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4 January 1991; accepted 15 February 1991

Spatial Control of Gut-Specific Gene Expression During *Caenorhabditis elegans* Development

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The nematode *Caenorhabditis elegans* was transformed with constructs containing upstream deletions of the gut-specific *ges-1* carboxylesterase gene. With particular deletions, *ges-1* was expressed, not as normally in the gut, but rather in muscle cells of the pharynx (which belong to a sister lineage of the gut) or in body wall muscle and hypodermal cells (which belong to a cousin lineage of the gut). These observations suggest that gut-specific gene expression in *C. elegans* involves not only gut-specific activators but also multiple repressors that are present in particular nongut lineages.

THE *ges-1* GENE OF THE NEMATODE *Caenorhabditis elegans* codes for a carboxylesterase that is expressed only in the intestine (or E) lineage (*1, 2*). *ges-1* expression is due to lineage-autonomous zygotic transcription that occurs when the embryo has 100 to 150 cells and the developing gut has only 4 to 8 cells (*1, 2*). Here we address the spatial control of *ges-1* expression during *C. elegans* development.

We have used DNA-mediated transformation to identify lineage-specific control sequences in *ges-1*. Exogenous transforming DNA can be integrated into the *C. elegans* genome (*3, 4*), but this is a rare event. *Caenorhabditis elegans* can also be "heritably transformed" by means of extra-chromosomal DNA that is passed through the germline with efficiencies of 20 to 90%, depending on the strain (*5–7*). The trans-forming DNA is usually co-injected with a second marker gene that allows transformants to be identified (*8*). We have also "transiently transformed" *C. elegans* by injecting exogenous DNA through the body wall of the mother worm into the oocyte cytoplasm (*9*). The oocyte is then fertilized naturally and stained for *ges-1* activity later in embryogenesis. A wild-type *C. elegans* embryo, stained to show normal *ges-1* activity in the developing gut, is shown in Fig. 1a. An embryo of the *ges-1* null strain JM1041 (*10*), in which the gut does not stain, is shown in Fig. 1b. An example of a *ges-1* null embryo in which gut-localized esterase expression has been accurately reconstituted by transient transformation with the wild-type *ges-1* gene (*11*) is shown in Fig. 1c.

We prepared a unidirectional deletion series beginning from the far upstream 5′ region of *ges-1* and proceeding toward the *ges-1*–coding region. Each deletion construct was introduced into the *ges-1* null worms by the use of both heritable and transient transformation; transformed embryos at roughly the 1½-fold stage of development were then stained for *ges-1* activity.

Six classes of *ges-1* spatial expression patterns were observed, depending on the size of the 5′ flanking *ges-1* DNA in the transforming construct. The top part of Fig. 2 shows examples of individual transformed embryos exhibiting these different *ges-1* expression patterns; the bottom part of Fig. 2 summarizes the pattern frequency (*12*). The different expression patterns were as follows.

I) Gut only. Transformation of *ges-1* null worms with a cosmid containing *ges-1* accurately reproduced the gut-specific pattern of wild-type esterase expression (Fig. 2, a, b, and c).

II) Either gut only or gut + pharynx. When *ges-1* null worms were transformed with deletion −1309, all staining embryos stained in all cells of the gut (Fig. 2, d, e, and f). However, 80 to 90% of the staining embryos also stained, albeit weakly, in the vicinity of the posterior bulb of the pharynx (Fig. 2, d and e). The proportion of embryos showing pharynx staining was reproducible, for example, 89% (n = 37), 89% (n = 19), and 84% (n = 96) in three independently transformed strains.

III) Either pharynx only or gut + pharynx. After transformation with deletion −1220, essentially all (98%) of the staining embryos stained in the gut, but 100% also stained in the pharynx. As more of the 5′ region was deleted, gut expression dropped dramatically, but pharynx expression remained high. With deletion −1140, all of the staining embryos stained in the pharynx, but only half (48%) stained in the gut. The all-or-none nature of these staining patterns was unexpected and striking; the intensity of gut-staining was not graded between individual embryos, but rather the entire pattern shifted (compare g and h in Fig. 2 with i) (*13*).

IV) Pharynx only. Embryos transformed with deletion −1069 expressed *ges-1* in the pharynx, but not in the gut. This pattern persisted down to deletion −1001 and was also found with deletions −521 to −473. In the absence of gut staining, a *ges-1*–expressing cell in the tail now became apparent (arrows in Fig. 2, j, k, and l).

V) Either pharynx or body wall muscle or hypodermis. With deletion −835, *ges-1* was now expressed in three distinct tissues: either the posterior pharynx (Fig. 2m), the posterior body wall muscles (Fig. 2n) or the posterior hypodermis (Fig. 2o) (*14*). As noted earlier, the expression patterns have an unexpected all-or-none character and few embryos stained in more than one of the three tissues. When the 5′ flanking region was deleted a further 10 bp to −825, the same three staining patterns could still be detected, but now the proportion of embryos that stained in the pharynx was increased to 97% and the proportion of embryos that stained in the hypodermis or body wall muscle was decreased to 13%.

VI) Pharynx + random. A predominantly pharynx staining pattern (class IV) was maintained by deletions extending to −473.

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Fig. 1. Embryos stained for esterase activity. (**a**) Wild-type embryo; (**b**) *ges-1* null mutant embryo (strain JM1041) (*10*); (**c**) *ges-1* null mutant embryo transiently transformed with a plasmid that contains *ges-1* (*9, 11*).

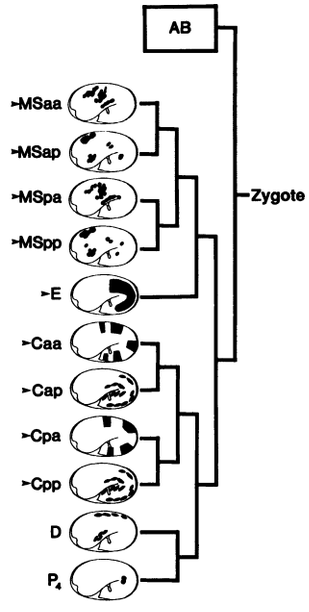
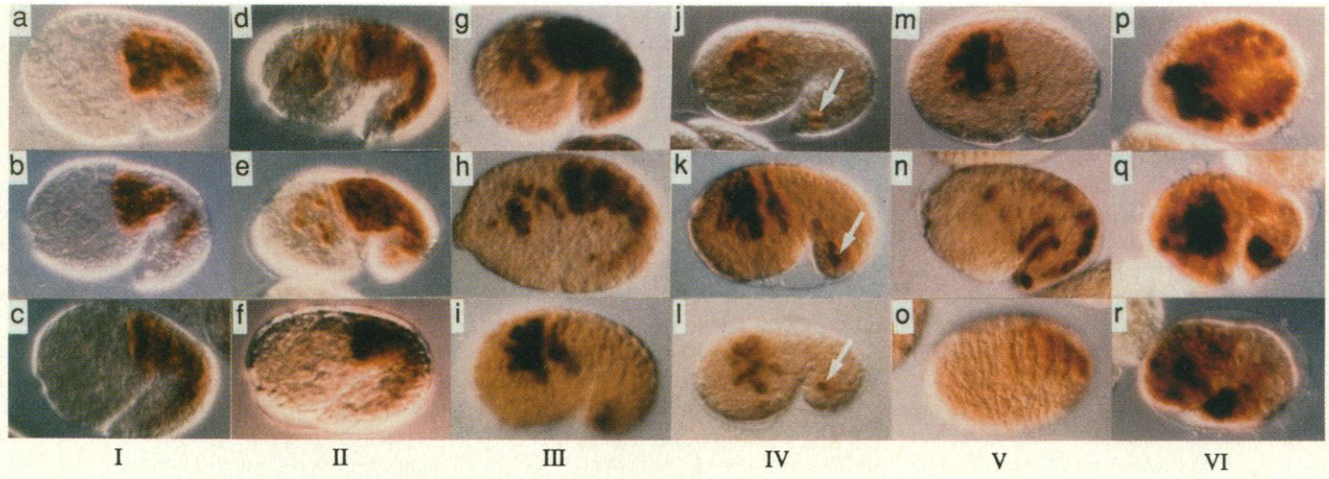


Fig. 3. The position of each cell in sublineages of the 1½-fold *C. elegans* embryo, as estimated from (*16*). The arrowheads indicate the sublineages where the *ges-1* deletions are expressed; only cells in the E lineage, its sister MS lineage, and cousin C lineage have been observed to express the *ges-1* deletions. For the sake of clarity, not all of the hypodermal cells are shown for lineages Caa and Cpa.

When the 5′ flanking region was deleted further, the *ges-1* staining pattern became patchy and variable, although an underlying pharynx staining could still be detected down to deletion −64 (Fig. 2, p, q, and r). *ges-1* esterase activity was no longer detected when part of the coding region had been deleted.

The lineages of cells that can be made to express *ges-1* ectopically may reflect the *ges-1* control circuits operating inside the intact embryo. These ectopically expressing lineages can be identified by comparing the observed staining patterns (Fig. 2) with the known positions of embryonic nuclei (Fig. 3). Cells that stain anterior to the gut in patterns II to VI can be identified as MS-lineage muscle cells of the posterior pharynx. In worms transformed with deletion −835 (class V pattern), the nongut nonpharynx cells that stain can be identified as either C-lineage muscle cells or C-lineage hypodermal cells. Both the MS-lineage and the C-lineage muscle expression can be identified by staining transformants later in development, after morphogenesis has occurred. The dorsal hypodermal cells of the C lineage can be identified by the distinctive striped pattern that arises because, in any one embryo, only derivatives of Caa or derivatives of Cpa (but not both) express the *ges-1* construct. We observed no staining patterns that could be ascribed to cells of the AB, D, or P4 lineage and hence not all muscle cells nor all hypodermal cells in the embryo express *ges-1*. We conclude that it is not cell type that determines whether *ges-1* can be expressed ectopically.

Given the predominance of cell lineage mechanisms in *C. elegans* early development (*15, 16*), it is natural to assume that the choice of ectopically expressing cells is also lineage based; such an assumption is supported by the fact that the MS lineage is a



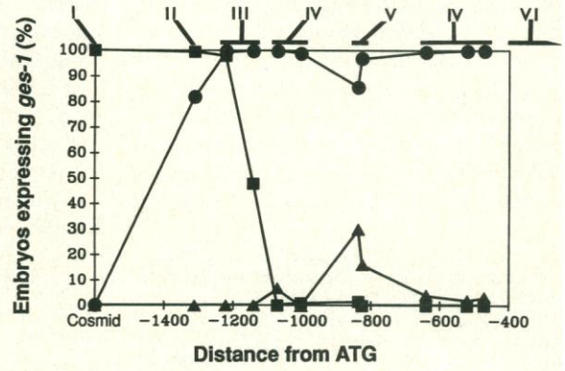


Fig. 2. Summary of the deletion analysis of *ges-1*. The bottom portion of this figure plots the percent of staining embryos that express *ges-1* in the gut (■), the posterior pharynx (●), or the posterior body wall muscles or hypodermis, or both (▲), as a function of the amount of 5′ flanking DNA in the transforming construct [expressed as base pairs upstream from the translation start codon (*12*)]. For each deletion, between 108 and 363 embryos (mean = 186) were analyzed from at least four separate strains. The upper part of the figure shows examples of the different *ges-1* staining patterns observed in individual transformed embryos. The roman numerals I to VI designate the examples in the different classes of patterns exhibited by the different deletions, as discussed in the text. We use the following abbreviations to describe the individual embryos: H or T refers to heritable or transient transformation, respectively; *unc-22* or *rol-6* refers to the phenotypic marker used in heritable transformations; the number (for example, −1309) refers to the transforming deletion. (**a** to **c**) H/*unc-22*, cosmid C29B10; (**d** to **f**) H/*unc-22*, −1309; (**g** and **h**) H/*rol-6*, −1220; (**i**) H/*rol-6*, −1140; (**j**) H/*unc-22*, −521; (**k**) T, −1069; (**l**) T, −473; (**m**) H/*rol-6*, −835; (**n**) T, −835; (**o**) H/*rol-6*, −835 (we show an earlier embryo in this case because the hypodermal stain is easiest to interpret at this stage); (**p** to **r**) H/*unc-22*, −232. Deletions were prepared by the method of Henikoff (*25*) and defined by sequencing.

sister lineage of the gut and the C lineage is a cousin (Fig. 3). However, the simplest lineage-based mechanism can only be a first approximation. The pharyngeal muscles that express *ges-1* are derivatives of the MSaa and MSpa cells (Fig. 3), but other MSaa or MSpa derivatives (for example, the muscles of the anterior ventral body wall) were not observed to express *ges-1*. Furthermore, only 2 of the approximately 30 cells in the MSap and MSpp lineages were observed to express *ges-1*. These two cells are (i) the positively staining cell that appears in the tail of worms staining with a class IV pattern (see arrows in Fig. 2, j, k, and l); we identify this cell as Mu int R, a derivative of the MSpp cell (*17*), and (ii) the M mesoblast cell, which occupies precisely the same position in the MSap lineage as Mu int R occupies in the MSpp lineage (*18*). We have considered a number of mechanisms whereby these particular lineages and not others are selected for ectopic *ges-1* expression but no single explanation appears adequate (*19*).

Our deletion data can be restated in terms of a simple molecular model (Fig. 4) that describes normal control of *ges-1* in terms of an activator restricted to the E lineage, a repressor and a (second) activator restricted to parts of the MS lineage, and finally a (second) repressor and a (third) activator restricted to the C lineage. Several predictions of this simple model have been fulfilled in preliminary experiments. Injection of multiple copies of *ges-1* 5′ flanking DNA into wild-type worms can lead to altered patterns of *ges-1* expression. We interpret these experiments in terms of a competition between the endogenous and the exogenous sequences for a limited number of activators and repressors. Furthermore, both deoxyribonuclease I footprinting and bandshift experiments have provided direct evidence that *C. elegans* embryos contain nuclear proteins that interact with *ges-1* DNA in the region of the proposed gut-activator binding site (*20*).

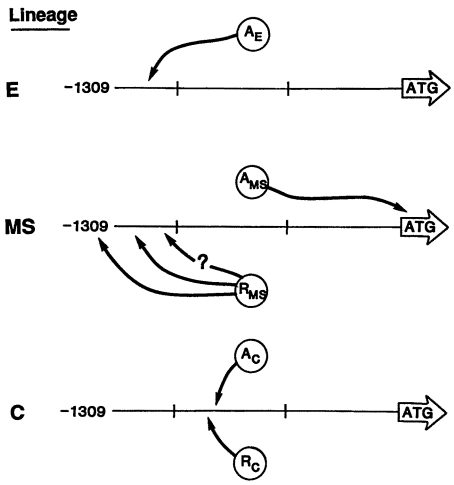


Fig. 4. Model for the spatial control of *ges-1* in *C. elegans*. Each horizontal line represents the 5′ flanking region of *ges-1* from nucleotide −1309 to the translation initiation ATG. The arrows indicate the approximate location on the DNA where activators (A) or repressors (R) are suggested to bind the *ges-1* DNA in cells of the E lineage, the MS lineage and the C lineage. In making this model, we have assumed that an increase or decrease in *ges-1* expression in a particular lineage is caused by removal of binding sites for repressors or activators, respectively. In the MS lineage, the arrow with a question mark is intended to indicate that the intensity of pharynx stain appears to increase below deletion −1220, as if further repressor binding sites are being deleted. We suspect that the site to which the MS-lineage activator binds is located downstream of translation initiation, since the *ges-1* gene can be deleted close to the coding sequence and yet pharynx expression remains.

How general are our conclusions that spatial regulation during development involves a series of repressors (at least two) present in normally nonexpressing lineages? Whereas most work on tissue-specific gene expression in different organisms has focused on the positive effect of gene activators, there are also a number of well-studied examples of negative elements and factors (*21*). It is often suggested that such negative factors are responsible for keeping a particular gene silent in nonexpressing tissues. However, transformation of a variety of different organisms with deleted gene constructs usually produces a simple decrease in gene activity in the particular organ or tissue, not a reappearance of activity in a lineage-related set of cells, as is observed here. Nonetheless, several studies have indicated that in organisms besides *C. elegans*, spatial regulation of gene activity can be under negative control. For example, the mouse transthyretin gene is normally expressed in the liver and the choroid plexus of the brain, but certain 5′ deletions of the gene lead to expression throughout the brain (*22*). Similarly, deletions in the 5′ end of the *Drosophila ftz* gene cause ectopic expression between the normal *ftz* bands (*23*). In both these cases, it was concluded that the deletions had removed binding sites for repressors that keep the gene silent in normally nonexpressing cells. Davidson and co-workers (*24*) have also concluded, on the basis of cross-species transformation and competition with exogenous binding sites, that spatial control of gene expression in the sea urchin likewise involves repressors.

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8. We used two such marker genes in these experiments: an *unc-22* antisense construct that confers a twitching phenotype [A. Fire, S. W. Harrison, D. D. Dixon, *Gene* **93**, 189 (1990)] and a *rol-6* gene that confers a dominant Roller phenotype (C. Mello, D. Stinchcomb, J. Kramer, V. Ambros, personal communication).
9. Injections were done essentially as described (*3*), except that the oocyte cytoplasm rather than the nucleus was injected with approximately 8 pl of DNA at a concentration of 0.1 mg/ml. Hermaphrodites containing the injected oocytes were grown overnight at 16°C, at which time all of the embryos (both injected and noninjected) were collected from the plate and stained for esterase activity as described previously (*1, 2*). If, as shown in Fig. 1c, ~10,000 molecules of DNA were injected per oocyte, gut expression of the *ges-1* esterase was accurately reconstituted. If tenfold less DNA was injected, *ges-1* expression was still confined to the gut but did not occur in all of the gut cells; injection of 10- to 100-fold more DNA resulted in ectopic *ges-1* expression in most, if not all, of the cells of the embryo. We have not yet investigated whether, in the latter case, this widespread expression arises from low-level readthrough transcription or whether it arises from nongut repressors being competed by the presence of excess binding sites; in light of preliminary direct competition experiments described in the text, we favor the latter explanation. In any case, we can use these results to argue that there are no overriding posttranscriptional controls on *ges-1* expression, that is, the *ges-1* protein can be produced in all cells of the early embryo.
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11. The wild-type *ges-1* genes (both genomic and cDNA versions) have been cloned and completely sequenced (B. P. Kennedy, F. L. Allen, J. D. McGhee, in preparation). The *ges-1* mRNA is trans-spliced, as are about 10% of messages in *C. elegans* [M. Krause and D. Hirsh, *Cell* **49**, 753 (1987)]; thus we do not yet know where *ges-1* transcription initiates and deletion sizes are expressed as base pairs upstream of the ATG protein initiation codon. Several considerations suggest that *ges-1* transcription initiates close to the ATG: (i) as a rule, *C. elegans* genes have short 5′-untranslated leaders; a survey of 15 genes in which the initiation site of transcription had been determined experimentally yielded an average leader length of 35 ± 19 (SD) nucleotides; (ii) the *C. elegans ges-1* gene can be deleted to within 64 bp of the ATG and yet still retains strong expression (although with deranged spatial control as is discussed in the text); and (iii) the *ges-1* homolog from the closely related nematode *Caenorhabditis briggsae* gives accurate gut expression in *C. elegans* with only 347 bp of upstream DNA (E. J. Aamodt, M. A. Chung, J. D. McGhee, in preparation).
12. Pattern frequency is plotted as the number of embryos that show a particular spatial pattern of *ges-1* staining, normalized to the total number of embryos that show any *ges-1* staining. This convention is necessary because heritable transformants do not pass the exogenous transforming DNA to all of their progeny and because, in transient assays, only a fraction of the embryos that have been laid have also been injected. This method of data analysis is unlikely to introduce ambiguities. For heritable transformants, the propagation rate of the visible marker closely approximates the fraction of transformants that show *ges-1* staining; for example, a particular strain transformed with the *unc-22* antisense plasmid and a *ges-1* construct passed the twitching phenotype to 73% (n = 248) of its offspring, whereas 71% (n = 31) of the embryos stained for *ges-1*. Thus, there is no evidence for a class of worms that are transformed but do not express *ges-1*. As seen in the transient assays, when fluoresceinated dextran is co-injected with the *ges-1* DNA, every fluorescent embryo also stains for esterase.
13. This all-or-none nature of the different *ges-1* expres-

sion patterns was demonstrated quantitatively with a microspectrophotometer used to measure stain intensity in the guts of embryos transformed with deletion −1140. Two distinct classes of stain intensity were evident: of 70 embryos showing pharynx staining, 40 had an average gut optical density of 0.51 ± 0.07 (SD) and 30 had an average gut optical density of 0.18 ± 0.05. For comparison, a typical *ges-1* null embryo has an average gut optical density of 0.16 ± 0.04. We do not have an adequate explanation for this all-or-none phenomenon. Explanations such as histochemical thresholds or loss of transforming DNA appear unlikely because closely spaced neighboring deletions show quite different behaviors and the distribution of the different expression patterns is a property of the particular deletion. The distribution is influenced only weakly, if at all, by the particular transformed strain, the generation of transformed worms, or the method of transformation. It is conceivable that the large number of transforming gene copies present in the extrachromosomal arrays (*5*–*7*) could somehow be the cause of this all-or-none behavior and it will be important to see if the phenomenon can be reproduced with single integrated copies of the appropriate *ges-1* construct. It is more puzzling to consider how entire arrays could somehow be marked for lineage-specific expression or lineage-specific repression. As an explanation for this exclusive choice between distinct expression patterns, cell-cell communications later in development seem unlikely. Rather, we suggest that the choice of patterns must be made early in development before the different *ges-1* expressing lineages diverge. Since the *ges-1* expressing lineages are identified later as the sister and the cousin lineage of the gut, the expression pattern must be decided at the two-cell stage or the four-cell stage of the embryo.

14. With deletion −835, the proportion of embryos that showed hypodermal expression appeared to be higher if *rol-6* rather than *unc-22* was used as a marker gene for the heritable transformants; the *rol-6* data are included in the bottom part of Fig. 2, but their omission would not alter our conclusions. This was the only case where we have detected any influence of the marker gene on the expression of a *ges-1* construct.
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17. Mu int R, a derivative of the MSpp cell, is born in the head, along with other MS derivatives, and then migrates posteriorly during early morphogenesis (*16*). In *C. elegans* strains that are defective in the *unc-39* gene, Mu int R migration is often aberrant and incomplete [E. M. Hedgecock, J. G. Culotti, D. H. Hall, B. D. Stern, *Development* **100**, 365 (1987)]. A stained tail cell was observed in only 58% of pharynx-staining *unc-39* embryos transformed with deletion −521, compared to 87% for the wild-type control. In addition, these *unc-39* transformants often had a stained cell at variable positions near the middle of the embryo.
18. The M mesoblast forms vulval and uterine muscles in the adult hermaphrodite and forms tail muscles in the adult male (*16*). In adult worms transformed with deletion −521, esterase staining was indeed detected in hermaphrodite vulval muscles and in male tail muscles. In adult worms of the untransformed *ges-1* null strain, the hermaphrodite vulva and the male tail do not stain.
19. Because the deletion series was unidirectional and spaced at 10- to 100-bp intervals, we cannot yet conclude that other *ges-1* constructs will not be expressed in other lineages. However, the amount of background staining in the system is low and only rarely did we detect stained cells that did not fit into the above patterns. We can rule out several artifactual explanations for the choice of ectopic *ges-1* expressing lineages. Integration into a MS- or C-specific gene can be ruled out, because the same expression patterns were observed with numerous independently constructed strains and each strain gave the same proportion of patterns in each generation. Similarly, *ges-1* expression in the body wall muscles did not derive from rearrangements that brought *ges-1* under control of the muscle specific *unc-22* promoter of the coinjected phenotypic marker plasmid; the hypodermally expressed *rol-6* marker gene gave the same patterns, as did transient transformation in which the marker gene was omitted altogether. The patterns did not result from a fortuitous activator in the vector, since removal of all vector sequences from, for example, deletion −1309 did not affect pharynx expression. The ectopic expression patterns are unlikely to reflect loss of the transforming DNA in particular lineages because the patterns were reproducible in successive generations and with numerous independently transformed strains in which the frequency of propagation of the transforming DNA ranged from 20 to 90%. As we noted above (*9*), the choice of ectopic expression patterns is unlikely to reflect posttranscriptional control. Finally, we have used isoelectric focusing gels to show that it was indeed the *ges-1* enzyme (and not some other esterase that had somehow been induced) that was present in pharynx-expressing strains. Both MS and C cells are relatively close to the embryonic gut precursor cells and it is conceivable that the ectopic expression patterns could reflect cell-cell interactions. However, it is difficult to see why some MS derivatives but not others equally close to the gut would be chosen. Similarly, the *C. elegans* cytoplasmic fate map does not provide an obvious explanation for the choice of ectopically expressing lineages. Genes involved in specifying particular cell fates in *C. elegans*, for example the *mec-3* gene [J. C. Way and M. Chalfie, *Genes Dev.* **3**, 1823 (1989)], can be present in highly restricted cell lineages. Thus it is conceivable that the various *ges-1* deletions, because of fortuitously rearranged *cis*-acting binding sites, could now have fallen under the influence of some quite unrelated gene control factors. However, all but one of the *ges-1* deletions expressed esterase activity at close to the time that it is normally expressed in the gut; this timing would not be expected if deleted *ges-1* genes were now under separate and unrelated control. In the one exceptional case, deletion −1140, gut (but not pharynx) *ges-1* activity appeared significantly later than normal, as if a gut-specific timing signal in the gene had been altered.
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26. Supported by the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research. We thank A. Fire, C. Mello, D. Stinchcomb, J. Kramer, and V. Ambros for sharing their unpublished results and for providing the marker genes used in this study. We also thank C. Berglind for preparation of the manuscript.

30 November 1990; accepted 19 February 1991