Cell Recognition and Synapse Formation

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Total: 11
Professional: 9
Other: 2

(a) Human subjects (b) Human tissues (c) Neither

- (a1) Mammals
- (a2) Interviews

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<sub>p</sub> 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 diffusible 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 oligolibraries from human brain were screened with 3 oligodeoxynucleotide probes for recombinants coding for α subunits of G protein 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 32P-probes used. DNA inserts from 13 positive clones were sequenced partially; 11 clones were identified as αs cDNA and 2 clones as αi. The DNA insert from one of the αs clones was sequenced completely and additional partial sequences were obtained for 10 αs clones. Four species of αs cDNA were found that differ in nucleotide sequence in the region that corresponds to αs amino acid residues 71-88. The clones differ in the codon for αs 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 αs mRNA by alternative splicing of precursor RNA transcribed from a single gene.

cDNA from one of the two human αi clones was sequenced completely (BG-4), and a partial sequence was obtained for the second clone. The first nucleotide residue of BG-4 αi cDNA corresponds to the 14th residue of the bovine αi coding sequence and the last residue of BG-4 (1251) is in the 3' -untranslated region. The amino acid sequence derived from the nucleotide sequence of human BG-4 αi cDNA is highly homologous to bovine and rat αi sequences reported by others. In addition, the 3' -untranslated region of BG-4 αi cDNA is highly homologous to the 3' -untranslated regions of bovine and rat αi cDNA. The 3' -untranslated nucleotide sequences of human, bovine, and rat αs cDNAs are also highly conserved, but differ markedly from αi 3' -untranslated sequences. These results suggest that the 3' -untranslated regions of αs and αi 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 [3H]-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 dibutylr 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 10-50 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 λgt11 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 β 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 λgt11 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. 35S-labeled-TOP-antibody complexes were purified by protein A-Sepharose column chromatography and subjected to NaDodSO4/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 Mr of approximately 47,000. A protein of Mr 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 diffusible 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 (IP3) and diacylglycerol. The newly synthesized IP3 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 Ca2+ 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:


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).