DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

SECTION OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

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Cell Recognition and Synapse Formation

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SECTION

Section of Molecular Biology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
11	9	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.)

λgt11 cDNA libraries derived from human brain poly A^+ RNA were screened for recombinants that code for α-subunits of G signal transduction proteins. Eleven α_s and two α_i clones were characterized. Four species of α_s cDNA were found. A mechanism for generating the four species of α_s mRNA by alternative splicing of precursor RNA was proposed.

Treatment of NG108-15 neuroblastoma-glioma hybrid cells cAMP for several days results in thee appearance of voltage-sensitive calcium channels and other ions channels. Twenty cDNA clones were obtained that hybridize to species of poly A^+ RNA that increase in abundance 10-90 fold due to treatment of cells with dibutyryl cAMP. Affinity-purified antibodies to the α or γ protein subunits of voltage-sensitive calcium channels were used to screen a λgt11 cDNA library. Twenty putative voltage-sensitive calcium channel α subunit cDNA clones and 29 putative γ subunit clones were found.

Antigenic molecules termed TOP, which are distributed in a dorsal > ventral concentration gradient in chicken retina, were purified. TOP was shown to be a protein with an M_r of 47,000. The gradient of TOP in the retina is formed early in embryonic development. Thereafter, perpetuation of the gradient does not depend on the continuous presence of an extracellular gradient of diffusable molecules or on maintenance of interactions between cells. Synapses and neurites in the retina of developing chick embryos were reduced markedly by injection of anti-TOP antibody into the eye.

Project Description:

Objectives:

Our objective is to discover basic mechanisms that regulate the expression of genes.

Major Findings:

Two λgt11 cDNA libraries from human brain were screened with 3 oligodeoxynucleotide probes for recombinants coding for α subunits of G signal transducing proteins, which couple receptors activated by hormones or light to effectors such as adenylate cyclase or cGMP phosphodiesterase. Fourteen of the 575,000 recombinant clones screened from a human basal ganglia cDNA library and 12 of the 400,000 clones screened from a human cerebral cortex library were detected with 2 or 3 of the ^{32}P-probes used. DNA inserts from 13 positive clones were sequenced partially; 11 clones were identified as α_s cDNA and 2 clones as α_i. The DNA insert from one of the α_s clones was sequenced completely and additional partial sequences were obtained for 10 α_s clones. Four species of α_s cDNA were found that differ in nucleotide sequence in the region that corresponds to α_s amino acid residues 71-88. The clones differ in the codon for α_s amino acid residue 71 (glutamic acid vs. aspartic acid), the presence or absence of codons for the next 15 amino acid residues, and the presence or absence of an adjacent serine residue. A mechanism was proposed for generating 4 species of α_s mRNA by alternative splicing of precursor RNA transcribed from a single gene.

cDNA from one of the two human α_i clones was sequenced completely (BG-4), and a partial sequence was obtained for the second clone. The first nucleotide residue of BG-4 α_i cDNA corresponds to the 14th residue of the bovine α_i coding sequence and the last residue of BG-4 (1261) is in the 3'-untranslated region. The amino acid sequence derived from the nucleotide sequence of human BG-4 α_i cDNA is highly homologous to bovine and rat α_i sequences reported by others. In addition, the 3'-untranslated region of BG-4 α_i cDNA is highly homologous to the 3'-untranslated regions of bovine and rat α_i cDNA. The 3'-untranslated nucleotide sequences of human, bovine, and rat α_s cDNAs also are highly conserved, but differ markedly from α_i 3'-untranslated sequences. These results suggest that the 3'-untranslated regions of α_s and α_i genes and/or mRNA are needed for functions that have not been identified thus far.

In previous studies we have shown that elevation of cAMP levels of NG108-15 neuroblastoma-glioma hybrid cells or neuroblastoma cells for several days results in 10-100 fold increases in the activity of voltage-sensitive calcium channels, 15-45 fold increases in spontaneous secretion of acetylcholine at synapses, and 5-15 fold increases in the abundance of synapses with cultured striated muscle cells. In addition, the number of molecules of the voltage-sensitive calcium channel protein subunit that binds [^{3}H]-nitrendipine increases 12-fold. We previously obtained about 100 cDNA clones that hybridize to species of mRNA that are more abundant in NG108-15 or NS20-Y cells that had been treated with dibutyryl cAMP for several days then in untreated control cells. Quantitative studies on the extent of increase in abundance of the

species of mRNA that respond to dibutyryl cAMP were performed using the cloned cDNA as probes. Twenty cDNA clones were obtained that hybridize to species of poly A^+ RNA that increase in abundance 10-90 fold due to treatment of cells with dibutyryl cAMP. Northern blots also were performed and the number of bands of poly A^+ RNA that hybridize to each cloned cDNA probe and their chain lengths were determined.

Affinity purified antibodies to the α, β, and γ protein subunits of voltage-sensitive calcium channels were used to screen a λgtll cDNA library prepared from poly A^+ RNA from rat skeletal muscle. Approximately 20 recombinant clones were found that were identified tentatively as calcium-channel α subunit cDNAs. Other cDNA clones were obtained that are putative γ subunit clones.

In previous studies a putative cDNA clone for choline acetyltransferase was found. We now have determined the nucleotide sequence of the 1118 bp DNA insert. Partial amino acid sequences of several peptides derived from choline acetyltransferase by the action of peptidases were obtained in collaborative studies by Lou Hirsh and his colleagues in Dallas. The λgtll cDNA library was screened again with 2 new oligodeoxynucleotide probes to different regions of choline acetyltransferase and cDNA clones were obtained that were recognized by both probes. Further studies with these clones are in progress.

Antigenic molecules termed TOP, which are distributed in a dorsal > ventral concentration gradient in chicken retina, are expressed early in development (by 48 hr after fertilization) in the optic cup of chicken embryos and continue to be expressed in retina thereafter. ^{35}S-labeled-TOP-antibody complexes were purified by protein A-Sepharose column chromatography and subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis and autoradiography. TOP also was purified from dorsal retina by anti-TOP IgG-Affigel 10 affinity column chromatography. Both purification methods yielded one major band of protein with an M_r of approximately 47,000. A protein of M_r approximately 47,000 also was purified from chicken embryo brain. Cultured cells dissociated from 8-day chicken embryo retinas accumulated the amount of TOP expected of cells in the intact retina, depending on the position of the cells in the retina. TOP accumulations by cells dissociated from dorsal or ventral retina, mixed in different proportions and cocultured were additive. These results show that TOP is a protein, that the gradient of TOP is established early in development, and that perpetuation of the gradient does not depend on the continuous presence of an extracellular gradient of diffusable molecules or on maintenance of interactions between cells. Synapses and neurites in the retina of developing chick embryos were reduced markedly by injection of anti-TOP antibody into the eye.

The addition of bradykinin to NG108-15 cells was shown in previous studies to increase cellular levels of inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol. The newly synthesized IP_3 in turn stimulates the release of stored calcium ions into the cytoplasm, thereby activating calcium-dependent K^+ channels. The increased efflux of K^+ ions results in cell hyperpolarization. This is followed by cell depolarization due to inhibition of M channels, thereby decreasing the rate of K^+ efflux from cells via M channels. Additional results now show that inhibition of M channels is due to diacylglycerol and Ca^{2+} dependent activation of protein kinase C. Several phosphoproteins were detected

by two dimensional gel electrophoresis whose synthesis is dependent upon the addition of bradykinin to cells. Whereas, injection of inositol 1,4,5-trisphosphate inside NG108-15 cells results in the release of stored calcium into the cytoplasm, injection of inositol 1,3,4-trisphosphate or inositol 1,3,4,5-tetrakisphosphate has little or no effect on calcium mobilization, but instead results in the activation of nonspecific cation channels. Calcium ions are not required for the activation of the nonspecific cation channels. The nature and significance of these findings warrant further investigation in light of recent reports that inositol 1,3,4-trisphosphate and inositol 1,3,4,5-tetrakisphosphate are present in some tissues and that inositol 1,3,4,5-tetrakisphosphate is synthesized by phosphorylation of inositol 1,4,5-trisphosphate, catalyzed by an appropriate kinase, and that inositol 1,3,4-trisphosphate is formed by dephosphorylation of inositol 1,3,4,5-tetrakisphosphate.

Publications:

1. Higashida, H., Streaty, R.A., Klee, W. and Nirenberg, M.: Bradykinin-activated Transmembrane Signals are Coupled via N_o or N_i to Production of Inositol 1,4,5-trisphosphate, a Second Messenger in NG108-15 Neuroblastoma-glioma Hybrid Cells. Proc. Natl. Acad. Sci., USA 83, 942-946 (1986).

2. Grunwald, G.B., Gierschik, P., Nirenberg, M., and Spiegel, A.: Detection of α-Transducin in Retinal Rods but not Cones. Science 231, 856-859 (1986).

3. Moskal, J.R., Trisler, D., Schneider, M.D., and Nirenberg, M.: Purification of a Membrane Protein Distributed in a Topographic Gradient in Chicken Retina. Proc. Natl. Acad. Sci., USA 83, 4730-4733 (1986).

4. Dubois, C., Magnani, J.L., Grunwald, G.B., Spitalnik, S.L., Trisler, G.D., M. Nirenberg, and Ginsburg, V.: Monoclonal Antibody 18B8, Which Detects Synapse-associated Antigens, Binds to Ganglioside G_{T3} ($II^3(NeuAc)_3LacCer$). J. Biol. Chem. 261, 3826-3830 (1986).

5. Dubois, C., J.L. Magnani, G.B. Grunwald, S.L. Spitalnik, D. Trisler, M. Nirenberg, and V. Ginsburg. 1985. Monoclonal antibody 18B8, which detects synapse associated antigens, binds to ganglioside GT3 [$II^3(NeuAc)_3LacCer$]. In Glycoconjugates: Proceedings of the VIIIth International Symposium, E.A. Davidson, J.C. Williams, and N.M. DiFerrante, eds. Vol.2, p. 5545, Praeger Publ., New York.

6. Bray, P., Carter, A., Simons, C., Guo, V., Puckett, C., Kamholtz, J., Spiegel, A., and Nirenberg, M.: Human cDNA Clones for Four Species of $G\alpha_s$ Signal Transduction Protein. Proc. Natl. Acad. Sci., USA (In Press).

7. Trisler, D. Synapse formation in retina is influenced by molecules that identify cell position. Current Topics in Developmental Biology (In Press).

8. Trisler, D. Synapse formation in avian retina is inhibited by antibody to molecules that identify cell position. Amer. Zool. (In Press).

9. Trisler, D., J. Bekenstein, and M.P. Daniels. Antibody to a molecular marker of cell position inhibits synapse formation in retina. Proc. Natl. Acad. Sci., USA, 83: 4194-4198 (1986)

10. Higashida, H. and Brown, D.A.: Two Polyphosphatidyl Metabolites Control Two K^+-Currents In A Neuronal Cell. Nature (In Press).

11. Higashida, H. and Brown, D.A.: Membrane Current Responses to Intracellular Injections of Inositol 1,3,4,5-tetrakisphosphate And Inositol 1,3,4-trisphosphate in NG108-15 Cells. (Submitted to FEBS Letters).