DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 0155-01 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.)

Regulation of Gene Expression

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

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LAB/BRANCH

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SECTION

Section on Molecular Biology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.5	7.5	1.0

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.)

A. Mouse Homeobox And POU-Domain Genes. A novel mouse homeobox gene NKx-1, was cloned and approximately 8 kb was sequenced. The deduced amino acid sequence of the NKx-1 homeodomain differs from the Drosophila NK-1 homeodomain by only 3 of 60 amino acid residues. Both NKx-1 and NK-1 proteins contain an acidic region before the homeodomain. Northern analysis revealed one major band of NKx-1 poly A^+ RNA in brain and trace bands in testes and spleen. The abundance of NKx-1 poly A^+ RNA is highest in 10 day mouse embryos and progressively decreases thereafter. In situ hybridization with sections of 14-day mouse embryos revealed NKx-1 RNA in the mesencephalon, myelencephalon, spinal cord, vertebrae, and ribs. Five additional novel mouse homeobox genes were cloned and partially sequenced. A novel mouse POU-domain gene related to Brain-3 POU-domain cDNA was cloned and about 3 kb was sequenced. The expression of Brain-1, Brain-2, Brain-4 and Skip POU-domain genes in the mouse nervous system was determined by in situ hybridization as a function of developmental age.

B. Regulation of a Calcium Channel a-1 Subunit Gene. A nucleotide sequence was found in the 5 -upstream regulatory region of a voltage-sensitive calcium channel α–1 subunit gene that is a powerful activator of an enhancerless chloramphenicol acetyltransferase reporter gene. A cDNA expression library was screened for recombinants that direct the synthesis of proteins that bind to this nucleotide sequence. Seven kinds of clones were found that encode proteins that bind to oligonucleotides with appropriate sequence specificity.

C. Promotor And Enhancer Selection. cDNA clones were obtained that encode proteins that specifically bind to oligonucleotide sequences by a method that selects for gene regulatory sequences. Partial sequences of the cDNA clones were obtained.

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REGULATION OF GENE EXPRESSION
PROJECT DESCRIPTION
MAJOR FINDINGS

Mouse Homeobox And POU-Domain Genes. Approximately 8 kb of a novel mouse homeobox gene, NKx-1, a homolog of the Drosophila NK-1 homeobox gene, was sequenced. The amino acid sequences of the NKx-1 and NK-1 homeodomains differ by only 3 of the 60 amino acid residues present. Both proteins also contain an acidic domain. However, most of the other regions of the protein that have been defined differ markedly. NKx-1 poly A^+ RNA was found to be most abundant in 10-day mouse embryos; the abundance progressively decreases thereafter. Northern analysis of poly A^+ RNA from adults revealed 1 major band of NKx-1 poly A^+ RNA in brain and trace bands in RNA from testes or spleen. The NKx-1 gene is expressed in discrete regions of the 14-day old mouse embryo mesencephalon and myelencephalon and also in spinal cord, vertebrae, and ribs.

A mouse genomic DNA library was screened with oligodeoxynucleotide probes for novel homeobox genes. Seventy-two positive recombinants were cloned. Thus far five novel homeobox genes have been found. Restriction site analysis of the 72 clones revealed additional classes of clones that have not yet been identified. In addition, a novel mouse POU-domain gene related to Brain-3 POU-domain cDNA was cloned and 3 kb was sequenced. Sites of expression of Brain-1, Brain-2, Brain-4, and SKIP POU-domain genes in the mouse nervous system were determined by *in situ* hybridization as a function of mouse embryo developmental age and also were defined in the adult mouse. Hox 4.1 cDNA and genomic DNA were cloned and the complete Hox 4.1 open reading frame was sequenced.

Regulation of a Calcium Channel α-1 Subunit Gene. The α-1 subunit of a voltage-sensitive calcium channel previously was shown to be inducible in NG108-15 cells and the expression of the gene was shown to control the ability of the cells to form synapses with striated muscle cells. The 5′-upstream regulatory region of the calcium channel gene was cloned and sequenced. A nucleotide sequence was found that activates an enhancerless chloramphenicol acetyltransferase reporter gene. A protein was found in NG108-15 nuclei that specifically binds to this sequence. A cDNA expression library in λgt11 was screened for recombinants that direct the synthesis of proteins that bind to the nucleotide sequence and 35 positive clones were obtained. Seven kinds of clones were found that encode proteins that bind to oligonucleotides with appropriate sequence specificity but differ in specificity for double-stranded DNA, or (+) or (-) single-stranded DNA. Further work is needed to determine whether one or more of the proteins regulate the expression of the Ca^{2+} gene channel.

Enhancer and Promotor Selection. During the past year further work has been done on the selective amplification of DNA clones that contain enhancer or promoter nucleotide sequences that activate gene expression. The method is based on the observation that the

synthesis of polyoma virus DNA in mouse cells requires viral enhancer sequences that also are required for the synthesis of mRNA from polyoma genes. Mouse genomic DNA fragments were ligated to polyoma DNA that lack the enhancer region of the virus. The E. coli origin of replication and β-lactamase gene also were inserted in the polyoma coat protein gene. Promoters or enhancers in the mouse genomic DNA inserts that activate plasmid DNA synthesis in mouse cells are able to replicate and hence are selectively amplified; whereas, plasmids that lack functional enhancer sequences do not replicate. Plasmid DNA was harvested from mouse cells that had been transfected and incubated for several days. Recovered DNA then was amplified in E. coli. The selection method is highly effective; some clones were shown to increase in abundance more than 100,000-fold. Fragments of the recovered DNA inserts were shown to bind nuclear protein and activate the expression of an enhancerless chloramphenicol acetyltransferase reporter gene. Previously, cDNA clones were obtained that code for proteins that specifically bind to oligonucleotide sequences that were identified by the oligonucleotide selection method. Partial sequences of some of the cDNA clones were obtained.