M. Kruse-Wolters, D. J. Ruiter, P. I. Schrier, *EMBO J.* **7**, 1023 (1988).
22. D. Wang *et al.*, *J. Virol.* **62**, 4173 (1988).
23. The expression of EBV LMP and EBNA-2 RNA was unchanged in c-*myc*– and control-transfected LCLs (CB-33, UH-1, and CPH-3 LCLs) analyzed by Northern blot hybridization with the use of probes specific for LMP (1.9-kb insert from plasmid pBSPcLMP 71 ΔN) and EBNA-2 (1.8-kb insert from plasmid pUC EBNA 2). LMP and EBNA proteins were also unchanged in SVmyc2.3-transfected CB-33 LCLs analysis by protein immunoblot and MAbs (CS 1–4) to LMP and human antisera to EBNA molecules [M. Rowe *et al.*, *J. Gen. Virol.* **68**, 1575 (1987)].
24. R. S. Larson, A. L. Corbi, L. Berman, T. J. Springer, *J. Cell Biol.* **108**, 703 (1989).
25. C. Clayberger *et al.*, *Lancet* **i**, 533 (1987); C. M. Rooney, M. Rowe, L. E. Wallace, A. B. Rickinson, *Nature* **317**, 629 (1985); M. Rowe *et al.*, *EMBO J.* **6**, 2743 (1987); M.-G. Masucci *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4567 (1987); G. Klein, *Cell* **58**, 5 (1989).
26. G. Inghirami and F. Grignani, in preparation. The ability of *myc*-transformed LCLs to elicit helper or cytotoxic recognition (or both) by autologous T cells in vitro was tested in mixed lymphocyte cultures and cytotoxicity assays of Cr release. The results indicate that c-*myc*–transformed LCLs are impaired in their ability to stimulate T cell proliferative responses and are resistant to T cell–mediated cytotoxic lysis.
27. L. C. Plantefaber and R. O. Hynes, *Cell* **56**, 281 (1989).
28. F. G. Giancotti and E. Ruoslahti, *ibid.* **60**, 849 (1990).
29. E. R. Fearon *et al.*, *Science* **247**, 49 (1990).
30. F. Grignani *et al.*, unpublished observations.
31. R. Akeson and R. Bernards, *Mol. Cell. Biol.* **10**, 2012 (1990).
32. P. Casali, G. Inghirami, M. Nakamura, T. F. Davies, A. L. Notkins, *Science* **234**, 476 (1986); G. Inghirami, M. Nakamura, J. E. Balow, A. L. Notkins, P. Casali, *J. Virol.* **62**, 2453 (1988).
33. F. Toneguzzo, A. C. Hayday, A. Keating, *Mol. Cell. Biol.* **6**, 703 (1986).
34. G. Inghirami *et al.*, *Blood* **72**, 1431 (1988).
35. D. L. Bentley and M. Groudine, *Nature* **321**, 702 (1986); E. Cesarman, R. Dalla-Favera, D. Bentley, M. Groudine, *Science* **238**, 1272 (1987).
36. K. Kurzinger *et al.*, *J. Immunol.* **127**, 596 (1981).
37. We wish to thank T. Springer for the gift of antibodies to LFA-1 and LFA-3 and LFA-1 probes, A. Krensky for antibodies to α_L, E. Kieft for the LMP and EBNA-2 probes, W. Lee for SpMoMT-1myc2.3, and P. Casali for advice during the development of the study and the preparation of the manuscript. Supported by National Institutes of Health grants CA 37165 and CA 37295 (to R.D.F.) and CA 48236 and EY 06337 (to D.M.K.).

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Table 1. Mutations in *pha-1*. Derivation of alleles is described in (*9*).

Allele	Mutagen	Embryos hatching (%)	Phenotype
e2123	EMS	4*	Strong‡, ts
t1001	EMS	5*	Strong‡, ts
e2468	EMS		Strong‡
e2275	EMS	5*	Strong at 25°C§
e2286	EMS	38*	Weak‖
t1002	EMS	95†	Weak‖
eDf20	X-ray		Weak‖
tDf2	X-ray		Strong‡

*All hatched embryos die as L1 larvae. †A few hatched individuals survive to late larval stages. ‡Pharynx cells are present and enclosed by a basement membrane; they are attached to the gut, but not extending to the tip of the head. No morphogenesis, differentiation, or pumping is visible. Prolonged incubation of mutant embryos beyond the time of normal hatching leads to gradual appearance of some differentiation markers, such as interior cuticle; ts, temperature sensitive. §Though lethal at any temperature, the phenotype of *e2275* is more severe at 25°C. ‖In embryos homozygous for weaker alleles, the pharynx is usually of full length but appears poorly differentiated. Pharyngeal pumping as it occurs in wild-type embryos shortly before hatching is generally not observed.

An Organ-Specific Differentiation Gene, *pha-1*, from *Caenorhabditis elegans*

HEINKE SCHNABEL AND RALF SCHNABEL

Embryonic lethal mutations in the nematode *Caenorhabditis elegans* were generated and screened for phenotypes that suggest regulatory functions in order to identify genes involved in the control of early development. In embryos homozygous for mutations in one such gene, *pha-1*, the pharynx fails to undergo late differentiation and morphogenesis. Early pharynx development is not affected; thus, *pha-1* controls the latter stages of this developmental process. All markers specific for differentiation in various pharyngeal cell types tested are affected, suggesting that *pha-1* acts in an organ-specific, rather than cell type–specific, manner. The temperature-sensitive phases of both temperature-sensitive mutations indicate that *pha-1* function is required solely during midembryogenesis, shortly before the onset of morphogenesis.

IN OUR STUDY OF THE EMBRYONIC development of the nematode *Caenorhabditis elegans*, we have attempted to identify developmental control genes by analyzing the phenotypes caused by embryonic lethal mutations; our experiments are similar to the work of Nüsslein-Volhard and Wieschaus in *Drosophila* (*1*). Here we report on one such developmental control gene, *pha-1*, which is specifically involved in the development of the pharynx, an organ required for the uptake and transport of food.

Shortly before the wild-type *C. elegans* embryo hatches (800 min after first cleavage) (*2, 3*), the pharynx has developed into a functional organ with characteristic morphology, enclosed by a basement membrane (*4*) (Fig. 1A). All pharynx cells, which come from a variety of unrelated lineages, are generated during the first half of embryonic development, the proliferation phase (0 to about 430 min). The pharynx primordium, a group of cells bounded by a basement membrane, becomes visible anterior to the gut toward the end of this phase. Subsequently, the pharynx elongates, attaches to the anterior tip of the embryo where a buccal cavity is formed, develops its characteristic morphology, and begins to pump shortly before hatching at 800 min (*2*). Embryos homozygous for mutations in *pha-1* (Table 1) lack a functional pharynx and instead have a group of cells anterior to the gut surrounded by a basement membrane (Fig. 1B). This structure is similar to that of the pharynx in wild-type embryos at an earlier developmental stage, the 1½-fold stage, just after initiation of morphogenesis (430 min), suggesting that pharyngeal development has arrested at this stage. The normal number, 80, of pharyngeal cells appears to be generated in mutant embryos as we counted 80 ± 4 nuclei (±SD, $n = 25$) by Nomarski microscopy in *pha-1(e2123)* embryos and 79 nuclei in a *pha-1(t1001)* embryo optically sectioned with a confocal microscope.

The pharynx of *C. elegans* contains five different cell types: muscle cells, marginal cells, epithelial cells, glands, and nerves. To determine whether the effect of *pha-1* mutations was limited to any single cell type, we stained mutant embryos with monoclonal antibodies (MAbs) to markers expressed in three of the five pharyngeal cell types. Antibody 3NB12 (Fig. 2A) recognizes an epitope expressed early (at about 400 min) in pharyngeal muscle cells, several hours before pharyngeal myosin is expressed (*5*). The staining pattern in terminal-stage *pha-1* embryos was similar to that in wild-type embryos of the 1½-fold stage (430 min) indicating, as suggested by the morphology observed in Nomarski microscopy (Fig. 1), that pharynx development in mutant embryos ceases at the 1½-fold stage of embryonic development. Furthermore, three late pharyngeal markers normally expressed during terminal differentiation—pharynx-specific myosin C, intermediate filaments, and a component of gland cells—were not observed in most *pha-1(e2123)* embryos (Fig. 2, B through D). Although we could not

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test the remaining two pharyngeal cell types with specific antibodies, these results together with the cessation in pharynx morphogenesis suggest that *pha-1* function is required not only for the differentiation of one specific cell type but also for all cells in the pharynx. We tested structures outside of the pharynx by MAb staining and found that the H-shaped excretory cell (Fig. 2C), certain nerve-associated cells in the head (Fig. 2D), neurons expressing GABA (γ-amino-*n*-butyric acid), and body wall muscle develop normally in *pha-1* embryos. Although embryos homozygous for the alleles *e2123*, *t1001*, *e2468*, and *e2275* are often shorter than wild-type embryos and have epidermal lumps, we found no abnormalities in the number and arrangement of hypodermal cells in mutant comma-stage (400 min) embryos. Because the pharynx fills the anterior third of the developing wild-type worm, the effect of *pha-1* on general body shape may be an indirect consequence of abnormal pharynx development.

The phenotypes of the six recessive *pha-1*

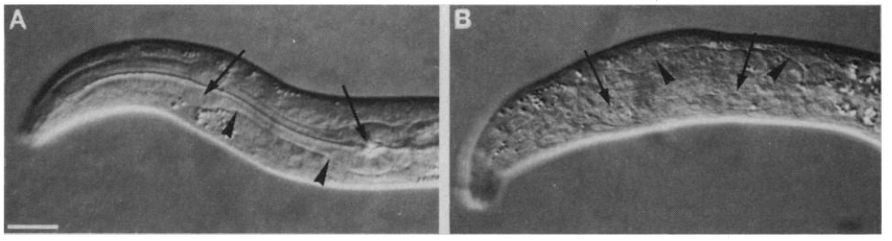

Fig. 1. Nomarski photomicrographs of the head regions of (**A**) a newly hatched wild-type larva and (**B**) a *pha-1(e2123)* embryo raised at 25°C. Arrows point to the pharynx in both animals, arrowheads to the basement membrane surrounding the pharynx. Animals were prepared for Nomarski microscopy and photographed as described by Sulston *et al.* (*2*). For ease of observation, *pha-1* embryos were released from the eggshell. Terminal-stage *pha-1(e2123)* embryos were treated with alkaline hypochlorite solution (1.6% NaOCl, 0.25 M KOH) (*10*) for 5 min, centrifuged for 2 min at 1500 rpm in a Heraeus Minifuge, washed several times in chitinase buffer (10 mM tris-HCl, pH 7; 120 mM NaCl), and then incubated with chitinase (Sigma, 1500 units per milliliter) at room temperature for 10 to 15 min. The eggs were sucked up and down through a pulled-out Pasteur pipette until most of the embryos were liberated. The released embryos were washed several times with M9 buffer (*10*) and then mounted for Nomarski microscopy. Bar, 10 μm.

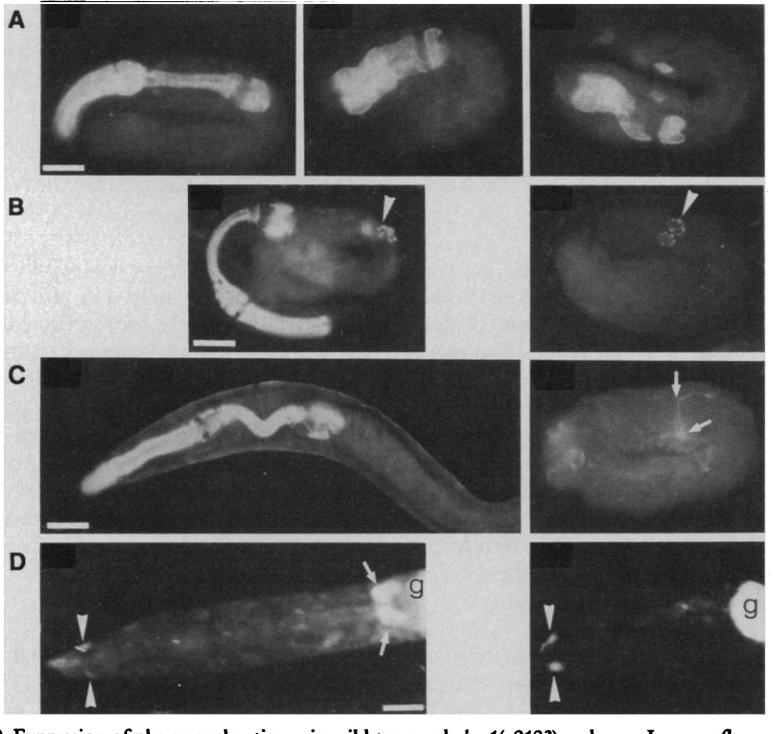

Fig. 2. Expression of pharyngeal antigens in wild-type and *pha-1(e2123)* embryos. Immunofluorescence micrographs of animals stained with various antibodies that recognize specific structures or antigens. (**A**) Pharyngeal muscle cells stained with MAb 3NB12 (*11*), which stains the pharynx of wild-type embryos from early morphogenesis (400 min) on (*5*). Wild-type embryos shortly before hatching (left) and at the 1½-fold stage (center); *pha-1* terminal-stage embryos (right). (**B**) Pharynx-specific myosin in pharyngeal muscles (stained with MAb 9.2.1.) (*12*), and P granules (arrowheads) in the two germline precursor cells as a control for permeabilization of embryos (stained with MAb to P granules) (*13*). (**C**) Staining with MAb to intermediate filaments (*14*) in marginal cells of a wild-type animal (left) and the excretory H cell outside of the pharynx (right), visible only in *pha-1* (arrows). (**D**) Staining with MAb ICB4 (*11*) of pharyngeal glands in the pharynx (left, arrows), visible only in wild type, and of gut (g) and two nerve-associated cells (arrowheads) outside of the pharynx in both wild type (left) and *pha-1* (right). Embryos were fixed and stained as described (*15*). Bar, 10 μm.

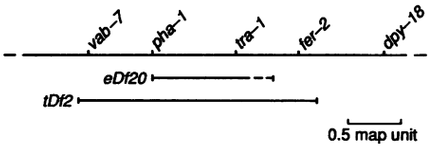

Fig. 3. Genetic map of a section of the right arm of chromosome III. The positions of the genes *pha-1*, *vab-7*, and *tra-1* and the deficiencies *eDf20* and *tDf2* are shown. Standard 2-factor and 3-factor crosses were used to map *pha-1* (*16*). Deficiency *tDf2* was isolated by crossing wild-type males that had been X-irradiated (7500 rad) with *vab-7* (*e1562*) *dpy-18* (*e499*) III; *dpy-7* (*e88*) X hermaphrodites at 20°C and screening the progeny for Vab non-Dpy hermaphrodites.

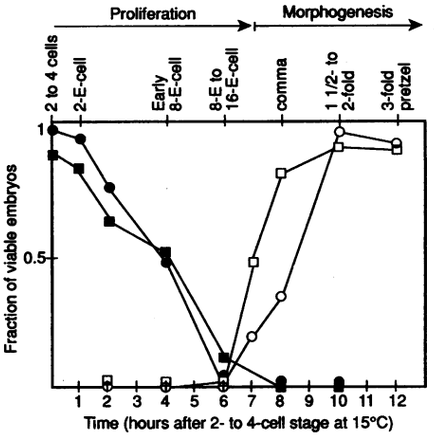

Fig. 4. Temperature-sensitive period of the two temperature-sensitive alleles of *pha-1* (*e2123* and *t1001*); (●) *e2123* down-shift, (○) *e2123* up-shift, (■) *t1001* down-shift, (□) *t1001* up-shift. Stages either refer to the number of E cells (gut precursor cells) in the embryo or are named as in (*2*). Embryos were cut from gravid adults either raised entirely at 15°C for up-shift or raised at 15°C and then at 25°C for 24 to 36 hours for down-shift experiments. Embryos with two or four cells were placed on agar plates kept at the same temperature as that of the adults and either were moved to an incubator at 25°C for up-shift after the indicated times of 15°C, or were moved to an incubator at 15°C for down-shift at times corresponding to half of those indicated, as development at 25°C proceeds at approximately twice the rate of development at 15°C. An average of 32 (10 to 63) embryos was shifted for each data point. Plates were examined for viability. Only those hatched embryos that grew up to adult worms were counted as viable.

mutations described here are probably the result of reduced or abolished function of the *pha-1* gene. In order to identify the phenotype presented by complete absence of *pha-1* (null phenotype), we isolated a deficiency, *tDf2*, which fails to complement not only *pha-1* but also both flanking genes, *vab-7* and *tra-1* (Fig. 3), and therefore has most likely completely lost the *pha-1* locus. The phenotype of homozygous *tDf2* embryos is very similar to that of the strong *pha-1* alleles (Table 1). Thus, complete removal of *pha-1* function results in defective pharynx differentiation. Another deficiency, *eDf20* (formerly *e1855*) (*6*), also fails to complement *pha-1* and *tra-1*. Its phenotype is similar to that of weak *pha-1* alleles, suggesting that some *pha-1* function is still present.

Animals homozygous for the temperature-sensitive *pha-1* alleles *e2123* and *t1001* grow to fertile, wild-type–looking hermaphrodites and males of wild-type morphology when shifted to nonpermissive temperature (25°C) after embryogenesis and hatching at permissive temperature (15°C). Temperature-shift experiments during embryogenesis show that the temperature-sensitive phases of both temperature-sensitive alleles begin during the proliferation phase and end at the 1½-fold stage of embryogenesis (Fig. 4).

The defect in pharynx development observed in *pha-1* mutants indicates that there are at least two steps in embryonic pharynx development. Early pharynx development does not require *pha-1* function since, in mutant embryos, pharyngeal cells are generated, a basement membrane is formed, and an early muscle cell–specific antigen is expressed. Later pharynx development, however, requires *pha-1* function, as expression of later markers in three of five pharyngeal cell types and morphogenesis of the pharynx are blocked in mutant embryos. Thus, cells that have already acquired their identities do not continue to differentiate but require an additional cue. The temperature-shift experiments indicate that *pha-1* only functions during embryogenesis and is not required for the continued expression of pharynx components such as myosin during postembryonic growth. Therefore, *pha-1* appears to be a gene involved in the control of the latter stages of differentiation and morphogenesis of the pharynx.

The identification of an organ-specific gene provides further evidence against the original thought that the *C. elegans* body is largely put together in a piecemeal manner by cell-autonomously determined cell lineages (*2, 5*). To our knowledge, organ-specific lack of differentiation has rarely been observed. The other examples of organ-specific genes that have been described are *cardiac lethal* (7) and *eyeless* (*8*) in the Mexican axolotl and may be involved in inductive interactions, as shown by transplantation experiments. The gene *pha-1* may similarly be involved in an inductive interaction. Alternatively, *pha-1* could be required autonomously in all pharynx cells—for example, to turn on expression of late differentiation functions in the pharynx of *C. elegans*.

REFERENCES AND NOTES

1. C. Nüsslein-Volhard and E. Wieschaus, *Nature* **287**, 795 (1980).
2. J. E. Sulston, E. Schierenberg, J. G. White, J. N. Thomson, *Dev. Biol.* **100**, 64 (1983).
3. All developmental times refer to time after the first cleavage at 20°C, unless otherwise indicated.
4. D. G. Albertson and J. N. Thomson, *Philos. Trans. R. Soc. London Ser. B* **275**, 299 (1976).
5. J. R. Priess and J. N. Thomson, *Cell* **48**, 241 (1987).
6. J. Hodgkin, *Genes Dev.* **1**, 731 (1987).
7. R. R. Humphrey, *Dev. Biol.* **27**, 365 (1972).
8. L. G. Epp, *Am. Zool.* **18**, 267 (1978).
9. A mutation in *pha-1*, *e2123*, was isolated from a screen of 22,000 F2 progeny of mutagenized, wild-type, strain Bristol *C. elegans* for animals that produced viable progeny at 15°C, but nonviable eggs at 25°C. By Nomarski light microscopic observation of terminal stage embryos, *pha-1(e2123)* was identified as a mutation resulting in an undifferentiated pharynx. Further alleles of *pha-1(t1001, e2275, e2286,* and *t1002)* were isolated from crosses of mutagenized wild-type or *tra-1(e1099)* males with hermaphrodites of genotype *pha-1(e2123) dpy-18(e499)* III, or *bli-3(e767) cib-1(e2300)* I; *vab-7(e1562) pha-1(e2123)* III. A total of 25,200 F1 cross progeny were tested for temperature-sensitive embryonic lethality. Allele *pha-1(e2468)* was isolated in an independent screen for embryonic lethal mutations (J. H. Rothman, personal communication). Deficiency *eDf20* was obtained (*6*).
10. J. Sulston and J. Hodgkin, in *The Nematode Caenorhabditis elegans*, W. B. Wood, Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988), pp. 587–606.
11. H. Okamoto and J. N. Thomson, *J. Neurosci.* **5**, 643 (1985).
12. H. F. Epstein, D. M. Miller III, L. A. Gossett, R. M. Hecht, in *Muscle Development*, M. Pearson and H. Epstein, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982), pp. 7–14.
13. S. Strome and W. B. Wood, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1558 (1982).
14. R. M. Pruss *et al.*, *Cell* **27**, 419 (1981).
15. D. Albertson, *Dev. Biol.* **101**, 61 (1984).
16. Map data were submitted to the Caenorhabditis Genetics Center.

17. Part of this work was supported by Otto Hahn fellowships of the Max-Planck-Gesellschaft and by EMBO fellowships. We thank F. Bonhoeffer, C. Nüsslein-Volhard, and the Deutsche Forschungsgemeinschaft for support of our work and T. Bogaert, W. Driever, and J. Sulston for comments on the manuscript. Some strains were provided by the Caenorhabditis Genetic Center.

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Changes in Sodium Channel Gating Produced by Point Mutations in a Cytoplasmic Linker

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Voltage-gated sodium channels are transmembrane proteins of approximately 2000 amino acids and consist of four homologous domains (I through IV). In current topographical models, domains III and IV are linked by a highly conserved cytoplasmic sequence of amino acids. Disruptions of the III-IV linker by cleavage or antibody binding slow inactivation, the depolarization-induced closed state characteristic of sodium channels. This linker might be the positively charged "ball" that is thought to cause inactivation by occluding the open channel. Therefore, groups of two or three contiguous lysines were neutralized or a glutamate was substituted for an arginine in the III-IV linker of type III rat brain sodium channels. In all cases, inactivation occurred more rapidly rather than more slowly, contrary to predictions. Furthermore, activation was delayed in the arginine to glutamate mutation. Hence, the III-IV linker does not simply act as a charged blocker of the channel but instead influences all aspects of sodium channel gating.

THE AMINO ACID SEQUENCES OF many voltage-gated ion channels have been identified, and segments responsible for channel gating and ion conduction have been proposed. A highly conserved segment links domains III and IV of the voltage-dependent Na^+ channel (*1–3*) (Fig. 1A). This linker has a role in Na^+ channel gating, since antibodies against a peptide sequence within this region delay Na^+ current inactivation in skeletal myoballs when applied from the cytoplasmic surface (*4*), and coinjection of mRNAs encoding domains I to III with mRNAs encoding only domain IV of the type II Na^+ channel produces slowly inactivating Na^+ currents in *Xenopus* oocytes (*5*). A physical basis for these findings was originally sug-

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