DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

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PERIOD COVERED

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

Cell Recognition and Synapse Formation

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
14	13	1

CHECK APPROPRIATE BOX(ES)

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

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mouse Hox 1.11 homeobox DNA was cloned and sequenced. Hox 1.11 poly A^+ RNA is expressed in 12 to 14 day-old mouse embryos in the hind brain up to, but not including, the pons. Hox 1.11 poly A^+ RNA also was expressed in the spinal cord, the VIIth and VIIIth cranial ganglia, spinal ganglia, larynx, lungs, vertebrae, sternum, and intestine. A mouse homeobox gene, mNK-1, was cloned and approximately 5.2 kb of DNA was sequenced. Comparison of the amino acid sequences of the mouse and Drosophila NK-1 revealed 95% homology. Hox 4.1 cDNA and genomic DNA were cloned and sequenced; comparison of Hox 4.1 and Hox 2.7 proteins revealed 59% homology. Hox 4.9 also was cloned and partially sequenced. Four Pou-domain genes expressed in embryonic and adult mouse brain were cloned and sequenced; Brain-1, Brain-2, Brain-4, and Scip. Similar amino acid sequences were found in various regions of the proteins. No introns were detected in the coding regions of the 4 Pou-domain genes which suggests that the genes originated by reverse transcription of an ancestral species of Pou-domain mRNA followed by insertion of cDNA molecules into germ cell DNA. The Drosophila homeobox gene, NK-2, is expressed initially by virtually all cells in the ventral neurogenic anlage as the neurogenic anlage appears. The nucleotide sequence of the DNA binding site for the NK-2 homeodomain was determined. Putative genes that are regulated by NK-2 protein were cloned and were sequenced partially. Drosophila genomic DNA corresponding to 20 genes identified in enhancer trap experiments, which for the most part are expressed specifically in the nervous system, were cloned. A nuclear protein was found that binds to a novel trinucleotide repeat sequence in the 5'-upstream region of a rat brain voltage-sensitive calcium channel α1 subunit that activates an enhancerless chloramphenicol acetyltransferase reporter gene. cDNA clones were obtained that encode 3 kinds of DNA binding proteins, each specific for a different novel mouse enhancer sequence were cloned.

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CELL RECOGNITION AND SYNAPSE FORMATION

PROJECT DESCRIPTION

MAJOR FINDINGS

Mouse Homeobox Genes. Homeobox and Pou-domain proteins bind to DNA and regulate gene expression. Hox-1.11 cDNA and genomic DNA were cloned and 5,856 bp were sequenced. The Hox-1.11 gene contains 2 exons separated by a small intron. The Hox-1.11 protein, 372 amino acid residues long, contains a conserved pentapeptide, a homeodomain, and an acidic region. The amino acid sequence of the Hox-1.11 homeodomain is identical to that of Hox-2.8, which suggests that both homeobox proteins may bind to the same or similar nucleotide sequences in DNA. In collaboration with C. Kozak the Hox 1.11 gene was mapped to mouse chromosome 6, which contains the Hox-1 cluster of homeobox genes. One species of Hox-1.11 poly A^+ RNA (1.7 kb) was found that is most abundant in 12-day-old embryos and progressively decreases thereafter. The most anterior expression of Hox-1.11 poly A^+ RNA in 12 to 14-day-old mouse embryos is the hind brain up to, but not including, the pons. Hox-1.11 poly A^+ RNA also is expressed in the spinal cord, the VIIth and VIIIth cranial ganglia, spinal ganglia, larynx, lungs, vertebrae, sternum, and intestine.

In collaboration with Y. Kim genomic DNA and cDNA for a novel mouse homeobox gene, mNK-1, were cloned and approximately 5.2 kb was sequenced. mNK-1 is the mouse equivalent of the Drosophila homeobox gene, NK-1, which we discovered previously. Fifty seven of the 60 amino acid residues of mNK-1 homeodomain are identical to the amino acid residues of the Drosophila NK-1 homeodomain (95% homology), which suggests that mouse and Drosophila homeodomain proteins bind to similar nucleotide sequences in DNA. Both mouse and Drosophila NK-1 proteins contain an acidic domain. mNK-1 poly A^+ RNA was detected in 12 to 18-day-old mouse embryos.

Hox 4.1 cDNA and genomic DNA were cloned and sequenced. The deduced amino acid sequence of Hox 4.1 protein consists of 417 amino acid residues. Hox 4.1 protein contains a conserved pentapeptide and a homeodomain that is similar to that of Hox 2.7 (97% homology). Comparison of Hox 4.1 and Hox 2.7 proteins revealed 59% homology, which suggests that these genes evolved from a common ancestor. Another homeobox gene, Hox 4.9, also was cloned and partially sequenced.

Pou-Domain Genes. The Pou-domain is a conserved amino acid sequence approximately 150 amino acid residues long that contains a Pou-specific domain and a homeodomain; both domains are required for high-affinity binding to DNA. Genomic DNA was cloned for four Pou-domain genes that are expressed in embryonic and adult mouse brain; Brain-1 (10.3 kb sequenced), Brain-2 (4.6 kb sequenced; Brain-2 cDNA also was cloned and sequenced), Brain-4, a novel

Pou-domain gene, (3.2 kb sequenced), and Scip (9.9 kb sequenced). The four proteins have similar Pou-domain amino acid sequences; sequences in other regions of the proteins also are similar, but to a lesser extent. Although the proteins contain between 361 to 495 amino acid residue, no introns were detected in the coding regions of the four Pou-domain genes. These results suggest that the 4 Pou-domain genes arose by duplication of an ancestral Pou-domain gene, which originated by reverse transcription of a molecule of Pou-domain mRNA followed by insertion of the cDNA into germ cell DNA.

Drosophila Homeobox Genes. Previously, we cloned a novel Drosophila homeobox gene, NK-2, which initially is expressed by virtually all cells in the ventral neurogenic anlage early in embryonic development when the ventral neurogenic anlage first appears. The results suggest that expression of the NK-2 gene results in cell commitment to the neuroblast pathway of differentiation, which is required for the development of a large portion of the CNS. During further development, only 20% of the cells continue to differentiate as neuroblasts; whereas 80% switch to the epidermoblast pathway of development and give rise to the ventrolateral epidermis of the embryo. Concomittantly, NK-2 mRNA levels decrease in some, but not all, neuroblasts resulting in a pattern consisting of clusters of neuroblasts with high levels of NK-2 mRNA surrounded by cells destined to become epidermoblasts with lower levels of NK-2 mRNA. During further development, expression of the NK-2 gene is extinguished in epidermoblasts and isolated precursors of neuroblasts with high levels of NK-2 mRNA can be seen surrounded by epidermoblasts with little or no NK-2 mRNA. It is likely that the positions in the embryo of the clusters of neuroblast precursors with high NK-2 mRNA are selected by a set of transactivating proteins. Then 1 neuroblast precursor per cluster is selected by lateral inhibition mediated by cell·cell interactions. Ten genes have been identified by others that are required for the neuroblast selection process. By defining the mechanisms that regulate NK-2 gene expression in future studies, we hope to understand how the number of neuroblasts and their relative positions are selected in a large part of the CNS.

A peptide that contains the NK-2 homeodomain was synthesized in E. coli and purified to apparent homogeneity. Most of the peptide was given to D. Tsao and J. Ferretti to determine the conformation of the NK-2 homeodomain in solution by nuclear magnetic resonance spectroscopy. The NK-2 homeodomain peptide also was covalently coupled to Sepharose and synthetic double-stranded oligodeoxynucleotides that contain random sequences in the middle were passed through the column under conditions that promote NK-2 homeodomain·DNA binding. Oligodeoxynucleotides with sequences that bind to the NK-2 homeodomain were purified by repetitive affinity-column chromatography, cloned, and sequenced, and a consensus binding sequence for the NK-2 homeodomain was defined. Putative NK-2 homeodomain binding sites were identified in the 5'-upstream region of the NK-2 gene, which suggests that NK-2 protein may regulate the expression of the NK-2 gene. Other putative genes regulated by NK-2 protein were purified by passing

Drosophila genomic DNA fragments through the NK-2 homeodomain affinity-column. DNA retained by the column was eluted, cloned, and 42 clones of genomic DNA were sequenced partially. Multiple binding sites for the NK-2 homeodomain were found in all sequences examined. Further work is needed to determine whether the cloned DNA fragments correspond to genes that are regulated by the NK-2 homeodomain protein in vivo.

P-Element Transposition. Transgenic lines of Drosophila were generated previously by transposition of a P-element that contains the β-galactosidase gene from one site in the genome to another. Some P-element insertions into genes expressed only in the nervous system were identified as homozygous lethal mutations. P-element insertions into some genes were found that result in gross morphological defects in the developing nervous system. The locations of about 60 genes with P-element insertions were mapped. P-elements with a few kb of adjacent Drosophila genomic DNA were cloned from 20 of the most interesting transgenic fly lines and the cloned Drosophila DNA genomic DNA fragments were used as probes to screen a Drosophila genomic DNA library for larger DNA fragments. Thus far, large genomic DNA clones were obtained that correspond to P-element insertion sites for 5 transgenic fly lines. Additional studies are in progress to characterize the genes that have been cloned that are expressed specifically in the nervous system.

Regulation of a Gene for a Voltage-Sensitive Calcium Channel α-1 Subunit. The efficiency of transynaptic communication between NG108-15 cells and cultured striated muscle cells previously was shown to be regulated by intracellular levels of cAMP or by retinoic acid, which in turn regulate the level of mRNA for a voltage-sensitive calcium channel α-1 subunit required for stimulus-secretion coupling. The 5'-upstream region of the calcium channel gene was cloned and sequenced and a novel trinucleotide repeat sequence was found that is a powerful activator of an enhancerless chloramphenicol acetyltransferase reporter gene. Synthetic oligodeoxynucleotides inserted upstream of the reporter gene in + or - orientations or inserted downstream of the reporter gene activate reporter gene expression. In collaboration with T. Kamp and E. Marban, a nuclear protein was found that binds to the novel enhancer sequence.

Enhancer Selection: Previously we devised a selection method for mouse genomic DNA clones that contain enhancer sequences based on the demonstration that the synthesis of polyoma virus DNA in mouse cells requires viral enhancer sequencers that also are required for the synthesis of mRNA from polyoma genes. An E. coli-mammalian cell shuttle vector was constructed that contains a library of mouse genomic DNA fragments ligated to the vector to replace the deleted polyoma virus enhancer region. In addition, the β-lactamase gene and E. coli origin of replication from pBR322 were inserted in the polyoma coat protein gene. Thus, only plasmids with mouse genomic DNA inserts that contain enhancers that activate plasmid DNA synthesis replicate in mouse cells and are selectively amplified. In collaboration with W. Odenwald, the enhancer selection method was used to clone genes that may be

regulated by mouse homeobox protein, Hox-1.3. Mouse fibroblasts were cotransfected with a mouse genomic DNA library inserted in the polyoma shuttle vector and Hox-1.3 cDNA under the control of constitutive enhancer sequences so that Hox 1.3 protein is synthesized. Two of the DNA clones that were obtained after several rounds of selection were sequenced. One clone contained 22 and the other 44 putative Hox-1.3 binding sites. Insertion of the cloned DNA upstream of a chloramphenicol acetyltransferase reporter gene was found to inhibit the expression of the reporter gene in cells that were cotransfected with the reporter gene construct and Hox-1.3 cDNA. Inhibition of reporter gene expression required the homeobox region of Hox-1.3 cDNA, which suggests that the inhibition of gene expression is dependent on Hox 1.3 homeodomain binding to DNA.

Previously, 3 novel mouse enhancer DNA sequences were found by the enhancer selection method. A cDNA expression library was screened for DNA binding proteins that bind with specificity to a ^{32}P-labeled oligodeoxynucleotide enhancer sequence. cDNA clones were obtained that encode proteins that bind to each enhancer sequence.