axons toward their targets. p75 is found not only on axons but also on pathways along which axons grow (*11*), including Schwann cells, where it could sequester and present neurotrophins to growing axons (*12*). The specificity of the lesion could arise from developmental differences between the abnormally innervated pineal and sweat glands and the other, normally innervated, sympathetic targets. The pineal gland and footpads are relatively distant from the ganglia and are not innervated until after birth (*9*, *10*), in contrast to the heart, salivary glands, and iris, which are innervated prenatally (*13*). This may account for the absence of pineal gland innervation in p75-deficient mice, but it is less easy to explain why sweat glands in adjacent footpads are innervated differently. Sympathetic innervation to the hind footpads originates from distinct distal branches of the sciatic nerve (*7*): The medial footpad is innervated predominantly by the medial plantar nerve, whereas the lateral pad is innervated predominantly by the lateral plantar nerve. Thus, successful innervation in p75-deficient mice is correlated with one nerve and unsuccessful innervation with another.

How p75 functions to support axon growth remains unclear. It could guide sympathetic axons. If all sympathetic neurons, however, use similar pathfinding mechanisms to reach their targets, the selective effect of the p75 mutation argues for p75 homologs that are differentially expressed in subsets of neurons, Schwann cells, or targets and that compensate for the lack of p75. Alternatively, p75, by sequestering and presenting NGF, could provide trophic support for neurons as they extend axons to targets. Finally, p75 may increase NGF sensitivity either independently or in concert with trkA (*14*). Consistent with this hypothesis, SCG neuron survival from p75-deficient mice at P3 is normal at saturating levels of NGF, but survival is reduced at subsaturating levels (*15*). If the concentration of NGF produced by Schwann cells and fibroblasts along axonal pathways is limiting, then by sequestering and presenting NGF or by increasing sensitivity to NGF (or both), p75 would contribute to neuronal survival. Accordingly, late-growing neurons with distant targets, such as pineal and sweat glands, would be uniquely affected.

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16. To localize TH, VIP, and p75, we perfused animals with 4% paraformaldehyde (*8*). Tissue was rinsed and equilibrated with 30% sucrose. Cryostat sections were thawed onto coated slides, labeled by incubating overnight with primary antiserum in dilution buffer (0.5 M NaCl, 0.01 M phosphate buffer, 0.01% sodium azide, and 5% bovine serum albumin), and rinsed and incubated in secondary antiserum. The VIP antiserum was generated in guinea pigs by use of porcine VIP. The p75 antiserum was generated in rabbits by use of peptide encoded by the third exon. The TH antiserum was purchased from Pel-Freez (Rogers, AR). All research on experimental animals was conducted according to NIH guidelines and in compliance with Massachusetts Institute of Technology Institutional Animal Care and Use Committee regulations, under protocol 89-054-3.
17. Pineal glands were dissected from P4 and adult mice that were perfused with 2% paraformaldehyde and 2% glutaraldehyde in phosphate buffer. Tissues were postfixed in osmium, stained with uranyl acetate, and embedded in Epon (Polysciences, Warrington, PA). Sections of 1 μm were stained with toluidine blue.
18. We assayed sweating by making a mold of the plantar surface with silicone elastic material (Denture Elasticon, Kerr, Romulus, MI) (*7*). Mice were anesthetized with Avertin (Aldrich, Milwaukee, WI) and injected with the muscarinic agonist pilocarpine (3 mg per kilogram of body weight, intraperitoneally; Sigma). The base material, mixed with hardener, was applied and allowed to polymerize. As the impression material hardens, sweat droplets form pores.
19. To establish co-cultures, we dissected pineal glands from adult mice and SCG from P3 mice. Pineal gland cells and SCG were embedded 5 mm apart in a collagen gel, and the cultures were incubated at 37°C for 15 min. We added 0.5 ml L15-CO_2 of medium (Specialty Media, Lovallette, NJ) supplemented with 5% fetal calf serum (Hyclone, Logan, UT) to each well, and the cultures were incubated at 37°C. Neurite outgrowth was scored 16 hours after medium addition.

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Autonomy and Nonautonomy in Cell Fate Specification of Muscle in the *Caenorhabditis elegans* Embryo: A Reciprocal Induction

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EMS, a blastomere of the *Caenorhabditis elegans* embryo, produces body wall muscle cell-autonomously in isolation. Within the embryonic context, however, the specification of body wall muscle derived from EMS depends on inductive interactions between its daughter MS and ABa descendants that are required to overcome inhibitory interactions with other cells. The inductive events between the MS and ABa descendants are reciprocal, specifying subsequent fates in both lineages. Both induction events are blocked by mutations in the gene *glp-1*, known to encode a Notch-like transmembrane receptor protein.

Several criteria are used in developmental biology to determine the mechanisms by which cells become committed to a fate. If cells in isolation from the embryo do not acquire the fate expected from the fate map, it is assumed that the normal fate of these cells is specified nonautonomously by means of an induction from other cells. If the cells acquire the fate expected from the fate map in isolation from the embryo, they are considered autonomously specified. Classical developmental biology introduced another criterion to distinguish the state of specification of cells. When grafted to ectopic positions in the embryo, only cells that are not reprogrammed to follow a new fate reflecting their new position in the embryo are considered to be terminally determined. The biological meaning of this test, however, remains obscure when the fate of cells can be changed even though they are already restricted to their

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normal fate according to the fate map. In vertebrates, the early specification pathway of most fates requires interactions (*1*). In invertebrates, especially in nematodes, some cell fates appeared to be specified cell-autonomously (*2–6*). As described in this work, the classical criteria used to distinguish the basic mechanisms of fate specification may lead to incorrect conclusions about the pathway by which a fate is acquired. With the example of body wall muscle specification in C. *elegans*, it is shown here that despite an underlying cell-autonomous potential to adopt a certain fate, cells are still subject to interactions occurring in the normal embryonic context.

During the embryonic development of C. *elegans*, 81 body wall muscle cells are produced. When considering a two-cell stage embryo, 80 body wall muscle cells are produced by descendants (EMS, C, and D) of the posterior blastomere P_1 and only one body wall muscle cell is derived from the anterior blastomere AB (*7*) (Fig. 1). If all body wall muscles derived from P_1 are specified autonomously, then the number of muscle cells produced by this blastomere should not be affected by the ablation of the blastomere AB. Embryos in which this blastomere was ablated, however, lack approximately 30 muscle cells (Figs. 2C and 3B). Controls show that the laser ablations per se do not affect neighboring cells unspecifically (Fig. 4B). The experiment therefore indicates that a signal from the AB lineage is required to permit the specification of the full equivalent of muscle cells in P_1. Further ablations show that ABa, the anterior daughter of AB, but not ABp, the posterior daughter, is required for normal muscle development. The ablation of ABa reduces the number of muscles from 81 to approximately 50, whereas the ablation of ABp results in the production of the normal number of muscle cells (Figs. 2E and 3B). The blastomere requiring a signal from the ABa lineage was identified by double ablations. If ABa and the blastomere requiring a signal for body wall muscle specification are ablated, the effect should be the same as if ABa alone was ablated. Thus, MS was identified as the blastomere requiring the signal, because the ablation of ABa and all MS descendants results in the same number of muscle cells (that is, approximately 50) as was observed with the ablation of ABa alone (Fig. 3C). This number of muscle cells corresponds to the sum of the muscle contributions of the blastomeres C (32 cells) and D (20 cells), which suggests that the lineages derived from these blastomeres do not depend on the activation by the ABa lineage. The ablation of ABa descendants at different developmental stages indicates that the activation of the MS blastomere occurs before the onset of gastrulation and starts immediately after MS is born (Fig. 3D).

These findings conflict with earlier experiments that were consistent with the notion that the MS lineage has a cell-autonomous

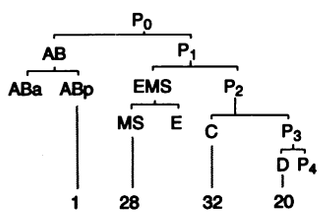

Fig. 1. Lineal descent of the 81 body wall muscle cells produced in the embryo [adapted from (*7*)]. The number of body wall muscle cells produced by the somatic founder cells is indicated below the lineage.

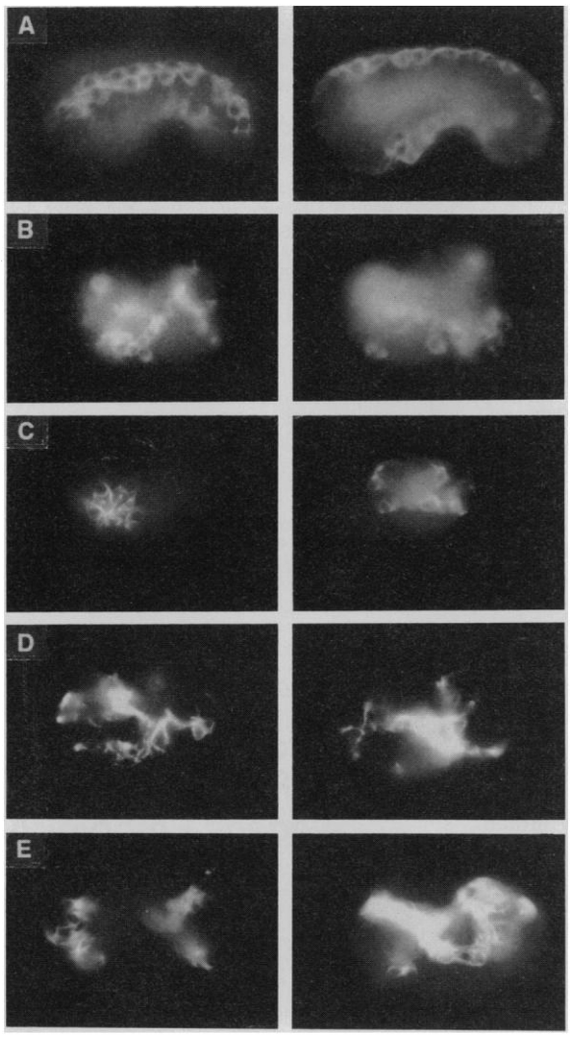

Fig. 2. Immunofluorescence micrographs showing body wall muscle cells in wild-type, *glp-1*, and laser-ablated embryos. All embryos are stained with mAb NE8 4C6.3 (see Table 1). The two panels in each row show (two) different focal planes of each embryo. (**A**) Wild-type embryo at approximately 400 min of development (*7*) shortly after elongation has started. Three of the four muscle rows formed in the embryo are seen. In normal embryos, 81 body wall muscle cells stain. (**B**) *glp-1* (*e2142*) embryo. A total of 55 muscle cells were observed in this embryo. (C to E) Laser-ablated wild-type embryos. (**C**) Partial embryo derived from EMS. All other blastomeres were ablated in this embryo. The number of muscle cells observed in this embryo was 28. (**D**) Embryo with an ablated ABa blastomere. A total of 47 muscle cells were observed in this embryo. (**E**) Embryo with an ablated ABp blastomere. The number of muscle cells observed in this embryo was 78. The antibody brightly stains the cytoplasm of muscle cells at approximately 400 min of development. The unstained nuclei surrounded by the stained cytoplasm can be identified and counted by focusing through the embryo. The eye can resolve regions that are overexposed—for example, those in (D) and (E). Bar, 10 μm. Methods are as described in Fig. 3.

Table 1. *glp-1* affects body wall muscle development. Body wall muscle cells in wild-type embryos and in embryos from *glp-1* mutant mothers (*8*) were counted in the immunofluorescence microscope after staining with two different antibodies. In embryos from the strong alleles *e2142* and *e2144*, approximately 28 body wall muscle cells are missing. The weak mutation *e2141* has no effect on muscle determination (*12*). Mean numbers of counts ± SD are shown. The number of embryos evaluated is shown in parentheses. ND, not determined. Monoclonal antibody (mAb) NE8 4C6.3 (antibody collection MRC LMB, Cambridge) recognizes a structural component of body wall muscles that colocalizes with paramyosin (*14*). Examples of the staining pattern are shown (Fig. 2). Shortly before hatching, the antibody recognizes faintly four muscles, which are not body wall muscles. These muscles are not considered further in this analysis. Polyclonal antibody MH1 recognizes the transcription factor MyoD expressed in all nuclei of body wall muscle cells (*15*). Methods are as given in (*15, 16*). Hermaphrodites were incubated at 25.5°C.

Antibody staining of body wall muscle	Wild-type (81 cells)	*glp-1* (*e2141*)	*glp-1* (*e2142*)	*glp-1* (*e2144*)
NE8 4C6.3	78 ± 3 (9)	78 ± 6 (20)	53 ± 5 (20)	54 ± 10 (20)
MH1 (MyoD)	80 ± 3 (6)	ND	52 ± 2 (21)	ND

potential to form body wall muscles (*3*, *5*). Therefore, a stringent test was reapplied for cell-autonomous development of muscle cells from EMS. The blastomere was isolated in the four-cell stage embryo by ablation of all other blastomeres present at this stage. Differentiated embryos contain 25 ± 3 body wall muscle cells (Figs. 2C and 3A) after this manipulation, showing that EMS has indeed a cell-autonomous potential to form the normal number of body wall muscle cells.

The demonstration that muscle development in the EMS lineage appears to be autonomous when the blastomere is completely isolated, but appears to be nonautonomous within the embryonic context, can only be resolved by making the following assumption. Although EMS has an autonomous potential to produce muscle, during normal development other cells have the potential to suppress muscle formation from this blastomere. Double and triple ablations indeed show that the blastomeres P_2 and ABp, or their descendants, act as inhibitors of muscle development in the EMS lineage (Fig. 3E). Thus, the ABa lineage is required to counteract interactions that inhibit muscle production from EMS.

These experiments were inspired by the lack of approximately 30 body wall muscle cells in embryos from mothers homozygous for strongly mutant alleles of *glp-1* (*8*) (Table 1). A genetic lesion affecting the determination of the AB lineage (*8*, *9*) has, therefore, the same effect on muscle development from EMS as the ablation of the AB lineage (Figs. 2, B and D, and 4A). The ablation of the blastomeres ABa, ABp, or P_2 shows that in *glp-1* embryos only the activation of muscle fate in the EMS lineage does not occur (Fig. 4A2),

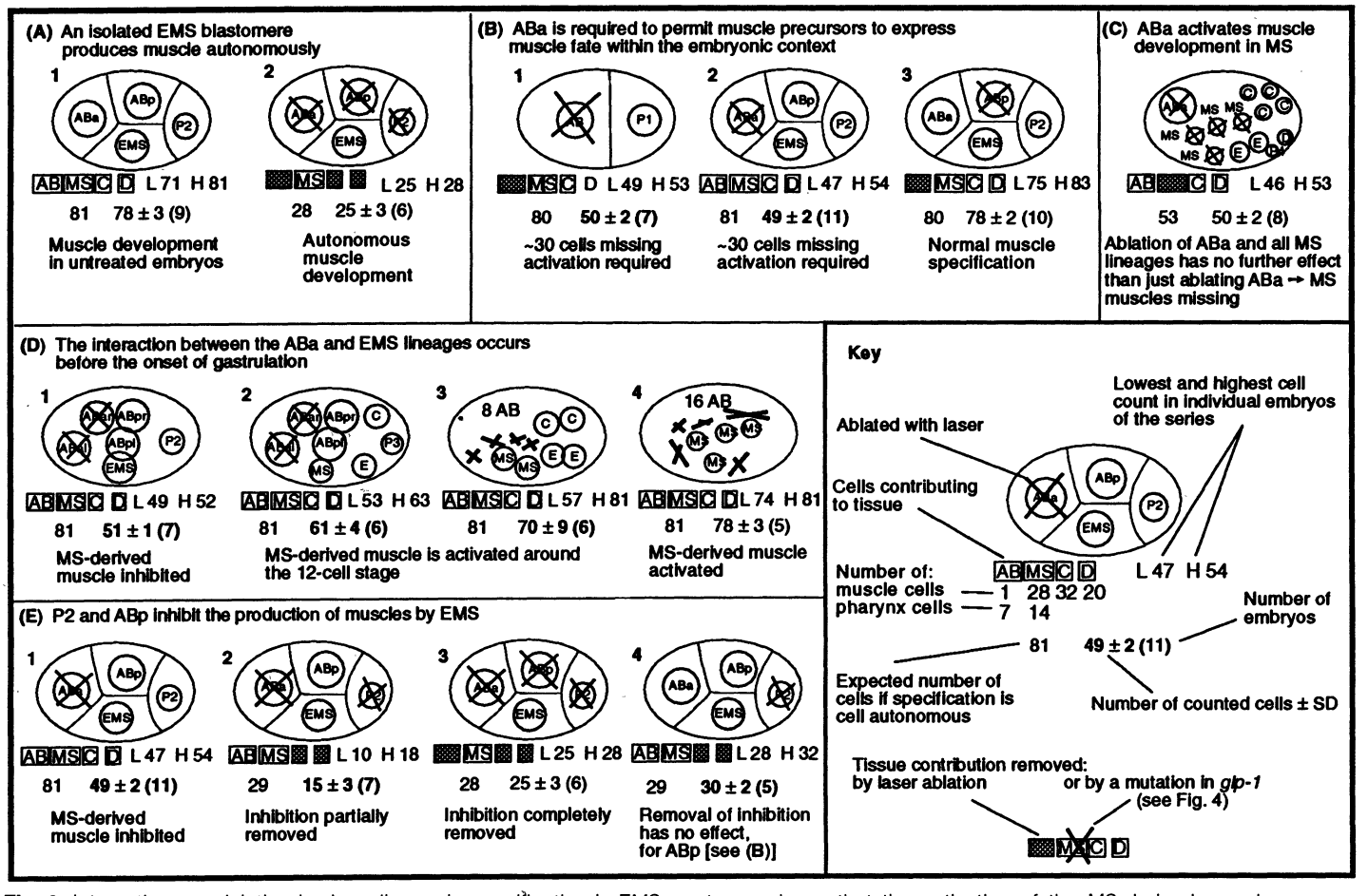

Fig. 3. Interactions modulating body wall muscle specification in EMS (key to figure in lower right panel). (**A1**) Muscle development in untreated embryos. (**A2**) An isolated EMS blastomere produces body wall muscle cell autonomously. (**B**) Within the embryonic context, the EMS lineage [see (C)] requires an activating induction from the ABa lineage. After the ablation of AB (**B1**) or ABa (**B2**), approximately 30 muscle cells are missing. (**B3**) The ablation of ABp does not affect muscle specification in the embryo. (**C**) ABa activates muscle development in MS. Ablation of ABa and the whole MS lineage has no further effect than just ablating ABa; therefore, the MS-derived contribution of the muscle is missing after the ablation of ABa. (**D**) The interaction between the ABa and EMS lineages occurs before the onset of gastrulation. The activation of muscle fate in the EMS lineage occurs only when the MS blastomere, producing the muscles derived from this lineage, is born. (**D1**) Before EMS has divided, the ablation of the two daughters of ABa (ABal and ABar) fully suppresses muscle formation in EMS. (**D2**) If the two blastomeres are ablated after MS is born, a partial activation of the body wall muscle fate occurs. (**D3** and **4**) Ablation of all ABa descendants touching MS descendants at the 8-AB or 16-AB cell stages shows that the activation of the MS-derived muscles occurs before the onset of gastrulation. (**E**) This activation is required to overcome the inhibition of muscle formation by the P_2 and ABp lineages. (**E1**) Ablation of ABa causes the inhibition of muscle production by EMS within the embryonic context. (**E2**) This inhibition is partially relieved by the additional ablation of the P_2 lineage and completely relieved if ABp also is ablated (**E3**). The removal of the inhibitory interactions by the ablation of only P_2 (**E4**) or ABp (B3) has no effect on the specification of body wall muscle in EMS. All muscle counts, which deviate from the numbers expected if muscle specification occurred only cell-autonomously, are printed in bold. Laser ablation of blastomeres and immunostaining of irradiated embryos were carried out essentially as described previously (*16*). Blastomeres were irradiated with 20 laser pulses per second. AB was ablated for 75 to 90 s, ABa or ABp for 60 to 90 s, EMS for approximately 60 s, and P_2 for approximately 45 s. Laser operations of wild-type embryos were carried out at 15.0°C. The irradiated embryos were incubated at 15°C for 10 to 15 hours. Untreated embryos developed normally under these conditions. All embryos were stained with mAb NE8 4C6.3. See Table 1.

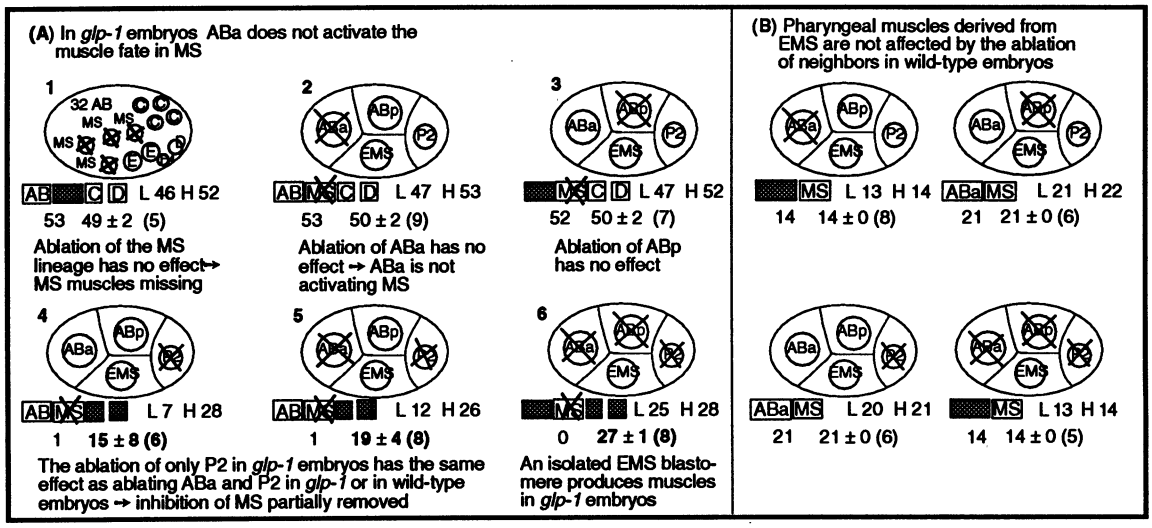

Fig. 4. *glp-1* affects body wall muscle development. (**A**) In embryos from mutant *glp-1* (*e2142*) mothers, ABa does not activate body wall muscle development in EMS. (**A1**) The body wall muscle derived from EMS (MS) is missing in mutant embryos, since the ablation of the MS lineage has no further effect on muscle development in the mutant embryos (Table 1). (**A2**) Ablation of ABa has no effect on muscle specification. The ABa lineage does not activate body wall muscle formation from EMS in the mutant embryos. (**A3**) Ablation of ABp has no effect. As in wild-type embryos, the P_2 lineage alone is sufficient to inhibit muscle formation in EMS (Fig. 3B1). The inhibitory inductions from P_2 and ABp are still present, causing the suppression of muscle fate in the EMS lineage. Ablation of only P_2 in *glp-1* embryos (**A4**) has the same effect as ablating P_2 and ABa in *glp-1* (**A5**) or wild-type embryos (Fig. 3E2). The inhibition of muscle production in EMS is partially removed. (**A6**) Ablation of ABa, ABp, and P_2 shows that an isolated EMS blastomere produces muscles cell-autonomously in mutant *glp-1* embryos. Embryos were stained with mAb NE8 4C6.3. (**B**) Control: Wild-type embryos. The embryos were stained with mAb 3NB12, which recognizes 21 pharyngeal muscle cells, 7 derived from ABa and 14 from EMS. The specification of pharyngeal muscles recognized by the mAb is not affected by the ablation of neighboring blastomeres. This demonstrates that the laser ablations per se do not influence the determination of EMS. As a result of the very pronounced staining of the antibody and the low number of cells, the counting of the number of cells is very exact. Key and methods are as presented in Fig. 3. Embryos from mutant *glp-1* (*e2142*) mothers were irradiated at 25.5°C and then incubated at the same temperature for 7 to 10 hours.

whereas the inhibitory signal from P_2 and ABp (Fig. 4, A4 to A6) or their descendants is still present. This results in the repression of muscle fate in EMS in the mutants. An EMS blastomere isolated from a *glp-1* embryo produces the same number of muscle cells as an EMS blastomere isolated from a wild-type embryo (Fig. 4A). This result excludes the possibility that *glp-1* also has a cell-autonomous function in muscle production by EMS. The muscle defect in *glp-1* embryos is therefore caused by the ablation of an inductive activation signal as a consequence of the mutation.

An intriguing aspect of muscle induction in the blastomere EMS (MS) by the AB lineage is the fact that it occurs at the very same time when the blastomere MS itself induces the left-right asymmetry in the AB lineage (Fig. 3D) (*10*). The inductions are therefore reciprocal, and both depend on the transmembrane receptor encoded by *glp-1*. There are two possible explanations for the reciprocity of the inductions: (i) One of the inductions could specify a state in the target cell (or cells), which in turn enables the reciprocal signal; or (ii) the induction could be truly reciprocal in the sense that the cells exchange inductive signals simultaneously. In the first case, the effect of *glp-1* may be indirect concerning one of the two inductions. In the second case, the gene would be directly involved in both inductions. It could be envisioned that the *glp-1* receptor, located on the AB descendants (*11*), and an unknown receptor located on MS activate each other on contact, and thus the corresponding cell fates are executed. Because there is no delay between the two inductions, I favor the possibility that the inductions are directly reciprocal [see also (*12*)]. Reciprocity of inductions was observed earlier in kidney development in vertebrates (*13*).

The EMS blastomere forms not only body wall but also pharyngeal muscles. The same set of laser ablations, which demonstrated the modulation of body wall muscle development by cell interactions, does not affect the specification of pharyngeal muscles (Fig. 4B). These experiments serve as a control showing that the laser ablations per se have no deleterious effects on the development of EMS.

It is unknown why the autonomous potential of EMS to form body wall muscles is subjected to conflicting cellular interactions. It appears futile to modulate a cell-autonomous potential to form a tissue by counteracting interactions. However, the interaction defined here as inhibitory may serve a different purpose. The activating signal is required to maintain the potential to form body wall muscles in a determinative pathway. The observed phenomenon may thus reflect the evolution of the determinative pathway for muscle specification, which did not necessarily produce the most simple solution. The interactions may reflect the most parsimonious solution for the modification of a network of interdependent pathways. The results shown in this work suggest that a classical criterion for cell autonomy may lead to wrong conclusions about the mechanism and the pathway of specification by which a tissue is formed.

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12. The weak mutation *e2141* (*8*), like the stronger mutations, affects the induction of the left-right asymmetry of the AB lineage, but in contrast to the strong mutations does not affect the activating induction required for body wall muscle development (Table 1). Thus, the induction of the AB lineage is not a prerequisite for the induction of muscles; it appears that the activity of *glp-1* itself is crucial. This is a further indication that the inductions are directly reciprocal.
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