DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

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PERIOD COVERED
October 1, 1987 - September 30, 1988

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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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10	8	2

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.)

1. Treatment of NG108-15 neuroblastoma-glioma hybrid cells results in marked increases in the abundance of certain species of RNA. Seventeen cDNA clones corresponding to these species of RNA were obtained and the nucleotide sequences of three clones were determined. DNA clone NG-32 corresponds to mRNA for ATP synthase subunit 6, which is transcribed from the heavy chain of mitochondrial DNA and codes for a protein that is part of the H^+ channel of the ATP-synthase complex. Clone NG-10 DNA corresponds to another mitochondrial RNA of unknown function, which is transcribed from the light chain of mitochondrial DNA and may be involved in the initiation of replication of mitochondrial heavy strand DNA.
2. Eleven cDNA clones were obtained that correspond to mRNA for the α-subunit of the L-type voltage-sensitive calcium channel of rat brain. Analysis of the DNA sequence and the deduced amino acid sequence reveals strong homology between brain and skeletal muscle calcium channel α-subunits. Approximately 75% of rat brain α-subunit amino acid residues that were defined are either identical to the amino acid residues of rabbit skeletal calcium channel α-subunit or are conservative amino acid replacements.
3. Four novel Drosophila homeobox genes NK-1, -2, -3, and -4 were cloned and partial nucleotide sequences were determined. One NK-1 cDNA clone was obtained from a cDNA library prepared from poly A^+ RNA from 3-12 hr Drosophila embryos, but none was detected in the 0 to 3 hr embryo library. Six NK-3 cDNA clones were obtained from a library prepared from Drosophila poly A^+ RNA from 0 - 3 hr embryos and 6 additional clones were obtained from a library prepared from 3 - 12 hr embryo poly A^+ RNA. The exon-intron structure of the NK-1 gene was determined. One of the two introns found resides within the homeobox.

PROJECT DESCRIPTION

MAJOR FINDINGS

Previously, many neuroblastoma and related somatic hybrid cell lines were shown to acquire voltage-sensitive ion channels and other neuronal properties when intracellular cyclic AMP levels were elevated for a number of days. Cells with elevated cAMP acquire new proteins such as the α-subunit of voltage-sensitive calcium channels and other proteins of unknown function. A cDNA library was constructed from poly A^+ RNA prepared from NG108-15 neuroblastoma-glioma hybrid cells that had been treated for 5 days with 1 mM dibutyryl cAMP. The library was screened and 17 cDNA clones were obtained that correspond to species of RNA that are 3 to 40 times more abundant in cells treated with dibutyryl cAMP than in cells cultured without this compound. Each cloned cDNA was used as a probe with Northern blots to determine the number of species of poly A^+ RNA responsive to dibutyryl cAMP and the chain length of each species of RNA. The results suggest that the 17 cDNA clones correspond to species of RNA transcribed from 10 genes. Partial nucleotide sequences of the cDNA inserts from 3 clones were obtained. Clone NG-32 corresponds to mouse mitochondrial mRNA for ATP synthase subunit 6, a mitochondrial gene. This protein is part of the H^+ channel portion of the mitochondrial ATP synthase complex. Treatment of NG108-15 cells with dibutyryl cAMP results in an 8-fold increase in the abundance of mRNA for this protein. The nucleotide sequence of clone NG-10 cDNA was identified as part of the D-loop region of mouse mitochondrial DNA that contains the origin of replication for the heavy strand of DNA. The 5'-terminal nucleotide sequence of some molecules of heavy strand mitochondrial DNA is known to consist of a short segment of RNA that is complimentary to a short light strand miochondrial DNA sequence nearby. Hence, NG-10 cDNA may correspond to an RNA transcript of the light strand of mitochondrial DNA that serves as a primer for the initiation of heavy strand mitochondrial DNA synthesis. Treatment of NG108-15 cells with dibutyryl cAMP results in a 40-fold increase in this species of RNA. These results show that treatment of NG108-15 neuroblastoma-glioma cells with dibutyryl cAMP results in marked increases in the abundance of RNA transcripts from heavy and light strands of mitochondrial DNA. Further work is needed to determine whether cAMP regulates mitochondrial biogenesis or the ability to synthesize ATP.

A λgt11 cDNA library was prepared from rat brain poly A^+ RNA and screened with oligodeoxynucleotide probes that correspond to the α-subunit of L-type voltage-sensitive calcium channels. Eleven positive clones were detected that have cDNA inserts 1.6-5.5 Kb in

length. Nucleotide sequence analysis reveals strong homology as well as differences in the deduced amino acid sequences of the α-subunits of rat brain and rabbit skeletal muscle L-type voltage-sensitive calcium channels.

To detect recombinant DNA clones that correspond to novel homeobox genes a Drosophila genomic DNA library was screened with multiple oligodeoxynucleotide probes, each designed to hybridize to multiple homeobox genes. Five clones that gave positive signals with 2 or more oligodeoxynucleotide probes exhibited specificities that could not be explained on the basis of known nucleotide sequences of Drosophila homeobox genes. Nucleotide sequence analysis of the homeobox regions of 4 clones revealed 4 new homeobox genes (NK-1,2,3,4). Two recombinant clones contained identical DNA inserts, each insert contained 2 new homeobox genes (NK-3 and NK-4). The deduced amino acid sequence of the NK-1 homeobox exhibits the highest homology to the homeobox regions of deformed, zen-2, and zen-1 (75, 72, and 71% homology, respectively). The relative homology of the NK-2 homeobox is as follows: NK-4 > NK-3 > NK-1 = IAB-7. The homology of NK-3 is: NK-2 > labial > NK-4 > NK-1; and NK-4 homology is NK-2 > zen 2 = NK-3 > labial. Genomic DNA fragments from the 4 new homeobox genes were used to screen cDNA libraries prepared from poly A^+ RNA from 0-3 hr Drosophila embryos or from 3 - 12 hr embryos. One NK-1 cDNA clone was obtained from the 3-12 hr embryo cDNA library, but none was detected in the 0-3 hour embryo libary. Comparison of the nucleotide sequences of NK-1 cDNA and genomic DNA clones showed that the NK-1 gene has 3 exons. One of the 2 introns detected resides within the homeobox region.

Significance of the Results

1. The demonstration of dibutyryl cAMP dependent regulation of 2 species of mitochondrial RNA raises questions that can be addressed in future studies; namely, does cAMP regulate mitochondrial biogenesis or the ability to synthesize ATP?

2. Voltage-sensitive calcium channels are known to play a central role in stimulus-secretion coupling and signal transmission both within cells and between cells. The DNA clones that were obtained for the α-subunit of rat brain L-type voltage-sensitive calcium channels can be used as probes to explore the mechanisms that regulate the α-subunit gene. The cloned DNA also can be used to direct the synthesis of the α subunit protein of the dihydropyridine-sensitive calcium channel from brain. Site directed mutagenesis can be used to alter the DNA and explore the relation between calcium channel structure and function.

3. Four novel Drosophila homeobox genes were cloned and partially sequenced. The homeobox family of genes code for proteins that regulate the expression of genes during development and some determine pathways of differention. These genes provide an

experimental system that can be used to define the mechanisms that regulate the expression of these homeobox genes as well as mechanisms for regulation of gene expression by homeobox proteins.

Publications

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2. Fitzpatrick, L.A., Chin, H., Nirenberg, M., and Aurbach G.D.: Antibodies to an α subunit of skeletal muscle calcium channels regulate parathyroid cell secretion. Proc. Natl. Acad. Sci., USA 85, 2115-2119, (1988).

3. Carter, A., Bardin, C., Collins, R., Simons, C., Bray, P., and Spiegel, A.: Reduced expression of multiple forms of Gs-α in pseudohypotarathyroidism type IA. Proc. Natl. Acad. Sci., USA 84, 7266-7269 (1987).

4. Brown, D.A., and Higashida, H.: Voltage- and calcium-activated potassium currents in mouse neuroblastoma x rat glioma hybrid cells. J. of Physiology 397: 149-165 (1988).

5. Brown, D.A. and Higashida, H.: Membrane current responses of NG108-15 mouse neuroblastoma x rat glioma hybrid cells to bradykinin. J. of Physiology 397: 167-184 (1988).

6. Brown, D.A. and Higashida, H.: Inositol 1,4,5-trisphosphate and diacylglycerol mimic bradykinin effects on mouse neuroblastoma x rat glioma hybrid cells. J. of Physiology 397: 185-207 (1988).

7. Higashida, H.: Acetylcholine release by bradykinin, inositol 1,4,5-trisphosphate and phorbol dibutyrate in rodent neuroblastoma cells. J. of Physiology 397: 209-222 (1988).

8. Trisler, D., and Collins, F.: Corresponding spatial gradients of TOP molecules in the developing retina and optic tectum. Science 237: 1208-1209 (1987).