Development 125, 1191-1205 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
DEV5135

daf-12 regulates developmental age and the dauer alternative in *Caenorhabditis elegans*

Adam Antebi[1,*,†], Joseph G. Culotti[2] and Edward M. Hedgecock[1]

[1]Department of Biology, Johns Hopkins University, Baltimore, Maryland 21218, USA
[2]Division of Molecular Immunology and Neurobiology, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto M5G 1X5, Canada
*Present address: Max-Planck-Institut fuer molekulare Genetik, Ihnestrasse 73, D-14195 Berlin, Germany
†Author for correspondence (e-mail: antebi@MPIMG-Berlin-Dahlem.MPG.DE)

Accepted 18 December 1997; published on WWW 26 February 1998

SUMMARY

From egg through adult, *C. elegans* has six life stages including an option for dauer formation and diapause at larval stage L3 in adverse environments. Somatic cells throughout the organism make consistent choices and advance in unison, suggesting a mechanism of coordinate regulation at these stage transitions. Earlier studies showed that *daf-12*, which encodes a nuclear receptor (W. Yeh, 1991, Doctoral Thesis. University of Missouri-Columbia), regulates dauer formation; epistasis experiments placed *daf-12* near the end of the dauer signaling pathway. Here we describe novel *daf-12* alleles that reveal a general role in advancing L3 stage programs. In these mutants, somatic cells repeat L2-specific cellular programs of division and migration at the L3 stage; epistasis experiments place *daf-12* between *lin-14* and *lin-28* within the heterochronic pathway. We propose *daf-12* and other heterochronic genes provide cellular memories of chronological stage for selecting stage-appropriate developmental programs. Endocrine factors could coordinate these stage transitions and specify developmental alternatives.

Key words: Dauer-formation, Heterochronic gene, Cell migration, Diapause, Aging, *Caenorhabditis elegans*, *daf-12*

INTRODUCTION

The life history of *Caenorhabditis elegans* has six recognized stages, embryo, first (L1) through fourth (L4) stage larvae, and adult, with options at specific points for alternative developmental programs or reversible arrest (diapause). From cell division to tissue morphogenesis, the sequence and timing of developmental events are nearly invariant (Sulston and Horvitz, 1977; Sulston et al., 1980, 1983; Kimble and Hirsh, 1979; Hedgecock and White, 1985). These descriptions of wild-type development in controlled conditions provide an integrated framework, spanning from the cellular to the organismal level, for studying the influence of environment and genotype on life history.

Physiological and environmental factors, including food, temperature and population density, can pause or divert postembryonic development at several points (Golden and Riddle, 1984; Johnson et al., 1984; Arasu et al., 1991). Most dramatically, larvae must choose between two mutually exclusive programs, continuous or dauer, for L3 stage development (Cassada and Russell, 1975). The dauer larva is specialized, both morphologically and behaviorally, for extended diapause and dispersal, allowing survival until conditions improve. Signals for this decision are integrated via the *da*uer-*f*ormation (*daf*) genes. Daf-defective mutants bypass dauer formation, while Daf-constitutive mutants select this option, regardless of environmental conditions. These genes identify multiple signaling pathways that converge on a nuclear hormone receptor gene, *daf-12*, possibly their ultimate target (Yeh, 1991; Riddle and Albert, 1997).

Heterochronic genes specify developmental age, i.e., select stage-appropriate developmental programs, in nematode tissues (Ambros, 1997). Mutations in these genes can advance or delay the expression of stage-specific developmental programs. The known heterochronic gene activities affect all extragonadal tissues, but have no known role in the somatic gonad and germline. Those genes that have been molecularly characterized encode presumptive transcriptional or translational regulators, generally distributed throughout the affected tissues (Ruvkun and Giusto, 1989; Lee et al., 1993; Rougvie and Ambros, 1995; Moss et al., 1997).

Here we describe novel heterochronic mutants, defining two loci, that delay gonadal development at L3 and later stages. Some *mig-7* alleles, and the one existing *mig-8* allele, delay gonadal maturation only, notably leader cell migrations. Other *mig-7* alleles also delay extragonadal development; epistasis experiments suggest *mig-7* mediates between *lin-14* and *lin-28* heterochronic activities in these tissues. Neither gene affects germline age. Unexpectedly, *mig-7* mutants prove to be novel alleles of *daf-12*. The various alleles reveal two separable gene activities, one specifying L3 dauer programs in adverse conditions, and another specifying L3 continuous programs in

favorable environments; in the absence of either activity, somatic cells repeat L2 programs as a default. We discuss how *daf-12* and related genes could act as selectors for these programs. We propose the hierarchy of heterochronic genes form cellular memories regulating these selector functions. In particular, endocrine factors could trigger stage transitions and simultaneously advance these memories.

MATERIALS AND METHODS

Nematode culture

Except where noted otherwise, nematodes were cultured at 20°C on NGM media inoculated with *Escherichia coli* strain OP50 (Sulston and Hodgkin, 1988). *him-5(e1490)* strains were used to examine male phenotypes (Hodgkin et al., 1979).

Mutant isolation

daf-12 alleles *rh61, rh62* and *rh84*, and *mig-8* allele *rh50*, were isolated after ethylmethanesulfonate (EMS) mutagenesis in F_2 screens for mutants with distal-tip cell migration (Mig) phenotypes (Hedgecock et al., 1987). Allele *rh193*, which was found segregating in strain CB3400, presumably also resulted from EMS mutagenesis. Alleles *rh62rh157* and *rh61rh411* were isolated as spontaneous non-Mig revertants of *rh62* and *rh61*, respectively. Seven additional, *daf-12* alleles were obtained after EMS mutagenesis in non-complementation screens for Mig phenotypes. Briefly, crosses of EMS-treated N2 males with *daf-12 unc-128(rh110)* hermaphrodites were screened for rare Mig non-Unc hermaphrodite progeny. Alleles *rh257* and *rh258* were obtained from 6000 mutagenized chromosomes tested over *daf-12(rh84)*. Alleles *rh273* and *rh274* were obtained from 20,000 chromosomes tested over *daf-12(m20)*. Finally, alleles *rh284, rh285* and *rh286* were obtained from 25,000 chromosomes tested over *daf-12(rh258)*.

Genetic mapping

Mapping data for *daf-12* and *mig-8*, summarized in Fig. 1, were deposited with the Caenorhabditis Genetics Center.

RESULTS

Postembryonic development is coordinated with the molt cycle

During postembryonic development, each tissue undergoes a sequence of unique or repeated stage-specific events for growth and maturation (Sulston and Horvitz, 1977; Sulston et al., 1980). In the hypodermis, a new cuticle is synthesized at the beginning of each stage, followed by seam cell divisions and molt of the old cuticle (Singh and Sulston, 1978). This entire sequence of events, or molt cycle, typically requires just 9 hours at 20°C. However, the sequence is modified in the first molt cycle, where there is no old cuticle, and the fifth, where the seam cells fuse together, exiting permanently from the cell cycle (see Fig. 4). At characteristic stages, some seam cells undergo additional divisions to increase their numbers or generate peripheral neuroblasts. Finally, the composition and structure of the cuticle itself is specialized in the L1, dauer larva and adult stages. Growth and maturation of other somatic tissues are closely coordinated with the hypodermal molt cycle. Intestinal nuclei, for example, endoreplicate just before each molt (Hedgecock and White, 1985).

The somatic gonad and germline also undergo a sequence of unique stage-specific events for reproductive maturation. Mesoblasts Z1 and Z4 divide in L1 stage to generate the distal and proximal leader cells, and various gonadoblasts (Kimble and Hirsh, 1979). The gonadal leader cells, which are postmitotic, organize gonadogenesis, and polarize germline maturation, along their distoproximal axis. In the male, the proximal leader, or linker cell, undergoes a multistage migration along the larval body wall, which determines the shape and position of the mature gonad, while, in the hermaphrodite, the two distal leaders, or distal-tip cells, lead such migrations. The gonadoblasts divide in L3 and L4 stage to generate specific regions of the gonad. Beginning in L3, the proximalmost germline cells cease proliferation and commit to meiosis. In hermaphrodites, meiocytes commit to spermatogenesis or oogenesis at L4 and adult stages, respectively (Kimble and White, 1981; Austin and Kimble, 1987).

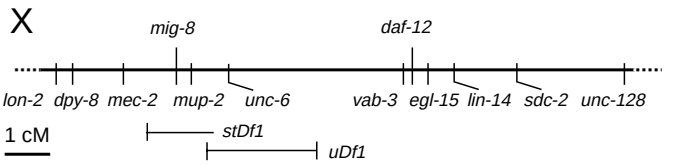

Fig. 1. Genetic map of chromosome X showing the positions of *daf-12* and *mig-8*. Regions deleted in deficiency chromosomes are shown by solid lines.

Development of gonadal and extragonadal tissues is closely coordinated over a range of environments. We examined the relative timing of gonadal development, notably leader cell migrations, under various culture conditions. Each leader cell describes a unique trajectory on the body wall which can be analyzed as a succession of unidirectional migrations on uniform substrata (Fig. 2). Under exhausted conditions, gonadoblast division, gonadal leader cell migration and germline proliferation cease during dauer diapause, indicating that germline, somatic gonad and extragonadal tissues all respond to a common signal for developmental arrest.

To describe the effects of genotype and environment on the relative timing of events, we must distinguish chronological and developmental stage. Following Ambros and Horvitz (1984), we reserve ‘L1’ through ‘L4’ for chronological stages as measured by elapsed molts, and use ‘S1’ through ‘S4’ for the corresponding developmental stages in wild type. In particular, ‘S3’ and ‘S3d’ refer to the characteristic developmental programs expressed in non-dauer or dauer larvae, respectively, during the third larval (L3) stage. Similarly, ‘A’ and ‘SA’ refer to chronological and developmental adult stages, respectively. Finally, we have adapted this notation to gonadal development, notably leader migrations (Fig. 2), with the proviso that some events occurring late within an intermolt are considered early events of the next developmental stage.

New *daf-12* mutants affecting developmental age

The original *mig-7* and *mig-8* alleles were isolated after ethylmethanesulfonate mutagenesis in screens for self-fertile hermaphrodites with abnormal distal-tip cell migrations (Hedgecock et al., 1987). Additional *mig-7* alleles were obtained in non-complementation screens or as spontaneous intragenic revertants (see Methods). As described below,

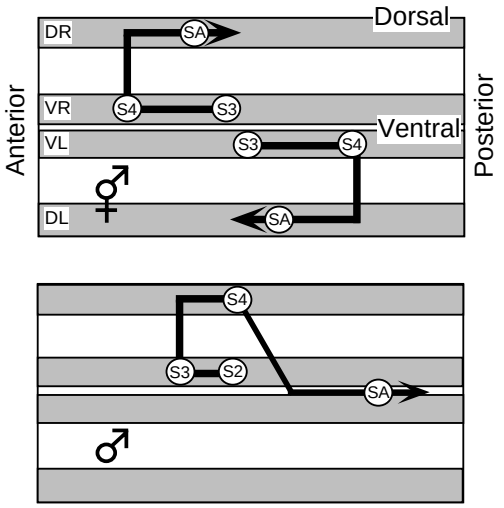

Fig. 2. Gonadal leader cell migrations. The body wall is shown as a cylindrical projection opened along the dorsal midline with the body wall muscles (DL, dorsal left, DR, dorsal right; VL, ventral left, VR, ventral right) shown by shading. Circles along the migration paths mark proposed transitions in the pathfinding programs of the gonadal leaders corresponding to S2, S3, S4 and SA, respectively, in the hypodermis. Times are averages (*n*=5) from larvae cultured at 20°C under replete conditions. (upper) The hermaphrodite distal-tip cells are born around the L1 molt (16 hours) but remain stationary until mid-L2 stage (21 hours). They then move in opposite directions along the ventral muscles, halting in mid-L3 (30 hours). These cells reorient over the next hour, and then move dorsally across the lateral hypodermis (31-32.5 hours). As they near the dorsal muscles, they quickly reorient (33 hours) and then migrate centripetally along these muscles (ca. 10 μm/hour), completing about 80% of this final segment by mid-L4 (39 hours). Active migration then slows and ceases entirely by the L4 molt (45 hours). (lower) The male linker cell begins moving anteriorly along the ventral right body muscle soon after its birth around the L1 molt (16 hour). It halts in mid-L2 (21 hours), reorients and then moves dorsally across the lateral hypodermis (22-23.5 hours). It reorients on the dorsal right muscle, and then migrates posteriorly along this muscle until mid-L3 (30 hours) where it turns obliquely and migrates across the lateral hypodermis (31-32.5 hours). Crossing ventral body muscles (33 hours), the linker cell continues posteriorly along the ventral midline. It reaches the developing cloaca by mid-L4 (39 hours) where it is engulfed.

genetic mapping (Fig. 1) and complementation show *mig-7* mutations represent new classes of *daf-12* alleles that perturb developmental age as well as dauer-formation. Cellular phenotypes, described below by tissue, vary greatly by allele and cell type. The most severe alleles delay expression of S3 and later developmental programs throughout the soma. In many cases, cells express essentially normal S3 programs after variable delays; in other cases, cellular development arrests completely. Cells also make inappropriate stage commitments in these mutants. In some cases, cells repeat S2 programs one or more times before expression of later programs; in other cases, they express abnormal programs, interpreted as hybrids of S2 and S3 programs.

Ectoderm

Hypodermal development appears normal in *daf-12(rh61)* through the L2 stage. At L3, however, seam cells frequently fail to express S3 programs, repeating S2 programs instead. In particular, Vn.pap and Vn.ppp undergo equational division followed by stem-cell division in early L3 (Figs 3A, 4). Occasional cells repeat S2 programs yet again at L4, but most express S3 or S4 programs (which are not easily distinguishable). Finally, a fraction of the seam cells delayed at L3 later fail to express SA programs at adult and repeat S4 programs instead. As a result, the alae of the adult cuticle have visible gaps (Figs. 3B, 4). In hermaphrodites whose V3 seam descendants are delayed, uterine, vulval and sex muscle cells fail to attach to the seam, allowing the uterus to evert at the onset of egg-laying (cf. Newman et al., 1996). Adults with many delayed seam cells may undergo an extra molt cycle in which delayed cells finally express SA programs, filling in any alae gaps. The penetrance of these seam phenotypes varies greatly by allele; the adult phenotype generally parallels the L3 phenotype, but is less penetrant (Table 1).

Vulval development is generally normal in *daf-12(rh61)* hermaphrodites. However, in another allele, *rh193hs*, the anchor-cell-dependent divisions of vulval precursors (cf. Kimble, 1981) are frequently delayed or absent. At 25°C, these divisions were delayed beyond the L3 molt in 6/26 larvae examined. Finally, in *rh61* males, there are frequent defects in tail hypodermal development (cf. Sulston et al., 1980), including delayed division of ray precursor cells in the L3 stage, and incomplete tail-spike retraction and fan morphogenesis in the L4 stage. In particular, the anteriormost ray sensilla often fail to join the developing fan.

Somatic mesoderm

In *daf-12(rh61)* hermaphrodites, development of the somatic mesoblast M, including sex-myoblast migrations, are normal through the L2 stage (cf. Sulston and Horvitz, 1977). However, the subsequent divisions of the sex-myoblasts are often delayed (Fig. 3C). At the L3 molt, only 2/22 cells had completed all three division rounds on schedule, while 13/22 had completed two divisions and 7/22 just one. Finally, all three division rounds are completed by mid-L4. Despite these delayed divisions, differentiation of sex-myoblast descendants, the vulval and uterine muscles, are usually normal. Some adults, however, retain their eggs.

Endoderm

In wild type, intestinal cells int3 through int9 undergo nuclear division at L1 lethargus, becoming binucleate (Sulston and Horvitz, 1977; Hedgecock and White, 1985). In *daf-12(rh61)*, intestinal development appears normal through the L2 stage. However, occasional cells become either tri- or tetranucleate as one or both nuclei undergo a second division at L2 lethargus (Fig. 3D). We interpret this phenotype as a repetition of S2 programs in the intestinal cells at the L3 stage (Table 1).

Gonadal mesoderm

In *daf-12(rh61)* males, gonadal development appears normal through mid-L2 stage. In particular, the compaction of gonadoblasts behind the linker cell and its initial anterior migration are normal. Beginning in late L2, the linker cell migration is frequently abnormal. We have classified these migration errors according to larval stage, i.e., L3 or L4, and direction of movement, i.e., dorsoventral or anteroposterior (Fig. 5, Table 2). The mutant trajectories may reflect repetitions

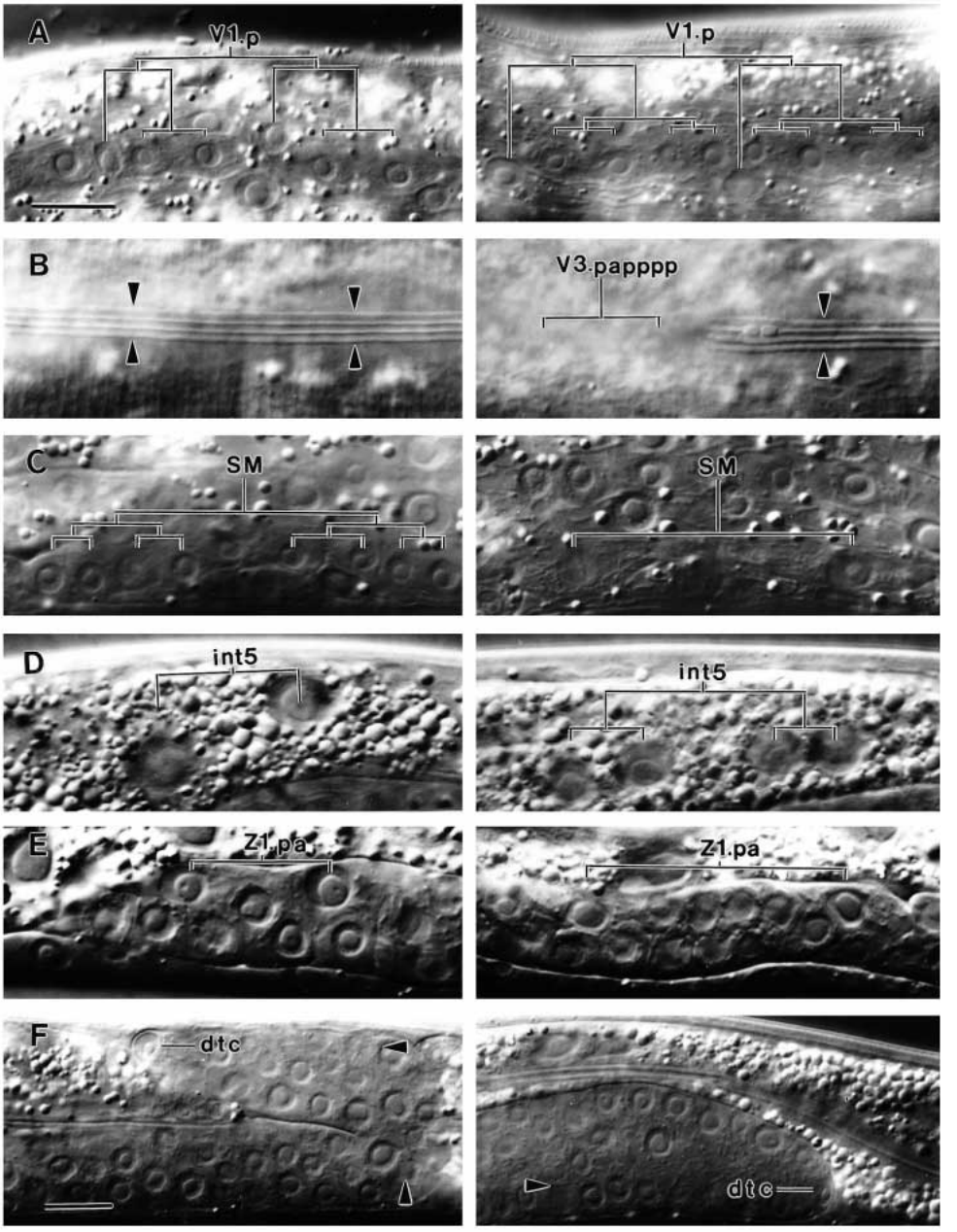

Fig. 3. DIC micrographs comparing normal and delayed development in (left) wild type and (right) *rh61* hermaphrodites, respectively. Lateral aspects; scale bars are 10 μm. (A) Seam cell V1p descendants in early L3 larvae. In wild type, seam cells V1.pap and V1.ppp express S3 programs, or stem-cell division (cf. Fig. 4). In *rh61*, these seam cells repeat S2 programs, i.e., equational division followed by stem-cell division. (B) Seam cells at the L4 molt. In wild type, these cells express the SA seam program of cell cycle exit, cellular fusion and formation of a distinctive, ridged cuticle or alae (arrowheads). In *rh61*, while most seam cells express the SA program, occasional cells (e.g., V3.papppp) repeat larval stage programs, i.e., stem-cell division. (C) Sex-myoblasts at the L3 molt. In wild type, three successive divisions of SM have generated 8 post-mitotic myoblasts. In *rh61*, only the first division is completed; two additional divisions follow in the L4. (D) Intestinal cell int5 soon after the L2 molt. In wild type, the S2 program creates a binucleate cell; no further nuclear divisions occur during the S3. In *rh61*, the S2 program repeats, creating a tetranucleate cell. (E) Gonad primordium in early L3 larvae. In wild type, the Z1.pa daughters have coalesced near the center of the primordium. In *rh61*, gonadoblast Z1.paa has been separated from its sister Z1.pap by proliferating germline cells. (F) Left distal-tip cell at the L3 molt. In wild type, the S4 movements of the distal-tip cell (dtc) have established the characteristic reflexed shape of the ovary (arrowheads). In *rh61*, the distal-tip cell continues posteriorly along the body ahead the column of proliferating germline cells.

of S2 or S3 pathfinding programs in the linker cell at L3 and L4, respectively (see Discussion). In cases where the linker cell completes its S4 movements, it generally halts short of the cloaca and/or fails to be engulfed, suggesting its SA program may be delayed as well. Adult males are invariably infertile.

In *daf-12(rh61)* hermaphrodites, gonadal development appears normal through mid-L2 stage. However, the compaction of gonadoblasts at L2 lethargus, and their divisions in early L3, are variably delayed (Fig. 3E). We interpret these phenotypes as delay or failure to express S3 gonadoblast programs (Kimble and Hirsh, 1979). Later abnormalities, including irregular queuing of oocytes, production of undersized eggs and sterility, may be consequences of these L3 phenotypes, or reflect direct defects in the regulation of S4 or SA programs in these cells. Beginning in late L3, the distal-tip cell migrations are frequently abnormal (Figs. 3F, 5; Table 2). The mutant trajectories may reflect repetition of S3 pathfinding programs in the distal-tip cells at L4 (see Discussion). Frequently, the basement membrane surrounding the distal ovary ruptures with escape of germline cells into the pseudocoelom. In most alleles, the distal-tip cells remain motile through mid-L4 as judged by their adherent morphology. In *rh193* cultured at 25ºC, however, these cells cease active migration during L3.

Germline

In wild-type hermaphrodites, pachytene stage meiocytes are first evident in L3 lethargus (33 hours), while sperm and oocytes become evident at the onset of L4 lethargus (42 hours) and at ecdysis (45 hours), respectively (Kimble and White, 1981; Austin and Kimble, 1987). In *daf-12(rh61)* hermaphrodites, sperm and oocytes become evident about 6

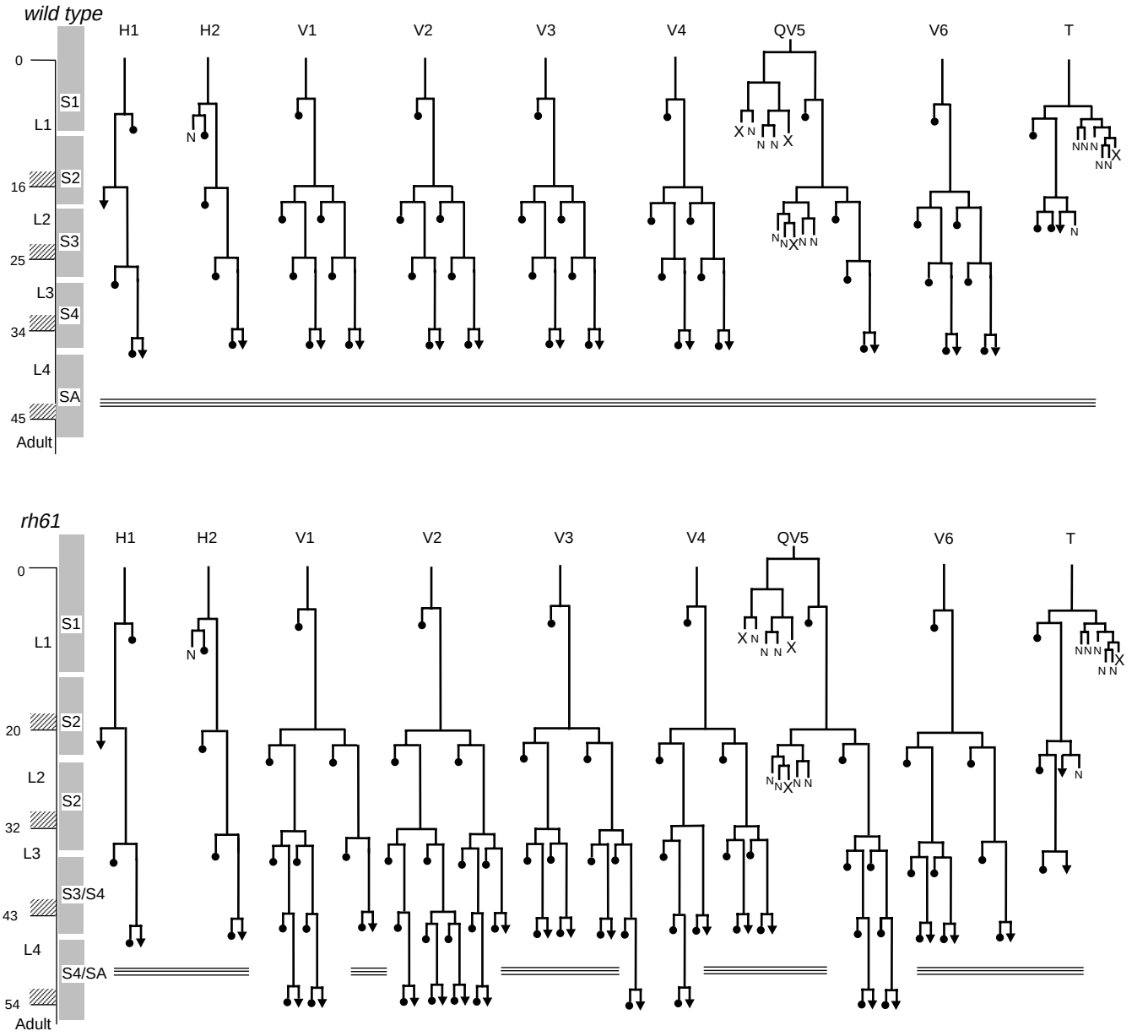

Fig. 4. Hypodermal seam development. Seam cells, arranged in longitudinal rows along either side of the animal, undergo a sequence of stage- and position-specific divisions coordinated with the molt cycle (Sulston and Horvitz, 1977). Chronological age is shown on the left as hours elapsed (at 20°C) since hatching. The extent of each chronological (e.g., L1) and proposed developmental stage (e.g., S1) is also shown. Lethargus, the period of suppressed feeding and reduced locomotion that culminates with ecdysis, is shown by hatched lines (Singh and Sulston, 1978). In wild type hermaphrodites, during S1, S3 and S4 programs of typical seam cells in midbody (e.g., V1 lineage), each cell divides just once, and then the anterior daughter endoreplicates and fuses with the general hypodermis, while the posterior daughter remains a stem cell (Sulston and Horvitz, 1977; Hedgecock and White, 1985). During S2 programs, each seam cell first undergoes an equational division. During SA programs, the seam cells fuse together, exiting permanently from the cell cycle, and synthesize a distinctive ridged cuticle, or alae (horizontal lines). In *rh61* hermaphrodites, seam cells frequently undergo additional divisions in early L3, interpreted as repeated S2 programs. Within affected cell lineages, subsequent cell commitments are also abnormal. For example, descendants sometimes repeat S2 programs again at L4 stage, e.g., seam cell V2.pappp in this individual. More commonly, descendants express larval programs at adult stage. The mean times of lethargus and ecdysis for *rh61* larvae cultured at 20°C (n=6) are also shown. *N*, neuron or neuronal support cell; *X*, programmed cell death.

and 2 hours, respectively, before L4 ecdysis. The brood size ranged from 0 to 41, with mean 10±12, for a sample of selfing hermaphrodites (n=22), compared with over 300 in wild type (Hodgkin et al., 1979).

daf-12 regulates dauer formation

The reference allele, *daf-12(m20)*, and similarly selected alleles are Daf-defective and, by genetic epistasis experiments, act near the end of the dauer pathway (Riddle et al., 1981; Vowels and Thomas, 1992; Thomas et al., 1993). Many of the new classes of *daf-12* mutations that we isolated affect the regulation of dauer formation; most alleles are Daf-defective, some are Daf-constitutive, while others have near normal dauer regulation. Epistasis experiments using these new allele classes

Table 1. Heterochronic and dauer formation phenotypes

Gene	Allele	°C	L3[1]	L3 seam cells[2]	Adult seam cells[3]	L3 intestinal cells[4]	L4 distal-tip cells[5]	L4 linker cell[6]	Dauer regulation replete[7]	Dauer regulation exhausted[8]
wild type	N2	20	S3	100	100	100	100	99±1	0	>10
		20	S3d	100	100	-	100	-	NA	NA
daf-12										
class 1	*rh61*	15	S3	-	63±3	-	0	-	0	0
		20	S3	7±2	81±1	80±2	7±2	1±1	0	0
		25	S3	-	69±2	-	29±4	-	0	0
	rh61/m20	20	S3	24±4	94±1	-	51±6	-	0	0
	rh61/+	20	S3	-	100	-	100	-	0	>10
	rh84	15	S3	-	80±1	-	25±4	-	0	0
		20	S3	5±2	80±1	80±3	56±3	7±3	0	0
		25	S3	-	72±2	-	90±3	-	0	0
	rh84/m20	20	S3	-	97±1	-	98±1	-	0	0
	rh84/+	20	S3	-	100	-	100	-	0	>10
	rh257	15	S3	-	50±2	-	24±5	-	0	<5[9]
		20	S3	5±2	63±1	85±3	16±2	2±2	0	<5[9]
		25	S3	-	55±2	-	74±4	-	0	<5[9]
		20	S3d	-	99±1	-	89±4	-	NA	NA
	rh257/+	20	S3	-	100	-	100	-	0	-
class 2	*rh62rh157*	20	S3	15±3	86±1	91±2	100	83±3	0	0
	rh62rh157/m20	20	S3	-	97±1	-	100	-	0	0
	rh62rh157/+	20	S3	-	100	-	100	-	0	>10
class 3	*m20*	20	S3	68±2	99±1	87±3	100	87±3	0	0
	rh61rh411	20	S3	79±3	99±1	86±3	100	90±3	0	0
	rh258	20	S3	89±1	100	86±3	100	86±3	0	0
	rh258/m20	20	S3	-	100	-	100	-	0	0
	rh258/+	20	S3	-	100	-	100	-	0	>10
	m583	20	S3	96±2	99±1	89±3	100	93±2	0	0
class 4	*rh193*	15	S3	87±3	95±1	-	94±2	-	0	>10
		20	S3	40±5	74±1	95±2	51±3	14±4	0	>10
		25	S3	67±4	12±1	-	4±2	-	<5	>10
		20	S3d	-	99±1	-	62±3	-	NA	NA
	rh193/m20	20	S3	36±4	89±2	-	81±5	-	0	>10
	rh193/+	20	S3	-	100	-	100	-	0	-
	rh285	15	S3	95±2	9±1	-	20±4	-	<5	>10
		20	S3	50±5	42±1	83±3	1±1	0	0	>10
		25	S3	35±5	56±3	-	30±6	-	0	>10
		20	S3d	100	99±1	-	80±3	-	NA	NA
	rh285/+	20	S3	-	100	-	100	-	0	-
class 5	*rh284*	15	S3	-	100	-	97±1	-	0	>10
		20	S3	100	100	97±1	28+3	53±7	0	>10
		25	S3	-	100	-	29±5	-	0	>10
		20	S3d	-	100	-	98±1	-	NA	NA
	rh284/m20	20	S3	87±3	100	-	29±5	-	0	>10
	rh284/+	20	S3	-	100	-	100	-	0	-
	rh286	20	S3	97±2	100	94±2	97±1	97±2	0	>10
		20	S3d	-	100	-	100	-	NA	NA
	rh286/+	20	S3	-	100	-	100	-	0	-
class 6	*rh62*	15	S3	-	99±1	-	90±4	-	13±2[9]	>10
		20	S3	100	99±1	96±2	6±2	80±4	3±1[9]	>10
		25	S3	-	98±1	-	12±4	-	8±2[9]	>10
		20	S3d	-	97±1	-	100	-	NA	NA
	rh62/m20	20	S3	99±1	100	-	16±4	-	69±5[9]	-
	rh62/+	20	S3	-	100	-	100	-	0	-
	rh273	15	S3	-	76±2	-	100	-	92±2[9]	>10
		20	S3	98±1	97±1	99±1	24±3	50±18	41±3[9]	>10
		25	S3	-	92±1	-	21±5	-	39±3[9]	>10
		20	S3d	-	100	-	99±1	-	NA	NA
	rh273/+	20	S3	-	100	-	100	-	0	-
	rh274	15	S3	-	100	-	74±3	-	53±2[9]	>10
		20	S3	100	100	99±1	17±3	84±5	7±3[9]	>10
		25	S3	-	95±1	-	10±4	-	38±2[9]	>10
		20	S3d	-	100	-	95±2	-	NA	NA
	rh274/+	20	S3	-	100	-	100	-	0	-
mig-8	*rh50*	20	S3	100	100	99±1	8±2	58±5	0	>10
		20	S3d	-	100	-	100	-	NA	NA
	rh50/+	20	S3	-	100	-	100	-	0	-
mig-8 daf-12	*rh50 rh84*	20	S3	-	67±2	-	39±6	-	0	<5[9]
	rh50 m20	20	S3	-	100	-	98±1	-	0	0

[1]Larvae were cultured contiuously under replete conditions (S3 alternative), or were transferred from exhausted cultures as dauer larvae (S3d alternative) to replete conditions to resume development.

[2,3,4,5,6,7,8]Results are reported in percentages as mean ± standard deviation for a binomial distribution with the observed mean and actual sample size.

[2]Percentage of seam cells from V1 to V6 that express S3 programs at L3 stage (ca. 10 larvae × 11 cells/larva).

[3]Percentage of seam cells from H1 to V6 that express SA programs at Adult stage (ca. 25 adults × 13 cells/adult).

[4]Percentage of intestinal cells int3 to int9 that express S3 programs at L3 stage (ca. 10 larvae × 14 cells/larva).

[5]Percentage of hermaphrodite distal-tip cells that migrate dorsally at L4 stage (ca. 100 larvae × 2 cells/larva).

[6]Percentage of male linker cells that migrate ventrally at L4 stage (ca. 100 larvae × 1 cell/larva).

[7]Percentage of dauers among larvae cultured at low population density on replete bacterial lawns (according to Larsen et al., 1995). NA, not applicable.

[8]Percentage of dauers among larvae cultured at high population density on exhausted bacterial lawns. For transheterozygotes and dominance tests, individual dauer larvae were scored for the segregation of parental genotypes. NA, not applicable.

[9]Partial-dauer larvae.

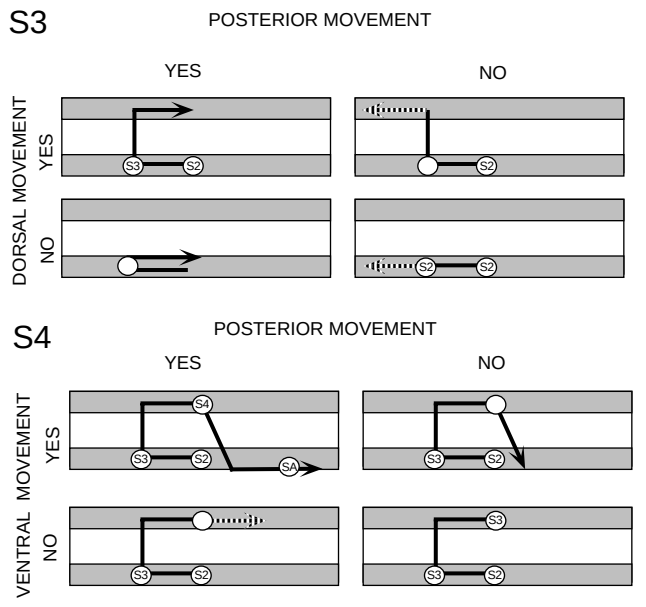

Fig. 5. A classification of pathfinding errors for the gonadal leader cells. For each larval stage, cells were scored separately (YES or NO) for expression of their normal circumferential, i.e., dorsal or ventral, and longitudinal, i.e., anterior or posterior, movements (Table 2). For simplicity, only the right body wall is shown. (top) Normal and mutant S3 movements of the male linker cell. The S4 movements of the hermaphrodite distal-tip cell are analogous. (bottom) Normal and mutant S4 movements of the male linker cell.

also place *daf-12* near the end of the dauer-signaling pathway, e.g. Daf-defective allele *rh84* completely suppresses dauer formation in doubles with Daf-constitutive mutants *daf-1(m40)* and *daf-7(e1372)*.

We compared dauer formation over a range of growth temperatures in both replete and exhausted cultures. Generally, no morphologically recognizable dauer larvae were ever observed in cultures of the various Daf-defective alleles. However, in exhausted cultures, allele *rh257* forms occasional larvae with cuticular alae, and some of the radial shrinkage of body and pharynx, characteristic of dauer larvae. Under these conditions, wild-type dauer larvae remain arrested in diapause, and neither feed nor undergo cellular development. By contrast, these 'partial-dauer' larvae attempt to resume feeding and, consequently, are sensitive to 1% sodium dodecyl sulfate (SDS) (Cassada and Russell, 1975).

Daf-constitutive alleles *rh62*, *rh273* and *rh274* form partial-dauer larvae under replete conditions at all temperatures, whereas *rh193* and *rh285* can produce occasional partial-dauer larvae when cultured at 25°C or 15°C, respectively (Fig. 6; Table 1). Again, these larvae resume feeding and are generally SDS-sensitive. Somatic development, but not germline proliferation, are arrested in the partial-dauer larvae. Whereas dauer larvae in exhausted cultures generally arrest with fewer than 40 germ cells in wild type, partial-dauer larvae often have twice this number. Most partial-dauer larvae eventually progress to fertile adults, but up to one-third remain arrested indefinitely even at 15°C. Occasionally, these larvae have been observed to synthesize dauer-specific cuticle at L3, and then again, at L4 stage. Finally, all five of these alleles can form normal dauer larvae when cultured under exhausted conditions.

A functional classification of *daf-12* alleles

We have placed available *daf-12* alleles into six, idealized classes based on their dauer-formation phenotypes, i.e., Daf-defective, -normal, or -constitutive, and secondarily, their heterochronic phenotypes in gonadal and extragonadal tissues

Table 2. Gonadal leader migration phenotypes[1]

		Distal-tip cell			Linker cell					
			Posterior[2]			Posterior			Posterior	
Gene	Allele	S4 Dorsal	Yes	No	S3 Dorsal	Yes	No	S4 Ventral	Yes	No
wild type	N2	Yes	100	0	Yes	100	0	Yes	100	0
		No	0	0	No	0	0	No	0	0
daf-12										
class 1	*rh61*	Yes	4±1	3±1	Yes	86±4	5±2	Yes	0	1±1
		No	1±1	92±2	No	0	9±3	No	1±1	84±4
	rh84	Yes	44±3	12±1	Yes	73±4	21±4	Yes	4±2	3±2
		No	2±2	42±3	No	0	6±2	No	3±2	63±5
class 2	*rh62rh157*	Yes	100	0	Yes	100	0	Yes	77±4	6±2
		No	0	0	No	0	0	No	9±3	8±3
class 3	*rh61rh411*	Yes	99±1	1±1	Yes	99±1	1±1	Yes	81±4	9±3
		No	0	0	No	0	0	No	5±2	4±2
class 4	*rh285*	Yes	1±1	0	Yes	45±6	35±6	Yes	0	0
		No	0	99±1	No	2±1	18±5	No	0	45±6
class 5	*rh284*	Yes	22±3	6±1	Yes	96±3	4±3	Yes	33±6	20±5
		No	0	72±3	No	0	0	No	6±3	37±7
class 6	*rh62*	Yes	3±1	3±1	Yes	98±1	0	Yes	74±4	6±2
		No	0	94±2	No	2±1	0	No	2±1	16±4
mig-8	*rh50*	Yes	7±2	1±1	Yes	98±1	2±1	Yes	55±5	3±2
		No	0	92±2	No	0	0	No	1±1	39±5

[1]Percentages of various pathfinding errors, classified as described in Fig. 5, are reported as mean ± standard deviation for a binomial distribution with the observed mean and actual sample size (ca. 100 larvae × 2 distal-tip cells or 1 linker cell/larva).
[2]Data from left and right distal-tip cells are combined.

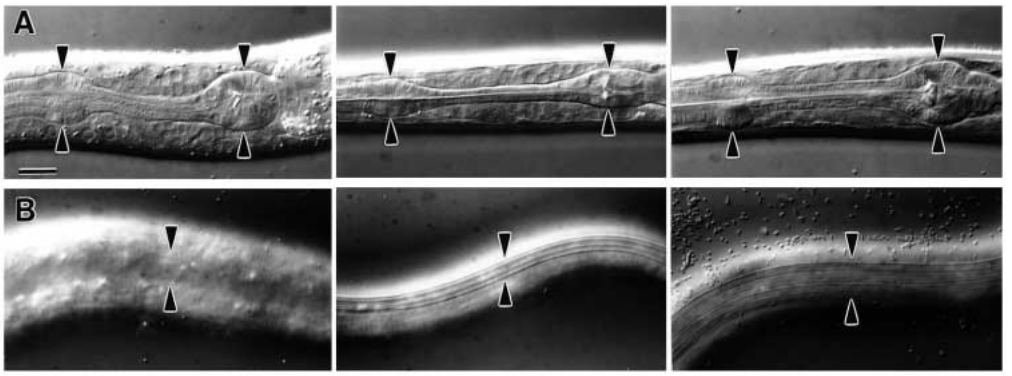

Fig. 6. DIC micrographs comparing (left) wild type L3 non-dauer larva from replete culture, (middle) wild type L3 dauer larva from exhausted culture, and (right) *daf-12(rh62)* L3 partial-dauer larva from replete culture. Lateral aspects; scale bar is 10 μm. (A) Larval head. The pharynx is indicated by arrowheads. (B) Larval body. The hypodermal seam is indicated by arrowheads. Cuticular alae characteristic of seam S3d programs are visible in the dauer and partial-dauer larvae. The dauer larva undergoes a dramatic radial shrinkage during S3d diapause; this process is not completed in the partial-dauer larva.

(Table 1). Class 1, 2 and 3 alleles are Daf-defective. Class 1 alleles (*rh61*, *rh84*, *rh257*) have delayed gonadal and extragonadal development. Class 2 comprises a single allele *rh62rh157* with nearly normal gonadal, but delayed extragonadal development; this mutation arose as a spontaneous non-Mig, intragenic revertant of *rh62*. Class 3 alleles (*rh258*, *rh61rh411*) have nearly normal gonadal and extragonadal development. Allele *rh61rh411* arose as a spontaneous non-Mig, intragenic revertant of *rh61*. The original, Daf-defective alleles isolated by D. Riddle and others (e.g., *m20*, *m25*, *m116*, *m583*, *sa156*) all fall within this, largest class (Riddle et al., 1981; Thomas et al., 1993; Larsen et al., 1995). Class 4 and 5 have nearly normal dauer regulation. Class 4 alleles (*rh193*, *rh285*) have delayed gonadal and extragonadal development, while class 5 alleles (*rh284*, *rh286*) have delayed gonadal development only. Finally, class 6 alleles (*rh62*, *rh273*, *rh274*) are Daf-constitutive. These mutants have delayed gonadal, but nearly normal extragonadal development.

All *daf-12* alleles examined are recessive to wild type for both dauer regulation and heterochronic phenotypes (Table 1). For dauer-formation, class 1, 2 and 3 alleles fail to complement, i.e., *trans*-heterozygotes are Daf-defective; moreover, these Daf-defective classes are recessive to class 6, i.e., *trans*-heterozygotes are Daf-constitutive. These mutations reveal a gene function required for S3d programs in adverse conditions designated *daf-12b*. We propose class 1, 2 and 3 alleles reduce or eliminate *daf-12b*, while class 6 alleles misregulate this activity (see Discussion).

For gonadal and extragonadal heterochrony, class 3 alleles partially complement class 1, i.e., the penetrance of these phenotypes follows the series *rh61/rh61>rh61/m20> m20/m20>rh61/+ = m20/+ = +/+* (Table 1). Class 6 alleles complement extragonadal, but not gonadal heterochrony, of other classes; finally, class 3 alleles only poorly complement gonadal heterochrony of class 6. These mutations reveal a gene function required for S3 programs in replete conditions designated *daf-12a*. We propose class 3 alleles reduce or eliminate *daf-12a* function arising from the *daf-12* locus, while class 1 alleles also antagonize *daf-12a* function arising from other sources (see Discussion).

Temperature-shift experiments

Heterochronic and dauer-formation phenotypes vary significantly with growth temperature in many *daf-12* alleles (Table 1). To learn when *daf-12(+)* activity is required during development, we performed temperature-shift experiments with two temperature-sensitive alleles. In *rh285*, seam cells express S3 programs at L3 stage when cultured at 15°C, but repeat S2 programs instead at 25°C (Table 1). Up until mid-L2, temperature upshifts and downshifts can reveal or rescue this phenotype, respectively (Fig. 7). In *rh193*, seam cells generally repeat S2 programs at L3 stage regardless of culture temperature; their descendants express SA programs at adult stage when cultured at 15°C, but repeat larval programs instead at 25°C (Table 1). Up until late L2, temperature upshifts can reveal the adult seam phenotype, while downshifts can rescue until about mid-L3 (Fig. 7). In *rh193* hermaphrodites, the distal-tip cells express S4 pathfinding programs at L4 when cultured at 15°C, but repeat S3 programs instead at 25°C (Table 1). Up until late L2, temperature upshifts can reveal this phenotype, while downshifts can still rescue until mid-L3 (Fig. 7).

Interaction with other heterochronic mutants

Using double mutants with class 1 allele *daf-12(rh61)*, we examined possible functional interactions with the heterochronic genes *lin-4*, *lin-14*, *lin-28* and *lin-29* (Ambros, 1997). Hypodermal phenotypes of these double mutants are summarized below; gonadal phenotypes generally resemble *daf-12* alone.

daf-12 is epistatic to *lin-14b*

In *lin-14(ma135)* null mutants, hypodermal cells express S2-S3-S4-SA programs in succession, bypassing S1 entirely (Ambros and Horvitz, 1984). This phenotype reflects two separable activities, *lin-14a*, which prevents precocious expression of S2 programs in L1 stage, and *lin-14b*, which prevents precocious expression of S3 programs in L2 stage (Ambros and Horvitz, 1987). In *lin-14 daf-12*, most seam cells express S2-S2-S3-S4-SA programs in succession, indicating *daf-12* acts downstream of *lin-14*; moreover, *daf-12* prevents precocious vulval development (Table 3). We propose *lin-14b* inhibits *daf-12a*, possibly indirectly, to prevent precocious expression of S3 and later programs.

In *lin-4(e912)* null mutants, hypodermal cells fail to advance to S2 programs, repeating S1 programs indefinitely (Chalfie et al., 1981; Ambros, 1997). *lin-4 lin-14* double mutants are indistinguishable from *lin-14* alone, suggesting *lin-4(+)* inhibits *lin-14(+)* to advance S2 and later programs (Ambros,

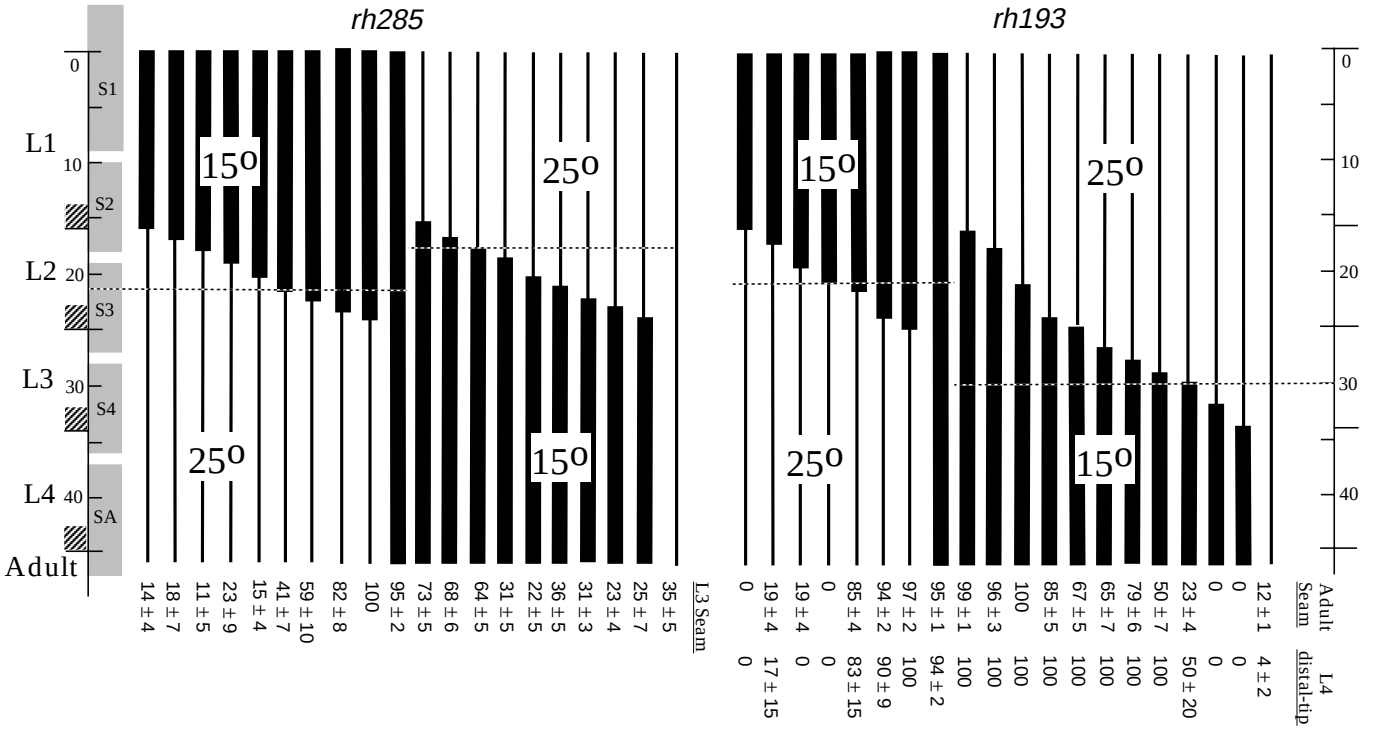

Fig. 7. Temperature-shift experiments with *daf-12* alleles *rh193* and *rh285*. Larvae were examined briefly by DIC microscopy for staging (Ambros and Horvitz, 1987; Sulston and Horvitz, 1977) and then transferred to culture plates pre-equilibrated at the post-shift temperature. These individuals were re-examined as L3 larvae or young adults to score seam and distal-tip cell phenotypes (see Table 1). Chronological age, normalized to the growth rate of wild type larvae at 20°C, is shown on the left. Thick and thin portions of the vertical lines indicate culture at permissive (15°C) and non-permissive (25°C) temperatures, respectively. Dotted horizontal lines bracket the critical period for these phenotypes.

1989). As expected, hypodermal development in *lin-4 daf-12* double mutants resemble *lin-4* alone (Table 3); also like *lin-4* alone, *lin-4 daf-12(rh62)* double mutants are Daf-defective (data not shown). In the absence of *lin-4(+)*, persistent *lin-14b* can evidently inhibit *daf-12a* and *daf-12b* indefinitely.

lin-28 is epistatic to *daf-12*

In *lin-28(n719)* null mutants, hypodermal cells express S1-S3-S4-SA programs in succession, bypassing S2 entirely (Ambros and Horvitz, 1984). In *lin-28 daf-12*, seam and vulval development are nearly indistinguishable from *lin-28* alone, indicating *lin-28* acts downstream of *daf-12* (Table 3). We propose *daf-12a* inhibits *lin-28(+)*, possibly indirectly, to advance S3 and later programs.

lin-4 enhances *daf-12* in a *lin-14* null background

lin-4 enhances *daf-12* hypodermal phenotypes in the *lin-14(ma135)* background, indicating *lin-4(+)* acts on a second target, downstream of *lin-14b*, to advance S3 programs. For example, seam cells often express SA programs at L4 stage in *lin-14 daf-12* doubles, but never advance to these programs before adult stage in *lin-14 daf-12 lin-4* triples (Table 3). Moss et al. (1997) have recently reported that *lin-4(+)* can inhibit *lin-28(+)* directly. Incorporating their observation, we propose *lin-4(+)* and *daf-12a* inhibit *lin-28(+)* through independent mechanisms to advance S3 and later programs.

Interactions with *mig-8*

mig-8 is represented by a single, recessive allele, *rh50*, with delayed expression of S4 gonadal programs but normal extragonadal development and dauer regulation (Tables 1, 2). In hermaphrodites, the compaction and divisions of gonadoblasts are normal through early L3, but thereafter, there are penetrant defects in distal-tip cell migrations and herniation of the distal ovary. The brood size ranged from 40 to 146, with mean 117±32, for a sample of selfing hermaphrodites (n=11). Similarly, in males, linker cell migrations are essentially normal through early L3, but then halt without undergoing their S4 movements.

mig-8 maps between *mec-2* and *mup-2* on chromosome X and fails to complement the deficiency *stDf1* (Fig. 1). *rh50/stDf1* hemizygotes are phenotypically indistinguishable from *rh50/rh50* homozygotes, suggesting that *rh50* could be null or near null. In particular, no enhancement of gonadal phenotypes, nor novel defects in extragonadal development or dauer regulation were observed. Using double mutants, we examined possible functional interactions with *daf-12(+)* (Table 1). Unexpectedly, *daf-12(m20)*, which has no gonadal phenotype itself, can partially suppress the L4 distal-tip cell migration phenotype of *mig-8*. Conversely, *mig-8*, which itself has normal dauer regulation, can partially suppress the Daf-defective phenotype of *daf-12(rh84)*. These results suggest that *mig-8(+)* somehow acts with *daf-12(+)* to regulate both life stage transitions and dauer formation.

Phenotypic mosaicism

In *daf-12* individuals, each tissue is generally a mosaic of cells expressing normal and delayed developmental programs (Fig.

Table 3. *daf-12* interactions with heterochronic genes

	Seam programs (SA%)[1]						Vulval programs[2]		
Genotype	L1	L2	L3	L4	A1	A2	L3	L4	A1
wild type N2	S1	S2	S3	S4	SA 100%	NA	S3	S4	SA
daf-12(rh61)	S1	S2	S2	S3/S4[3]	S4/SA 81% (±1)	SA 100%	S3	S4	SA
lin-14(ma135)[6]	S2	S3	S4	SA 100%	NA	NA	S4	SA	NA
lin-14 daf-12	S2	S2	S3	S4/SA 41% (±2)	SA 99%	NA	S3	S4	SA[4]
lin-4(e912)[6]	S1	S1	S1	S1/S2	ND	ND	NA	NA	NA
lin-4 daf-12	S1	S1	S1	S1/S2	ND	ND	NA	NA	NA
lin-14 lin-4[6]	S2	S3	S4	SA 100%	NA	NA	S4	SA	NA
lin-14 lin-4 daf-12	S2	S2	S2/S3	S3/S4[3]	S4/SA 47% (±2)	SA 100%	S3	S4	SA[4]
lin-28(n719)[6]	S1	S3	S4/SA 61% (±4)	SA 100%	NA	NA	S4	SA	NA
lin-28 daf-12	S1	S3	S4/SA[5] 72% (±3)	SA 99% (±1)	NA	NA	S4	SA	NA
lin-29(n333)[6]	S1	S2	S3	S4	S4	S4	S3	S4	SA
lin-29 daf-12	S1	S2	S2	S3/S4	S4	S4	S3	S4	SA

Individual larvae were followed intermittently by DIC microscopy and scored for seam and vulval development through the stages.

[1]Seam cell programs are classified as in Fig. 4 (cf. Ambros and Horvitz, 1984). S2 seam programs were determined by equational seam divisions, SA programs by the presense of adult alae. Percentages of cells expressing SA programs (ca. 20 individuals) are reported as in Table 1. A1,A2, first and second adult molts respectively; ND, not determined; NA, not applicable.

[2]Vulval cell programs are classified: S3, multipotential precursors; S4, committed precursors, and SA, postmitotic cells (cf. Chalfie et al., 1981; Euling and Ambros, 1996b). NA, not applicable. In *lin-4* and *lin-4 daf-12*, cells express abnormal or no late larval programs.

[3]Occasional cells repeat S2 programs.

[4]Morphogenesis is occasionally delayed.

[5]For 3/20 animals examined, there were no further molts.

[6]Related data contained in Ambros and Horvitz (1984), Chalfie et al. (1981), or Ambros (1989).

8). For example, individual seam cells may express S3 programs at L3 stage while adjacent cells within the same larva repeat S2 programs instead. Similarly, phenotypic mosaicism is observed for expression of seam SA, intestinal S3 and distal-tip S4 programs. In the partial-dauer larvae formed in Daf-constitutive alleles under replete culture, occasional seam cells express S3 rather than S3d programs. Whereas most seam cells produce dauer-specific alae and undergo mitotic arrest, these exceptional cells leave gaps in the alae and divide, bypassing S3d diapause. Interestingly, in both hypodermis and intestine, the frequency of delayed phenotypes is highest for cells in the mid-body and declines toward both the head and tail (Fig. 8).

In *daf-12(rh84)* hermaphrodites (n=100), 74% ± 4 of the left (posterior) and 39% ± 4 of the right (anterior) distal-tip cells undergo S4 dorsal movements (Fig. 8). The frequency of larvae in which both distal-tip cells migrate dorsally, 33% ± 5, is consistent with probabilistic independence of these events, 74%×39% = 29%. In *mig-8(rh50)* hermaphrodites (n=201), by comparison, 6.5±2% of the left and 7±2% of the right distal-tip cells migrate correctly. In this case, the high frequency of larvae in which both distal-tip cells migrate dorsally, 4.5±1%, appears inconsistent with probabilistic independence, 6.5%×7% = 0.5%.

DISCUSSION

From a cellular perspective, the multicellular organism is a colony of cells whose coupled programs of gene expression collectively direct development. Few steps in these programs are strictly cell-autonomous; rather most are contingent upon intercellular signals or environmental factors encountered during development. Inductive signals, for example, coordinate cellular programs in adjacent tissues, while morphogens and hormones coordinate development over much greater distances. In special cases, hormones can advance cellular programs throughout the organism; such events are usually recognized as life stage transitions. Without such endocrine regulation, each individual would eventually become a mosaic of cells and tissues of incommensurate developmental ages, or incompatible life strategies. *daf-12* regulates L3 stage development in *C. elegans*. We propose this gene coordinates cellular commitments throughout the soma, and subordinates them to organismal commitments, through an endocrine mechanism.

daf-12 regulates L3 commitments in somatic tissues

In *daf-12* mutants, development is essentially normal until L3 commitment but, thereafter, somatic cells throughout the larva fail to express S3 and later programs on schedule (Tables 1, 2). In hypodermis and intestine, cells undergo extra proliferative divisions interpreted as repeated S2 programs. In the gonad, altered cell migrations likely reflect delays or repetitions in pathfinding programs of leader cells themselves (Antebi et al., 1997; M.-W. Su, M. Killeen, E. Hedgecock and J. Culotti, unpublished data). For example, the male linker cell often continues along the ventral body muscles without changing direction at L2 lethargus, interpreted as a repetition of its S2

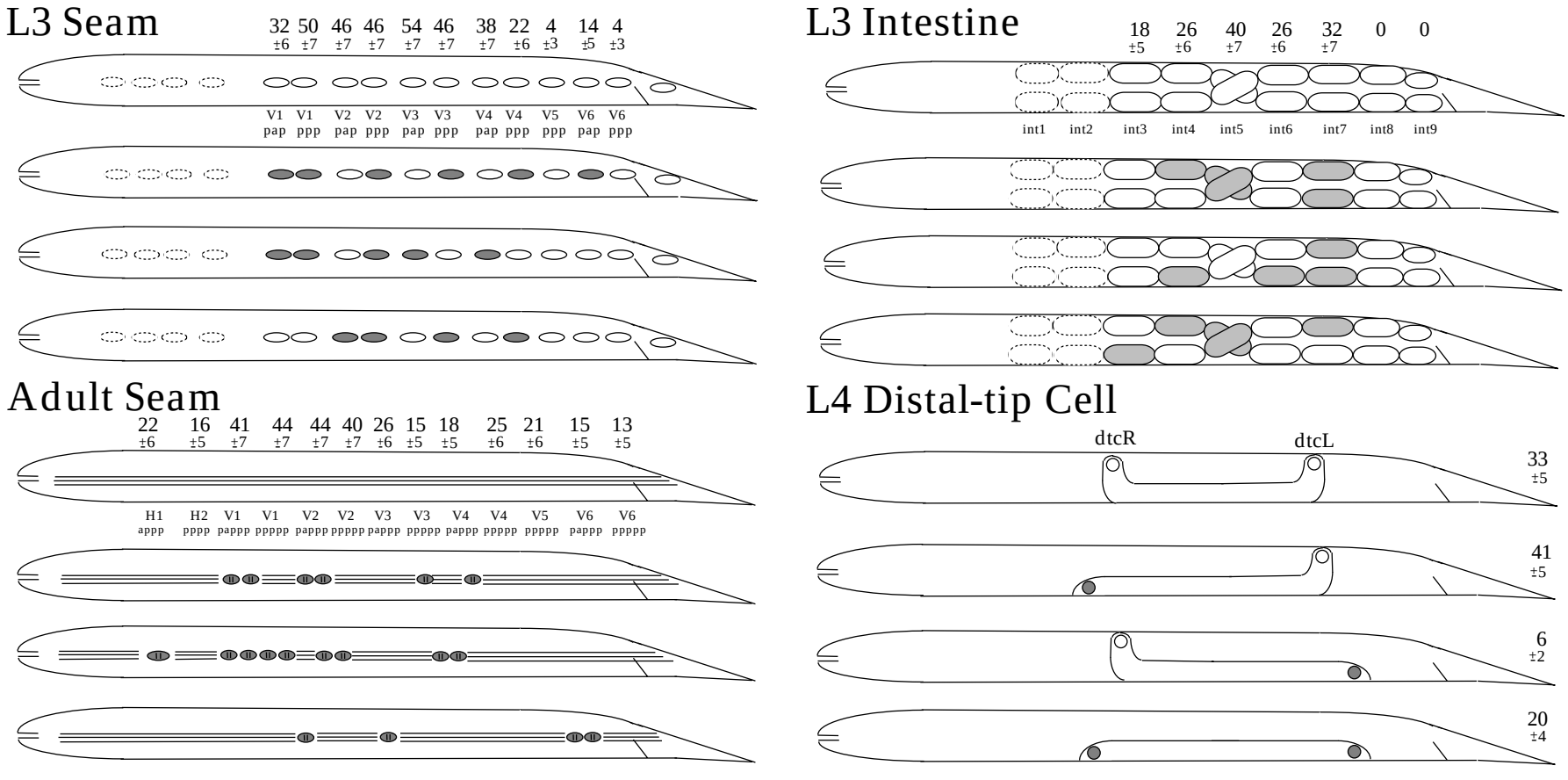

Fig. 8. Cell-autonomous delays in post-embryonic development. (upper left) The top panel shows the percentage of seam cells in *daf-12(m20)* larvae (*n*=50) that repeated S2 programs at L3 stage. The bottom panels show the distributions of seam cells that repeated S2 programs (shaded) in three representative individuals. (lower left) The top panel shows the percentage of seam cells in *daf-12(rh61)* hermaphrodites (*n*=50) that repeated larval programs at adult stage; descendants of repeated S2 programs have been pooled. The lower panels show the distributions of seam cells with repeated larval programs (shaded) in three representative individuals. (upper right) The top panel shows the percentage of intestinal cells in *daf-12(rh61)* larvae (*n*=50) that repeated S2 programs at L3 stage. The lower panels show the distributions of intestinal cells with repeated S2 programs (shaded) in three representative individuals. (lower right) The panels show the percentage of *daf-12(rh84)* hermaphrodites (*n*=100) that failed in the dorsal movements of either one or both distal-tip cells at L4 stage.

pathfinding program (Fig. 5). In other cases, this cell executes dorsal movement but then fails to turn posteriorly, interpreted as hybrid S2/3 programs.

A few *daf-12* phenotypes, e.g., the repeated execution of S2 programs in the intestine or linker cell, are already evident by L2 lethargus. Presumably, the cellular commitments for these programs occur even earlier. Indeed, in temperature-sensitive *daf-12(rh285hs)* larvae, the critical period for seam cell commitments to S3 programs occurs around the middle of the L2 intermolt (Fig. 7). Later stage phenotypes seen in some *daf-12* mutants, e.g., failure to express S4 distal-tip cell programs at L4, or SA seam cell programs at adult stage, might be purely consequences of errors made at L3 commitment. In *daf-12(rh193hs)*, however, the critical periods for these phenotypes occur within the L3 intermolt itself (Fig. 7), indicating either a direct requirement for *daf-12(+)* at L4 commitment, or inappropriately late gene activity in these mutants.

In wild type, germline and somatic development are closely coordinated. For example, both germline and somatic cell divisions are arrested during the dauer diapause. Like other known heterochronic genes, *daf-12* probably has no direct role in germline development. The onsets of meiosis and gametogenesis, which roughly coincide with S4 and SA somatic programs in wild type, are not delayed in these mutants. Moreover, while somatic tissues are arrested in partial-dauer larvae arising in Daf-constitutive alleles, germline proliferation appears unaffected. Signals directly from the somatic gonad, which determine the spatial arrangement of germline maturation, might control germline cell age as well (Schedl, 1997).

daf-12 regulates the S3/S3d decision

Dauer larvae express an alternative program for L3 development, designated S3d, adapted for diapause and dispersal (Cassada and Russell, 1975). Several environmental factors, predictors of future starvation, regulate both the choice between S3 and S3d programs, and within the S3d program itself, the decision to exit from diapause (Golden and Riddle, 1984). Exudates from soil microbes, indicating potential food sources, bias L3 commitment toward S3 programs, while warm temperatures and pheromone, indicating potential overcrowding, bias this decision toward S3d programs. Committed dauer larvae cease feeding at L2 lethargus and undergo morphological adaptations for diapause, e.g., radial shrinkage of the body and maturation of a dauer-specific cuticle. On exit from diapause, these larvae resume feeding and reverse their radial shrinkage. At this time, cells throughout the animal resume programs of division, migration and differentiation that are indistinguishable from their S3 counterparts.

Environmental and physiological factors regulating dauer commitment and recovery are integrated via the dauer formation genes (Riddle and Albert, 1997). Of the known genes in this signaling pathway, *daf-12* appears unique in three respects. First, this locus has both Daf-defective and Daf-constitutive alleles, and may therefore have an instructive role in the dauer decision. The Daf-constitutive alleles are dominant to Daf-defective alleles, and both classes are recessive to wild type, suggesting that *daf-12(+)* can promote or inhibit S3d programs under adverse and favorable growth conditions, respectively. Second, the other genes only affect the choice between S3 and S3d programs, not the advance from S2 to either S3 or S3d programs. Finally, epistasis experiments suggest that *daf-12* is possibly the ultimate target of the dauer signaling pathway (Riddle et al., 1981; Thomas et al., 1993; Gottlieb and Ruvkun, 1994; Riddle and Albert, 1997). The terminal branches of this pathway are shown in Fig. 9B.

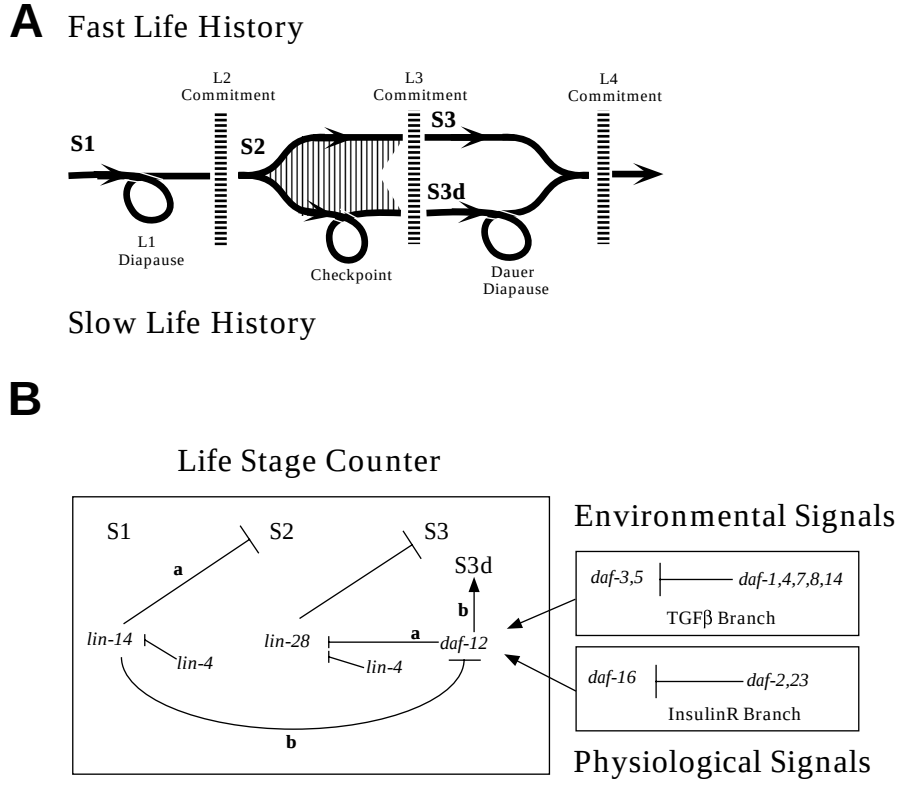

Fig. 9. (A) A model for *C. elegans* life history regulation (cf. Golden and Riddle, 1984). After hatching, starvation threats can arrest S1 programs and delay L2 commitment indefinitely (Arasu et al., 1991). This same checkpoint, re-initialized at L2 commitment, can delay L3 commitment by up to several hours and bias the S3/S3d decision toward dauer formation. The decision itself can be reversed at any time before L3 commitment by changing environmental conditions. Finally, the starvation threat checkpoint, re-initialized at L3 commitment, maintains dauer-associated diapause. Within the S1 and S3d programs, the exits from diapause may coincide with L2 and L4 commitment, respectively. (B) A model of the life stage counter including environmental and physiological inputs (see Discussion). Terminal branches of the dauer signaling pathway are named here after the TGFβ homolog DAF-7 (Ren et al., 1996) and the insulin-like receptor homolog DAF-2 (Kimura et al., 1997), respectively. Gene activities required for specifying S4 and SA programs are not shown.

The 'partial-dauer' larvae formed in Daf-constitutive alleles of *daf-12* under replete conditions never complete radial shrinkage, but instead resume feeding several hours after the onset of L2 lethargus. Paradoxically, developmental programs in the soma and gonad, excepting germline proliferation, remain arrested. These observations might be explained if commitment to S3d programs is incomplete in these individuals, affecting some but not all aspects of dauer formation. Instead, we suggest that these larvae initiate all S3d programs at L3 commitment, but sensing no threat of starvation, attempt to exit from the dauer-associated diapause at the earliest opportunity (cf. Vowels and Thomas, 1992; Gottlieb and Ruvkun, 1994). The continued developmental arrest of these larvae after resumption of feeding suggests *daf-12* has a role in maintaining growth arrest during dauer diapause.

Environmental and physiological factors also regulate adult life history traits, e.g., size at maturity, reproductive schedule and lifespan, and behaviors, e.g., egg-laying and thermotaxis. In particular, the insulin-like receptor branch of the dauer signaling pathway (Fig. 9B) has been implicated in the regulation of adult lifespan (Kenyon et al., 1993; Dorman et al., 1995; Larsen et al., 1995; Morris et al., 1996; Kimura et al., 1997, Ogg et al., 1997, Lin et al., 1997). This pathway is proposed to integrate physiological signals regulating larval and adult life histories. We suggest these signals converge on an endocrine tissue, yet unidentified, making stage commitments for the entire organism, and then relayed hormonally throughout the soma.

Functional complexity of *daf-12*

The functional organization of the *daf-12* locus is surprisingly complex (Table 1). In class 1 alleles, somatic cells cannot select S3 or S3d programs, but instead repeat S2 programs, in L3 stage. In class 4 alleles, these cells cannot select S3 programs, but express S2 or S3d programs in favorable and adverse environments, respectively. In class 3 alleles, they cannot select S3d programs, even in adverse environments. In class 6 alleles, they cannot select S3 programs, but express S3d programs even in favorable environments.

The various *daf-12* mutants reveal two separable gene activities, i.e., *daf-12a*, which selects S3 programs in favorable environments, and *daf-12b*, which selects S3d programs in adverse environments (Fig. 9B). In the simplest model, these mutant alleles, which are all recessive to *daf-12(+)* for the phenotypes examined (Table 1), reflect a reduction of one or both gene activities. In particular, class 3 and 4 alleles selectively reduce *daf-12b* or *daf-12a* activity, respectively, while class 1 alleles reduce or eliminate both activities. Finally, in class 6 alleles, *daf-12b* is active even in favorable environments; whereas these Daf-constitutive alleles are dominant to the Daf-defective classes, they are fully recessive to *daf-12(+)*.

Preliminary molecular studies suggest that class 3 alleles, rather than some other class, make no functional products (A. Antebi, M. Snow, W. Yeh, E. Hedgecock, D. Riddle, and P. Larsen, unpublished data). If so, *daf-12b* activity reflects a simple loss-of-gene-function in class 1 and 3 alleles, while *daf-12a* reflects a recessive gain-of-function in class 1 and 4 alleles. This could explain why class 3 alleles are relatively common among *daf-12* mutants, and how class 1 alleles sometimes mutate spontaneously to class 3 (Table 1). Class 1 and 4 alleles are recessive to wild type, indicating *daf-12(+)* products can compete effectively against the mutant products to advance S3 programs; moreover, class 3 homozygotes have impenetrant heterochronic phenotypes, indicating *daf-12(+)* products must themselves provide some *daf-12a* activity. An obvious explanation is that *daf-12a* reflects partially redundant actions of *daf-12(+)* and another, unknown gene; only a genetic analysis of both genes can establish the relative contributions of each locus.

Heterochronic genes specify developmental age

We have incorporated epistasis relationships (see Results) for both *daf-12a* and *daf-12b* activities into existing models of the heterochronic and dauer signaling pathways (cf. Moss et al., 1997; Riddle and Albert, 1997). In particular, *daf-12a* acts between *lin-14* and *lin-28* in extragonadal cells. In our model (see Fig. 9B), S1, S2 and S3/S3d programs form a default hierarchy favoring the highest available program. In extragonadal tissues, the regulatory circuit is initialized by embryonic expression of *lin-14* and *lin-28*. S2 and S3 programs are repressed by *lin-14a* and *lin-28*, respectively, while *lin-14b* provides feed-forward inhibition of *daf-12a* and *daf-12b*. Consequently, at L1 commitment, cells must adopt S1 programs. By L2 commitment, inhibition of *lin-14* by *lin-4* has derepressed S2 programs. By L3 commitment, inhibition of *lin-28* by *lin-4* and *daf-12a* has derepressed S3 programs. As discussed above, *daf-12* may also act at L4 commitments. Finally, *lin-29* (not shown) is required to initiate SA programs at adult commitment (Ambros, 1989).

The molecular mechanisms for several heterochronic gene activities have been identified. *lin-14* and *lin-28* encode unstable nuclear and cytoplasmic proteins, respectively, expressed throughout the extragonadal tissues in L1 larvae (Ruvkun and Giusto, 1989; Moss et al., 1997). LIN-28, which has a cold-shock domain and CCHC zinc-finger motifs, is a presumptive RNA-binding protein, while LIN-14 appears entirely novel. After hatching into a replete environment, *lin-4* expresses small RNAs that base pair with the 3′ UTR of *lin-14* and *lin-28* mRNAs, inhibiting their translation (Lee et al., 1993; Wightman et al., 1993; Moss et al., 1997). As a consequence, these protein levels decline significantly from mid-L1 through mid-L2.

Less is known about the molecular mechanisms for other heterochronic gene interactions. The critical period for *lin-14b* activity, which prevents precocious expression of S3 programs, occurs in mid-L1 (Ambros and Horvitz, 1987). We propose LIN-14 is required at this time to inhibit *daf-12a* and *daf-12b* (Fig. 9B); the mechanism of this inhibition is unknown. Similarly, the critical period for *daf-12a* activity, which prevents repeated expression of S2 programs, occurs in mid-L2. We propose *daf-12a* acts at this time to either lower LIN-28 levels or inhibit its actions; unlike the translational inhibition of *lin-28* mRNA described above, this *daf-12a* action does not require *lin-4(+)*. Indeed, in extragonadal tissues, overexpression of LIN-28 causes repeat of S2 programs at L3 stage, phenocopying *daf-12* mutants (Moss et al., 1997). Finally, *lin-29* encodes proteins with CCHH zinc-finger motifs that accumulate at later stages in various somatic nuclei, both gonadal and extragonadal (Rougvie and Ambros, 1995; Bettinger et al., 1996). In the hypodermis, LIN-29 accumulation is regulated by LIN-14 and LIN-28.

Under adverse conditions, *daf-12b* activity specifies the S3d alternative at L3 commitment. We favor a one-step model for commitment to dauer formation (see Fig. 9A). Under favorable conditions, the insulin-like receptor and TGF-β signaling pathways together select *daf-12a*, while under adverse conditions, when one or both pathways are inactive, *daf-12b* acts as a default (Fig. 9B). This model also explains why *lin-14*, but not *lin-28* mutants, cause precocious expression of *daf-12b* activity and selection of S3d programs at L2 commitment (Liu and Ambros, 1989). Interestingly, the late stage phenotypes in *daf-12*, *mig-8* and other heterochronic mutants are suppressed by dauer development (Table 1; Liu and Ambros, 1991; Euling and Ambros, 1996a). We suggest these phenotypes reflect misregulation of the *daf-12a* activity but are bypassed during dauer development by *daf-12b*.

A variety of evidence suggests that the hierarchy of heterochronic genes is initialized differently, and operates independently, in different tissues and, perhaps, individual cells. For example, *lin-29* is expressed throughout the soma (Bettinger et al., 1996), whereas *lin-14* and *lin-28* appear inactive in the gonad (Ambros and Horvitz, 1984; Ruvkun and Giusto, 1989; Moss et al., 1997). Such differences could contribute to the tissue specificity of some *daf-12* alleles, e.g., in class 5 and class 6 *daf-12* alleles, and in *mig-8(rh50)*, gonadal somatic cells, but not extragonadal cells, fail to express S3 or later programs on schedule (Table 1). Interestingly, mutations changing the relative timing of gonadal and extragonadal development are proposed to play a major role in the evolution of metazoan life histories (Gould, 1977).

Life history regulation

Heterochronic genes select stage-appropriate developmental programs in nematode tissues (Ambros, 1997). In *daf-12* and other mutants, individual cells within a tissue can vary in developmental age (cf. Ambros and Horvitz, 1984, 1987). Moreover, these differences, precocious or retarded, can propagate through lineal descendants over successive stages. By inference, each somatic cell maintains a partly autonomous memory of its developmental age. Heterochronic genes are likely components of these memory circuits as well as temporal selectors per se. We suggest these circuits, distributed throughout the soma, advance one step with each new life stage so that the heterochronic activities enumerate the chronological stages (Fig. 9B). At the same time, these activities are used to select stage-appropriate developmental programs. In this view, the heterochronic genes are analogous to cyclin-dependent protein kinases in cell-cycle regulation whose activities both specify a progression of stages and select stage-appropriate events.

The hypodermal molt cycle provides several convenient landmarks for staging nematode development (cf. Fig. 4). Ecdysis, the traditional boundary between molt cycles, occurs properly within a life stage, not between stages. A variety of evidence suggests that organismal and cellular commitments to new life stages precede each lethargus by about 5 hours (corrected to 20°C culture). First, new stage-specific programs, observed as changes in gene expression, cell-cycle commitment, or cell migration, commence several hours before lethargus (cf. Euling and Ambros, 1996b; Johnstone and Barry, 1996; Antebi et al., 1997). Cellular commitments are presumably completed before any execution of these programs. Second, critical periods of several heterochronic gene activities precede the lethargus by several hours. For example, *lin-14b* and *daf-12a* act several hours before L1 or L2 lethargus, respectively (Ambros and Horvitz, 1987); these critical periods likely reflect cellular commitment itself. Finally, a change of environmental conditions can reverse any bias toward S3 or S3d alternatives until up to 5 hours before L2 lethargus (Swanson and Riddle, 1981; Golden and Riddle, 1984). Phenotypic mosaicism has not been observed in wild type, suggesting that organismal commitment to a particular L3 alternative strictly precedes cellular commitments.

The nervous system, integrating several environmental factors, releases growth factors modulating the *C. elegans* life history (Riddle and Albert, 1997; Ren et al., 1996). Presumably, these graded neural signals are evaluated by an endocrine tissue, yet unidentified, that makes life history decisions for the entire organism. If heterchronic genes provide cellular memories of developmental age, a single hormone from this tissue could suffice to periodically coordinate cellular programs and advance their memories. In insects, for example, ecdysteroids periodically coordinate cellular programs (Thummel, 1995), but it is unknown if they also advance memories.

Switching circuits that step through a sequence of unique states in response to a repeating input are called counters. We suggest that nematode heterochronic genes form a *life stage counter* that advances at each new stage in response to a repeating endocrine input (Fig. 9B). Presumably, two distinct hormones are required at the L3 branchpoint to coordinate cellular programs as well as specify an S3/S3d alternative (Fig. 9A). A hormone released only in adverse environments, for example, might complement or replace the hormone released in favorable environments.

The discovery that *daf-12* encodes a nuclear receptor subunit (Yeh, 1991; Riddle and Albert, 1997) suggests hormonal activation of nuclear receptor complexes could regulate cellular commitments for L3 and perhaps other stages. Selective activation of receptor complexes mediating *daf-12a* activity could select S3 programs in favorable environments, while activation of *daf-12b* could select S3d programs in adverse environments. Within the S3d program itself, endocrine signals for L4 commitment could also coordinate dauer recovery by activating *daf-12a* (cf. Fig. 9A). Without *daf-12a* or *daf-12b*, cells recommit to S2 programs as a default, suggesting that distinct nuclear receptor complexes could act as selectors for S2, S3 and S3d programs, respectively. It will be important to determine the molecular composition of *daf-12* and related receptor complexes, and compare them to selector activities defined genetically.

We wish to thank V. Ambros, P. Larsen, W. H. Yeh and D. Riddle for sharing unpublished results and strains, D. Brown, V. Corces, A. Fire, H. Hutter, M. Montgomery and C. Norris for helpful comments on the manuscript, and S. Wimbuish for technical assistance. The Caenorhabditis Genetics Center, which is supported by National Institutes of Health National Center for Research Resources, provided strains for this work. A. A. received postdoctoral fellowships from the Damon Runyon-Walter Winchell Cancer Research Fund and the National Institutes of Health. This research was supported through a grant from the National Institutes of Health (NS26295) to E. H.

REFERENCES

Ambros, V. (1989). A hierarchy of regulatory genes controls a larva-to-adult developmental switch in *C. elegans*. *Cell* **57**, 49-57.

Ambros, V. and Horvitz, H. R. (1984). Heterochronic mutants of the nematode C. elegans *Science* **226**, 409-416.

Ambros, V. and Horvitz, H. R. (1987). The *lin-14* locus of *Caenorhabditis elegans* controls the time of expression of specific post-embryonic developmental events. *Genes Dev*. **1**, 398-414.

Ambros, V. (1997) Heterochronic Genes. In *C. elegans II* (ed. D. Riddle, B. Meyer, J. Priess and T. Blumenthal). Cold Spring Harbor: Cold Spring Harbor Press.

Antebi, A., Norris, C. R., Hedgecock, E. M., Garriga, G. (1997) Cell and growth cone migrations. In *C. elegans II* (ed. D. Riddle, B. Meyer, J. Priess and T. Blumenthal). Cold Spring Harbor: Cold Spring Harbor Press.

Arasu, P., Wightman, B. and Ruvkun, G. (1991). Temporal regulation of *lin-14* by the antagonistic action of two other heterochronic genes *lin-4* and *lin-28*. *Genes Dev.* **5**, 1825-1833.

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

Bettinger, J. C., Lee, K. and Rougvie, A. E. (1996). Stage-specific accumulation of the terminal differentiation factor LIN-29 during *Caenorhabditis elegans* development. *Development* **122**, 2517-2527.

Cassada, R. C. and Russell, R. L. (1975). The Dauer-Larva: A Post-Embryonic Developmental Variant of the Nematode C. elegans. *Dev. Biol.* **46**, 326-342.

Chalfie, M., Horvitz, H. R. and Sulston, J. E. (1981). Mutations that lead to reiterations in the cell lineages of *C. elegans*. *Cell* **24**, 59-69.

Dorman, J. B., Albinder, B., Shroyer, T. and Kenyon, C. (1995). The *age-1* and *daf-2* genes function in a common pathway to control the lifespan of *Caenorhabditis elegans*. *Genetics* **141**, 1399-1406.

Euling, S. and Ambros, V. (1996a). Reversal of cell fate determination in *Caenorhabditis elegans* vulval development. *Development* **122**, 2507-2515.

Euling, S. and Ambros, V. (1996b). Heterochronic genes control cell cycle progress and developmental competence of *C. elegans* vulval precursor cells. C*ell* **84**, 667-676.

Golden, J. W. and Riddle, D. L. (1984). The *C. elegans* dauer larva, developmental effects of pheromone food and temperature. *Dev. Biol.* **102**, 368-378.

Gottlieb, S. and Ruvkun, G. (1994). *daf-2, daf-16* and *daf-23*: genetically interacting genes controlling dauer formation in *Caenorhabditis elegans. Genetics* **137**, 107-120.

Gould, S. J. (1977). *Ontogeny and Phylogeny*. Cambridge MA: Belknap Press.

Hedgecock, E. M., Culotti, J. G., Hall, D. H. and Stern, B. D. (1987). Genetics of cell and axon migrations in *Caenorhabditis elegans. Development* **100**, 365-382.

Hedgecock, E. M., and White, J. G. (1985) Polyploid tissues in the nematode C. elegans. *Dev. Biol.* **107**, 128-133.

Hodgkin, J., Horvitz, H. R. and Brenner, S. (1979). Nondisjunction mutants of the nematode C*aenorhabditis elegans*. *Genetics* **91**, 67-94.

Johnson, T. E., Mitchell, D. H., Kline, S., Kemal, R. and Foy, J. (1984). Arresting development arrests aging in the nematode *C. elegans*. *Mech. Ageing Dev.* **28**, 23-40.

Johnstone, I. L. and Barry, D. J. (1996) Temporal reiteration of a precise gene expression pattern during nematode development. *EMBO J.* **15**, 3633-39.

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

Kimble, J. and Hirsh, D. (1979). The post-embryonic cell lineages of the hermaphrodite and male gonads in *C. elegans*. *Dev. Biol.* **70**, 396-417.

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

Kimble, J. and White, J. G. (1981). On the control of germline cell development in *C. elegans*. *Dev. Biol.* **81**, 208-221.

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

Larsen, P. L., Albert, P. S. and Riddle, D. L. (1995). Genes that regulate both development and longevity in *C. elegans. Genetics* **139**, 1553-1566.

Lee, R. C., Feinbaum, R. L. and Ambros, V. (1993). The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* **5**, 843-854.

Lin, K., Dorman, J. B., Rodan, A. and Kenyon, C. (1997) *daf-16*: An HNF-3/forkhead family member that can function to double the life-span of Caenorhabditis elegans. *Science* **278**, 1319-1322

Liu, Z. and Ambros, V. (1989), Heterochronic genes control the stage-specific initiation and expression of the dauer larval developmental program in Caenorhabditis elegans. *Genes Dev.* **3**, 2039-2049.

Liu, Z. and Ambros, V. (1991). Alternative temporal control systems for hypodermal cell differentiation in *C elegans*. *Nature* **350**, 162-165.

Morris, J. Z., Tissenbaum, H. A. and Ruvkun, G. (1996) A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. *Nature* **382**, 536-539.

Moss, E. G., Lee, R. C. and Ambros, V. (1997) The Cold Shock Domain Protein LIN-28 Controls Developmental Timing in C. elegans and Is Regulated by the *lin-4* RNA. *Cell* **88**, 637-646.

Newman, A. P., White, J. G. and Sternberg, P. W. (1996) Morphogenesis of the *C. elegans* hermaphrodite uterus. *Development* **122**, 3617-3626.

Ogg, S., Paradis, S., Gottlieb, S., Patterson, G. I., Lee, L., Tissenbaum, H. A. and Ruvkun, G. (1997) The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. *Nature* **389**, 994-999.

Ren, P., Lim, C. S., Johnsen, R., Albert, P. S., Pilgrim, D. and Riddle, D. L. (1996) Control of *C. elegans* Larval Development by Neuronal Expression of a TGF-β Homolog. *Science* **274**, 1389-91.

Riddle, D. L. and Albert, P. S. (1997) Genetic and environmental regulation of dauer larva development. In *C. elegans II* (ed. D. Riddle, B. Meyer, J. Priess and T. Blumenthal). Cold Spring Harbor: Cold Spring Harbor Press.

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

Rougvie, A. E. and Ambros, V. (1995). The heterochronic gene *lin-29* encodes a zinc finger protein that controls a terminal differentiation event in *Caenorhabditis elegans*. *Development* **121**, 2491-2500.

Ruvkun, G. and Giusto, J. (1989). The *C. elegans* heterochronic gene *lin-14* encodes a nuclear protein that forms a temporal developmental switch. *Nature* **338**, 313-319.

Schedl, T. (1997) Developmental genetics of the germ line. In *C. elegans II* (ed. D. Riddle, B. Meyer, J. Priess and T. Blumenthal). Cold Spring Harbor: Cold Spring Harbor Press.

Singh, R. N. and Sulston, J. E. (1978). Some observations on moulting in *Caenorhabditis elegans*. *Nematologica* **24**, 63-71.

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

Sulston, J. E., Albertson, D. G. and Thomson, J. N. (1980). The *C. elegans* male: post-embryonic development of non-gonadal structures. *Dev. Biol.* **78**, 542-576.

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

Sulston, J. and Hodgkin, J. (1988). Methods. In *The Nematode C. elegans* (ed. WB Wood). pp 587-606 Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Swanson, M. M. and Riddle, D. L. (1981). Critical periods in the development of the *Caenorhabditis elegans* dauer larva. *Dev. Biol*. **84**, 27-40.

Thomas, J. H., Birnby, D. A. and Vowels, J. J. (1993). Evidence for parallel processing of sensory information controlling dauer formation in *C. elegans. Genetics* **134**, 1105-1117.

Thummel, C. S. (1995). From embryogenesis to metamorphosis: the regulation and function of *Drosophila* nuclear receptor superfamily members. *Cell* **83**, 871-877.

Vowels, J. J. and Thomas, J. H. (1992). Genetic analysis of chemosensory control of dauer formation in *C. elegans*. *Genetics* **130**, 105-123.

Wightman, B., Ha, I. and Ruvkun, G. (1993). Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. *Cell* **75**, 855-862.

Yeh, W. H. (1991). Genes acting late in the signaling pathway for *Caenorhabditis elegans* dauer larval development. Doctoral Thesis. University of Missouri-Columbia.