specific conformation to serve as a substrate for a signal peptidase. Since the second function is not required for the first, a secretory protein can be translocated across the cytoplasmic membrane without the cleavage of the signal peptide. Therefore, inhibition of signal peptide cleavage results in the accumulation of a secretory precursor protein, which is attached to the cytoplasmic membrane through the signal peptide. However, if the precursor protein has a strong affinity for the outer membrane, as in the case of the pC_3 mutant prolipoprotein, it may be pulled out of the cytoplasmic membrane and assembled in the outer membrane with the signal peptide uncleaved. Similar results have been observed in a mutant in which the cysteine residue was replaced with a glycine residue (*6*) and in a case in which prolipoprotein accumulated in the presence of globomycin (*13*).

SUMIKO INOUYE
Department of Biochemistry,
State University of New York,
Stony Brook 11794
CHIAN-PIN SHERMAN HSU
KEIICHI ITAKURA
Department of Biology,
City of Hope Research Institute,
Duarte, California 91010
MASAYORI INOUYE
Department of Biochemistry,
State University of New York

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Induction of Neuronal Branching in *Caenorhabditis elegans*

Abstract. *The two postembryonic touch receptor neurons in the nematode* Caenorhabditis elegans *arise from essentially identical cell lineages and have the same ultrastructural features. The cells are found in different positions in the animal, however, and differ in neuronal branching, connectivity, and function. These structural and functional differences are not seen when cells are placed in similar positions by mutation or laser-induced damage. Thus, some, but probably not all, of the differentiated properties of these cells are a consequence of their cellular environment.*

Little is known about how much of neuronal structure is the product of an intrinsic developmental program within a given cell and how much is the result of cellular interactions during maturation (*1*). In this report we examine the consequences of ectopic placement of one set of neurons, the touch receptor cells, in the nematode *Caenorhabditis elegans* on their subsequent development. The touch cells were repositioned by two methods. In the first, advantage was taken of a mutation that affects the migration of one of the touch cell precursors, and in the second the migration of the precursor was blocked by a barrier of laser-produced debris. We found that some, but not all, of the structural features of the cells depended on cell position. Specifically, neuronal branching appeared to be induced only when the cells were in the correct position within the animal, but the length of the neuronal process and ultrastructural features of the process were not affected by cell position.

The development and structure of the *C. elegans* nervous system have been extensively studied over the past few years (*2–10*). As a result of these studies the cell lineage origins (*9*) and structure (*10*) of all of the 302 neurons of the adult hermaphrodite have been described. Almost all of the nerve cells are structurally simple; cells are either monopolar or bipolar and lack extensive arborizations. Most neuronal processes are unbranched, although a few show one or two branches.

The touch receptor cells (the microtubule cells) have a simple branching pattern (*11, 12*) (Fig. 1). Each cell has a single long receptor process that projects anteriorly from the cell body. This process is filled with a unique class of microtubules that give the cells their name. In addition, the receptor process has associated extracellular material (the mantle) that is also characteristic of the cells. These features are probably important for sensory transduction along the entire length of the receptor process (*12, 13*). Near the end of the receptor process in most touch cells is a branch (the synaptic branch) on which a number of synapses are made. There are six microtubule cells in *C. elegans*, but in this paper we will discuss primarily the two ventral cells—the anterior ventral microtubule (AVM) cell and the posterior ventral microtubule (PVM) cell.

The AVM and PVM arise postembryonically from essentially identical cell lineages (*7*). The AVM is produced on the right side of the animal from divisions of cell Q2, and PVM is produced

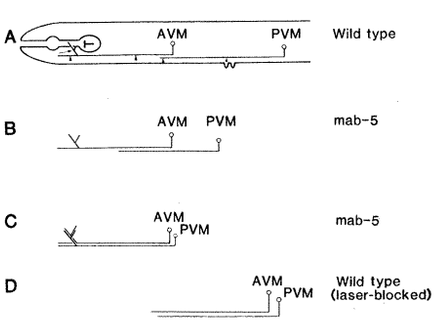

Fig. 1. Positions of AVM and PVM under various conditions. The position of the receptor processes (triangles) and the AVM synaptic branch (arrow) are indicated. Except in wild type (A), the complete structure of the synaptic branches is not known; electron micrographs of serial sections (*2*) of the region of the wild-type synaptic branch have been examined only to the extent of determining whether branching had occurred. The lengths of the receptor processes were estimated from the extent of microtubule-containing processes in electron microscopic sections (a short segment at the end of the process lacks microtubules, but is ignored here). (A) Wild type (*C. elegans* var. Bristol; strain N2). (B) Mutant *mab-5* animals in which PVM is located in the middle of the animals. The *mab-5* stock used in all these experiments (CB3531) contained, in addition to the *mab-5(e1239)III* mutation, a *him-5(e1490)V* mutation (*22*). (The *mab-5* mutation affects male tail structure, and the *him* mutation increases the frequency of males so that this effect is more apparent.) (C) Mutant *mab-5* animals in which PVM is located in the anterior ventral cord. (D) Wild-type animals in which the migration of Q2 (the precursor to AVM) has been blocked by debris produced by laser damage to the hypodermis (*21*). It was difficult to block the migration of Q2 without damaging the cells. The best results were obtained when cell V4 and the adjacent hypodermal nucleus (*7*) were killed in newly hatched larvae.

on the left side from divisions of Q1. Although Q1 and Q2 are initially found in the same position on the two sides of the animal, they migrate in different directions (Fig. 2). Cell Q1 migrates a short distance posteriorly, whereas Q2 and its progeny migrate a considerable distance anteriorly. The difference in position of AVM and PVM in adults results from these different migrations.

Although AVM and PVM share a number of features—for example, both have morphologically identical receptor processes and both show the same cellular defects in touch-insensitive mutants (*12*)—they are not identical cells. Aside from the positions of their cell bodies, the most striking structural difference is that AVM, but not PVM, has a synaptic branch. On this branch AVM makes specific synapses onto interneurons that are required for touch-induced movement and also makes gap junctions to other anterior touch cells [anterior lateral microtubule (ALM) cells] (*14*). Lacking the branch, PVM does not make these connections. A behavioral correlate of these synaptic differences is seen when all the touch cells but AVM or PVM are killed with a laser microbeam (*12*). When only AVM remains, the animals are partially sensitive to touch in the head. Therefore, AVM alone can support a touch response, albeit a weak one. However, PVM alone cannot support a touch response.

Special properties of a pleiotropic mutant, *mab-5(e1239)* (*15*), have allowed us to examine whether the differences between AVM and PVM result from the different locations of the cells. In *mab-5*, the migration of Q2 is normal, but that of Q1 is unusual (Fig. 2). In the mutant, Q1 begins to migrate posteriorly, but then moves anteriorly. The extent of this anterior migration varies from animal to animal, but in all cases PVM resides much further forward than in the wild type, in some cases as anterior as AVM.

We have examined the structure and function of PVM in a number of *mab-5* animals (Fig. 1). Infrequently, PVM was located in the middle of the animal. When it was, it acted much the same as in the wild type; that is, it did not support a touch response when the other touch cells were killed (one animal). Moreover, the PVM receptor process, although it extended farther anteriorly than in the wild type (two animals), did not extend into the region of the nerve ring, the area where AVM normally branches (Fig. 1B). These cells presumably lack a synaptic branch. More often, however, PVM was located in a more anterior position, where it acted like AVM: alone, it would mediate a touch response (three animals). Electron micrographs of these animals reveal that the PVM receptor branch extended through the area of the nerve ring (12 animals) and that the cell grew a synaptic branch (three animals, Figs. 1C and 3). Thus, the more anterior position of PVM resulted in the generation of the synaptic branch and the formation of the specific interneuron synapses required for touch-induced movement.

It is possible that PVM in *mab-5* differentiates like AVM not as a consequence of its altered position but because it has been homoeotically transformed into an AVM-like cell. This possibility seems less likely as a result of a reciprocal experiment in which AVM was produced in an abnormal position in the wild type. The anterior migration of Q2 can be prevented by killing some of the cells in the path of the migration. In these animals, AVM was produced more posteriorly than usual (two animals) and did not support a touch response. Thus, as in the *mab-5* animals, the position of the cells appears to be important for the production of the synaptic branch (*16*).

Although the induction of the synaptic branch requires the correct localization of the touch cells, other properties of the cells do not. Receptor process length appears to be independent of cell position; the length of the PVM receptor process is relatively constant regardless of the position of the cell body in *mab-5* (Fig. 1). [The position-independence of process length of the *C. elegans* neurons contrasts with the marked changes in length seen in grafted wind-sensitive hair neurons in locusts (*17*) and in chimeric retinular cells in cockroaches (*18*).] A similar relationship was also seen for AVM in laser-blocked wild types (Fig. 1D; two animals). Moreover, all the cells contained the microtubules and mantle regardless of position. Therefore, not all

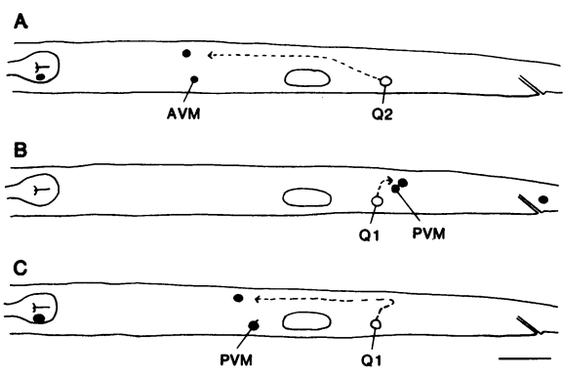

Fig. 2. Migrations of Q1 and Q2 cells in wild-type and *mab-5* mutants. Q1 (the precursor to PVM) and Q2 (the precursor to AVM) are indicated by open circles, and their progeny (three for each) by filled circles. The direction of migration (dashed arrows) was determined by direct observation of living animals with Nomarski differential interference contrast microscopy (*7*). (A) Migration of Q2 in the wild type and *mab-5*; no differences are seen. (B) Migration of Q1 in the wild type. (C) Migration of Q1 in *mab-5*. Q1 in *mab-5* and Q2 in both strains divide in the course of their migrations; the posterior daughters of these cells ultimately give rise to PVM and AVM (*7*). Calibration bar, 50 μm.

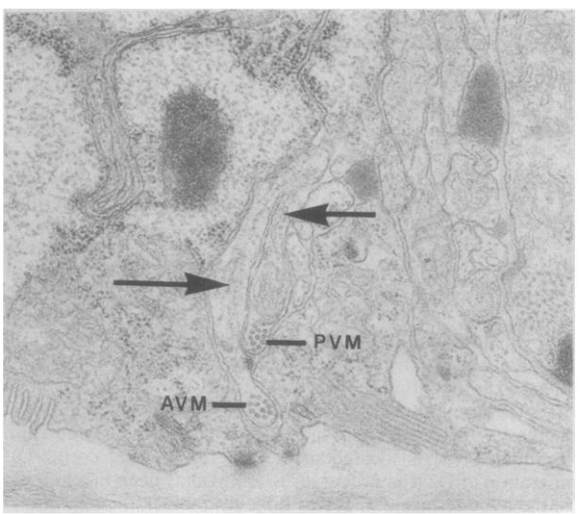

Fig. 3. Electron micrograph showing the beginning of synaptic branches (arrows) from both AVM and PVM in a *mab-5* animal. The receptor processes (labeled with the cell names and containing the bundles of microtubules) are perpendicular to the plane of this transverse section. In this series the synaptic branches were followed through 143 serial sections (approximately 7.1 μm), but neither had branched or ended. Thus, the exact structure of the branch is not known. Although a few chemical synapses are made by cells in various series examined, the identity of the postsynaptic cells has not been determined. The animal was fixed with osmium tetroxide and prepared for electron microscopy (*1*). Magnification, ×30,000.

aspects of the differentiation of these neurons need be the result of cell-cell interactions.

Our results suggest that local cell interactions affect neuronal branching. Indirect support for this hypothesis comes from experiments in insects in which patches of integument have been transplanted. Anderson and Bacon (*19*) found that neurons from transplanted wind-sensitive hairs in locusts projected according to their original location. Similarly, Murphey *et al.* (*20*) found that most neurons from transplanted cerci in crickets produced their normal arborizations despite the unusual position of the cerci. Thus, when neurons and their local environments are transplanted together, neuronal differentiation is essentially unaltered.

Other laser ablation studies in *C. elegans* have shown that disruption of cellular interactions results in a range of effects on neuronal development. For example, the absence of neighboring cells prevents the production of the cell lineage that gives rise to the dopamine-containing postdeirid neurons (*21*). In addition, the ventral cord–precursor P1 will produce a motor neuron (VA1) if the more anterior precursor, cell W, is present, but P1 will generate an interneuron (AVF) by the apparently identical lineage if W is absent (*21*). Thus, cell-cell interactions can influence a range of differentiated characteristics: the production of specific cell lineage patterns, the total structure and function of a single cell, or, as in the case of the touch cells, the substructure of a particular neuron.

MARTIN CHALFIE*
Department of Biological Sciences, Columbia University, New York 10027, and *Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, England*
J. NICHOL THOMSON
JOHN E. SULSTON
Medical Research Council Laboratory of Molecular Biology

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23. This work was done while M.C. held a British-American research fellowship from the American Heart Association and the British Heart Foundation, a research fellowship from the Muscular Dystrophy Association of America, and grants GM30997 and AI19399 from the Public Health Service.
* Send requests for reprints to Department of Biological Services, Columbia University, New York 10027.

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Gamma Interferon Synthesis by Human Thymocytes and T Lymphocytes Inhibited by Cyclosporin A

Abstract. *Cyclosporin A depresses the synthesis of gamma interferon by human thymocytes and T lymphocytes in vitro. This observation is of potential clinical significance because the long-term treatment of transplant patients with cyclosporin A, a widely used immunosuppressive agent, can give rise to B-cell lymphoma resulting from Epstein-Barr virus activation.*

Cyclosporin A (CsA), a fungal metabolite of *Trichoderma polysporum* is a cyclic polypeptide containing 11 amino acids (molecular weight, 1206.6). It is an effective immunosuppressant with low myelotoxicty (*1*) and has been used successfully as the primary drug to suppress the rejection of transplants of nonmatched cadaver kidneys, bone marrow transplants, and liver transplants (*2*). One of the risks of allogeneic organ transplants and long-term treatment with immunosuppressive agents is the development of B-cell lymphomas as a result of the activation of Epstein-Barr virus (EBV)-infected B lymphocytes (*3*). Crawford *et al.* (*4*) provided evidence that lymphocytes treated in vitro with CsA are unable to respond to the challenge of EBV-infected B cells and permit their polyclonal proliferation.

Cyclosporin A prevents the response of T lymphocytes to allogens and certain mitogens (*5*) apparently by binding to receptor sites that are in close association with the receptors for concanavalin A (ConA), phytohemagglutinin (PHA), and the histocompatibility antigen DR (*6*). The synthesis of the T-cell growth factor interleukin 2 and the expression of receptors for interleukin 2 on the cell membrane are inhibited by CsA (*5*).

In view of the potential importance of gamma interferon (γ-IFN) in the immune response, we designed a study to investigate whether CsA also inhibits the synthesis of γ-IFN by human T lymphocytes and thymocytes. Studies carried out in our laboratory have shown that γ-IFN increases T-cell–dependent antibody synthesis by B cells (*7*). We also demonstrated that thymocytes, although not yet immunocompetent, can be induced to synthesize γ-IFN in vitro in cultures containing Con A or PHA and B lymphoblastoid cells. The requirement for B lymphoblastoid cells could be replaced by phorbol myristate acetate (PMA). Neither PMA nor B lymphoblastoid cells alone could induce γ-IFN synthesis by human thymocytes (*8*). This is in contrast to T lymphocytes, which can be induced to synthesize γ-IFN with only one inducing agent (*9*). The effect of CsA on human thymocytes has not, to our knowledge, been reported.

We provide evidence that CsA, in concentrations corresponding to those in the serum of patients treated with CsA, inhibits γ-IFN synthesis in vitro by human thymocytes and T lymphocytes. We investigated the role of CsA on thymocyte and T-lymphocyte functions by determining its effect on γ-IFN synthesis induced by a number of agents in vitro and by assessing its effect on cellular proliferation.

Thymocytes were isolated from sections of thymus obtained in the course of cardiac surgery of infants and young children. Isolated thymocytes were washed in medium and immediately cultured with the appropriate inducing agents (*8*). Mononuclear cells, separated by density centrifugation on a Ficoll-Hypaque gradient (density, 1.077 g/cm^3), were depleted of adherent cells by incubation on a plastic surface for 3 hours