GENETIC STUDIES OF CHEMOTAXIS MUTANTS IN NEMATODES [4]

The precision of neuronal development is programmed genetically. The genes involved must be expressed in an orderly sequence so that their products appear in the right cell at the right time. By studying mutants in which this sequence is altered, it should be possible to dissect the development and recognize the steps controlled by individual genes.

The choice of experimental organism is crucial for using mutants to study neural development. Neuronal mutants have been obtained in many organisms ; the best characterized are the cerebellar mutants in mice. However, organisms with smaller genomes and simpler nervous systems than the mouse have substantial experimental advantages. Some of the essential mechanisms for genetic control of neuronal development must have been established early enough in evolution to be revealed by the study of simple organisms-in the same way that basic features of gene structure and expression were revealed by the study of bacteria and viruses.

One such organism is the soil nematode *Caenorhabditis elegans:* it is easy to culture, it has only a few thousand genes, it has a generation time of 3.5 days, it reproduces hemaphroditically, and it has a nervous system of only 300 neurons. The organism was selected by Sydney Brenner ten years ago as an experimental system for applying the tools of molecular genetics to study the nervous system. Since then more than 1500 mutants have been isolated and characterized genetically (11, 12).

Ward has carefully investigated the nematode's chemotactic behavior. The nematode is attracted by at least four different classes of attractants cyclic nucleotides, anions, cations, and hydroxyl ions (78). It is repelled by acid, aromatic compounds, and bicarbonate (26). The response to gradients of attractants includes orientation up the gradient, accumulation at the peak of the gradient, and then habituation. Several classes of mutants have been obtained which fail to respond to one or more of the attractants. The behavior of these mutants is normal in all respects except for the absence of a chemotactic response.

[4] This section is based on the presentation by Dr. Samuel Ward.

The analysis of mutants was undertaken by electron microscopy. In order to recognize lesions caused by the mutations, it was first necessary to reconstruct the neuroanatomy of the wild type from serial transverse sections. The anatomy was found to be highly reproducible among several wild type animals examined, thus permitting the detection of small alterations in mutants (79). In the central nervous system, similar cells were connected in each animal examined but the exact number of synapses varied slightly.

The anatomical analysis of mutants has concentrated on the sensory endings because these are located at the tip of the head and their favorable geometry makes reconstruction relatively easy.

Approximately 15 sensory mutants have been analyzed and anatomical alterations were found in more than half of these (41, 80). Some sensory mutants were found with a single aberrant neuron. Others were found defective in the terminals of 11 neurons. The defects were reproducible in several organisms of identical genotype, and similar defects were found among different mutant alleles of the same gene. The correlation of anatomical and behavioral defects is not simple, but all the nonchemotactic mutants were defective in some neurons which terminate in the presumed chemoreceptive sensilla. The observations that most mutants are altered anatomically establishes C. *elegans* as a useful organism for studying how genes program neural development.

Future work will involve analysis of development of these neurons in wild type and mutants ; and development of methods for identifying molecular lesions in mutants, both biochemically and immunologically. The difficulties of such work are related to the difficulty of isolating nervous tissue and the inherent difficulty of determining that the observed chemotactic mutants are primarily mutations of the nerve cell. The advantages of such a system are the ease of genetic and anatomic analysis and the presence of a large number of mutants, many of which have already been anatomically defined. It is hoped that a concentrated study of mutants in the simple organism may result in a better understanding of how genes can program the development of a more complex nervous system.