# Cilia in Nematode Sensory Organs

Abstract. *Electron microscopic studies revealed the presence of true cilia in nerve processes connected with sensory organs of a nematode. These structures are important in evaluating the relation between nematodes and the other aschelminths, from which they were separated partially on the basis of the supposed total absence of cilia.*

Examination of ultrathin sections of *Xiphinema index* has led to the discovery of ciliated structures in some sensory organs of this nematode (amphids, somatic, and cephalic papillae). Structures in the nematode intestine have been reported as cilia (*1*), but Wright (*2*) demonstrated that the evidence presented was inadequate and that the observed structures were intestinal microvilli. Hope (*3*) described similar sensory organs in *Thoracostoma californicum* as containing structures that may be modified cilia, but, as he had no evidence for the presence of basal bodies or central fibrils, he did not think his evidence was conclusive.

The observations reported here definitely establish the existence of cilia in nematodes, complete with peripheral and central fibrils and a basal body (Fig. 1). That they have not been discovered before is partially due to the fact that very few detailed histological studies have been made of these sensory organs (*4*).

The nematodes used for this study were reared under greenhouse conditions on roots of grape or fig seedlings. They were separated from the soil by wet-sieving and handpicked from the sievings into tap water. The nematodes were then processed for electron microscopy by transferring them to 15 percent dimethyl sulfoxide (*5*) in distilled water for 2.5 minutes and then fixing in 2 percent $OsO_4$ in physiologic saline for 30 minutes. This procedure allows a good and rapid penetration of the $OsO_4$. The tissue was then treated by the method of Wright and Jones (*6*).

The sensory organs consist in part of dendritic nerve processes having the structure of a cilium. These cilia have a typical basal body with a ninefold symmetry from which central and peripheral fibrils extend into the shaft. The number of these fibrils is variable and several configurations have been observed: $9 + 2$, $9 + 4$, $8 + 2$, and $8 + 4$. The fact that these cilia are not all of the $9 + 2$ type is not incompatible with their ciliary nature, as several instances of irregular and modified cilia have been reported (*7*).

The combination of dendrites and cilia, as we have found in *Xiphinema index*, has been described in Crustacea (*8*), Insecta (*9*), and Ctenophores (*10*). The resemblance between the insect receptors and the sensory papillae in *X. index* is striking. Indeed, in both groups the dendrites have a ciliated part and are enclosed in a vacuolar space with a highly folded wall. As the nematodes have no direct relationship to the arthropods, this similarity must be understood as being the result of common function rather than of common ancestry.

These ciliated structures probably can be compared to the ciliated sensory

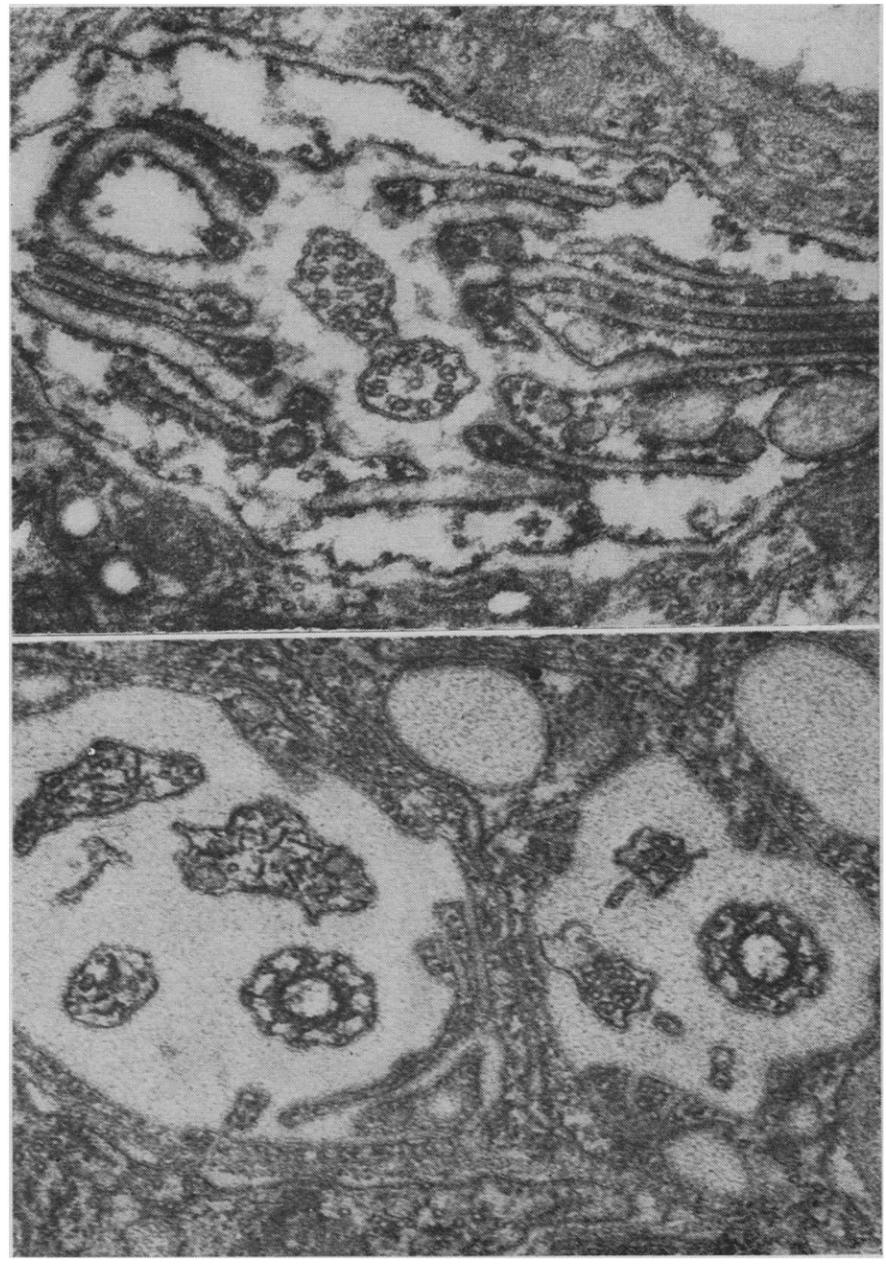

Fig. 1. (Top) Slightly oblique section of a sensory organ, showing two cilia enclosed in a vacuolar space with highly folded wall. The whole structure is surrounded by epidermis (× 75,600). (Bottom) Transverse section through two other sensory organs, showing basal bodies and irregular cilia (× 64,500) (RCA EMU 3 G electron microscope, 50 kv, 50-micron objective aperture; dimethylsulfoxide–osmium tetroxide fixation; finally stained in Watson's lead hydroxide).

organs that are widely distributed in the other classes of the Aschelminthes (*11*), but as long as no electron-microscopic studies of these organs in the other groups are available all comparisons should be considered premature. Nevertheless, the demonstration of true cilia in nematodes lessens the gap that exists between the nematodes and the remainder of the aschelminths.

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**References and Notes**

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## Synthesis of Nonribosomal RNA by Lymphocytes: A Response to Phytohemagglutinin Treatment

Abstract. *When lymphocytes are stimulated to enlarge and divide by treatment with phytohemagglutinin, most of the rapidly synthesized RNA is nonribosomal. The phenomenon is a response by lymphocytes to stimulation by phytohemagglutinin, rather than a general concomitant of lymphocyte growth.*

When phytohemagglutinin (PHA), extracted from kidney beans (*1*), is added to suspensions of human peripheral blood lymphocytes, there occurs a prompt increase in the rate of RNA synthesis (*2*). A period of growth ensues, and during the succeeding 60 to 72 hours most of the cells undergo progressive enlargement and mitosis (*3, 4*). We have reported (*5*) that under these conditions most of the rapidly synthesized RNA in lymphocytes appears to be nonribosomal. This material sediments in a sucrose gradient as a broad band with sedimentation constants from 6*S* to more than 20*S*. Our findings differed from reported observations with other cultured cells, where the largest fraction of rapidly synthesized RNA is a 45*S* precursor which is subsequently converted to ribosomal RNA (*6, 7*). We also observed this fraction in PHA-stimulated lymphocytes (*5*), but it constituted only a minor portion of the pulse-labeling RNA. Our previous studies did not permit us to distinguish between two possible interpretations of our findings: (i) whether the type of RNA metabolic pattern observed was an obligatory property of growing lymphocytes or (ii) whether PHA specifically stimulated a high rate of production of nonribosomal RNA in lymphocytes.

To clarify this problem, we compared the RNA synthetic activity of PHA-stimulated lymphocytes with that of cells exposed to streptolysin O, another substance which provides a mitogenic stimulus to lymphocytes (*4, 8*). Streptolysin O is effective only in lymphocytes from subjects previously exposed to hemolytic streptococci, and it apparently acts as a specific antigenic stimulus to sensitized cells (*4, 8*). Phytohemagglutinin requires no prior exposure, and stimulates a much larger proportion of the lymphocyte population.

We found that stimulation of lymphocytes by a specific antigen resulted in the production of ribosomal precursor as the largest fraction of rapidly synthesized RNA, but confirmed that the major component of pulse-labeling RNA produced by PHA-treated cells is nonribosomal. The pattern of RNA metabolism of lymphocytes treated with growth stimulating agents, therefore, is not fixed, but depends on the stimulating agent used.

Human peripheral lymphocyte suspensions were prepared (*2, 5*) in Eagle's minimum essential medium (spinner modification) containing 10 percent autologous plasma and antibiotics. Phytohemagglutinin P and streptolysin O obtained from Difco were added as growth stimulants to appropriate cultures. Newly synthesized RNA was labeled with uridine-$H^3$, and whole-cell RNA was extracted. The technique for RNA extraction, based on that of Scherrer and Darnell (*6*), has been modified from that previously used (*2, 5*) to provide increased yields and to minimize RNA degradation. Labeled cells were chilled and washed in Earle's salt solution. All steps were carried out at 0° to 4°C, except as noted. The cells were suspended in .01*M* acetate buffer, *p*H 5.1, containing 0.1*M* NaCl, 0.001*M* $MgCl_2$, and 1 percent bentonite and then made 0.34 percent to sodium dodecyl sulfate. The suspension was treated with high-frequency sound for 3 to 5 seconds and then shaken manually for 1 minute. An equal volume of redistilled, buffer-saturated phenol, containing 0.1 percent of 8-hydroxyquinoline, was added; the emulsion was then mixed at 60°C for 5 minutes and rapidly chilled. The emulsion was broken by centrifugation and the aqueous phase was reextracted with hot phenol for 3 minutes. To the final aqueous phase,

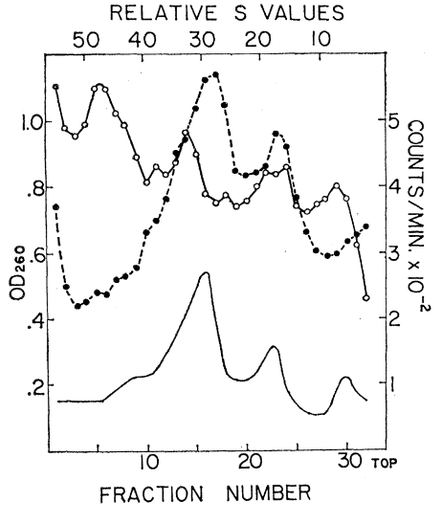


Fig. 1. Effect of treatment with actinomycin D on rapidly synthesized RNA from lymphocytes stimulated with streptolysin O. Parallel cultures, 5 × $10^7$ lymphocytes each, 2 × $10^6$ cells/ml, were incubated for 7 days with streptolysin O (4 ml of standard streptolysin O reagent per culture). Both cultures were incubated with uridine-$H^3$ (10 $\mu$/ml, 4 to 7 c/mmole) for 30 minutes. One culture was then harvested ("pulse"), and actinomycin D (10 $\mu$g/ml) and unlabeled uridine (2 mg) were added to the other. After 1 hour of further incubation this culture was harvested ("chase"). RNA was extracted and sedimented in a sucrose gradient (5 to 20 percent) at 25,000 rev/min for 11 hours in a Spinco SW25 rotor. The gradient was pumped through a recording spectrophotometer to determine ultraviolet absorbancy ($OD_{260}$), and 30 to 35 serial fractions were collected and analyzed for radioactivity in a liquid-scintillation counter. ———, $OD_{260}$; ○———○, count/min for pulse; ●———●, count/min for chase. Relative sedimentation constants were calculated (*13*).