A. Beckenbach, E. J. Gilchrist and D. L. Baillie, 1995 Molecular cloning and characterization of the *dpy-20* gene of *Caenorhabditis elegans*. Mol. Gen. Genet. **247:** 367–378.

Ferguson, E. L., and H. R. Horvitz, 1985 Identification and characterization of 22 genes that affect the vulval cell lineages of the nematode *Caenorhabditis elegans*. Genetics **110:** 17–72.

Ferguson, E. L., and H. R. Horvitz, 1989 The multivulva phenotype of certain *Caenorhabditis elegans* mutants results from defects in two functionally redundant pathways. Genetics **123:** 109–121.

Ferguson, E. L., P. W. Sternberg and H. R. Horvitz, 1987 A genetic pathway for the specification of the vulval cell lineages of *Caenorhabditis elegans*. Nature **326:** 259–267.

Greenwald, I. S., and H. R. Horvitz, 1980 *unc-93(e1500)*: a behavioral mutant of *Caenorhabditis elegans* that defines a gene with a wild-type null phenotype. Genetics **96:** 147–164.

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

Hajnal, A., C. W. Whitfield and S. K. Kim, 1997 Inhibition of *Caenorhabditis elegans* vulval induction by *gap-1* and by *let-23* receptor tyrosine kinase. Genes Dev. **11:** 2715–2728.

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

Han, M., and P. W. Sternberg, 1991 Analysis of dominant negative mutations of the *Caenorhabditis elegans let-60 ras* gene. Genes Dev. **5:** 2188–2198.

Han, M., R. Aroian and P. W. Sternberg, 1990 The *let-60* locus controls the switch between vulval and nonvulval cell fates in *Caenorhabditis elegans*. Genetics **126:** 899–913.

Han, M., A. Golden, Y. Han and P. W. Sternberg, 1993 *C. elegans lin-45 raf* gene participates in *let-60 ras*-stimulated vulval differentiation. Nature **363:** 133–140.

Hill, R. J., and P. W. Sternberg, 1992 The gene *lin-3* encodes an inductive signal for vulval development in *C. elegans*. Nature **358:** 470–476.

Hodgkin, J., 1983 Two types of sex determination in a nematode. Nature **304:** 267–268.

Hodgkin, J., 1984 Switch genes and sex determination in the nematode *C. elegans*. J. Embryol. Exp. Morphol. **83**(Suppl.): 103–117.

Horvitz, H. R., and J. E. Sulston, 1980 Isolation and genetic characterization of cell-lineage mutants of the nematode *Caenorhabditis elegans*. Genetics **96:** 435–454.

Horvitz, H. R., and J. E. Sulston, 1990 Joy of the worm. Genetics **126:** 287–292.

Jongeward, G. D., T. R. Clandinin and P. W. Sternberg, 1995 *sli-1*, a negative regulator of *let-23*-mediated signaling in *C. elegans*. Genetics **139:** 1553–1556.

Kauffman, S. A., 1973 Control circuits for determination and transdetermination. Science **181:** 310–318.

Kimble, J., 1981 Alterations in cell lineage following laser ablation of cells in the somatic gonad of *Caenorhabditis elegans*. Dev. Biol. **87:** 286–300.

Lee, M. G., and P. Nurse, 1987 Complementation used to clone a human homologue of the fission yeast cell cycle control gene cdc2. Nature **327:** 31–35.

Lewis, E. B, 1994 Homeosis: the first 100 years. Trends Genet. **10:** 341–343.

Rogalski, T. M., D. G. Moerman and D. L. Baillie, 1982 Essential genes and deficiencies in the *unc-22 IV* region of *Caenorhabditis elegans*. Genetics **102:** 725–736.

Ruvkun, G., V. Ambros, A. Coulson, R. Waterston, J. Sulston *et al.*, 1989 Molecular genetics of the *Caenorhabditis elegans* heterochronic gene *lin-14*. Genetics **121:** 501–516.

Sawa, H., L. Lobel and H. R. Horvitz, 1996 The *Caenorhabditis elegans* gene *lin-17*, which is required for certain asymmetric cell divisions, encodes a putative seven-transmembrane protein similar to the Drosophila frizzled protein. Genes Dev. **10:** 2189–2197.

Simon, M. A., D. D. Bowtell, G. S. Dodson, T. R. Laverty and G. M. Rubin, 1991 Ras1 and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the sevenless protein tyrosine kinase. Cell **67:** 701–716.

Sternberg, P. W., and H. R. Horvitz, 1984 The genetic control of cell lineage during nematode development. Annu. Rev. Genet. **18:** 489–524.

Sternberg, P. W., and H. R. Horvitz, 1988 *lin-17* mutations of *C. elegans* disrupt asymmetric cell divisions. Dev. Biol. **130:** 67–73.

Sulston, J. E., and H. R. Horvitz, 1977 Post-embryonic cell lineages of the nematode, Caenorhabditis elegans. Dev. Biol. **56:** 110–156.

Thomas, R., 1973 Boolean formalization of genetic control circuits. J. Theor. Biol. **42:** 563–585.