more distally. During this process, the nerve cell bodies appear normal.

An attractive hypothesis to explain the impairment of axonal transport produced by IDPN is a direct toxic effect on some element of the slow transport mechanism. Alternatively, IDPN could produce a postsynthetic alteration in the axonal cytoskeleton, rendering it incapable of transport. Such an alteration could affect neurofilaments primarily, with secondary effects on tubulin and actin transport. In this regard it may be instructive to study the effect of IDPN on slow transport in neurons containing relatively few neurofilaments, asking if transport of tubulin and actin is impaired. Our data can exclude two considerations: first, since slowly transported constituents synthesized before (as well as after) IDPN administration are arrested, the transport defect cannot be due to intrinsic abnormalities of the proteins acquired during their synthesis. Second, since the impairment of slow transport can be detected shortly after injection of IDPN, it cannot be due to preexisting structural pathology of the axon.

The neurofibrillary pathology in IDPN intoxication appears to result directly from the abnormality in neurofilament transport. Focal accumulation of neurofilaments is a prominent pathologic feature of a number of other clinical and experimental disorders. Neurofilamentous swellings in proximal axons, similar to those induced by IDPN, have been found in human amyotrophic lateral sclerosis (*12*) and in hereditary canine spinal muscular atrophy in Brittany spaniels (*13*). Neurofilamentous swellings in the distal axon are found in a variety of "dying back" neuropathies, including a childhood neuropathy in man and the human and experimental toxic neuropathies induced by hexacarbons, acrylamide, and carbon disulfide [for a review, see (*14*)]. While the mechanisms underlying these changes may vary, IDPN intoxication represents the first model in which the pathogenesis of neurofibrillary changes can be reconstructed, and raises the possibility of abnormalities in slow axonal transport in other disorders.

Finally, this pathologic process provides information pertaining to the biology of axonal transport. First, the ultrastructural and biochemical correlation between the accumulation of neurofilaments in the proximal axon and accumulation of the 68,000-, 145,000-, and 200,000-dalton proteins in the same region strongly supports the identification of these proteins as components of neurofilaments. This identification has been based on inference from previous axonal transport studies (*3*) and (in the case of the 68,000-dalton protein) by ultrastructural and immunological approaches (*10*). Second, our observation that IDPN selectively impairs the transport of the neurofilament proteins, as well as actin and tubulin, lends support to the concept that these cytoskeletal proteins share a common transport system that is separate from that of fast and retrograde axonal transport.

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9. Injection of IDPN produced hyperactivity and circling movements within 48 hours, as previously described (*4*, *5*). Administration in the drinking water produced no neurologic abnormalities in the first several months, and weight gain was normal. The IDPN was from lots A6A and B6A. It is clear and colorless; a yellow discoloration develops on storage at room temperature. This change is minimized by storage at 4°C.
10. The association of the 68,000-dalton proteins with neurofilaments has been confirmed by immunologic and electron microscopy studies [W. W. Schlaepfer, *J. Cell Biol.* **74**, 226 (1977); ——— and R. G. Lynch, *ibid.*, p. 241]. Whether the higher molecular weight proteins are integral components of the neurofilament or are neurofilament-associated proteins remains to be determined.
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Temperature Sensitivity: A Cell Character Determined by Obligate Endosymbionts in Amoebas

Abstract. *A strain of* Amoeba proteus *has lost its ability to survive at temperatures above 26°C as a result of becoming dependent on endosymbiotic bacteria that are psychrophile-like. The observed temperature sensitivity develops in fewer than 200 host cell generations (18 months of culture) after the host cells are experimentally infected with the symbionts.*

We previously reported a case of newly established bacterial endosymbiosis in amoebas, where initially harmful bacterial parasites changed to stable symbionts that are now required by the hosts for their survival (*1*). The establishment of the mutually dependent symbiosis took several years during which the harmful effect of infection gradually diminished and the hosts became dependent on the symbionts. The hosts' dependence was first demonstrated by transplanting the nuclei of symbiotic amoebas into enucleated cytoplasm of the nonsymbiotic counterparts (*1*). Such nucleocytoplasmic hybrids were nonviable unless live bacteria obtained from symbiotic amoebas were transferred into the hybrid cells. Later, the hosts' dependence was also demonstrated by removing the symbionts with chloramphenicol (*2*) or trimethoprim (*3*). Symbiotic amoebas died after losing their symbionts at concentrations of these drugs which were harmless to nonsymbiotic amoebas. The strains of symbiotic and

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nonsymbiotic amoebas are referred to as xD and D, respectively (*4*). Stock cultures of these amoebas have been kept at 20°C.

To study the relationship between the growth rate of the amoebas and that of the symbionts, we grew xD amoebas at 13°, 20°, and 27°C. We knew that amoebas multiplied faster at higher temperatures. We grew individual amoebas singly in Syracuse watch glasses to determine their mean generation time, and groups of cells in 60 by 15 mm plastic petri dishes. The amoebas were fed every other day with axenically grown and washed *Tetrahymena* (*5*). To determine the average number of symbionts per amoeba, we lysed 20 amoebas taken from petri dish cultures in 20 μl of 2*N* NaOH after washing the amoebas five times to rid them of any adhering bacteria, and counted the intact bacteria by using a Petroff-Hausser bacteria counter (*2*).

The growth rates of amoebas at different temperatures and the average number of symbionts per amoeba are shown in Fig. 1, A and B, respectively. It is seen that amoebas grown at 27°C divided faster than those grown at lower temperatures during the first 2 days, but they ceased to divide after an average of two divisions and all cytolyzed by the 12th day. At 27°C, the average number of symbionts per amoeba declined rapidly, and the symbionts disappeared completely from all amoebas by the 7th day. The depletion of symbionts appeared to be the cause of death of the amoebas, which closely followed the disappearance of the symbionts. The death of amoebas could not be attributed to the elevated culture temperature alone, since nonsymbiotic amoebas (D strain) kept increasing in number until 2 weeks of culture at 27°C, having divided more than four times on the average (not shown in Fig. 1.)

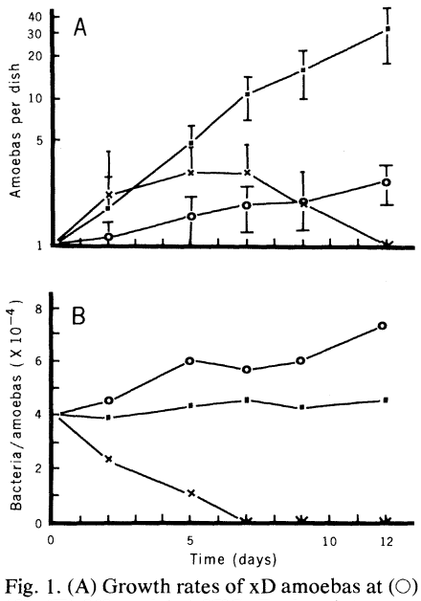

Fig. 1. (A) Growth rates of xD amoebas at (○) 13°, (■) 20°, and (×) 27°C. Each point represents the average (± standard deviation) from 24 cells grown singly in two different experiments. During the 12-day culture period, the mean generation times of cells grown at 13° and 20°C were 8.1 and 2.3 days, respectively. Cells grown at 27°C disappeared completely by the 12th day. (B) Change in the average number of endosymbionts per amoeba grown at three different temperatures (the symbols are the same as in A). Each point represents the average from two different experiments. The average number of endosymbionts per amoeba at 20°C remained 42,500 ± 2,920 during the culture period.

Table 1. Growth pattern of amoebas at 26°C that have been in symbiosis for varying periods of time.

Amoebas	Time in symbiosis	MGT at 26°C* (days)	Cells failing to form clones	
			%	No.†
D	Nonsymbiotic	2.08	5.3	4/76
xD_1	1 week	2.03	4.2	1/24
xD_2	6 months	1.82	18.2	6/33
xD_3	12 months	1.86	34.2	13/38
xD_4	18 months	2.22	90.4	47/52
xD_5	> 10 years	2.47	100	116/116

*Mean generation time (MGT) during the first 10-day culture period. †The numbers represent cells cultured in two to five separate experiments at different times.

To determine the critical temperature at which the symbiotic amoebas lost viability, we grew both D and xD amoebas at several different temperatures between 20° and 27°C. We found 25°C to be the highest temperature at which xD amoebas grew normally. At 26°C, the symbiotic amoebas died out within 2 weeks after having divided more than four times (Fig. 2). All the symbionts disappeared from the amoebas by the 7th day of culture at 26°C, as determined by the usual method. The nonsymbiotic D amoebas continued to multiply at 26°C indefinitely.

It was evident that the inability of the xD amoebas to grow at or above 26°C was due to the loss of their symbionts, whatever the actual mechanism for the disappearance of the symbionts was. Using the loss of viability at 26°C as a criterion for the amoebas' dependence on the symbionts, we attempted to determine how long it took for newly infected amoebas to become dependent on their symbionts. We chose five groups of xD amoebas with different lengths of symbiotic history (Table 1); one group consisted of the progeny of the original xD amoebas cultured continually for more than 10 years, and the other four groups had been experimentally infected at different times by methods described elsewhere (*6*). For each experiment, 12 to 24 amoebas from different groups were cultured singly for up to 3 weeks. The progeny of any amoeba that continued to divide during this period was considered to be forming a viable clone.

It is seen from Table 1 that more than 90 percent of amoebas that had been in symbiosis for 18 months or longer had developed dependence on their newly acquired endosymbionts. Many of the xD amoebas that had been in symbiosis for shorter periods of time (1 week to 12 months) were able to grow even when their symbionts were lost during growth at 26°C.

The reason for the disappearance of the endosymbionts from amoebas at 26°C or higher is not known. Meanwhile, our preliminary electron microscopic studies show that the endosymbionts that are not usually digested by the host amoebas (*1*, *6*) become enclosed in digestive vacuoles and are destroyed when amoebas are grown at 26°C. Their inability to remain viable at the high temperatures suggests that the symbiotic bacteria are like psychrophiles, whose optimum and maximum growth temperatures are below 25°C (*7*). Although the biological basis of psychrophily is not fully known (*7*), it is interesting to note that a prokaryote is more sensitive to elevated temperatures than its eukaryotic partner in the present case of symbiosis.

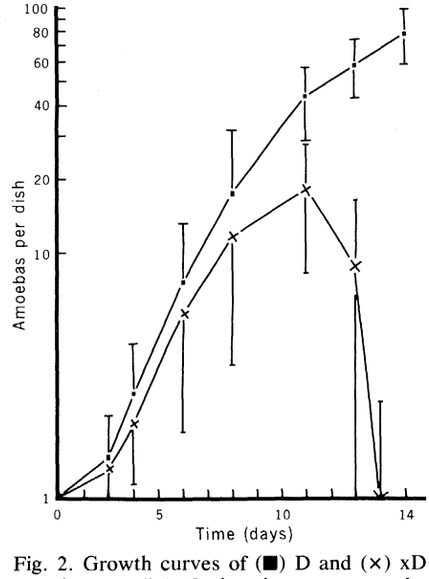

Fig. 2. Growth curves of (■) D and (×) xD amoebas at 26°C. Each point represents the average of 44 and 87 singly grown cells for D and xD amoebas, respectively, in four different experiments. No intact xD amoebas were found at 26°C on the 13th day.

We do not know if the dependence of the symbiotic amoebas on their symbionts involves any genetic interactions between the genomes of the hosts and symbionts. We have attempted to detect plasmid DNA's, which, if present in the symbionts, might play a role in the establishment of stable symbiosis, as by conferring resistance against the amoebas' digestive enzymes to the symbionts. Our recent results obtained by CsCl density-gradient centrifugation and gel electrophoresis have shown the presence of one plasmid group that migrates slower than the bands representing the chromosomal DNA's in 0.7 percent agarose gel (*8*). The molecular weights of these plasmids have not yet been determined.

There are other examples in which intracellular infective agents or symbionts are known to cause changes in cellular phenotypic characters in free-living cells (*9*) as well as in multicellular organisms (*10*). However, the change in a phenotypic trait reported here, the acquisition of temperature sensitivity, is unique in that it is brought about by newly acquired endosymbionts that are indispensable to the host's survival. Furthermore, an apparent integration of the symbionts into the cell has occurred within a relatively short period of time; that is, in fewer than 200 host cell generations. This contrasts with the conventional belief that the establishment of stable symbiosis and the possible integration of symbiotic partners occurs over a very long period of time (*11*). Once they are integrated into the cell as obligate components, such as the required endosymbionts in the dependent amoebas, it is difficult to decide whether such components should be regarded as cell organelles or foreign bodies. A further study of the amoeba-bacteria symbiosis might provide insight into the origin of eukaryotic cell organelles and contribute to our understanding of the mechanism for nonchromosomal inheritance.

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Origin of the Retina from Both Sides of the Embryonic Brain: A Contribution to the Problem of Crossing at the Optic Chiasma

Abstract. *Cells originating from one of the first two blastomeres of the frog embryo were labeled either by injecting them with horseradish peroxidase or by changing the ploidy of one blastomere. Both methods show the labeled cells confined to the same side of the brain as the labeled blastomere except for cells that have moved from the opposite side into the ventral diencephalon and ventral part of the retina. Reciprocal movement of cells from each side of the prospective forebrain into the prospective retina on the opposite side starts before the neural tube closes and results in the formation of an incipient optic chiasma which may provide the pathway for optic axons to grow from the retina to the opposite side of the brain.*

The origin of the optic vesicles as a pair of outgrowths from the sides of the embryonic forebrain has been described in all vertebrate species that have been examined (*1*). Fate maps of the prospective eye region in the neural plate of amphibian embryos also show a bilaterally symmetrical pair of prospective eye-forming zones (*2*), although the two prospective eye-forming zones appear to be connected across the midline in the newt (*3*). Experiments in which cyclopia resulted from the action of various agents on the embryos, apparently because of failure of segregation of a single median eye rudiment, have provided indirect evidence that eyes may arise from a single median anlage from which cells segregate to form the two optic primordia during the closure of the neural tube (*4*). We now provide what we believe to be the first direct evidence showing that each neural retina originates from both sides of the prospective forebrain in the frog.

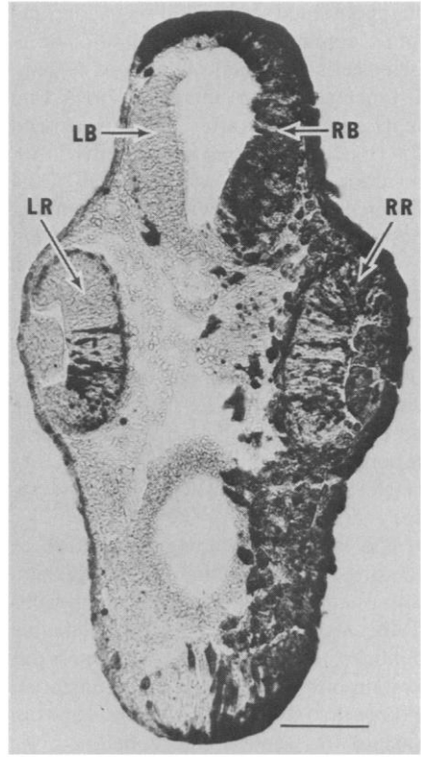

Fig. 1. Coronal section through *Xenopus* at stage 37 showing the localization of the dark reaction product for horseradish peroxidase, a label which was injected into the right blastomere at the two-cell stage. The label is seen in the right side of the brain (*RB*), right retina (*RR*), and ventral half of the left retina (*LR*), while the dorsal half of the left retina is unlabeled. Note the absence of label from the left side of the brain (*LB*), and the reduced labeling of the ventral half of the right retina. The bar is 100 μm.

Two methods of marking cells were used: by inducing a change in ploidy as a result of temperature shock to the fertilized egg, and by injecting horseradish peroxidase (HRP) into one blastomere at the two-cell stage.

Eggs were expressed from mature female *Xenopus laevis* and immediately fertilized in vitro with fresh macerated testes. Twenty minutes after fertilization, the eggs were heated to a temperature of 35.5°C for 6 minutes and then quickly cooled to 20°C. Similar treatment has been shown to result in heteroploidy and, in a small percentage of cases, to produce chromosome mosaics in which different parts of the animal are composed of cells with different chromosome numbers (*5*, *6*). Eggs were placed in individual petri dishes in a 5 percent Steinberg solution. The cleavage pattern was observed and recorded for each embryo to the 16-cell stage. At the neurula stage, embryos were selected in which

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