

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00009-19 LBG

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cell Recognition and Synapse Formation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Biochemical Genetics

SECTION

Section on Molecular Biology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:

6.5

PROFESSIONAL:

5.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

A. NK-2 Homeobox Gene. The NK-2 homeobox gene is expressed in nuclei in the ventral half of the ventrolateral neurogenic anlage very early in the development of part of the CNS of *Drosophila*. The distribution of NK-2 RNA in various mutants was determined to identify genes that regulate NK-2 expression. Four genes were found that encode DNA binding proteins that regulate NK-2 gene expression. In addition, the 5'-flanking region of the NK-2 gene was shown to contain many binding sites for NK-2 protein, which suggests that NK-2 protein may be required to maintain NK-2 gene expression. These results suggest that the NK-2 gene receives and integrates information from the ventral-dorsal and anterior-posterior gradients of gene regulators to generate a pattern of clusters of neuroectodermal cells that synthesize NK-2 RNA and are precursors of different types of neuroblasts. The NK-2 homeodomain was shown by NMR to have a novel secondary structure.

B. Gene Expression in the Developing Nervous System. A *Drosophila* gene was found that encodes a novel zinc finger protein that is restricted to the CNS. Homozygous P-element insertions are lethal and are accompanied by massive morphological defects in the ventral nerve cord. Another cDNA clone was identified as a DNA binding protein which is widely distributed during early embryonic development, but is expressed exclusively in the nervous system during later embryonic development. Another cDNA clone was found that corresponds to a *Drosophila* gene that encodes a novel member of the kinesin heavy chain gene family. Other cDNAs were found that correspond to genes that encode novel proteins that are specifically expressed in the developing nervous system.

CELL RECOGNITION AND SYNAPSE FORMATION
PROJECT DESCRIPTION
MAJOR FINDINGS

NK-2 Homeobox Gene. The first known step in the zygotic development of a considerable portion of the Drosophila CNS is the expression of the NK-2 gene. During the past year, proteins that regulate the expression of the NK-2 gene were identified by determining the expression of the NK-2 gene in embryos with mutations in various genes. The results show that the NK-2 gene is activated in the ventral half of the embryo, presumably by dorsal protein, which is distributed in nuclei in a ventral-dorsal concentration gradient. The NK-2 gene is activated but not expressed in the most ventral horizontal stripe of nuclei, the mesodermal anlage, due to repression by snail, a zinc finger protein, or in the adjacent horizontal stripe of nuclei, the mesectodermal anlage, due to repression by single-minded and Enhancer of split m8, which are basic, helix-loop-helix proteins. However, the NK-2 gene is expressed by nuclei in the ventral half of the ventrolateral neurogenic anlage early in Drosophila embryonic development as the nuclei undergo commitment to the neuroblast pathway of differentiation, or soon thereafter. Initially, the NK-2 gene is expressed fairly uniformly in a horizontal stripe of nuclei about 7 nuclei in width on each side that extends over 90% of the length of the embryo. During gastrulation, the horizontal stripe of cells expressing NK-2 is converted to 12 vertical stripes by repression of the NK-2 gene in some cells. Later, 26 clusters of cells that express the NK-2 gene are formed on each side, presumably by repression of the NK-2 gene in additional cells. Therefore, 2 clusters of neuroectodermal cells that synthesize NK-2 RNA are formed per hemisegment that are the precursors of many neuroblasts in the ventral nerve cord.

Twenty high-affinity and 13 low-affinity NK-2 binding sites were found in 2.2 kb of DNA from the 5' -upstream region of the NK-2 gene, which suggests that NK-2 protein may be required to maintain the expression of the NK-2 gene. Other putative sites for proteins that overlap or are adjacent to the NK-2 protein binding sites were found. The conversion of neuroectodermal cells to neuroblasts is accompanied by activation of the snail gene in the neuroblasts, thereby repressing activation of the NK-2 gene by dorsal protein. The results suggest that the NK-2 gene receives and integrates information from the ventral-dorsal and anterior-posterior gradients of gene regulators, which is needed to generate a pattern of clusters of neuroectodermal cells that synthesize NK-2 RNA that are precursors of different types of neuroblasts.

One of the major goals in neurobiology is to understand how the nervous system is assembled. Studies on the NK-2 homeobox gene led to some novel ideas and to a hypothesis which predicts the overall strategy of the gene program (that is the rules) for the early

development of part of the CNS of Drosophila. Every aspect of the hypothesis can be tested experimentally using the NK-2 gene. With a slight modification the hypothesis also applies to the assembly of part of the mammalian CNS.

Circular dichroism measurements and 1D NMR spectra showed that the t_m for denaturation of the NK-2 homeodomain, NK-2H, is approximately 25°C at pH 4.4 and that denaturation is fully reversible. NK-2H was shown to have relatively little α -helical content. No dramatic change in the CD spectra was observed on addition of an oligodeoxynucleotide with a high-affinity NK-2 binding site. The results show that NK-2H has an unusual homeodomain secondary structure.

Genes Expressed In The Developing Nervous System. Transposition of a P-element that contains the β -galactosidase gene from 1 site in the Drosophila genome to another yielded many transgenic fly lines that express β -galactosidase only in the nervous system during embryonic development. The developmental time and location of β -galactosidase expression then is determined by regulatory signals of the genes that contain the inserted P-element DNA. DNA flanking the P-element insertion sites were cloned from 15 of the most interesting transgenic fly lines and corresponding cDNA clones were obtained and were sequenced partially. Clone 393C-2 was shown to encode Drosophila high-mobility-group protein D (HMG-D), a DNA binding protein. A homologous mammalian protein, HMG-1, recognizes DNA conformation rather than nucleotide sequence; HMG-1 binds to cruciform DNA and to DNA with axial distortion due to cisplatin. The functions of HMG-1 and HMG-D proteins have not been identified; however, the proteins are thought to play a role in chromatin structure. Also the HMG domain has been found in many DNA binding proteins that regulate transcription. We find that the HMG-D gene is expressed ubiquitously during early embryonic development but later in development is expressed exclusively in the nervous system. The homozygous P-element insertion is a lethal mutation and is accompanied by striking morphologic defects in the central nervous system.

Clone 367C-3 DNA corresponds to a novel gene that encodes a zinc finger protein that is expressed in the CNS and anterior sensory organs. The homozygous P-element insertion is a lethal mutation that results in extraordinary morphologic defects in the ventral nerve cord of developing embryos. Clone 7D3C-1 corresponds to a novel Drosophila gene that encodes a member of the kinesin heavy chain gene family. Kinesin functions as a molecular motor for axonal fast transport of organelles or cell membranes on microtubule tracks from soma of neurons towards axon tips. Clone 314-4C-2 encodes a protein that is similar to the human QM protein, an apparent suppressor of Wilm's tumor, a pediatric nephroblastoma. Sequence analysis of other cDNA clones suggest that the cDNAs correspond to novel genes that are expressed in the nervous system.