Development 111, 921-932 (1991)
Printed in Great Britain © The Company of Biologists Limited 1991

Control of cell fate in the tail region of *C. elegans* by the gene *egl-5*

ANDREW CHISHOLM*

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

*Current address: Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

Summary

The tail region of *C. elegans* contains a number of blast cell and neuron types that either are found only in the tail, or are different from more anterior homologues. In *egl-5* mutants, the fates of many of these tail cells are abnormal or transformed to those of anterior homologues. The affected cells are related only by position and not by ancestry. *egl-5* is also required for normal development of the somatic gonad and sex muscles in males. The function of *egl-5* in all these tissues is cell autonomous. By genetic mapping, *egl-5* lies very close to *mab-5*, a gene with an analogous role in the immediately anterior body region. *egl-5* and *mab-5* may constitute a 'mini-cluster' of regional determination genes, similar to those described in other animal phyla.

Key words: *C. elegans*, lineage, regional specification.

Introduction

An early step in animal development must be the specification of the various regions of the body plan. What are the mechanisms by which body regions are initially delineated, and how do they subsequently become different? Genetic analysis of regional differentiation in two invertebrates, the fruit-fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans*, has cast light on such mechanisms of regional specification.

The determination of regional differences within the segmented body of *Drosophila* has been analysed in detail, beginning with the work of Lewis (1978) on the Bithorax complex of genes, and of Kaufman *et al.* (1980) on the Antennapedia complex. The action of genes within these complexes, termed selector genes, determines the identity of particular metameric units. The two complexes contain both protein-coding genes (sharing the homeobox motif) and extremely large regulatory regions involved in positional regulation of selector gene expression (Duncan, 1987).

The embryonic development of the nematode *C. elegans* has no overt similarity to that of *Drosophila*, but the mechanisms of regional specification may share important features. Evidence for regional mechanisms of determination comes from the analysis of the gene *mab-5* (Kenyon, 1986). In *mab-5* mutants, the cells in the posterior body region of the worm lose their region-specific differentiation; some are clearly transformed to the fates of anterior homologues. *mab-5* encodes a homoeodomain protein, whose messenger RNA appears to be localised to the region defined by the mutant phenotype (Costa *et al.* 1988). These observations suggested mechanistic similarities between regional determination in *C. elegans* and the selector genes of *Drosophila* mentioned above.

Here I describe the phenotypes caused by mutations in the gene *egl-5*, and show that *egl-5* is required in a variety of embryonic and postembryonic cell types in a local region of the tail. The cells affected in *egl-5* mutants lose their specific fates; in some instances, they take on the fates of their anterior homologues. The role of *egl-5* appears analogous to that of *mab-5*, in that the cells affected are not closely related by lineage ancestry or cell type, but only by position: the two genes act cell autonomously in adjacent regions of the posterior body and tail. Moreover, *egl-5* lies very close to *mab-5* by recombinational mapping. The analogies between the *mab-5*– *egl-5* 'cluster' and the gene complexes of insects strengthen the possibility that, at least in one situation, the problem of regional specification has been solved in a similar way in the two phyla.

Materials and methods

Strains and alleles used; strain construction

Nematode strains were cultured by standard techniques (Brenner, 1974; Sulston and Hodgkin, 1988). Mutations used are described in Hodgkin *et al.* (1988) or below. Alleles used were: LGII: *exp-1(sa6)* (Thomas, 1990); LGIII: *dpy-17(e164), dpy-18(e499), dpy-20(e2017*am*), lon-1(e185), unc-36(e251), unc-32(e189), sma-3(e491), sma-4(e729), ncl-1(e1865), mab-5 (e1239)*; LGIV: *him-8(e1489)*; LGV: *him-5(e1490)*; LGX: *deg-1(u38*sd*)*. Rearrangements used were: LGIII: *nDf16, nDf17* (V. Ambros and M. Finney, personal communication), *sDp3* (Rosenbluth *et al.* 1985).

Strain construction was by standard methods, except as

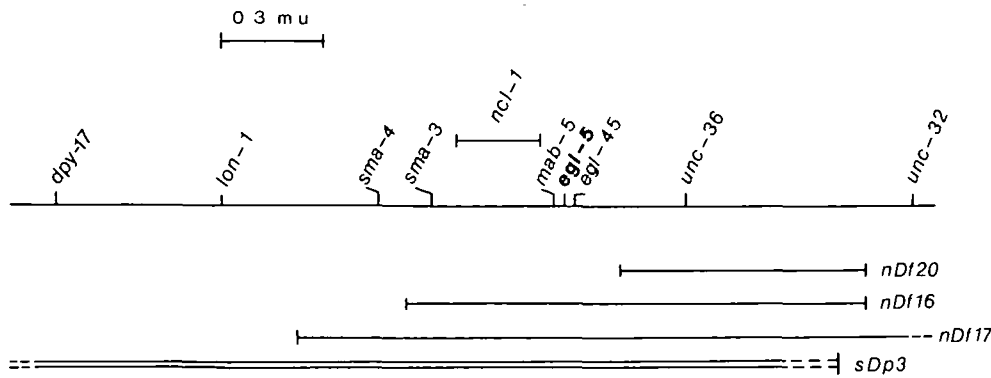


Fig. 1. Genetic map of the *mab-5–egl-5* region. By recombinational mapping *egl-5* is about 0.03 map units to the right of *mab-5* (see Materials and methods); the nearest marker to the right of *egl-5* is *egl-45* (M. Basson, personal communication). Other genetic map data are taken from Hodgkin *et al.* (1988).

described below. To study male phenotypes, strains containing *him-5* or *him-8* mutations, which increase the frequency of spontaneous male progeny, were used. The *mab-5 egl-5* double mutant was constructed by recombination: heterozygotes of genotype *lon-1 + egl-5(n945) +/+ mab-5 (e1239) + dpy-18* were cloned and Egl non-Lon recombinant F_1s picked and allowed to self for 24h; they were then tested for complementation with *mab-5* by mating with *mab-5/+* males.

Genetic mapping

egl-5 had previously been mapped to the left of the *nDf20* breakpoint and to the right of *lin-13* (C. Desai, personal communication). Four-factor crosses place *egl-5* very close to the right of *mab-5*: from heterozygotes of genotype *sma-3 mab-5 + unc-36/+ + egl-5(n1439) +* 44 Sma non-Unc and 46 Unc non-Sma recombinants were picked and tested by complementation for presence of *mab-5* and *egl-5*. The order was: *sma-3* (50/90) *mab-5* (3/90) *egl-5* (37/90) *unc-36*. Two-factor crosses had established the *sma-3–unc-36* distance as 0.9 map units (m.u.) (R. Rosenbluth, personal communication); by three-factor interpolation, the *mab-5–unc-36* distance is about 0.4 m.u., and *egl-5* 3/40 along this interval from *mab-5*, i.e. approximately 0.03 m.u. to the right (Fig. 1).

Isolation of egl-5 *alleles and establishment of null phenotype*

All twelve alleles are ethyl methanesulphonate induced and fully recessive. The allele *n486* was isolated in a general screen for Egl mutants (Trent *et al.* 1983). The alleles *n945, n988, n989,* and *n1066* were isolated in a selective pharmacological screen for HSN-defective Egl mutants, while *u202* and *n1439* were isolated in screens for Mec and HSN Mig mutants, respectively (Desai and Horvitz, 1989). Three different screens were used to isolate new *egl-5* alleles by noncomplementation. (1) *e2399* was isolated in a screen for mutations that fail to complement *n1439* for the Egl phenotype: L4 hermaphrodites of genotype *+ sma-3 mab-5 egl-5(n1439)/ lon-1 +++unc-36* were mutagenised and the F_1 screened (n= no. of F_1 chromosomes=11 800) for Egl hermaphrodites. (2) *e2495, e2502* and *e2506* were isolated in a noncomplementation screen against *n945*: L4 hermaphrodites of genotype *dpy-17 + egl-5(n945) unc-36 +/+ sma-4 ++ unc-32* were mutagenised and the F_1 screened (n=150 000) for Unc Egl worms. (3) *e2508* was isolated by noncomplementation against *n945*: mutagenised N2 males were mated with *egl-5(n945) unc-36* hermaphrodites and the F_1 screened (n=3850) for Egl Unc worms. The frequency of isolation in the second screen is much lower than the standard frequency of 5×10^{-4} (Brenner, 1974); there is no obvious explanation for this inefficiency.

What is the evidence that the strong alleles have resulted in a complete loss of *egl-5* gene function? The strong allele *n945* is a nonsense mutation (Desai and Horvitz, 1989) and is thus likely to give a severe loss of gene function. The Egl, Unc and Mab phenotypes of strong alleles are not significantly enhanced when in *trans* with a deficiency spanning the locus. In *trans* to spanning deficiencies, the Exp phenotype of *n945* and *n988* becomes less severe; this appears to be a nonspecific dominant effect of the deficiency chromosomes, since an equivalent suppression of the Exp phenotype of *exp-1* was observed in worms of genotype *exp-1; nDf16/+* (data not shown). It is possible that a true null mutation would be stronger for Exp than existing alleles; however, precomplementation screening for new alleles has failed to produce mutations with a more extreme phenotype than preexisting strong alleles. These lines of evidence together make it likely that the strong alleles result in complete or almost complete loss of *egl-5* function.

Lineage analysis

Cell lineages were followed by Nomarski microscopy as described by Sulston and Horvitz (1977). Male tail lineages were usually followed from about 8–10 h after hatching to after the L3 moult (34 h). Some male somatic gonad lineages were followed from the initial divisions of Z1 and Z4; most were followed in the late division period only.

Scoring of deg-1 *suppression*

The characteristic vacuole resulting from *deg-1*-induced PVC degeneration was scored in larvae by Nomarski microscopy at ×400 magnification. Scoring appearance of the vacuole may underestimate the actual frequency of degeneration, but allows consistent quantification of the phenotype. Approximately equal numbers (at least 100) were scored for each larval stage. The onset of *deg-1* degenerations is temperature-sensitive (Chalfie and Wolinsky, 1990); all scoring was carried out at 25°, at which most PVCs have degenerated by L3. The apparent reduction in frequency of PVC degeneration could be an artefact of the scoring procedure if, for example, *egl-5* mutations caused PVCs to degenerate more quickly. Therefore, for *deg-1* and *egl-5(n945); deg-1* the degenerations were followed in individual worms, counting the total number of neuronal nuclei in the tail ganglia in each larval stage. In *deg-1*, PVC degenerated on 29/32 sides, while in *egl-5(n945); deg-1* PVC degeneration was seen on only 16/27 sides (65 % of *deg-1*; cf. 48 % by scoring vacuoles); the scoring procedure therefore appears to reflect fairly accurately the degeneration frequency.

Isolation and analysis of genetic mosaics

Genetic mosaics were generated using the strain CB4816, of genotype *dpy-17 ncl-1 egl-5(n945); sDp3[dpy-17(+) ncl-1(+)*

egl-5(+)]; him-5. Worms from this strain are phenotypically wild-type; their progeny are either wild-type or, if the duplication was lost in the mother's germ cells or in the zygote, Dpy Ncl Egl. *sDp3* is also lost in somatic mitosis at a rate estimated to be about 1 in 400 cell divisions (Kenyon, 1986). The Dpy-17 phenotype is only produced if the duplication is lost in both P_1 and AB; a slightly weaker phenotype is produced by loss in P_2 (Yuan and Horvitz, 1990). Such mosaics were not required in this analysis, so non-Dpy animals were selected. *ncl-1* mutant animals have enlarged nucleoli in each cell nucleus; this phenotype is scorable in most cell types, and results from a cell-autonomous requirement for *ncl-1(+)* (E. Hedgecock, personal communication). Since *ncl-1* and *egl-5* are both covered by *sDp3*, the Ncl phenotype can be used as an independent marker of duplication loss.

Non-Dpy L3s and L4s from CB4816 were screened by Nomarski microscopy for mosaicism of the Ncl phenotype. For loss in AB, the pharynx, anterior ganglia, lateral ganglia and tail region were assessed for clones of Ncl^- nuclei corresponding to loss up to the AB^{16} stage; later mosaics were selected if of particular interest. For each lineage the most easily scored nuclei were screened for Ncl: if the marker nuclei were Ncl^-, other nuclei from that lineage were scored to check the loss point. Loss in P_1 and progeny was scored using the body muscle cells, coelomocytes and pharyngeal neurons.

Out of 3500 larvae screened, 105 mosaics were selected in which the point of duplication loss could be inferred (this does not represent the true frequency of mosaics observed, as many were discarded). In most cases, the loss could be pinpointed to a particular cell, in some to a cell or its daughter: for instance, losses at EMS and MS cannot be distinguished because Ncl is not scorable in the progeny of E. In such cases, the earlier point of duplication loss was assumed. In a small number of cases, the observed patterns could only be accounted for by a double loss of the duplication.

Results

egl-5 mutants have pleiotropic defects. Mutants of both sexes are *mec*hanosensory-defective (Mec), *unc*oordinated (Unc), and have abnormal *exp*ulsion movements in defecation (Exp). Mutant hermaphrodites have abnormal *egg-la*ying behaviour (Egl). Mutant males have abnormal tails and somatic gonads (*m*ale *ab*normal: Mab). The penetrances of some of these defects are shown in Table 1. I describe the phenotypes of *egl-5* mutants in turn, starting with the postembryonic lineage alterations, and then the behavioural defects.

The lineage defects in egl-5 *mutants*

egl-5 mutations cause several blast cells in the tail region to produce abnormal postembryonic lineages. In the hypodermal blast cell series, the most posterior member of each series (P12, V6.ppp) takes on the fate of its anterior neighbour; other tail blast cells execute lineages unlike those seen elsewhere in the wild-type. For those blast cells that divide in both sexes, the same defect is seen in both sexes. Development of two male structures, the somatic gonad and sex muscles, is also abnormal.

Ventral hypodermis: P12 transformed to P11

The cells P11 and P12 are the most posterior pair of cells in the series of ventral hypodermal blast cells. P11 and P12 are initially left–right homologs but in mid-L1 they migrate to lie in the ventral midline; they are developmentally equivalent, as shown by ablation experiments (Sulston and White, 1980). In *egl-5* mutants of both sexes, P12 undergoes a P11-like lineage.

In wild-type males, P9.p, P10.p and P11.p constitute the preanal equivalence group, which generates the hook. The three cells in the group undergo lineages designated 3°, 2°, or 1° respectively, based on the replacement hierarchy seen in ablation experiments (Sulston and White, 1980); P12.p divides in L1 and does not participate in the equivalence group. In *egl-5* males, the preanal equivalence group is effectively shifted posteriorly by one Pn.p cell, since P12.p fails to divide in L1 but instead survives until L3 and undergoes a 1° (P11.p-like) lineage; P11.p regulates to a 2° fate and P10.p to a 3° (Table 2).

Lateral hypodermis: V6.ppp transformed to V6.ppa

Only the most posterior member of the lateral hypodermal blast cell series (V6) is affected in *egl-5* males; the defect is confined to V6.ppp, which behaves like its anterior neighbour, V6.ppa. The division of V6.pppp is unequal (resembling the other V ray precursor parents), instead of being equal (Fig. 2B). V6.ppppa, instead of giving rise to the sixth ray sublineage (R6), is hypodermal and the morphologically unique sixth ray is not made. The positions of the ray papillae made by R4 and R5 are abnormal: instead of being lateral (next to the R6 papillus), they lie ventrally, next to the R2 and R3 papillae (Fig. 2D). The transformations are fully penetrant. Thus, both the lineage and terminal fates of V6.ppp are transformed to those of V6.ppa.

Abnormal development of internal blast cells

The lineages of all the four male-specific internal blast cells (B, Y, U and F) are abnormal in *egl-5* males. None of the structures normally formed by the descendants of these cells (spicules, proctodeum, *etc.*) are made. The blast cells appear to retain some aspects of their normal identity: for instance, the timing and axes of the initial divisions of B and Y are usually preserved, although the lineages thereafter become very abnormal. Typical mutant lineages of B and Y are shown in Fig. 3B,D. The mutant lineages do not resemble lineages seen elsewhere in the wild-type.

The fate of Y in *egl-5* hermaphrodites is abnormal. Normally the hermaphrodite Y undergoes a gradual transformation in its fate during L2–L3, from a rectal epithelial cell to a neuron (PDA). In *egl-5* mutants this transformation does not occur, and Y remains epithelial (as judged by Nomarski morphology). In 10–20 % of animals Y may divide in L2 or L3; the daughter cells appear to be structural rather than neuronal. The sister cells K and K′ make up part of the dorsal rectal epithelium in the L1; in wild-type L1s of either sex, K

Table 1. *Penetrance of* egl-5 *phenotypes*

(A) Behavioural phenotypes*

Allele	%Egl(*n*)	%Mig	%Mec	%Tab	%Exp^W	%Exp^-
n945am	100(918)	28	100	12	32(23/71)	47(33/71)
n988	100(960)	32	84	20	5(4/78)	81(63/78)
n1066	100(804)	25	94	30	38(26/69)	26(18/69)
u202	100(896)	24	98	20	43(29/67)	37(25/67)
e2399	100(745)	34	96	26	20(15/77)	57(44/57)
e2502	97(1275)	29	81	41	52(38/73)	37(27/73)
e2506	99(1168)	27	86	30	12(8/69)	47(28/69)
e2508	99(854)	30	84	28	14(10/73)	59(43/73)
n989	98(912)	38	84	30	9(7/78)	77(60/87)
n486	97(888)	46	72	20	6(4/78)	67(46/68)
e2495	87(1099)	52	73	30	3(2/74)	43(32/74)
n1439	92(1074)	52	6	2	3(2/66)	15(10/66)

(B) Male phenotypes†

Allele	% Tail and gonad Mab	% extra dorsal coelomocyte	% ectopic hooks
n945am	100	9.6	3.3
n988	100	5.5	2
n1066	100	3.6	5.5
u202	100	7	2
e2399	100	3.5	3
e2502	100	7	3.5
e2506	100	4.5	0
e2508	100	3.5	1.5
n989	100	2	2.5
n486	100	0	9
e2495	100	10.5	1.5
n1439	0	0	0

*Scoring of behavioural phenotypes: % Egl, the percentage of adults retaining eggs, as scored under the dissecting microscope (*n*=number of animals scored); % Mig, distance migrated by the HSN, relative to wild-type, scored by Nomarski microscopy in L1s (see Desai *et al.* 1988 for details of scoring HSN migration; *n*>100); Egl and HSN data (except for *e2399*, *e2495*, *e2502*, *e2506* and *e2508*) from Desai *et al.* (1988); Mec and Tab, scored in 50 adult hermaphrodites, testing the response to light touch (eyebrow hair) and heavy touch (worm-pick) on the tail; Tab worms are also Mec. Defecation (Exp): for scoring the expulsion defect in defecation, eight L4 hermaphrodites of each genotype were picked; 48 h later, defecation was scored. Each animal was observed for 8–10 cycles (about 8 min). No striking variability between individuals was observed. Exp^w: % of defecation cycles with only an dep contraction missing (fraction of cycles in parentheses); Exp^-, % of cycles with both intestinal and an dep contractions missing.

†Male phenotypes: for each allele, 200 young adult males were examined under Normarski at ×1000, scoring abnormal male tails and somatic gonads, extra dorsal coelomocytes and ectopic hooks.

The alleles fall roughly into a single allelic series. The eight strongest alleles (*n945*, *n988*, *u202*, *n1066*, *e2399*, *e2502*, *e2506* and *e2508*) all have similar phenotypes; *n486* and *n989* are slightly weaker and *e2495* weaker still. The male phenotypes of *e2495* are clearly slightly weaker than those of the other alleles; a larger gonad is made, and fan morphogenesis is marginally better. The higher penetrance of the ectopic hook phenotype in the weak allele *n486* is consistent with this phenotype being the result of an intermediate transformation. One unusual allele, *n1439* is about as Egl and Mig as the other weak alleles, but is only very rarely Unc or Mec; *n1439* males are never Mab. Heterozygotes for *n1439* in *trans* to a deficiency for the locus are Egl, weakly Exp, occasionally Mec and Unc, but not Mab (Desai and Horvitz, 1990; data not shown); the strong alleles *n945* and *n988* behave similarly to the deficiency when in *trans* to *n1439*. *n1439* may indicate that some functions of *egl-5* are mutationally separable.

divides, K.a taking on the function of K and K.p becoming the neuron DVB. In mutants for the strong alleles of *egl-5*, in both sexes, K never divides and DVB is not made. Thus, all five rectal blast cells behave abnormally in *egl-5* mutants.

Male-specific defects in somatic gonad and sex muscles

The somatic gonad is grossly abnormal in *egl-5* males, as a result of delays and defects in the late somatic gonad lineages. The abortive somatic gonad forms a sac-like structure into which a seemingly normal germline proliferates; some sperm, of normal appearance, are made (Fig. 4A–D). The lineages of the somatic gonad blast cells Z1 and Z4 are initially normal, producing an L1 somatic primordium which appears wild-type. A leader cell is selected, and functions normally in leading the migration of the gonad. The initial division of the vas deferens and seminal vesicle precursors is at about the usual time, but thereafter the lineages are delayed and defective. Instead of the wild-type modified stem-cell-like lineages, symmetrical proliferative patterns are seen (Fig. 4E,F). The extent to which the terminal fates are altered is difficult to decide on grounds of Nomarski morphology. In 5 to 10 % of *egl-5* males some of the somatic cells form a small enclosed lumen with highly infolded luminal membranes (Fig. 4H,I). This appearance is characteristic of the uterine tissue in hermaphrodite somatic gonads (Fig. 4G). *egl-5* hermaphrodite somatic gonads are wild-type. The function of *egl-5* in the somatic gonad is therefore sex-specific; it is possible

Table 2. *Ventral hypodermal transformations in* egl-5 *and* mab-5

Genotype	n	Fate of P11.p	Fate of P12.p
WT ♂		1°	D
WT ⚥		F	D
egl-5 ♂	9*	**2°**	**1°**
egl-5 ⚥	9†	F	**F**
mab-5‡	20	F	D
	15	F	**anterior, F**
	7	**posterior, D**	D
mab-5 egl-5	6§	**posterior, F**	F

Cell fates are notated as follows: F, fuses with hypodermal syncytium; D, divides, posterior daughter undergoes programmed cell death; 1°, 2° stand for the characteristic fates of the preanal equivalence group (Sulston and White, 1980). Mutant fate choices are in bold type. *n*, number of worms lineaged.

* Ventral hypodermal lineages were followed in males of the following genotypes: *egl-5(n945)* (*n*=3), *egl-5(n945)/nDf16* (2), *egl-5 (n486)* (3), *egl-5(e2399)* (1). In one *egl-5(n486)* male P12.p divided and P12.pp died; P12.pa executed a 2° lineage. The division of the cell P12.aap in L3 shows that the transformation is of P12 itself and not P12.p alone. Normally cells generated by a 2° lineage make the hook, but in some *egl-5* males hook morphogenesis does not occur. Sometimes the fate regulation is incomplete: P10.p may be 2° or may execute a hybrid 2°/3° lineage (2/3 *n486* males, 1/3 *n945*). The incomplete regulation presumably accounts for the formation of ectopic hooks (resulting from extra 2° fates) at low frequency (Table 1).

† Hermaphrodite ventral hypodermal lineages were followed in *egl-5(n945)* (*n*=2), *egl-5(n486)* (4), *egl-5(n1066)* (3). In one *egl-5 (n945)* hermaphrodite P12.p divided; both daughters appeared to fuse with the hypodermal syncytium.

‡ Data taken from Kenyon (1986), pooling results for animals of either sex and genotype *mab-5(e1239)*, *mab-5(e1936)* and *mab-5 (e1239)/nDf16*. In the cases where the fates of P11,12.p were abnormal, this correlated with the misplacement of the mutant cell towards the cell whose fate it assumed.

§ Data from 4 *mab-5(e1239) egl-5(n945)* hermaphrodites and 2 males. Hermaphrodite double mutants are fully viable, fertile and have additive hypodermal neuron migration defects. Males are viable; the somatic gonad and tail phenotypes are no worse than expected from simple additivity. Thus, it is unlikely that *mab-5* and *egl-5* have any redundant functions.

that the phenotype results from a partial and variable transformation of the sexual fate of the somatic gonad cells.

The arrangement of the male sex muscles is very abnormal, with most of the anterior diagonal muscles misattached or missing; some posterior diagonals are usually present. The lineages of the sex muscle precursors appear normal at least until the penultimate divisions. A small proportion of *egl-5* males possess an extra dorsal coelomocyte, of unknown ancestry, positioned next to the usual one (10 % of *n945* males; see Table 1 for other alleles). Wild-type males only have one dorsal coelomocyte, whereas wild-type hermaphrodites have two. The sex muscles of *egl-5* hermaphrodites are normal. It may be that, as in the somatic gonad, the male sex muscles are improperly specified in *egl-5* mutants, occasionally leading to a switch in sexual fate.

Behavioural phenotypes of egl-5 *mutants*

egl-5 mutants show many defects in behaviour. In two of these defects (egg-laying and touch), the deficit can be traced to dysfunction of specific neurons generated in the embryonic tail region. The other defects (expulsion, backwards movement) may arise from localised abnormalities in the defecation muscles and tail nervous system.

Egg-laying: loss of HSN identity

The effect of *egl-5* mutations on hermaphrodite egg-laying has been studied by Trent *et al.* (1983) and Desai *et al.* (1988). The phenotype results from defective development of the two serotonergic neurons involved in egg-laying (the HSNs). In *egl-5* mutants HSNs partially lose their characteristic terminal fates: the mutant HSNs do not migrate the full distance from their birthplace in the tail to the midbody (Table 1), and completely lack serotonin. These defects in the HSNs are sufficient to explain the fully penetrant Egl phenotype seen. The coordinate loss of these and other HSN characteristics has been interpreted to mean that *egl-5* functions in assigning the normal HSN identity (Desai *et al.* 1988). However, the mutant HSNs in *egl-5* retain some wild-type fate: the full specification of HSN fate must require the function of other genes in combination with *egl-5*.

Tail touch: defective interneuron identity

egl-5 mutants are touch-insensitive in the tail only. This insensitivity to light touch (Mec) is highly penetrant (Table 1). 10 to 30 % of mutant worms do not respond to heavy touch (the *t*ouch *ab*normal or Tab phenotype). Touch in the tail is sensed by the mechanosensory PLM cells, which synapse onto the PVC interneurons (Chalfie *et al.* 1985; White *et al.* 1986). A tail Mec phenotype can be produced by ablation of the PLMs, while a Tab phenotype is seen if the PVC interneurons are killed (Chalfie *et al.* 1985; Chalfie and Wolinsky, 1990). The low penetrance Tab phenotype in *egl-5* suggested that the Mec and Tab phenotypes could be the result of partial loss of PVC function. This was tested by making double mutants of *egl-5* with a mutation that specifically affects PVCs, *deg-1*. In mutants carrying the *deg-1(u38)* mutation, the PVCs degenerate postembryonically, causing a progressive Tab phenotype (Chalfie and Wolinsky, 1990). In double mutants between *egl-5* alleles and *deg-1*, PVC degenerations are partially suppressed, the frequency being reduced to 10 to 50 % of that of *deg-1* mutants (data not shown). Interestingly, the amber allele *egl-5(n945)* is the weakest suppressor of PVC degenerations (48 % of *deg-1*): the weakest allele *egl-5(n1439)* is about as effective (42 %), although *egl-5(n1439)* worms are only very rarely Mec; *egl-5(n1439); deg-1* double mutants are still Tab. This suggests that PVC identity as assayed by susceptibility to *deg-1* requires *egl-5* function, although as with the HSNs, *egl-5* cannot be the sole agent specifying PVC fate.

Other behavioural phenotypes: backwards movement and defecation

egl-5 mutations cause two other behavioural phenotypes, abnormal backwards movement and abnormal

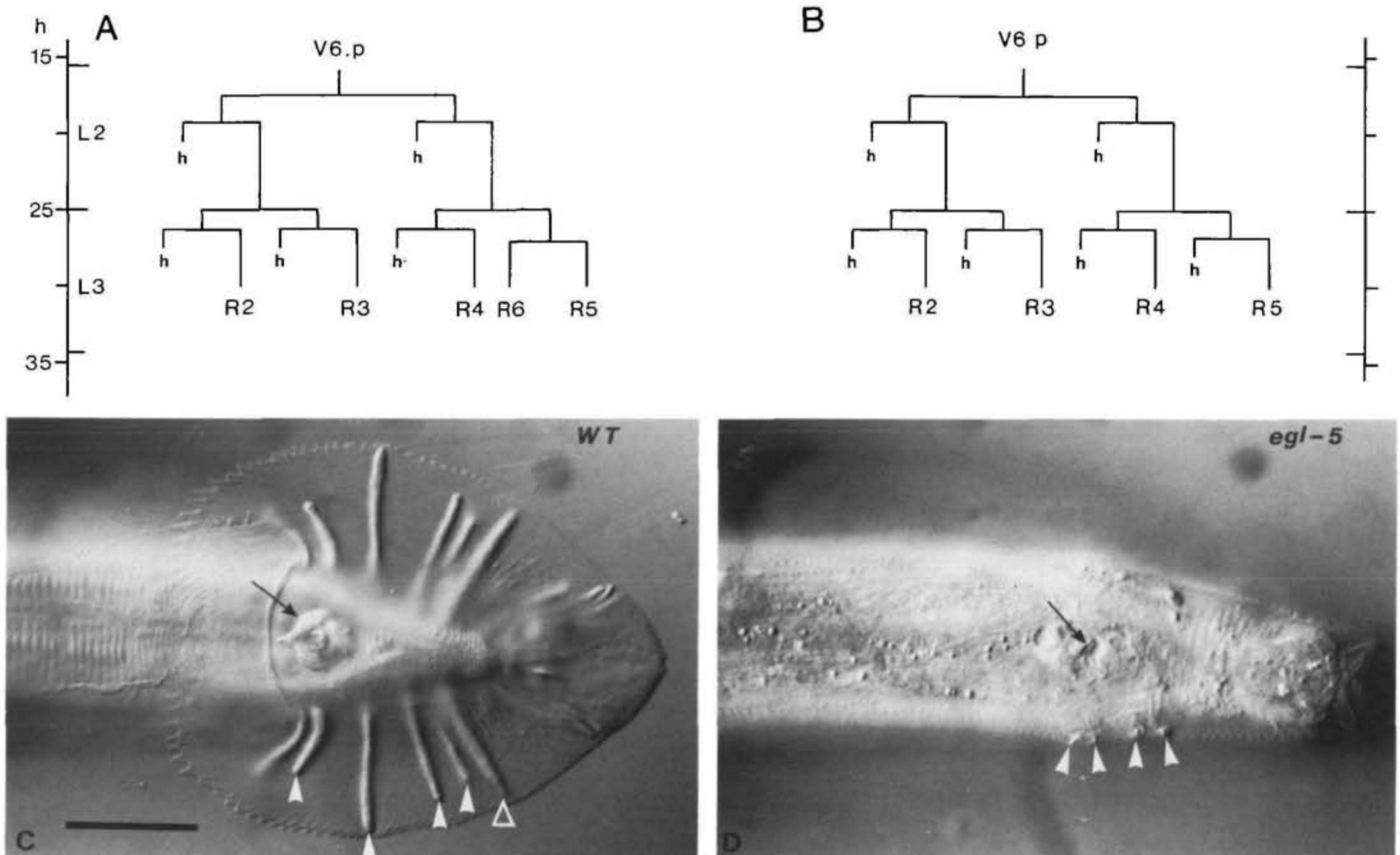


Fig. 2. Lateral hypodermal and tail phenotype of *egl-5* males. (A) The wild-type V6.p lineage (this, and all other wild-type postembryonic lineages, are based on those in Sulston *et al.* 1980). Vertical axis is time in hours since hatching, at 20°C, divided into the four larval stages L1–L4. Branchings represent cell divisions in the anteroposterior axis unless labelled otherwise (anterior to the left). Cells are named by their lineage ancestry from a founder cell by adding a series of letters representing the sequence of cell divisions giving rise to that cell. Division axes are described relative to the main axes of the worm: anteroposterior (a/p), dorsoventral (d/v), left–right (l/r) or any combination of the above. Ends of lines represent terminal differentiation. h=hypodermal cell; Rn=nth Ray sublineage precursor. Each ray precursor executes an identical lineage but the terminal fates and locations of the cells differ characteristically. (B) The V6 lineage in *egl-5* males. V6.ppppa, instead of dividing, is hypodermal (its exact fate has not been determined, but it probably joins the hypodermal syncytium since the tail seam contains the usual 5 nuclei in *egl-5*). The lateral hypodermal lineages were followed from L1 to early L4 in males of the following genotypes (number of animals is given in brackets): *n486* (1), *n945* (4), *n945/nDf16* (4), *e2399* (1). The same phenotype was seen in all cases except one *n945/Df* male, in which V6.pppap did not divide and thus only 7/9 ray precursors were made (on one side; on the other, 8 were made). (C) Nomarski photograph of young adult wild-type male tail, ventral view, anterior to the left. Rays 2–5 (solid arrowheads) and ray 6 (hollow arrowhead) are shown; the hook is visible (black arrow). The two small bumps just posterior to the hook are the postcloacal sensilla. Scale bar=20 μm. (D) *egl-5(n945)* young adult male tail, view and scale as in C. The papillae of rays 2–5 are indicated (arrowheads); all four lie in a ventral row on either side. Ray 6 is not made. The position of the cloaca is shown (black arrow); no hook was made by this worm, although misshapen hooks are sometimes seen; no postcloacal sensilla are made. The morphogenesis of the tail fan in *egl-5* males is rudimentary. This may be a secondary result of the many tail lineage abnormalities, although ablation experiments show that the absence of most descendants of the internal blast cells does not produce such an extreme fan morphogenesis phenotype (Chisholm and Hodgkin, 1989).

defecation. These will be described only briefly since their causes have not been fully established.

egl-5 worms of both sexes coil, in either a ventral or dorsal direction, when attempting to move backwards. This highly characteristic coiler phenotype is seen from the L1 onwards. In L1 worms the direction of coiling is biased ventrally (roughly a 5:1 ratio), but by L4 this bias has almost vanished. The Unc phenotype has variable expressivity: some worms can move back quite well before coiling up, others kink slightly. The connectivity of the posterior ventral nervous system of an adult *egl-5* *(n945)* hermaphrodite was reconstructed from electron micrographs (data not shown) and found to be highly abnormal: of the 13 cell bodies in the preanal ganglion, only 4 (PVT, PVPL, PVPR, DD6) could be confidently assigned. Other cells of the ganglion could be roughly identified, but all had abnormal connectivity to varying extents.

In the wild-type, defecation is accomplished by a cycle of stereotyped movements: the final act of each cycle is an almost simultaneous contraction of the intestinal (mu int) and anal muscle (anal depressor, an

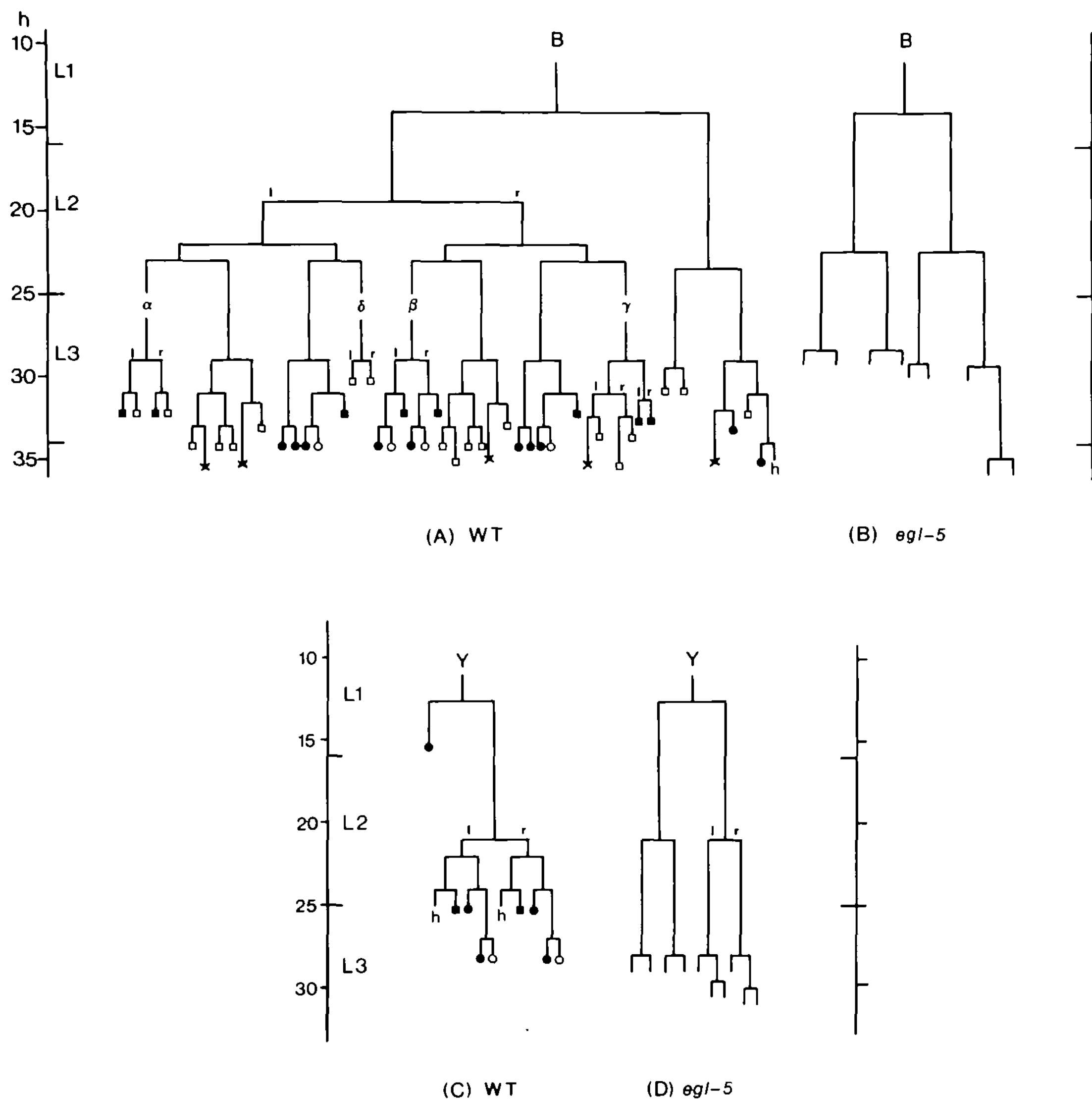
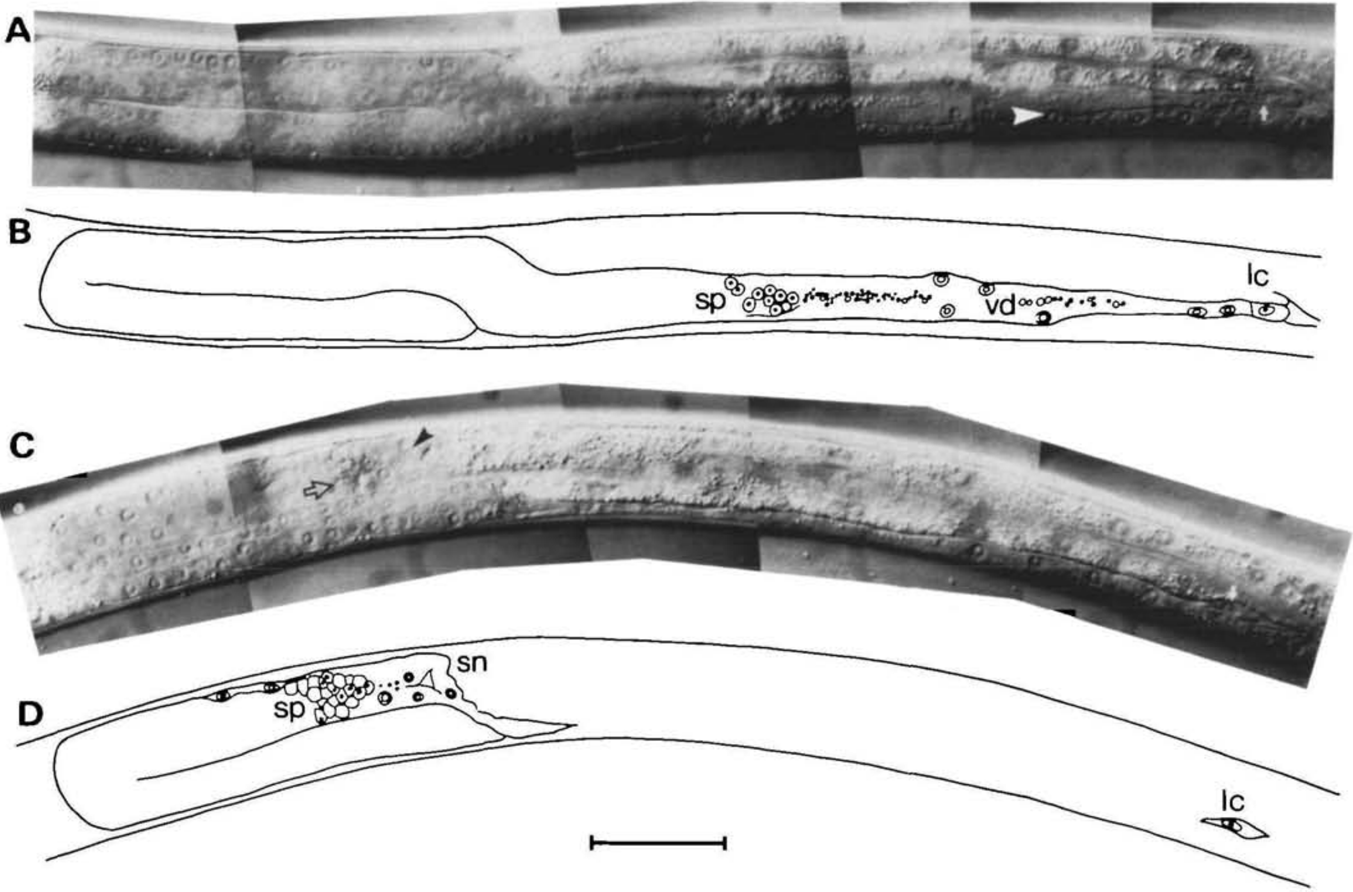


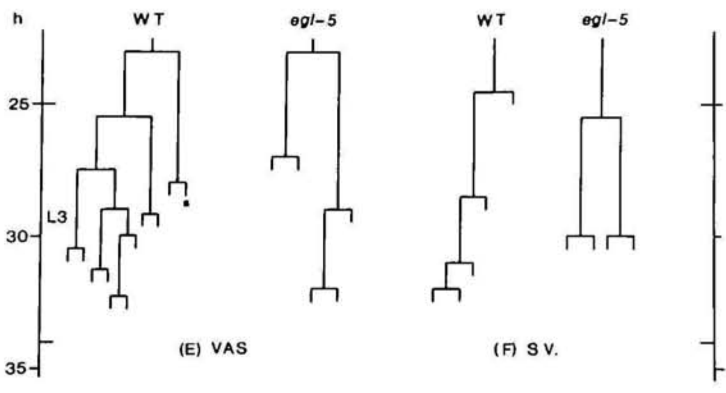
Fig. 3. B and Y lineages in wild-type and *egl-5* males. (A) Wild-type B lineage. Terminal fates are: closed circles, neuronal; open circles, sheath cell of sensillum; closed squares, socket cell of sensillum; open squares, structural cell of proctodeum; h, tail hypodermis; x, cell death. The allocation of fates in some l/r homologue pairs is interchangeable in the wild-type: one possible arrangement is shown. (B) Typical *egl-5* mutant B lineage, from an *n945* male; similar patterns were seen in animals of the following genotypes, lineaged from mid-L1 to the L3 moult: *n486* (3), *n945* (2), *n945/nDf16* (4), *e2399* (1). Several partial B lineages were also obtained for each genotype. The nuclei of B and the other rectal blast cells increase in size in wild-type L1 s, but not in *egl-5* mutants. The initial division is on time, and has the normal ad/pv division axis, but subsequent divisions become variably delayed and are variably oriented. By early L4, the wild-type B lineage has generated 47 cells (of which 5 die); in *egl-5* males B usually produces about 5–10 cells of variable morphology. In most *egl-5* males, B.a makes more cells than B.p (*i.e.*, some of the WT asymmetry is preserved); in one *n486,* one *n945* and one *n945/Df* B.a and B.p executed similar lineages. The terminal fates of the cells made by the mutant lineages are variable, as judged by Nomarski morphology, and can appear compact (neuronal) or structural; none of the B lineage cell deaths, normally occurring in early L4, was seen. (C) Wild-type Y lineage. Fates are shown as in A. (D) Typical *egl-5* mutant Y lineage, based on the same animals as for the B lineage data, although Y was followed fully in only 2/4 *n945/Df* animals. Y.a and Y.p usually execute similar lineages, except in one *n945* worm, when Y.a did not divide (wild-type asymmetry). The terminal fates, as in the mutant B lineages, appear variably compact or structural by Nomarski.

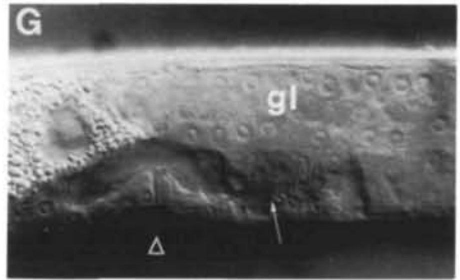
dep) allowing the contents of the intestine to be expelled (exp: Thomas, 1990). In mutants for strong *egl-5* alleles only 1/4 to 1/8 of cycles have a wild-type expulsion (Table 1). The other cycles either completely lack exp or have a partial exp in which mu int but not an dep contracts (and nothing is expelled). The mutant phenotype is expressed progressively: early larvae are Exp^{+}, and only become fully Exp^{-} by the second day of adulthood.

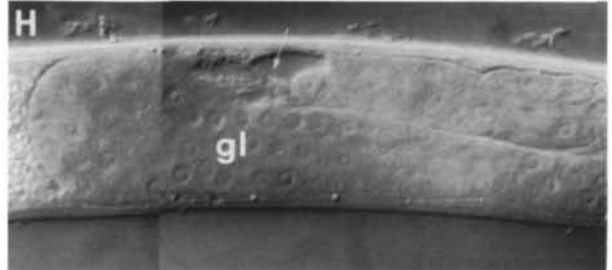
Analysis of egl-5 *genetic mosaics*

To address the question of whether the phenotypes seen


A
B
sp
vd
lc
C
D
sp
sn
lc


h
WT
egl-5
WT
egl-5
25
30
35
L3
(E) VAS
(F) S V.


G
gl


H
gl

I

Fig. 4. The *egl-5* male somatic gonad phenotype. (A) Montage of Nomarski photomicrographs of a wild-type male gonad in late L4 (about 42 h). Lateral view; anterior to the left and dorsal up. The first sperm have been made (hollow arrow). A nucleus of the vas deferens is indicated (solid arrowhead). The linker cell (arrow) has reached the cloaca but has not been killed yet. Scale bar=40 μm. (B) Tracing of A: sp, sperm; vd, vas deferens; lc, linker cell. (C) *egl-5(n945)* male gonad, stage and view as for A. Sperm are being made (hollow arrow); the somatic gonad nuclei (solid arrowhead), although disorganised, make a sac containing the sperm. The linker cell (arrow) has migrated normally, reflexing at the correct time (L2 moult), and has subsequently broken free of the rest of the somatic gonad, migrating alone to the cloaca. Scale as for A. (D) Tracing of C. sn, somatic gonad nucleus. (E) and (F) Wild-type and mutant vas deferens and seminal vesicle precursor lineages. Wild-type lineages are taken from Kimble and Hirsh (1979). Division axes are drawn relative to the proximal–distal axis of the gonad: left is proximal, right is distal. The mutant lineages shown are typical, although the phenotype is variable in detail. The somatic gonad lineages were followed from mid-L2 to late L3 for males of genotype: *n945* (2) and *n945/nDf16* (2); for one of the *n945/Df* males the lineage was followed from mid-L1. The L1 somatic primordium was normal in those animals lineaged in the late phase; several L1 gonad lineages for males of each genotype were normal, although one *n945/Df* L1 primordium was abnormally arranged. The late lineages have not been completely followed, and further divisions may occur in late L3/early L4. (G) Wild-type hermaphrodite somatic gonad, late L4, lateral view. Detail of uterus (arrow) and vulva (hollow arrowhead). gl, distal germline. Scale bar=40 μm. (H) *egl-5(n945)* male gonad, lateral view, mid L4; detail of anterior gonad. There is a small patch of uterine-like tissue dorsally (arrow): the location and extent of this tissue are typical; gl, distal germline. Scale as for G. (I) *egl-5(n945)* male gonad, dorsal view, detail of uterine-like tissue. This patch is more extensive than typically seen; the appearance of the cells is characteristic. Scale as for G.

in *egl-5* mutants result from autonomous requirements for *egl-5*, or from a non-autonomously acting signal, genetic mosaics were analysed. These were found using a strain carrying a chromosomal duplication of *egl-5(+)* that is lost at low frequency in somatic cell division. The duplication also carries the cell-autonomous marker gene *ncl-1(+)* (E. Hedgecock, personal communication). From the pattern of cells with the Ncl^- phenotype, the point at which the duplication was lost in embryogenesis can be deduced; the effects of the clone of *egl-5*$^-$ cells are then scored (see Materials and methods). For all the phenotypes scorable in this analysis, the clonal loss of the *egl-5(+)* duplication had effects only within that clone, with one expected exception. 54 male mosaics, from 24 loss points, were examined (Table 3); the results are summarised here.

Loss of the duplication in an ancestor of the blast cell V6 gave males in which ray 6 was not made and rays 2–5 were fused into a single large ray in the normal ventral position of rays 2 and 3; in one male, this had occurred only on the left-hand side, while the right-hand side was wild-type. Fan morphogenesis was normal. The ray

Table 3. *Morphology of* egl-5 *mosaics*

Loss point	*n*	Phenotype
AB	2	Spicules, rays and fan mutant; gonad and muscles WT
AB.a	2	WT except for absence of R6, fusion of R2–5
AB.arp	2	ditto
AB.arppa	1	Absence of R6L, fusion of R2–5L only.
AB.p	4	No spicules; fan vestigial; ray positions WT, gonad and muscles WT
AB.pl	3	Small, abnormal spicules; fan small, partial hook; Y groups made.
AB.plp	1	Small abn spicules; fan small.
AB.plpp	1	Small abn spicules; fan small; hook made.
AB.pr	5	Refractile blob in rectum; fan small.
AB.prp	1	No spicules; fan small.
AB.prpp	5	No spicules; fan small; hook made.
AB.prpppp	1	No spicules; fan small; Y cell groups made
P_1	4	Gonad, muscles mutant; crumpled spicules
EMS	3	ditto
MS.a	2	Partial Egl-5 gonad; muscle pattern abn, crumpled spicules
MS.ap	2	ditto
MS.app	2	partial Egl-5 gonad; muscle pattern WT, spicules WT
MS.p	2	partial Egl-5 gonad
MS.pp	2	ditto
MS.ppp	1	ditto

Loss point: cell in the embryonic lineage at which the duplication was lost (see Materials and methods); *n*: number of mosaics examined. Only mutant mosaics are tabulated; losses at the following points gave wild-type males: AB.al (n=2), AB.pla (1), AB.plpa (2), AB.pra (3). Loss at AB.p gave fully mutant tail fan morphogenesis, but single losses within AB.p could not give the full phenotype, although losses at AB.prp and AB.plp gave small fans. The sum of these effects is probably sufficient to produce the full phenotype; this is supported by one double loss mosaic in which AB.prp and AB.pl were mutant, producing a fully mutant fan. Since the AB.plp and prp lineages make both the internal blast cells and the tail hypodermis, this analysis does not distinguish secondary effects of the internal defects from a primary role for *egl-5* in the tail hypodermis.

defects seen in *egl-5* are therefore autonomous to the ray lineages. Duplication loss in an ancestor of F and U (which are sisters) resulted in a male tail with small, crumpled spicules as expected from the minor contributions of F and U to the spicule lineages. Loss in Y and B resulted in the complete absence of spicules. In one male where the duplication had been lost in B but not in Y, the progeny of Y were positioned normally. The effects of loss in the male specific blast cells are therefore cell-autonomous. Duplication loss in both somatic gonad precursors Z1 and Z4 gave a fully mutant gonad, while loss in either produced a partly mutant gonad. Loss in the sex muscle lineage gave a fully mutant sex muscle pattern, showing that this phenotype is intrinsic to the muscle lineage; in such mosaics, the spicules were made but were crumpled, a nonautonomous effect expected because the sex muscles are required for proper spicule morphogenesis (Sulston and White, 1980). The requirements for *egl-5* in the ventral hypodermis were not assessed, as mosaics could not be scored early enough in development (L1) for these lineages to be followed.

51 hermaphrodites mosaic for *egl-5* were also isolated and their behaviour scored (not shown). The analysis is consistent with the Egl and Mec phenotypes resulting from defective HSN and PVC function, respectively; the foci for the Unc and Exp phenotypes are within AB.p.

Discussion

The wild-type function of egl-5

The genetic analysis of *egl-5* has shown that its function is required for the correct development of a variety of neurons and blast cells. Most of these cells are not related closely by ancestry (Fig. 5) or by cell type, but by their position, being confined to a small region around the rectum (Fig. 6A). The requirements for *egl-5* in the male sex muscles and somatic gonad differ in that the effects are sex-specific, and in some cases can be interpreted as partial sexual transformations.

The lineage alterations in the ventral hypodermis (both sexes) and the lateral hypodermis (in males only) can be interpreted as causing the posteriormost member of each series of blast cells (P12, V6.ppp) to take on the fate of the next anterior neighbour (P11, V6.pap). For the internal blast cells of the male tail, interpretation is less simple: the mutant lineages do not resemble any other seen in wild-type development. Two of the behavioural phenotypes (Egl and Mec) can be traced to defects in the fates of neurons made in the tail, the HSNs and the PVCs. The Unc and Exp phenotypes may result from the abnormal wiring seen in the preanal ganglion, although the defects are too profound to make any simple correlation.

Many cells affected in *egl-5* mutants do not completely lose their wild-type fates: for example, the

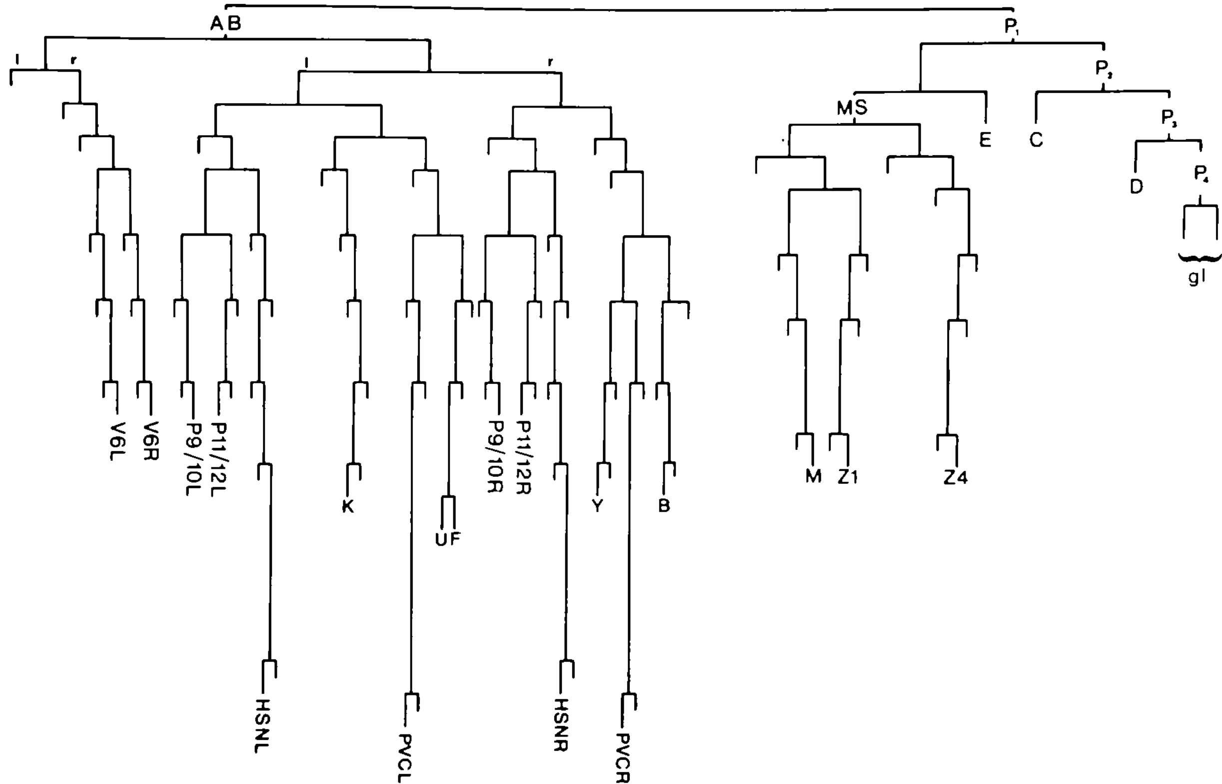


Fig. 5. The origin of cells affected by *egl-5* in the embryonic lineage. An abbreviated version of the embryonic lineage is shown, taken from Sulston *et al.* (1983). The male internal blast cells derive from AB.plppp (U and F) and AB.prppp (Y and B). Rays 2–6 are produced by descendants of V6L and V6R, derived from AB.arppa and AB.arppp, respectively. The MS founder cell gives rise to the sex muscle precursor M (MS.apaapp) and the somatic gonad precursors Z1 and Z4 (MS.($^{a}_{p}$)ppaap). The HSNs are AB.p($^{l}_{r}$)apppappa; the PVCs are AB.p($^{l}_{r}$)pppaapaa.

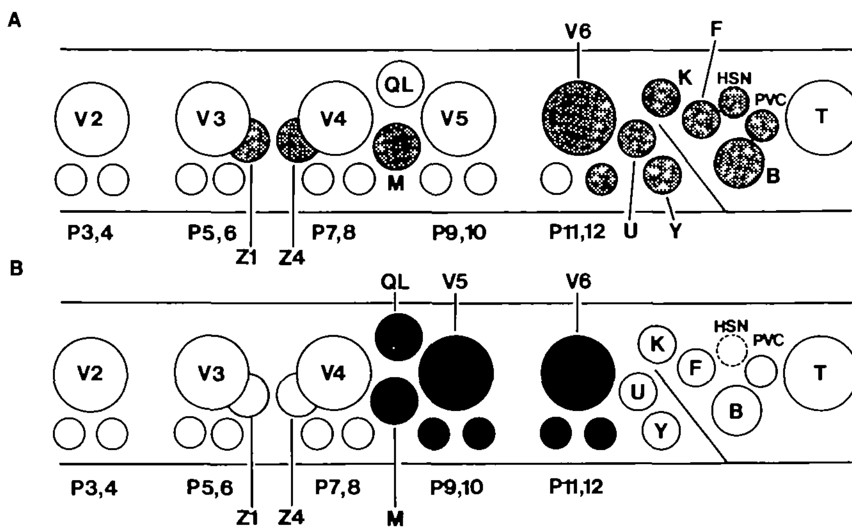


Fig. 6. Regional requirements for *egl-5* and *mab-5*. The cells affected by *mab-5* and *egl-5* are shown on idealised maps of the nuclei of the L1 larva; this arrangement is not seen in development because of differences in timing of cell division and migration. (A) Cells affected by *egl-5* mutations are stippled. (B) Cells affected by *mab-5* are hatched. The HSN birthplace is extrapolated from its position at 400′ into embryogenesis, next to its sister cell PHB (not shown). The hermaphrodite M lineage is normal in *egl-5*, but variably abnormal in *mab-5*. In *mab-5* males, the sex myoblasts (SMs) migrate anteriorly instead of posteriorly, and undergo aberrant divisions; in *egl-5* the male SM lineages are normal but the final dispositions of the muscle cells are abnormal. The progeny of QL always migrate anteriorly in *mab-5*, instead of posteriorly. Very occasionally in *egl-5* (<10 %) QL migrates anteriorly.

HSNs retain some ability to migrate, and the internal blast cells perform their initial divisions at the normal time. If the strong *egl-5* alleles cause a complete loss of *egl-5* function, then *egl-5* must act in combination with other genes to determine these cells. To test the possibility that *mab-5* might act synergistically with *egl-5* to specify these cell fates, a *mab-5 egl-5* double mutant was examined (see Table 2). The phenotype of the double mutant was as expected from simple additivity of effects, arguing against such a synergistic role. Alternatively, *egl-5* may not be involved with the specification of these fates, but rather with their execution.

The effects on the somatic gonad and sex muscles in males may be distinct from this regional function. The *egl-5* phenotype in these tissues is enigmatic: some individuals show an apparent partial sexual transformation (uterine-like gonad tissue, extra dorsal coelomocytes), but in most these tissues are simply disorganised. The blast cells generating the somatic gonad and sex muscles do not lie within the very limited domain in which all the other cells affected by *egl-5* mutations reside. The normal function of *egl-5* in the male somatic gonad, and possibly in the male sex muscles, may be to constrain cells to the male fates; in the lack of such constraint, the cells have an intersexual identity and occasionally veer towards the hermaphrodite fate.

The functions of mab-5 *and* egl-5

Most of the cells affected by mutations in *egl-5* fall into a local region or domain in the tail of the worm. The lineage defects are confined to the tail blast cells P12, V6.ppp, B, Y, U, F and K. The HSN and PVC neurons, which are born in the tail region, are abnormal. The Unc and Exp phenotypes cannot be fully accounted for, but probably result from localised alterations in the tail nervous system, defecation muscles and rectal cells. If the cells known to be affected by *egl-5* mutations are plotted out on a map of the nuclei of the larval worm (Fig. 6A), it is clear that most of them lie in a small region of the tail surrounding the rectum. The effects on the male somatic gonad and sex muscles are spatially, and perhaps qualitatively, distinct, in that the cells affected do not lie in the rectal region, and are only affected in males.

The phenotype of *egl-5* mutants may be dramatically compared with that of mutants of the gene *mab-5*. Mutations in *mab-5* similarly affect a variety of cell types in a local domain, the posterior body region (Kenyon, 1986). Cells affected by *mab-5* lose their characteristic regional differentiation: some become transformed towards their anterior homologues, others simply lose part of their wild-type identity (e.g., the M lineage is abnormal). Similarly, some of the lineage abnormalities in *egl-5* can be seen as transformations from posterior to anterior fates (for cells that are members of a series of homologues along the body axis), but others cannot be so construed.

The domains defined by mutations in the two genes are adjacent and overlap slightly (Fig. 6A,B). Both genes are required in the posterior ventral and lateral hypodermis. In the ventral hypodermis, *egl-5* mutants cause a fully penetrant transformation of P12 to P11. In *mab-5* mutants, the behaviour of P11 and P12 is variable: usually P12 is wild-type, but occasionally it is transformed to P11, and occasionally *vice versa* (Table 2). In the lateral hypodermis, *mab-5* function must be required at least in V5.ppp, V6.pap and V6.ppp for their extra doubling divisions in males. The effects of *egl-5* mutations are confined to V6.ppp. A double mutant between *mab-5* and *egl-5* was examined; additive effects of the two mutations were seen (Table 2). Both genes effect their functions cell-autonomously.

The HSN and PVC neurons are born at about 400 min in embryogenesis. Soon after their birth, the HSNs migrate anteriorly to the prospective midbody. Therefore, HSN fate must be partly specified soon after birth; *egl-5* function is thus required in the embryonic tail at least from this point. Newly hatched *egl-5* larvae are already Unc and Mec, and the internal blast cells in males appear smaller than normal. Similarly, a role for *mab-5* in the embryo is implied by the displaced posterior hypodermal nuclei seen at hatching in *mab-5*

mutants (Kenyon, 1986). Thus, *egl-5* and *mab-5* act during embryogenesis in the correct assignment of the fates of some of the cells generated in the tail region.

How are the initial domains of action of the two genes defined? Cells in the two regions are not clonally related, so an intercellular signal may locally activate *mab-5* and *egl-5*. *mab-5* has been cloned and has been found to encode a protein with a homoeodomain (Costa *et al.* 1988). The *mab-5* transcript is confined to the posterior body in L1 worms, and preliminary evidence suggests that it is also localised in embryos (Costa *et al.* 1988; D. Cowing and C. Kenyon, personal communication).

Comparison with other species

The genetic characteristics of *mab-5* and *egl-5* together appear reminiscent of the clusters of regional selector genes known from genetic studies of the insects *Drosophila* (Lewis, 1978; Kaufman *et al.* 1980) and *Tribolium* (Beeman *et al.* 1989). *mab-5* at least also shares one physical characteristic with the *Drosophila* selector genes, namely a homeobox. Do these limited genetic similarities between worms and insects imply diverged versions of a gene cluster present in a common ancestor of the two phyla, or are they convergent solutions to the common problem of regional differentiation? The former is appealing for reasons of parsimony, but the latter cannot be dismissed. The relationships of the Nematoda to the Arthropoda are not understood (Willmer, 1990): whether a common ancestor would require such mechanisms of regional differentiation is an open question. Resolution of this point will require a comparative analysis of mechanisms of embryogenesis in other phyla.

I thank my supervisor, Jonathan Hodgkin, for suggesting this project, and for much advice and inspiration along the way. Chand Desai and Gian Garriga (M.I.T.) generously provided strains and information on *egl-5*. The EM reconstruction could not have been done without the skills of Nichol Thomson; I am also indebted to Eileen Southgate and John White for their help with the reconstruction. Leon Avery, Tom Barnes, Siegfried Hekimi, Roger Hoskins, Andrew Spence and Michael Shen contributed help and ideas during this work. I thank Jonathan Hodgkin, Peter Lawrence, Joel Rothman and John White for their comments on the manuscript. For part of this work I was the recipient of a Medical Research Council (UK) Research Studentship.

References

Beeman, R. W., Stuart, J. J., Haas, M. S. and Denell, R. E. (1989). Genetic analysis of the homoeotic gene complex (HOM-C) in the beetle *Tribolium castaneum*. *Devl Biol.* **133**, 196–209.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71–94.

Chalfie, M., Sulston, J. E., White, J. G., Southgate, E., Thomson, J. N. and Brenner, S. (1985). The neural circuit for touch sensitivity in *Caenorhabditis elegans*. *J. Neurosci.* **5**, 956–964.

Chalfie, M. and Wolinsky, E. (1990). The identification and suppression of inherited neurodegeneration in *Caenorhabditis elegans*. *Nature* **345**, 410–416.

Chisholm, A. and Hodgkin, J. (1989). The *mab-9* gene controls the fate of B, the major male-specific blast cell in the tail region of *Caenorhabditis elegans*. *Genes and Dev.* **3**, 1823–1833.

Costa, M., Weir, M., Coulson, A., Sulston, J. E. and Kenyon, C. (1988). Posterior pattern formation in *C. elegans* involves position-specific expression of a gene containing a homoeobox. *Cell* **55**, 747–756.

Desai, C., Garriga, G., McIntire, S. L. and Horvitz, H. R. (1988). A genetic pathway for the development of the *Caenorhabditis elegans* HSN motor neurons. *Nature* **336** 638–645.

Desai, C. and Horvitz, H. R. (1989). *Caenorhabditis elegans* mutants defective in the functioning of the motor neurons responsible for egg-laying. *Genetics* **121**, 703–721.

Duncan, I. (1987). The bithorax complex. *A. Rev. Genet.* **21**, 285–319.

Hodgkin, J., Edgley, M., Riddle, D. R., Albertson, D. G. et al. (1988). In *The nematode* Caenorhabditis elegans (ed. W. Wood) pp. 491–584. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Kaufman, T. C., Lewis, R. and Wakimoto, B. (1980). Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*: the homoeotic gene complex in polytene chromosome interval 84A-B. *Genetics* **94**, 115–133.

Kenyon, C. J. (1986). A gene involved in the development of the posterior body region of *C. elegans*. *Cell* **46**, 477–487.

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

Lewis, E. B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature* **276**, 565–570.

Rosenbluth, R. E., Cuddiford, C. and Baillie, D. L. (1985). Mutagenesis in *Caenorhabditis elegans*. II. A spectrum of mutational events induced with 1500 R of γ-radiation. *Genetics* **109**, 493–511.

Sulston, J. E., Albertson, D. G. and Thomson, J. N. (1980). The *Caenorhabditis elegans* male: postembryonic development of nongonadal structures. *Devl Biol.* **78**, 542–576.

Sulston, J. E. and Hodgkin, J. (1988). Methods appendix. In *The Nematode* Caenorhabditis elegans (ed. W. Wood) pp. 587–606. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

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

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

Sulston, J. E. and White, J. G. (1980). Regulation and cell autonomy during postembryonic development of *Caenorhabditis elegans*. *Devl Biol.* **78**, 577–597.

Thomas, J. (1990). Genetic analysis of defecation in *Caenorhabditis elegans*. *Genetics* **124**, 615–625.

Trent, C., Tsung, N. and Horvitz, H. R. (1983). Egg-laying defective mutants of the nematode *Caenorhabditis elegans*. *Genetics* **104**, 619–647.

White, J. G., Southgate, E., Thomson, J. N. and Brenner, S. (1986). The structure of the nervous system of *Caenorhabditis elegans*. *Phil. Trans. R. Soc. Lond.* B **314**, 1–340.

Willmer, P. (1990). *Invertebrate Relationships: Patterns in Animal Evolution*. Cambridge University Press, Cambridge.

Yuan, J. and Horvitz, H. R. (1990). The *Caenorhabditis elegans* genes *ced-3* and *ced-4* act cell autonomously to cause cell death. *Devl Biol.* **138**, 33–41.

(*Accepted 14 January 1991*)