MOLECULAR NEUROBIOLOGY

Organizers: Tom Curran and James Morgan

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Plenary Sessions Page

Poster Sessions

Neurogenetics

CQ 001 THE ROLE OF THE VERTEBRATE ENGRAILED-LIKE GENES IN DEVELOPMENT OF THE CNS. Clay Davis, Cairine Logan, Anna Auerbach, Janet Rossant and Alexandra Joyner, Division of Molecular and Developmental Biology, Mount Sinai Hospital Research Institute, 600 University Ave. Toronto, M5G 1X5.

We cloned the mouse and human homeo box-containing genes, En-1 and En-2, based on their extensive sequence homology to the Drosophila segmentation gene engrailed (en). In the fly, en appears to play a role late in development in formation of the CNS in addition to a role earlier in pattern formation. In the mouse, using in situ hybridization and antibody localization analysis, we have found that the En genes are first expressed during gastrulation in a spatially restricted band of cells in the neural folds at the one somite stage. Both genes continue to be expressed in this midbrain region throughout development, however as cells become postmitotic, En expression becomes limited to several groups of neurons. En-1 is also expressed during organogenesis in cells within the spinal cord, hindbrain, somites, and limbs. In the hindbrain, expression begins in a rhombomere specific pattern after the rhombomeres form and in the spinal cord En-1 expression seems to be limited to the motor neurons. We are currently mapping the anterior and posterior borders of expression of the En genes in the midbrain region using scanning electron microscopy and element analysis of antibody stained embryos to determine whether they correlate with surface features of the neural plate. The DNA regulatory sequences responsible for these patterns of expression are being analyzed using lacZ reporter constructs introduced into mouse embryonic stem (ES) cells. Chimeric mouse embryos derived from such cell lines are being directly analyzed for appropriate En-like lacZ expression. Finally, in order to better understand the function of the En genes in the CNS, we are making mice carrying En null mutations via homologous recombination in ES cells. To this end we now have mice that are homozygous for the En-2 mutation which appear healthy and we are currently analyzing them for any defects in brain development. (Funded by the MRC and NCI of Canada).

CQ 002 NEURAL SPECIFIC EXPRESSION OF PROTOONCOGENES IN MURINE DEVELOPMENT

Dionisio Martin-Zanca*, Rudiger Klein+, Mariano Barbacid+, and Luis F. Parada*, *Molecular Embryology Group, NCI-Frederick Cancer Research Facility, Frederick, MD 21701, +Department of Molecular Biology, Squibb Institute for Medical Research, Princeton, NJ 08543

Protooncogenes are presumed to code for regulatory molecules that are central to cell growth or differentiation. We have undertaken a systematic survey of protooncogene expression in the mouse embryo by RNA *in situ* hybridization. Two protooncogene families, *myc* and *Trk*, display striking patterns of expression in the developing nervous system. N*myc*, is expressed in all CNS and PNS structures from neural tube formation onwards and is not constrained to mitotic cells. Direct comparison of N*myc* and c-*myc* expression indicates the exclusion of c-*myc* expression in the developing CNS and PNS and thus suggests a specific role for N*myc* in the development of nervous structures. The *Trk* protooncogene and a closely related gene, *Trk*b, encode putative tyrosine protein kinase receptors as defined by structural criteria. The *Trk* proto-oncogene is expressed uniquely in neural crest derived sensory neuroblasts and neurons of the trigeminal, jugular, superior, and spinal ganglia. In contrast, the *Trk*b gene is found expressed in all developing CNS and PNS structures. Both genes maintain expression in the adult and their ligands are as yet uncharacterized. The above results suggest that N*myc*, *Trk* and *Trk*b are role players from the earliest stages of neural development. Further study of these genes, their regulatory domains and their products will enhance our understanding of the molecular basis of neural development. Research sponsored in part by the National Cancer Institute, DHHS, under contract no. NO1-CO-74101 with BRI.

Molecular Neurobiology

Proto-Oncogenes

CQ 003 Proto-oncogenes As Components of the NGF and b-FGF Signal Transduction Cascades, Simon Halegoua, Norbert Kremer, Robert Armstrong, Gabriella D'Arcangelo, Haiqing Qi, Gail Mandel, Dawn Shepard, Paul Brehm, Shiela Thomas* and Joan Brugge*. Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, NY 11794 and *Howard Hughes Medical Institute, Department of Microbiology, University of Pennsylvania, Philadelphia, PA 19104.

The neuronal growth factors, NGF and b-FGF, induce the differentiation of the PC12 cell line into sympathetic neuron-like cells. The differentiation program elicited by NGF involves the stimulation of multiple second messenger pathways. Resulting post-translational modifications result in functional changes in cellular proteins which lead to changes in the cellular phenotype. Some of these changes directly result from the phosphorylation of physiologically important molecules as will be shown for tyrosine hydroxylase, the rate limiting enzyme for catecholamine neurotransmitter synthesis. In response to NGF, this protein is phosphorylated at two sites, one directed by C-kinase and the other directed by A-kinase. FGF elicits the phosphorylation of only the C-kinase directed site. Second messenger pathway stimulation also leads to changes in gene expression by transcriptional or post-transcriptional means. As will be discussed for the proto-oncogene *c-fos*, rapid transcriptional activation by NGF and FGF is mediated through C-kinase and other pathways. Longer term gene inductions include that of the Na channel gene(s), the result of which is the establishment of the action potential mechanism.

Because the NGF receptor does not encode a protein with known enzymatic activity, we have attempted to identify the signal transduction molecules responsible for mediating the differentiation program induced by NGF and FGF. From a series of experiments using neutralizing antibody microinjection into fused PC12 cells, we have found that both *c-src* and *c-ras* proto-oncogene products are necessary for both NGF and FGF induced differentiation. Both *src* and *ras* oncogenic forms mimic growth factor induced differentiation in PC12 cell lines transfected with inducible oncogenes. Using these cell lines and antibody microinjection, we have determined the sequential functional ordering of src and ras in eliciting the neuronal phenotype. These results will be presented with respect to the elicitation of neurite outgrowth and the specific second messenger and gene induction events described above.

CQ 004 MEMBRANE-ASSOCIATED PROTO-ONCOGENES IN NEURAL SIGNAL TRANSDUCTION,Michael R. Hanley, MRC Molecular Neurobiology Unit,MRC Centre,Hills Road,Cambridge CB2 2QH,England.

A number of genes with transforming potential are now recognised to be expressed at high levels in mammalian brain. However, there are a few, if any, neural functions that are firmly established for proto-oncogenes, although they are candidates for discrete components of signalling cascades in development or cell communication. The proto-oncogenes that have been identified in nerve cell populations belong to all the major structural classes: nuclear, growth factor-like, receptor-like, GTP-binding, and kinases. We have emphasised two classes of membrane-associated proto-oncogenes; those which have sequence similarity to either surface receptors or those which are monomeric GTP-binding proteins. In the former context, we have recently provided evidence that the MAS oncogene encodes a neuronal angiotensin receptor. Functional analysis suggests that activation of MAS by angiotensins couples through a G-protein to hydrolysis of inositol lipids, leading to the expected consequences of intracellular calcium discharge and stimulation of protein kinase C. Ligand activation of MAS is ultimately coupled to acute and long-lived changes in gene expression, and also leads to alterations in neuronal excitability. These results suggest that MAS, and perhaps other receptor-like proto-oncogenes, may have multiple functions such as regulating proliferation and maturation of nerve cell precursors and regulating ion channel and genetic activity in the fully differentiated state. Some of the neural responses to MAS depend upon cellular RAS, as neutralising anti-RAS p21 antibodies can block, for example, MAS-mediated entry into DNA synthesis. This result raises the interesting possibility that a number of proto-oncogenes may interact in a functional network which takes information from the neural environment to the nucleus. We are also exploring other possible functions of the RAS-related superfamily of GTP-binding proteins such as intracellular trafficking, secretion, and control of cytoskeletal architecture. In particular, members of the RAB family appear to be expressed preferentially in brain and are localised to the endoplasmic reticulum. We are examining whether RAB species are involved in GTP effects on calcium compartmentation and release.

CQ 005 NERVE GROWTH FACTOR INDUCES TRANSCRIPTION OF GENES ENCODING ZINC-FINGER PROTEINS, Milbrandt, J. Departments of Pathology and Internal Medicine, Washington University School of Medicine, St. Louis, MO 63110

Nerve growth factor (NGF) is a neurotrophic factor that is essential for the differentiation and survival of sympathetic and neural crest-derived sensory neurons. Our laboratory is investigating the changes in gene expression that occur during the NGF-mediated differentiation of the rat pheochromocytoma cell line PC12. Using differential hybridization several cDNAs have been identified which correspond to mRNAs that are rapidly induced by NGF. Transcriptional runoff analysis has shown that the elevated levels of these mRNAs results from the transcriptional activation of their respective genes. The nucleotide sequence of two of these cDNAs, NGFI-A and NGFI-B, has been determined and analysis of this sequence revealed that both of these early response genes encode zinc finger proteins. The NGFI-B gene product is homologous to members of the steroid/thyroid receptor gene family. Analysis of its gene structure shows that it constitutes yet a third digression from a postulated ancestral hormone receptor gene. The NGFI-A gene encodes a protein which contains three prototypical Cys-His zinc fingers in tandem. These zinc fingers are all encoded by a single exon and are almost identical to those of another early response gene, Krox 20. Antibodies to the NGFI-A protein have been used to demonstrate that NGFI-A is rapidly synthesized by PC12 cells in response to NGF and by neurons of the hippocampus after a Metrozol induced seizure. The transcriptional activation of the NGFI-A gene is mediated by sequence elements located in a fragment extending from nt -532 to nt +100 of the NGFI-A 5′ flanking region. This fragment contains multiple copies of the CarG box, a nucleotide element closely related to the serum response element. The effects of deletions and mutations in this region on the NGF-mediated transcriptional activation of this gene, and, further characterization of the NGFI-A protein will be presented.

CQ 006 EVOLUTIONARY AND DEVELOPMENTAL EXPRESSION OF THE PROTOONCOGENE C-SRC IN THE NERVOUS SYSTEM, Gernot Walter, Ximing Yang, Otmar Wiestler, Jean Le Beau, and Dagmar Roeckel, Department of Pathology, University of California, San Diego, La Jolla, CA 92093.

The protooncogene c-src is expressed in vertebrates and invertebrates, and the sequence of the src protein, a protein-tyrosine kinase, is phylogenetically highly conserved. Two forms of the src protein, referred to as pp60+ and pp60, have been observed in neurons. In mouse and chicken, pp60+ and pp60 differ by a stretch of 6 amino acids that is inserted by alternative splicing in the N-terminal half of pp60+ and is absent in pp60. pp60+ has only been detected in cells of neuronal origin. It is highly expressed in the CNS and at low levels, if at all, in the PNS. The highest expression of pp60+ takes place in the adult hippocampus. When assayed in various animal species, pp60+ is readily detectable in the brain of mammals, birds and reptiles (higher vertebrates), but not amphibians and fish (lower vertebrates). In embryonic mouse brain development, pp60+ is highly expressed after day 11 and peaks on day 18. By contrast, pp60 is expressed in all cells (neuronal and nonneuronal), at all stages of brain development, and in the brain of all animal species tested. Furthermore, similar quantities of pp60 are expressed in the CNS and PNS. These findings are compatible with the idea that pp60+ may play a role in events associated with higher brain function such as neuronal plasticity.

Several observations indicate that pp60+ and/or pp60 are involved in neuronal differentiation. In pheochromocytoma cells (PC12) infected with recombinant retroviruses expressing pp60 or pp60+, or the kinase-activated Tyr-->Phe527 mutants of pp60 and pp60+, only the latter induce differentiation. The possible role of pp60/pp60+ in the differentiation of PC12 cells will be discussed.

Signal Transduction in Neurons

CQ 007 NUCLEUS-ZONE CALCIUM SIGNALS AND ASSOCIATED *c-fos* INDUCTION IN NEURONS. J.A. Connor, A. Hernandez-Cruz, and S.C. Sun. Roche Institute of Molecular Biology, Roche Research Center, Nutley, N.J. 07110.

Acutely isolated CA1 neurons from the hippocampus of young guinea pigs and rats have been studied using the calcium indicator fura-2 in conjunction with digital imaging techniques. Following a period of stimulus with excitatory amino acids or action potential firing, Ca^{2+} levels in the region of the nucleus remained significantly higher than in other regions of the neuron for many minutes. In unstimulated neurons the levels of Ca^{2+} generally appeared to be somewhat lower in the nucleus than other cellular regions, and during the initial phase of stimulation, dendrites and non-nuclear regions of the soma showed the highest levels of Ca^{2+}. The Ca^{2+} iontophore, ionomycin, in low Ca saline abolished the nucleus-zone gradient. Extended washing in buffered, low Ca saline without the iontophore was relatively ineffective in lowering the high Ca^{2+} levels in the nuclear zone. In the guinea pig preparation the Ca^{2+} distribution almost never recovered to resting levels and the neurons showed poor survival after the gradient had appeared. In the, generally younger rat neurons a slow recovery over a 3 to 10 minute period was often observed. Of the guinea pig neurons with high nucleus-zone Ca^{2+}, approximately 50% showed nuclear staining by *c-fos* antibody. Unstimulated neurons that did not show nuclear-zone Ca^{2+} did not stain (> 95%). We are running a similar immunostain on the rat preparation.

We have demonstrated a much more rapidly developing and recovering nuclear gradient in amphibian spinal neurons, induced either by caffein or electrical stimulation. This preparation should prove very useful in studying the mechanisms of Ca^{2+} signalling to the nucleus.

Genes and Development

CQ 100 Single neuroepithelial cells become restricted to expression of either L-*myc* or N-*myc* at embryonic days 13-14 in the mouse. Ora Bernard, John Drago and Hui Sheng. The Walter and Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, Victoria, 3050, Australia.

The three well studied proto-oncogenes of the *myc* family, c-*myc*, L-*myc* and N-*myc*, share extensive regions of amino acid homology. The normal patterns of expression of these genes are however very different. While c-*myc* is expressed in essentially all dividing cells, N-*myc* and L-*myc* are expressed mainly in the developing embryos, where all three genes are expressed at relatively high levels within the developing brain. In contrast to the restriction of c-*myc* expression to the dividing cells, the expression of L-*myc* and N-*myc* correlates with cellular differentiation in the brain. Since the two genes are expressed in the developing central nervous system at the time when differentiation from neural stem cell into neurons and glia takes place, L-*myc* and N-*myc* genes might play a vital role in the development and differentiation of these cell types. To test the proposition that commitment of the stem cell is associated with restriction of expression to either L-*myc* or N-*myc* we have used the polymerase chain reaction [PCR] to monitor expression of the two genes in single neuroepithelial precursor cells from mouse embryos taken during the commitment period. At least 300 individual cells were examined for the expression of L-*myc* and N-*myc* for each embryonal age ranging from embryonal day 10 [E10] to E14. The results can be summarized as follows: [1] Individual cells from E10-E12 expressed both genes. [2] At E13 only a few cells expressed both L-*myc* and N-*myc*. At this stage 70% of the cells expressed either L-*myc* or N-*myc* while 30% expressed both of these genes. [3] At E14 95% of the cells are restricted to either L-*myc* or N-*myc* expression. These results indicate that at E10-E12 the neuroepithelial cells are very similar to each other and probably are bipotential. However at E13-E14, when cellular differentiation takes place and the cells become committed to either the glial or the neuronal lineage, they express L-*myc* or N-*myc*.

CQ 101 PATTERNS OF PROTEIN EXPRESSION OF *CUT*, A LOCUS REQUIRED FOR EXTERNAL SENSORY ORGAN DEVELOPMENT, IN WILD-TYPE AND *CUT* MUTANT *DROSOPHILA* EMBRYOS, Karen Blochlinger, Rolf Bodmer, Lily Yeh Jan and Yuh Nung Jan, Howard Hughes Medical Institute and the Departments of Physiology and Biochemistry, University of California, San Francisco, CA 94143

In the embryonic peripheral nervous system of *Drosophila* the loss of *cut* activity results in the transformation of the neurons and support cells of external sensory (es) organs into those of chordotonal (ch) organs. The *cut* locus encodes a homeodomain-containing protein (*Cut*) which is expressed in the cells of es organs but not in ch organs. We here describe the development of the embryonic *cut* expression pattern and show that *Cut* protein is expressed in sensory precursor cells which divide to give rise to es organs. Similar transformations of es organs into ch organs were observed in adult mosaic flies, when the *cut* gene activity was lost in marked mutant patches. We show that *Cut* is also expressed in the precursors of the adult es organs in the developping imaginal wing, eye-antenna, and leg discs of wild-type flies. In addition, we describe the changes in *Cut* expression pattern of several mutant alleles of the complex *cut* locus.

CQ 102 IGF-1 RECEPTOR GENE EXPRESSION DURING DEVELOPMENT: CORRELATION WITH IGF-2 PEPTIDE GENE EXPRESSION. Carolyn Bondy, Charles Roberts, Derek LeRoith and Haim Werner, NINDS AND NIDDK.

The insulin-like growth factors (IGF-1 and IGF-2) are mitogenic peptides thought to be involved in embryonic as well as post-natal growth and development. We have used *in situ* hybridization histochemistry to analyze the developmental pattern of the IGF-1 receptor (1-R) gene expression in parallel with that of IGF-1 and IGF-2 peptide genes in the rat. During organogenesis (embryonic days 14-18), 1-R mRNA was co-expressed with IGF-2 mRNA in mesenchymal derivatives such as muscle, connective tissue, and liver. During this period, levels of IGF-2 mRNA were extremely high, 1-R mRNA moderately abundant and IGF-1 mRNA barely detectable. In view of the ability of the transfected IGF1 receptor to bind IGF-2 with an affinity approaching that of IGF-1 itself, the presence of IGF-2 in great excess over IGF-1 in developing tissues suggests that it is primarily the IGF-2 peptide interacting in an autocrine or paracrine manner with the 1-R to promote their growth. Interestingly, the highest levels of 1-R mRNA during this early period were found in the notochord, where IGF-2 mRNA expression was also high. In the developing nervous system, the relationship of 1-R mRNA expression to that of IGF-2 was very different. The IGF receptor mRNA was abundantly expressed in the proliferative ventricular zones of neuroepithelium in developing brain and spinal cord and in cranial and dorsal root ganglia, while IGF-2 mRNA was heavily expressed in primordial meningeal and choroidal structures but specifically not in neural tissue. IGF-1 mRNA was undetectable in the nervous system. Later during neural development, 1-R mRNA is expressed diffusely throughout the grey matter of brain, spinal cord and associated ganglia and also in the choroid plexus and meninges. IGF-2 mRNA, while declining elsewhere, continues at high levels of expression in the choroid plexus and meninges. These findings suggest that the IGF-2 peptide, released into the embryonic circulation from the liver or into the CSF from choroid plexes, acts in an endocrine manner via the IGF-1 receptor to function in the early development and later maintanence of the rat nervous system.

CQ 103 TARGETED REPLACEMENT OF THE HOMEO-GENE HOX-3.1 BY THE E.COLI β-GALACTOSIDASE IN MOUSE CHIMERIC EMBRYOS. Philippe Brûlet, Hervé Le Mouellic and Yvan Lallemand, Unité de Génétique cellulaire du Collège de France et de l'Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France.
Through gene targeting in embryonic stem (ES) cells, a chosen gene can be inactivated and eventually a strain of mutant mice created. We have devised a new procedure to specifically replace a targeted gene by another gene. A murine homeo-gene was disrupted with a high frequency in ES cells by its replacement with lac Z. In chimeric embryos, the β-galactosidase activity was then driven by the Hox-3.1 promoter. This technique will allow the visual assessment at the cellular level of gene inactivation effects in transgenic mice.

CQ 104 DEVELOPMENTAL EXPRESSION OF MAJOR MYELIN PROTEIN GENES IN MYELIN-DEFICIENT MICE, Gardinier M.V., Tosic M., and Matthieu J.-M., Laboratoire de Neurochimie, Service de Pédiatrie, Centre Hospitalier Universitaire Vaudois, CH-1011 Lausannne, Switzerland
Myelin-deficient mutant mice (mld) possess a tandem duplication of the myelin basic protein (MBP) gene, whereby MBP gene transcripts are generated from both the partially inverted upstream MBP gene and the "normal" downstream MBP gene. Currently, we are analyzing not only effects on MBP gene expression, but also pleiotropic effects involving the other major myelin protein genes (proteolipid protein, PLP; myelin-associated glyoprotein, MAG; cyclic nucleotide phosphodiesterase, CNP). Both nuclear and total cellular RNA from mld and normal brain tissue (5-80 days of age) was analyzed by Northern and dot blot studies. MBP mRNAs from the "normal" downstream MBP gene of mld mice are transcribed in greatly reduced amounts. In contrast to maximal MBP mRNA expression in normal mice around 20 days of age, mld MBP mRNAs peak around 40 days of age. This peak was noted for both "normal" downstream and antisense upstream transcript populations. Thus, both gene copies are coordinately regulated during development. During peak myelination (in mice, 15-25 days of age), PLP mRNAs showed about a 50% reduction; whereas MAG and CNP mRNAs were at or near normal concentrations in mld mice. No significant differences were seen for mld PLP mRNA levels both before and after peak myelination. However, slightly increased MAG and CNP mRNA levels were evident by 10 days of age in mld mice, prior to peak myelination. In addition, MAG and CNP mRNA concentrations were significantly higher at 40 days of age in mld mice. Presently, we are determining if the transcriptional activity of the MBP (each gene copy in mld mice), PLP, MAG, and/or CNP genes reflect the perturbations indicated above.

CQ 105 *trk*B, A NOVEL TYROSINE PROTEIN KINASE GENE CODES FOR TWO TYPES OF PUTATIVE NEUROGENIC CELL SURFACE RECEPTORS: Rüdiger Klein[1], Dorothy Conway[1], Luis F. Parada[2] and Mariano Barbacid[1], [1]Department of Molecular Biology, Squibb Institute for Medical Research, Princeton, NJ 08543, [2]Molecular Embryology Section, BRI, NCI-FCRF, Frederick, MD 21701.
We have recently identified a novel tyrosine protein kinase gene, designated *trk*B, that is widely expressed in the central and peripheral nervous systems of fetal and adult mice (EMBO J., 8:3701,1989). The *trk*B locus exhibits a complex pattern of transcription. At least six mRNA species ranging in size from 9 kb to 2 kb have been identified in brain. Molecular analysis of cDNA clones derived from these *trk*B transcripts revealed that only two mRNA species (9 kb and 5.5 kb) code for a cell surface receptor containing a tyrosine protein kinase catalytic domain (TK+ mRNAs). Expression of cDNA clones derived from these TK+ transcripts revealed a membrane-associated glycoprotein of 145 kd which displays the expected tyrosine protein kinase activity. In contrast, the other four transcripts (TK− mRNAs) code for glycoproteins of 95 kd that contain the *trk*B ligand-binding domain anchored to the cellular membrane, but lack a catalytic cytoplasmic domain. Both of these receptor-like molecules have been identified in mouse brain extracts by Western blot analysis. *In situ* hybridization analysis of adult mouse brain indicates that transcripts encoding the TK+ gp145^{trkB} product are preferentially expressed in the cerebral cortex and the pyramidal cell layer of the hippocampus. In contrast, those transcripts coding for the TK− gp95^{trkB} protein could only be identified in the choroid plexus. These results illustrate for the first time, that a tyrosine protein kinase locus may code for two structurally and functionally distinct neurogenic receptors.

CQ 106 GENOMIC, cDNA AND PUTATIVE PROTEIN STRUCTURE OF SCIP, A GLIAL POU-DOMAIN GENE, Rainer Kuhn, Edwin S. Monuki, and Greg Lemke, The Salk Institute, La Jolla, CA 92138. We have recently described the identification of SCIP ("skip"), a gene expressed by myelin-forming glia of the central and peripheral systems, whose structure is closely related in its putative DNA binding domain to those of POU-domain transcription factors. The complete cDNA as well as genomic clones have been isolated. The sequence of several overlapping cDNAs totals 2977 nucleotides (excluding the poly(A) tail), which is consistent with the 3.1 kb size of the SCIP mRNA. The composite cDNA encodes a deduced protein of 451 amino acids, and can be divided into a glycine/alanine-rich amino terminal domain, a POU domain, and a short proline-rich carboxy terminal domain. Genomic Southern analyses, restriction mapping of genomic clones and sequence analyses demonstrate that SCIP is encoded by a single copy gene devoid of introns. We are currently analyzing the ability of SCIP to bind to its own promoter, and to the promoters of myelin-specific genes. Experiments to identify regulatory elements of the SCIP promoter are underway.

CQ 107 EXPRESSION OF THE β-S100 GENE IN ASTROGLIAL PROGENITOR CELLS AND CHONDROCYTES OF THE EMBRYONIC RAT BRAIN, Charles F. Landry, John H. Youson and Ian R. Brown, Department of Zoology, Scarborough Campus, University of Toronto, Toronto, Ontario, Canada, M1C 1A4

The β-subunit of the calcium-binding protein S100 is found in astrocytes within the mammalian central nervous system. We have cloned the cDNA for β-S100 (Dunn et al. J Biol Chem 262 (1987) 3562-3566) and have applied Northern blotting and *in situ* hybridization histochemistry to an analysis of β-S100 mRNA distribution during postnatal development of the rat brain (Landry et al. Mol Brain Res (1989) in press). We have now extended this study to embryonic day 13 (E13) in order to delineate early patterns of β-S100 mRNA distribution. At E13, β-S100 mRNA was confined to cells bordering the ventricle within the region of the myelencephalon. These cells were intensely labeled with β-S100 probe but remained unlabeled following hybridization with probe to the astrocyte-specific marker, Glial Fibrillary Acidic Protein. At E13, β-S100 mRNA was also observed within cartilage of the developing neurocranium. Signal corresponding to β-S100 mRNA was confined to chondrocytes and appeared to be expressed during differentiation of this cell type. Chondroblasts within the perichondrium remained unlabeled. In addition, signal was absent from regions of neurocranium undergoing ossification. These results identify both the presence of β-S100 mRNA in putative astroglial progenitor cells in the embryonic brain and within chondrocytes of the developing neurocranium. (Supported by NSERC)

CQ 108 SENSORY GANGLION-SPECIFIC EXPRESSION OF THE *Trk* PROTO-ONCOGENE IN MOUSE DEVELOPMENT, D. Martin-Zanca and L.F. Parada, Molecular Embryology Group, NCI-Frederick Cancer Research Facility, Frederick, MD 21701

We have isolated and characterized genomic clones encompassing the mouse *Trk* protooncogene and flanking 5′ sequences, and *Trk* cDNA clones from a rat embryonic library. Mouse *Trk* exonic sequences and rat cDNA clones were used as probes in Northern analysis of mouse embryo RNAs as well as of RNAs from adult mouse and rat tissues. All probes identify a single 3.2kb *Trk*-specific transcript which appears in mouse embryos at 9.5 days of gestation and reaches a maximum at 13.5 days. By RNA *in situ* analysis, *Trk* specific hybridization is first detected by E9.5, when morphogenesis of the peripheral nervous system has begun as a faint signal confined to the site of condensing DRG. At E13.5, when *Trk* mRNA levels reach a maximum, the *in situ* analysis shows the continued presence of *Trk* transcripts in the trigeminal ganglion and DRG and appearance in the IXth/Xth cranial ganglion complex during late gestation. Finally, *in situ* analysis of adult trigeminal ganglion shows that *Trk* expression is confined to a subset of neurons and is absent in associated glial cells. This result identifies *Trk* as an early molecular marker for trigeminal, spinal and the superior/jugular ganglia, suggesting the existence of cell types unique to these neural-crest-derived structures. Using our *Trk* genomic clones, we are in the process of *in vivo* mapping the regulatory sequences responsible for *Trk*'s striking pattern of expression. Anti-peptide antibodies are being used to identify the *Trk* protein(s), as well as to perform immunocytochemical analysis to establish its cellular localization as a first step towards elucidating physiological function.

CQ 109 SCIP: A GLIAL POU-DOMAIN GENE REGULATED BY CYCLIC AMP, Edwin S. Monuki, Rainer Kuhn, Gerry Weinmaster and Greg Lemke, The Salk Institute, La Jolla, CA 92138. Full-length cDNA and genomic clones have been isolated for SCIP, a POU-domain gene expressed by myelin-forming glia of the central and peripheral nervous sytems. In purified Schwann cell cultures, expression of SCIP is suppressed. This suppression is relieved by cyclic AMP, and induction of SCIP by this second messenger precedes induction of the myelin-specific genes. SCIP expression *in vivo* similarly precedes peak myelin gene expression in the developing CNS and sciatic nerve. This temporal sequence of transcription events during development is in direct contrast to that seen during peripheral nerve transection, in which SCIP up-regulation coincides with myelin-specific gene *down-regulation*. Transient cotransfection experiments in Schwann cells with the complete SCIP coding region reveal that SCIP acts to specifically repress CAT reporter constructs containing the P_0, MBP, and NGF receptor promoters. These results demonstrate that SCIP is expressed by myelin-forming glia and acts to regulate myelin-specific gene expression, suggesting a central role for SCIP in the progressive determination and phenotypic plasticity of these cells.

CQ 110 THE ROLE OF *MYC* ONCOGENES IN NEURONAL DEVELOPMENT, *IN VIVO* AND *IN VITRO*, Sharon D. Morgenbesser, James Horner, Lin Xu , Fung-Chow Chiu, and Ronald DePinho, Departments of Microbiology and Immunoigy and of Neurology, Albert Einstein College of Medicine, Bronx, New York 10461.
Myc family genes (c-, N-, and L-*myc*) are believed to encode trans-acting, transcriptional regulators that make important decisions affecting mammalian development. Consistent with their putative developmental role, each *myc* family member is expressed in a distinctive developmental-stage specific and tissue-specific manner. To understand the different roles of these oncogenes in neuronal development and the mechanisms by which they are regulated, we have studied their expression during *in vivo* and *in vitro* neurogenesis. Examination of the terminal stages of murine forebrain development in newborn and three-week old mice indicates that all three *myc* genes are developmentally regulated, exhibiting significant decreases in steady-state message levels. In order to study the differential expression of these genes in a single cell type, we have utilized a murine teratocarcinoma stem cell line which has the potential to pursue retinoic acid-induced neurogenesis and DMSO-induced myogenesis. Within each developmental pathway, each *myc* gene displays a different expression profile. In early stages of neuronal differentiation, N-*myc* is rapidly and significantly downregulated, while L-*myc* shows a transcient five-fold increase, followed by a fifty-fold decrease. In later stages, N-*myc* remains downregulated whereas L-*myc* returns to its baseline level. In myogenesis, alterations in *myc* expression are highlighted by L-*myc* downregulation, whereas N-*myc* undergoes a five-fold increase, followed by a gradual return to baseline. We will present these data in the context of phenotypic changes of differentiation. We have also examined the regulation of *myc* gene expression *in vivo* and *in vitro* and have demonstrated that transcriptional attenuation is the dominant mechanism responsible for the downregulation of *myc* expression. We are currently examining the physiological relevance of these events *in vitro* by determining whether constitutive expression of a *myc* family gene can block terminal differentiation and whether abrogation of *myc* activity by antisense can produce developmental consequences.

CQ 111 CELLULAR SPECIFICITY OF GAP JUNCTION GENE EXPRESSION IN THE MAMMALIAN NERVOUS SYSTEM, Christian C.G. Naus, Daniel J. Belliveau and John F. Bechberger, Dept. of Anatomy, University of Western Ontario, London, Canada N6A 5C1
Gap junction proteins are encoded by a family of genes, and many of the products of these genes are present in the mammalian nervous system. However, the cell specificity of gap junctions in the nervous system remains to be clarified. We have examined various neural cell types for the expression of the gap junction genes encoding connexin32 and connexin43. While both of these connexin mRNAs are present in RNA isolated from whole brain, the level of connexin43 is several fold higher than connexin32. In contrast, only connexin43 mRNA is detectable in cultures of primary astrocytes. Following dibutyryl cAMP treatment, the level of connexin43 mRNA does not change significantly. C6 glioma cell cultures also contain only connexin43 mRNA, but the level is much lower than in the primary astrocytes. Similarly, endothelial cells isolated from cerebral cortex contain predominantly connexin43 mRNA. In RNA isolated from optic nerve, which contains astrocytes and oligodendrocytes, but no neuronal somata, levels of both connexin43 and connexin32 mRNA are higher than those observed in whole brain. Primary cultures of oligodendrocytes contain relatively low levels of connexin43 and connexin32. LAN2 neuroblastoma cell cultures do not appear to express detectable amounts of connexin43 or connexin32. We are pursuing in situ hybridization to obtain cellular resolution of connexin gene expression. Supported by the Ontario Mental Health Foundation.

CQ 112 ANALYSIS OF MURINE *Nmyc* FUNCTION IN NERVOUS SYSTEM DEVELOPMENT BY IN SITU HYBRIDIZATION AND HOMOLOGOUS RECOMBINATION, Brian R. Stanton, David Sassoon and Luis F. Parada, Molecular Embryology Group, NCI-Frederick Cancer Research Facility, Frederick, MD, 21701

Nmyc mRNA expression is extremely limited in the adult and has been implicated in the regulation of murine embryogenesis. We have analyzed the sites of *Nmyc* expression during mouse development using *in situ* hybridization and shown that this gene is expressed at the highest levels in the developing nervous system. These studies have included the bulk of embryogenesis beginning at E6.5 and extending through E17.5. A high degree of *Nmyc* expression is maintained in the nervous system during the entirety of its formation. These observations have led us to propose a formative role for the *Nmyc* gene product in establishment of neural structures. Comparative studies of *Nmyc* and *c-myc* expression has also revealed a mutually exclusive pattern of expression for these two proto-oncogenes. To establish the precise role of *Nmyc* in neural development, we have performed site directed mutagenesis via homologous recombination in Embryonic Stem (ES) cells to introduce a null mutation into one allele of the *Nmyc* locus. Several such cell lines have been generated. Introduction of the null mutation into the germline of recipient animals will allow us to discern the phenotype of animals lacking *Nmyc* function and to ascertain the necessity for a functional *Nmyc* gene product during neural development. This approach should also allow us to gain insight into the co-regulation of the *Nmyc* and *c-myc* as well as establish whether or not *c-myc* is capable of functionally substituting for *Nmyc* in nervous system development. Research sponsored by the National Cancer Institute, DHHS, under contract no. NO1-CO-74101 with BRI- Basic Research Program.

CQ 113 **Identification of a Novel Neuronal c-*src* Exon Expressed in Human and Mouse Brain**

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Neuronal cells are known to express at least two different forms of the c-*src* proto-oncogene as a consequence of alternative splicing events which add an 18-nucleotide exon (the NI exon) between c-*src* exons 3 and 4. Here we report that a second neuronal exon of c-*src* is also present between c-*src* exons 3 and 4 in human and murine transcripts but not in avian transcripts. This 33-nucleotide neuronal exon (the NII exon) of c-*src* was isolated from human adult and fetal and murine adult brain-derived cDNAs. The locations of the NI and NII exons relative to exons 3 and 4 were found to be similar for both human and mouse genomic DNA: the NI exon is ~390 nt (human) or ~300 nt (mouse) from exon 3 and the NII exon is ~1000 nt (human) or ~900 nt (mouse) from exon 4. Analysis of human brain RNA revealed that the NII exon is utilized primarily in conjunction with the NI exon to yield transcripts capable of encoding C-*SRC* products possessing 17 additional amino acids. The splice between exons NI and NII is predicted to alter the sixth amino acid encoded by the NI exon from an arginine to a serine residue, producing a potentially novel phosphorylation site. Analysis of RNA from adult and fetal human brain indicated that the expression and splicing pattern of the C-*SRC* gene is developmentally regulated in the human brain.

CQ 114 DEVELOPMENTALLY REGULATED ALTERNATIVE SPLICING PRODUCES A TRUNCATED FORM OF GLUTAMIC ACID DECARBOXYLASE IN THE RAT EMBRYO, Russell J. Wyborski, Richard W. Bond and David I. Gottlieb, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110. In the adult rat brain the gene for glutamic acid decarboxylase (GAD) is expressed predominantly as a 3.7 kb transcript. Sequencing of the adult rat GAD cDNA and comparison to feline GAD demonstrates a high degree of homology (97% identity) at the amino acid level between the two species. Earlier data (Bond et al. (1988) PNAS 85, 3231-3234) demonstrated that the embryonic brain expresses an RNA transcript distinct from the adult form; however the exact structure of this form was not elucidated. We have isolated a rat embryonic cDNA which contains an 86 bp exon inserted into the coding sequence and results in an in frame stop codon. The exon is found in genomic DNA within the GAD gene where it is flanked by consensus intronic splice sites. This exon is desiginated the GAD ES (for embryonic stop) exon. On the basis of this strucutural data we propose that, early in brain development, transcripts coding coding for a truncated form of GAD are expressed. The deduced protein cannot function as a decarboxylase because the stop codon occurs upstream of the binding site for pyridoxal phosphate, an essential cofactor. Thus alternative splicing plays a crucial role in the pathway leading to the development of functional GABAergic neurons. The CNS derived cell lines B65 and C6 express a mixture of the adult and embryonic forms of GAD mRNA. They therefore are useful models of CNS cells in the early phases of differentiation.

CQ 115 EXPRESSION OF THE *neu* GENE IN DEVELOPING PERIPHERAL NERVE AND PERIPHERAL NERVE TUMORS, Anthony T. Yachnis and Jeffrey A. Cohen, Department of Neurology, University of Pennsylvania, Philadelphia, PA 19104. The *neu* gene, which encodes a putative tyrosine kinase growth factor receptor termed p185, has been implicated as a transforming gene in rat ethylnitrosourea-induced neurogliomas and schwannomas. The present studies were undertaken to determine the expression pattern of the *neu* gene in peripheral nerve and in peripheral nerve-derived neoplasms. Total tissue RNA was isolated from rat sciatic nerves on postnatal days 1, 7, 14, 21, 28 and from adults. Northern blot analysis demonstrated prominent *neu* mRNA expression on postnatal days 1 and 7 with substantially lower expression at later times. Immunoperoxidase studies confirmed the expression of p185 by Schwann cells (SC) in developing sciatic nerve. No p185 immunoreactivity was detected in mature myelinated nerves of adults. The cellular specificity of *neu* expression was determined by northern blot and automated cytofluorometric studies of neonatal rat sciatic nerve SC and several SC-derived cell lines. All cells tested expressed *neu* mRNA and surface p185. We have examined several human SC-derived neoplasms for p185 expression. Immunoperoxidase studies of twelve schwannomas and five cutaneous neurofibromas demonstrated prominent staining in all cases. Although the level of staining varied from region to region, immunoreactivity was seen throughout the tumors. These studies demonstrate that the *neu* gene and its protein product, p185, are expressed by SC during peripheral nerve development and suggest that p185 plays a role in normal and neoplastic SC proliferation.

CQ 116 MOLECULAR ANALYSIS OF THE FUNCTION AND REGULATION OF ELAV IN D. MELANOGASTER. Kwok-ming Yao and Kalpana White, Department of Biology, Brandeis University, Waltham, MA 02254.

elav (embryonic lethal, abnormal visual system) is a gene required for the development and maintenance of the nervous system in *D. melanogaster*. *in situ* hybridization analysis and antibody localization study showed that the gene is expressed in all developing and mature neurons. DNA sequence data suggested that the gene product is an RNA binding protein, leading to the proposal that this protein is involved in the RNA metabolism of neurons. Enhancer trap experiments showed that a neural specific enhancer-like element is present near *elav*. Such an element was localized by studying the expression of transformants carrying elav-ß-galactosidase transcriptional fusion constructs. Both the 5' untranscribed sequence and the first intron were found to contain elements important in specifying the neural specific expression. The homologous gene from a distantly related species *D. virilis* was cloned and sequenced. Sequence comparison allowed the identification of conserved regions of functional and regulatory significance within the coding and regulatory sequences, respectively.

Molecular Approaches to Neurobiology

CQ 200 CORRELATION BETWEEN ECOTROPIC MURINE LEUKEMIA VIRUS PROVIRAL CONTENT OF C58 AND AKR MICE AND SUSCEPTIBILITY TO AGE DEPENDENT POLIOMYELITIS, Grant W. Anderson and Peter G.W. Plagemann, Department of Microbiology, University of Minnesota, Minneapolis, Minnesota 55455. Development of murine age dependent poliomyelitis (ADPM) is mediated by the infection and subsequent killing of motor neurons by neurovirulent strains of murine lactate dehydrogenase-elevating Virus (LDV). Susceptibility of mice to ADPM requires the presence of several well defined parameters including old age, cyclophosphamide or X-irradiation treatment, homozygosity at the permissive allele for N-tropic retroviral replication FV-$1^{n/n}$ and the presence of several copies of endogenous ecotropic murine leukemia virus (MuLV). The expression of ecotropic MuLV RNA in the motor neurons of susceptible mice has been spatially and temporally correlated with susceptibility of the cells to infection with LDV. Expression of this endogenous retrovirus has been shown to be induced in spinal cord motor neurons of C58 and AKR mice in an age dependent manner and via treatment with cyclophosphamide or X-irradiation. We will present evidence showing substrains of AKR and C58 mice differ greatly in their susceptibility to ADPM as well as in their endogenous ecotropic MuLV proviral profiles. ADPM susceptible AKR/Boy mice carry one more provirus then ADPM nonsusceptible AKR/J mice suggesting a possible role for this ecotropic provirus in determining an ADPM susceptibility phenotype.

CQ 201 ALTERNATIVE SPLICING GENERATES NEUROPEPTIDE DIVERSITY IN INDIVIDUAL NEURONS, J. Bogerd, W.P.M. Geraerts, H. van Heerikhuizen*, Biological Laboratory and *Biochemical Laboratory, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

The central nervous system (CNS) of the freshwater snail *Lymnaea stagnalis* is a simple CNS, consisting of a small number (15,000) of large identifiable neurons which are clustered in 11 ganglia and can be readily manipulated.
Neurophysiological studies showed that two neurons (one positioned in the visceral ganglion, VD_1, the other in the right parietal ganglion, RPD_2) play an important role in the modulation of cardio-respiratory functions.
A number of VD_1/RPD_2 specific cDNA clones have been isolated after differential screening of a λgt10 cDNA library of the CNS of *Lymnaea stagnalis*. *In situ* hybridization shows that in addition to VD_1 and RPD_2 a number of small neurons contain homologous transcripts. The full length cDNA clone codes for a 129 amino polypeptide, exhibiting the characteristic features of a neuropeptide precursor. The architecture of this precursor molecule shows a high level of homology with the two R15 neuropeptide precursors of *Aplysia*. A peptide (α1) derived from one of the *Aplysia* precursors has been shown to be involved in osmoregulation and modulation of heart rate. The homologous *Lymnaea* peptide may serve similar functions.
The two different *Aplysia* precursors are translated from mRNAs generated by alternative splicing. To investigate whether the same mechanism is used in *Lymnaea* to generate transcript diversity, we have performed PCR experiments in which the pertinent transcripts were specifically amplified, subcloned and sequenced. In this way we identified at least 10 different, alternatively spliced, transcripts. As a result, at least 10 different polypeptide precursor molecules could in principle be generated leading to the formation of 10 different α peptides (homologous to the *Aplysia* R15 α peptides). However, in each precursor the putative β and γ peptides (homologous to the *Aplysia* R15 β and γ peptides) are not changed. Thus, if individual peptides within a polyprotein govern individual components of a behavioural or physiological program, regulated alternative splicing would provide a mechanism by which distinct but overlapping sets of peptides could generate distinct but overlapping behavioural or physiological programs.

CQ 202 NEURONAL CELL EXPRESSION OF THE β-AMYLOID PRECURSOR IN THE DEVELOPING MOUSE EMBRYO. MD Carman, MS Blakeley, D Games, I Lieberburg. Athena Neurosciences Inc, So. San Francisco, CA. Deposition of a 42-aa fragment of the β-amyloid precursor protein (β-APP) in plaques is the hallmark of Alzheimers Disease. To gain insight into the function of β-APP we studied the expression of β-APP in the developing mouse embryo. Sagital and parasagital sections from e11.5, e12.5, e13.5, e14.5, and e16.5 embryos were stained with antisera raised against a bacterial fusion protein containing aa 444-592 of the human β-APP. Sections were lightly pretreated with proteinase K, and detected using a Vectastain kit. No consistent pattern of staining was evident at e11.5 and e12.5. Specific, cell-associated staining was detected in the developing dorsal root ganglion (DRG) at e13.5. This pattern of staining was much more prevalent at e14.5. Staining of the g. Gasseri and the trigeminal nerve tract was also evident at e14.5. The immunoreactivity was most intense at e14.5, less at e15.5 and essentially nonexistent at e16.5. Expression was limited to the neuronal cells in the DRG and g. Gasseri. These ganglia are involved in extending neurites to target organs at e13.5 to e15.5 Thus β-APP, which is expressed at e13.5 to e15.5, may play a role in cell adhesion and/or neurite outgrowth in these cells. We are currently examining which splicing variants are expressed during embryogenesis using S1 protection assays and Western blots.

CQ 203 ESTABLISHMENT OF OCULAR TISSUE CELL LINES BY USE OF PROTOONCOGENES K. Dutt,* M. Scott,* P. Sternberg,** M. Delmonte,*** N. Aggarwal,**** A. Srinivasan.***** Morehouse School of Medicine, Atlanta, Ga,* Emory University, Atlanta, GA,** Kellogg Eye Center, Ann Arbor, Michigan,*** University of Texas Health Science Center at San Antonio, Texas,**** Wistar Foundation, Philadelphia, PA.*****

In our effort to develop tissue culture models for various ocular diseases, we have attempted to establish continuous cell lines in vitro using viral oncogenes and protooncogenes. Human primary retinal pigmented epithelial (RPE) cells (4-6th passage) were transfected with simian virus 40 virion DNA, simian virus 40 large T antigen gene, H-ras (Val 12), c-myc protooncogene and adenovirus EIA gene. These genes were transfected alone or in various combinations into RPE cells. Colonies of cells were noted in the transfected cultures within two weeks after transfection. Cell lines were generated from foci of cells. We have compared the biologic properties of the RPE cell lines established with the help of oncogenes, cell lines established spontaneously in vitro and the primary RPE cells. The parameters used for comparison include growth kinetics, serum independence, phagocytosis of rod outer segments, polarity (by Ouabain binding) and expression of specific cellular genes.

CQ 204 THE POSTSYNAPTIC 43K PROTEIN CAUSES CLUSTERING OF NICOTINIC ACETYLCHOLINE RECEPTORS IN *XENOPUS* OOCYTES. Stanley C. Froehner*, Charles W. Luetje#, and Jim Patrick#, *Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03756 and #Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.
A critical step in the formation of a synapse is the accumulation of neurotransmitter receptors in the postsynaptic membrane. Clusters of nicotinic acetylcholine receptors (AChR) appear on developing myotubes within hours of innervation and eventually accumulate in adult skeletal muscle on the crests of the postjunctional folds at very high densities. A peripheral membrane protein of Mr 43,000 (43K protein) is known to be associated with AChR in the synaptic membrane and has been implicated in the process that anchors AChR at synaptic sites. To assess the role of the 43K protein in the induction of receptor clustering, we have expressed AChR in *Xenopus* oocytes alone or with the 43K protein and examined the receptor distribution by immunofluorescence microscopy. Oocytes injected with *in vitro* synthesized RNA encoding the muscle AChR α, β, γ, and δ subunits expressed functional surface receptors that were diffusely distributed. On oocytes in which injection of RNA transcripts for the AChR subunits was accompanied by RNA encoding the muscle 43K protein, AChR were organized into clusters approximately 0.5 to 1.5 microns in diameter. 43K protein-induced clustering of AChR was not caused by an increase in the receptor concentration in the oocyte membrane. Furthermore, lectin labeling showed that the 43K protein does not cause clustering of all surface membrane proteins. These results provide the first direct evidence that the 43K protein causes clustering of nicotinic receptors. The AChR clusters formed on oocytes are much smaller than those that arise spontaneously on cultured skeletal muscle myotubes and might constitute precursors destined to coalesce into larger complexes through participation of cytoskeletal or other peripheral membrane proteins.

CQ 205 **BRAIN CAM KINASE IV AND TESTIS CALSPERMIN APPEAR TO BE DIFFERENTIAL SPLICING PRODUCTS OF THE SAME GENE, John Glod, David A. Jones, and James M. Sikela, Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262. A variety of important functions in brain and testis are thought to be regulated through calcium-dependent mechanisms. Calcium/calmodulin-dependent protein kinase type IV (CaM kinase IV) and calspermin are calcium-dependent calmodulin binding proteins found in brain and testis, respectively. Comparison of data from two independent reports on rat cDNAs for these proteins suggested that the proteins could be derived from the same gene. We have confirmed this in mouse by comparing the sequences of a brain CaM kinase IV cDNA with a calspermin cDNA from mouse testis. Comparison of the mouse cDNA sequences to each other and to a mouse genomic clone indicates that 1) the cDNAs are identical over most of the calspermin sequence but abruptly diverge near the 5' end of calspermin, and 2) an intron occurs precisely where the calspermin and CaM kinase IV cDNA sequences diverge. Northern blot experiments indicate that the 5' end of calspermin appears to be produced by an exon that is expressed only in testis, and that an mRNA encoding CaM kinase IV is also present in testis. Therefore it appears that transcripts of the CaM kinase IV gene are differentially processed in brain and testis to produce a kinase in both tissues and also a calmodulin binding protein in testis that lacks a kinase catalytic domain.**

CQ 206 A MAJOR AMYLOID RNA IN HUMAN BRAIN IS SPECIES SPECIFIC AND MAY ENCODE A NOVEL SOLUBLE PROTEIN, J. Steven Jacobsen, Arthur J. Blume and Michael P. Vitek, Molecular Neurobiology Group, CNS Research, Lederle Laboratories Division, American Cyanamid Company, Pearl River, NY 10965

Using an S1 nuclease protection assay, we have identified a new form of Amyloid Peptide Precursor (APP) RNA in human brain which does not comigrate with the 695, 751 and 770 forms. The sequence of a 1.6 Kbp cDNA clone corresponding to this new form predicts the synthesis of a 365 aa protein that is similar to the amino-terminal end of APP 770 but lacks the Beta-Amyloid-Peptide and any hydrophobic transmembrane spanning regions. Theses features suggest that an Amyloid Related Protein of 365 aa (ARP 365) is soluble, contains a Kunitz protease inhibitor domain and a novel carboxy-terminus.

S1 analysis of ARP 365 RNA shows it to be more abundant than APP 770, APP 751 and ARP 563, less abundant than APP 695 and virtually absent from mouse and rat brain RNAs. We have confirmed our S1 analysis results by RPC analysis which uses Polymerase Chain reaction to amplify cDNAs resulting from Reverse transcription of target RNAs. With RPC, we have found that human brain, but not mouse or rat brain RNAs, program the synthesis of a 420bp DNA corresponding to an ARP 365 transcript. Northern blots of human brain and HL60 RNAs probed with ARP 365 specific riboprobes yield complex patterns. These data suggest that humans express ARP 365 RNA and perhaps its cognate protein which have not been detected in rodents. Thus, amyloid plaques, a hallmark lesion of Alzheimer's disease brains which have not been reported in mouse and rat brains may require the participation of ARP 365 for plaque formation.

CQ 207 DOG AND POLAR BEAR AMYLOID PEPTIDE SEQUENCE IS IDENTICAL TO THAT OF HUMAN ALZHEIMER'S DISEASE AMYLOID PEPTIDE, E. M. Johnstone[1], M. O. Chaney[2], F. Norris[1], R. Pascual[3] and S. P. Little[1], Molecular Genetics Research[1] and Organic Chemistry Research[2], Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285 and University of California[3], Berkeley, California 94720.

Neuritic plaque and cerebrovascular amyloid deposits have been detected in aged monkeys, dogs and polar bear, and have rarely been found in aged rodents. To determine if the primary structure of the 42-43 residue amyloid peptide is conserved in species that accumulate amyloid plaques, the region of the beta amyloid precursor that encodes the A4 peptide was amplified using polymerase chain reaction, sequenced, and the predicted amino acid sequence compared to those species where amyloid accumulation has not been detected. The sequences of rabbit, cow, sheep, pig and guinea pig were also obtained and compared. We conclude that the primary amino acid sequence of dog and polar bear and other mammals is conserved and that the species where amyloid has not been detected (rat, mouse) may represent a divergence. Furthermore the predicted secondary structure of rat and mouse amyloid differs from that of amyloid bearing species by its lack of propensity to form a beta sheeted structure.

CQ 208 "SITE-SELECTED" TRANSPOSON MUTAGENESIS OF *Drosophila* BRAIN-SPECIFIC GENES. Kim Kaiser, Stephen F. Goodwin and Colin D. Milligan, Department of Genetics, University of Glasgow, Church St., Glasgow G11 5JS, U.K.

We are using subtraction cloning methods to isolate genes expressed specifically in the brain of the fly, *Drosophila melamogaster*. A criticism of such approaches has been that it can be difficult to establish gene function. "Site-selected" transposon mutagenesis (Kaiser and Goodwin, PNAS in press) circumvents this criticism. In brief: The polymerase chain reaction (PCR) allows the detection, even within small amounts of DNA, of specific DNA sequences and configurations of sequences. Thus a transposon within any genetic locus can be detected by PCR using one primer specific for the transposon and one primer specific for the genetic locus. Due to the sensitivity of PCR, one such individual can be detected within a large excess of flies without a transposon at the locus. We have exploited these findings in order to detect and isolate rare individuals in a population of mutagenised flies that carry a P-element inserted into a target gene. Rather than sacrifice the flies themselves we carry out PCR on eggs laid by mutagenised flies. Then, by sub-division of the population in successive stages, we can "home-in" on a rare fly with the desired characteristics merely by identifying at each stage the sub-population in which at least one individual is laying eggs with the appropriate chromosomal configuration. The method is fast, simple and general and is being used by us for the mutagenesis of brain-specific genes.

CQ 209 A COLLECTION OF cDNA CLONES WITH UNIQUE EXPRESSION PATTERNS IN MOUSE BRAIN, Kikuya Kato, MRC Molecular Genetics Unit, Hills Road, Cambridge, CB2 2QH, England

A total of 950 cDNA clones were randomly selected from mouse cerebellar cDNA libraries, and of these, about 130 clones were found to correspond to mRNAs whose expression was unequally distributed between the cerebellum and other parts of mouse brain. Their distribution patterns in adult mouse brain were determined by *in situ* hybridization, and eight clones were found to be restricted to specific regions of the brain. Four clones were specific to cerebellar granule cells, and one clone was specific to Purkinje cells. Another 27 clones were preferentially expressed in a diverse but distinctive subpopulation of brain cells. Among them seven clones were especially abundant in specific nuclei, and three in specific fibre bundles. DNA sequence analysis of these clones is in progress. One of them has significant homology with *Torpedo californica* 43 kd postsynaptic membrane protein.

CQ 210 CHARACTERIZATION OF A NOVEL SYNAPSE SPECIFIC PROTEIN IN THE MURINE CNS, Eileen M. Lafer, Rui J. Sousa, Shibin Zhou and Nancy Hrinya Tannery, University of Pittsburgh, Pittsburgh, PA 15260.

To further investigate the molecular biology of the synapse, we prepared a monoclonal antibody library against a protein preparation enriched in synaptosomal proteins, and screened this library immunohistochemically to identify synapse/nerve-terminal associated antigens. The antibodies were then used to characterize the antigens biochemically by western blot analysis, to determine their distribution in tissues by immunohistochemistry, and to isolate cDNA clones by screening cDNA expression libraries. The primary structures of these antigens were then deduced from the sequences of the isolated cDNA clones. The cDNA clones were also used to generate probes for northern blot and *in situ* hybridization analyses of the expression patterns of these antigens. Here we report the characterization of the antigen reactive with monoclonal antibody F1-20. Immunohistochemistry, immunoelectron-microscopy, and western blot analysis reveal that the F1-20 antigen is a synapse associated, brain specific, 190,000 M_r protein. Northern blot analysis indicates that probes generated from a cDNA clone hybridize to a single brain specific mRNA of approximately 4.8 kb. F1-20 mRNA levels increase abruptly at postnatal day 4 and protein levels increase abruptly at postnatal day 7. *In situ* hybridization experiments demonstrate that F1-20 mRNA expression is neuronal specific. Analysis of its deduced amino acid sequence reveals that it has not been described previously at the primary structure level.

CQ 211 CLONING AND EXPRESSION OF RAT CATECHOL-O-METHYLTRANSFERASE (COMT) Kenneth Lundström, Marjo Salminen, Anu Jalanko, Pertti Panula, Eero Castren, Tuula Karhunen, Carola Tilgmann, Nisse Kalkkinen and Ismo Ulmanen, Orion Corporation, Orion Pharmaceutica, Helsinki, Finland, Department of Anatomy and Institute of Biotechnology, University of Helsinki, Finland

We have cloned the cDNA from rat liver and the gene of catechol-O-methyl-transferase, the enzyme inactivating catechol neurotransmitters and several neuroactive drugs in mammals. The DNA sequence revealed an open reading frame of 663 nts coding for a 221 aa long, apparently nonglycosylated and cytoplasmic, polypeptide (MW 24747). The accuracy and identity of the DNA sequence was verified by direct amino acid sequencing of tryptic peptides of purified rat liver COMT. RNA blot analysis showed that COMT transcript in liver is 1.8-2.0 kb long, which by in vitro translation in rabbit reticulocyte lysate yielded a 24 kDa immunoreactive polypeptide. The COMT gene was found in a 8 kb genomic DNA fragment consisting of four exons. Transfection of the COMT coding region in an expression vector into mammalian cells induced the synthesis of specific mRNA and protein as well as the increase of COMT activity. DNA blotting indicated that there is only one single COMT gene in rodent genomes. The distribution of COMT protein in rat tissues was studied by immunohistochemistry using polyclonal anti-COMT and anti-peptide antisera. In rat brain COMT-specific staining was found both in neurons and glial cells.

CQ 212 EBV COSMIDS ARE USEFUL EXPRESSION VECTORS FOR GENOMIC DNA IN MAMMALIAN CELLS. Susan K. McLaughlin and Robert F. Margolskee, Department of Neurosciences, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110

Epstein-Barr virus based shuttle vectors have been used to introduce cDNA libraries into lymphoblastoid cells with efficiencies of 10-15%. cDNA libraries are useful for isolating single copy genes, but problems may be encountered if mRNA is difficult to isolate from a particular tissue, or if the gene of interest is encoded by a very large mRNA. We have therefore modified an existing EBV cosmid vector which has been previously used in the construction of a genomic library. The original vector contains all the viral genes necessary for EBV replication and maintenance (EBNA and *ori* P), as well as a selectable marker (hygromycin), a polylinker cloning site, and a *cos* site for packaging into bacteriophage λ capsids. We added one or two SV40 enhancers adjacent to a flanking cloning site. To test the vector, a 14 kb fragment of genomic DNA containing the gene for the T-cell surface marker Leu-2 (CD 8) was inserted into the polylinker. EBV-Leu-2 plasmids were used to transfect human lymphoblastoid cells. Expression of the Leu-2 gene was monitored using flow cytometry and a fluorescent Ab against Leu-2. Addition of a SV40 enhancers increased both the transfection efficiency and Leu-2 expression, compared to that of the plasmid with no enhancers. After selection for hygromycin resistance, virtually all of the cells expressed Leu-2, and ≥ 70% expressed at high levels. Transfection efficiencies of 15% were obtained, which will allow the ready introduction of an entire genomic library, into mammalian cells. We will be using this cosmid vector to screen a genomic library for the expression of several receptor and channel genes. Genomic expression libraries in this vector should be generally useful as a source for many different types of genes where sequence is unavailable but ligands are know.

CQ 213 **EXPRESSION OF E. COLI *LACZ* IN TRANSGENIC MICE USING THE DOPAMINE β-HYDROXYLASE GENE PROMOTER**, Eric H. Mercer, †Gary W. Hoyle, †Ralph L. Brinster, and Richard D. Palmiter, Howard Hughes Medical Institute and Dept. of Biochemistry, Univ. of Washington, Seattle, WA 98195; †Laboratory of Reproductive Physiology, School of Veterinary Medicine, Univ. of Pennsylvania, Phila. PA 19104

The relative simplicity and accessibility of the peripheral sympathetic nervous system allows it to serve as a model for more complex neural systems. We sought to isolate a gene promoter that would enable us to direct the production of physiologically and developmentally potent proteins in sympathetic neurons of transgenic mice. The dopamine β-hydroxylase (DBH) gene promoter is a good candidate since roughly 90% of peripheral sympathetic neurons express this enzyme. DBH catalyzes the conversion of dopamine to norepinephrine, and is found in adrenal chromaffin cells and in neurons that use norepinephrine or its derivative epinephrine as neurotransmitters.

The 5' end of the human DBH gene was isolated using probes based on the published cDNA sequence. Approximately 6 kb of DBH 5' flanking region was fused to the E. coli *lacZ* structural gene, and this construct was used to generate transgenic mice. The transgene was active in the appropriate cells of the brain, adrenal glands, and sympathetic ganglia of 10.5 and 14.5 day old embryos and in adults. In addition, we detected *lacZ* product in the embryonic myenteric plexus, in which endogenous DBH is transiently present. Unexpected transgene expression was seen in the snout at E10.5 and E14.5, and in a subset of spinal cord and brain stem cells on day E10.5. We are currently doing experiments to determine if endogenous DBH is transiently present in these sites.

CQ 214 REGIONAL DISTRIBUTION OF 5-HT1$_A$ RECEPTOR mRNA IN THE RAT BRAIN BY IN SITU HYBRIDIZATION, Marie-Christine Miquel, Edith Doucet, Claudette Boni, Salah El Mestikawy, Henri Gozlan and Michel Hamon, INSERM U. 288 , Faculté de Médecine Pitié Salpêtrière, 91 Boulevard de l'Hôpital, 75634 PARIS Cedex 13, FRANCE,

5-HT1$_A$ receptor mRNA was detected in the rat brain by in situ hybridization using a radiolabelled oligo probe of forty bases, corresponding to a highly selective portion of the third intracellular loop of the rat receptor (Albert et al., 1989). In situ hybridization was realized on 20 µm thick brain sections. The detection of the signal was achieved by exposure to an X-ray film. The external cell layer of the dentate gyrus was highly labelled, as well as, to a slightly lesser extent, the external cell layer of the CA$_1$, CA$_2$ and CA$_3$ hippocampal regions. The abundance of the 5-HT1$_A$ receptor mRNA was lower in the dorsal raphe nucleus and finally, the lateral septum and the frontal cortex appeared to be uniformly slightly labelled. Those results were confirmed by Northern blot analysis. Both autoradiography and radioimmunohistochemistry have shown the 5-HT1$_A$ receptor protein to be distributed in the same regions of the rat brain but with some different intensities. A study of the distribution of the 5-HT1$_A$ receptor mRNA at the cellular level, as well as that of the 5-HT1$_A$ receptor protein, is presently in progress.

CQ 215 GENETICALLY ENGINEERED PC12 CELLS OVEREXPRESSING β-AMYLOID., F.A. Sandhu, C.B. La Bonne, R.E. Majocha*, B. Tate-Ostroff*, E. Walcott*, M. Ventosa-Michelman*, W.G. Chou, C.A. Marotta*, and S. Zain. Department of Biochemistry and Cancer Center, University of Rochester School of Medicine, Rochester, NY 14642 and *Harvard Medical School, Massachusetts General Hospital and The McLean Hospital, Boston, MA 02114.

Studies were initiated to establish genetically engineered cell lines that overexpress the β-amyloid (A4) region and that may be relevant to amyloid accumulation in the AD brain. We used cloned amyloid cDNA that contains a region encoding β-amyloid amino acids, along with newly developed tumor virus vectors derived from SV40, to prepare transformed cells (PNAS 86:337, 1989). i. There are morphological and immunological differences between PC12 cells transfected with amyloid DNA and control cells. ii. Certain transfected PC12 cell lines show no morphologic response to NGF stimulation. iii. Conditioned media from transfected PC12 cells contain growth-factor-like activity. We conclude that the presence of amyloid accumulation in PC12 cells causes cellular alterations that affect morphology and functioning and which may affect cellular survival. The complexity of the changes strongly implies that amyloid A4 overexpression, and/or the overaccumulation of the peptide, may initiate a cascade of events that result in the expression of new unidentified genes. Supported by AG02126 and American Health Assistance Foundation.

CQ 216 ISOLATION OF NA CHANNEL GENES BY PCR GENE AMPLIFICATION, K.L. Schaller and J.H. Caldwell, Department of Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO 80262.

Gene amplification is ideally suited for isolating members of multi-gene families. Using the Polymerase Chain Reaction, regions of Na channel genes were amplified from RNA isolated from rat brain and skeletal and cardiac muscle. Sequence analysis of these partial genes revealed that some Na channels originally discovered in rat brain (RB I and RB III) are not brain specific. A third brain cDNA (RB II) was not found in either cardiac or skeletal muscle and is possibly brain specific. Cardiac muscle expresses two forms of RB I in addition to a cardiac-specific channel found by Rogart et al. Skeletal muscle expresses two forms of RB I (NaC I and NaC Ia) and three forms of RB III (NaC III, IIIa, and IIIb). These are in addition to the skeletal muscle-specific genes found by Trimmer et al and Kallen et al. The variable gene products found here are likely due to alternative splicing. Genomic clones are being sequenced to confirm this.

CQ 217 **CO-OPERATIVE CHANGES IN PRESYNAPTIC MEMBRANES OF HIPPOCAMPAL SLICES UNDER GLUTAMATE CONCENTRATION INCREASED, Galina G. Skibo and Dmitri A. Rusakov, Department of Physical and Chemical Biology of Cell membranes, A.A.Bogomoletz Institute of Physiology, Acad.Sci. of the Ukraine SSR, Kiev 252601, USSR. Normal hippocampal slice preparations (14-d-old rats, CA1 area) and the ones after monitored increase of extracellular L-Glutamate concentration (up to 500 mcM during 0.5 hour) were studied morphometrically using an electron microscope. With the use of statistic stereological approach worked out ad hoc the distributions of main spatial parameters of the presynaptic terminals (PTs) are obtained. Sharp rise in the number of exo- and endocytotic caveolae (up to 15 per a PT), increase of their mean radius (about 1.7 time), increase of axolemma surface total area (from 2.25 to 3.39 sq.mcm, in average) are indicated for control preparations. Sufficient alterations of PT volumes are not found. The data obtained allowed to estimate the neurotoxicity-mediated changes of some PT membrane physical and chemical parameters (ductility-elasticity, binding energy density of over-membrane polymolecular clasters with glycocalix etc.) connected with vesicular recycling. The role of such changes in the developing of general neurotoxicity fenomena (e.g. synaptic plasticity and kindling) is discussed.**

CQ 218 EXPRESSION OF PCD5 REGULATORY ELEMENT-LAC Z FUSION GENES IN THE NERVOUS SYSTEM OF TRANSGENIC MICE, Sylvie F. VANDAELE*, Daniel T. NORDQUIST° , Harry ORR°, Alan C. PETERSON*, *Ludwig Institute for Cancer Research, 687 Pine avenue W, H3A1A1 Montreal, °University of Minnesota Box 198 UHMC, Minneapolis, Minnesota 55455. Numerous genes expressed in discrete neuronal subpopulations have now been isolated. One such gene, PCD-5 (also referred to as L7) encodes a 99 aa protein expressed by cerebellar Purkinje cells and retinal bipolar neurons. In an attempt to define the regulatory elements conferring this highly restricted expression pattern, we have analyzed the expression of chimeric constructs composed of Lac Z and PCD-5 flanking sequences in transgenic mice. One line bearing 4kb of 5'flanking DNA expresses Lac Z in Purkinje cells and in superior colliculus neurons. Three lines bearing 0.4kb of 5' flanking DNA also express Lac Z in Purkinje cells and in a set of different "ectopic" neuronal populations including subpopulations of hippocampal and dorsal root ganglia neurons. Unexpectedly, three of these lines have a variable mosaic pattern of Lac Z expression in the Purkinje cell population in some cases reminiscent of but not identical to Zebrin mosaicism. These results indicate that truncated PCD-5 regulatory elements are recognizable in both Purkinje cells and in further neuronal populations subserving a diversity of nervous system functions. It therefore seems probable that part of the mechanism conferring cell type specific expression to genes such as PCD-5 involves DNA regulatory elements that function to block expression in "ectopic" neuronal populations

CQ 219 MOLECULAR ANALYSIS OF A COLLECTION OF NOVEL STRIATUM-ENRICHED mRNAS IDENTIFIED BY SUBTRACTIVE cDNA CLONING. Joseph B. Watson, Mental Retardation Research Center, Department of Psychiatry and Biobehavioral Sciences, UCLA School of Medicine, Los Angeles, CA 90024. While the vast majority of striatal neurons appear morphologically to fall into the medium-spiny class, there exist numerous subclasses of medium-spiny cells based on extensive analyses of known neurotransmitters and their cognate receptors in the striatum. Yet, these types of neurotransmitters and receptors are not specific to the striatum. A key question that remains is whether there exists an even more specialized class of striatal neurons that express proteins limited to striatal motor functions. I have identified cDNA clones of nine rare striatal mRNAs (0.02-0.001% abundance) by screening a rat caudate putamen cDNA library with a caudate putamen-minus-cerebellum cDNA probe generated by PERT-enhanced subtractive hybridization. Partial DNA sequence analysis indicates that the nine CPu cDNA clones correspond to novel mRNA sequences. Northern blot analysis for each CPu cDNA clone indicates that only CPu 1 detects an mRNA that may be specifically expressed in striatum. Cpu 1 mRNA (3.5 Kb) is expressed at comparatively high levels in dissected striatal tissue but barely detectable or absent from other dissected brain regions. DNA sequence analysis and In Situ hybridization studies are underway to determine the nature of the CPu 1 protein and in which striatal cells it is expressed.

Gene Regulation

CQ 300 TRANSCRIPTIONAL REGULATION OF VASOPRESSIN AND OXYTOCIN GENES BY SIGNAL TRANSDUCTION, Adan, R.A.H., Burbach, J.P.H., Rudolf Magnus Institute, University of Utrecht, Vondellaan 6, Utrecht, the Netherlands.

Vasopressin (VP) and oxytocine (OT) mRNA levels in the hypothalamo-neurohypophyseal system are critically regulated during development, lactation, pregnancy, and during changes in water balance. The aim of our studies is to characterize the mechanisms underlying the regulation of these mRNAs. One of our approaches is to determine the responsiveness of the VP and OT promoters to activation of signal transduction pathways and cytoplasmic receptors. To this end, the 5' flanking regions of rat OT (-363/+16), human OT (-382/+41) and VP (-175/+43) genes were cloned in front of the firefly luciferase enzyme which was used as reporter gene. These fusion genes, carried in a pSV2 vector, were transiently expressed in: 1. the estrogen sensitive breast tumor cell line MCF-7, 2. the neuroblastoma cell line SK-N-MCIXC and 3. the small cell lung carcinoma cell line GLC-8. The latter cell line contains VP transcripts (Verbeeck et al. submitted). Various signal transduction pathways, coupled to membrane as well as intracellular receptors, were stimulated in these cells, and luciferase activity determined. For example we found that ß-estradiol (400 µM, 16h) induced luciferase activity in MCF-7 cells transfected with plasmids containing the rat or human OT promoters. This potential, however, appeared not to be used in vivo by oxytocinergic neurons of the supraoptic and paraventricular nuclei of the rat. It was shown by immunohistochemistry that this in vivo insensitivity in the OT gene expression was due to the absence of the estradiol receptor (in collaboration with Van Leeuwen and Axelson). Future experiments concern the further characterization of sequences, including further up and down stream regions, and transcription factors involved in the regulation of VP and OT expression.

CQ 301 SELECTION OF NUCLEOTIDE SEQUENCES THAT ACTIVATE GENE TRANSCRIPTION IN MAMMALIAN CELLS, Sadamitsu Asoh, Mary Maral Mouradian#, Wha Seon Kwon, Alvaro Puga* and Marshall Nirenberg, Laboratory of Biochemical Genetics, NHLBI, #Experimental Therapeutics Branch, NINDS, and *Laboratory of Developmental Pharmacology, NICHD, NIH, Bethesda, MD 20892

Replication of polyoma virus in mouse cells is known to depend on enhancer sequences. The shuttle vector , pPyE0, was constructed, consisting of the pBR322-derivative, pAT153 (β-lactamase gene and *ori*, which both function in *E. coli* cells), and polyoma virus DNA that lacks the enhancer region. Insertion of mouse genomic DNA fragments containing putative enhancer element(s) into the pPyE0 vector thus was expected to confer upon the recombinant DNA the ability to replicate in appropriate mouse cell lines. Selection using mouse neuroblastoma or fibroblast cells as replication hosts yielded clones with inserts that stimulate DNA replication. All of the clones examined stimulated the expression of the chrolamphenicol acetyl transferase (CAT) gene (2- to 16-fold range) when inserted upstream of the CAT gene of the enhancerless CAT vector, pA10CAT2. Some, but not all of the clones tested stimulated CAT gene expression when inserted downstream from the CAT gene. DNA sequence analysis showed that one of the recombinant clones is homologous to the LTR of intracisternal A-particles, a second clone to the 5'-flanking region of the human vimentin gene, and a third clone has a 40-nucleotide repetitive TG sequence, which is expected to have the conformation of Z DNA.

CQ 302 CLONING AND STRUCTURAL ANALYSIS OF THE PORCINE CHOLINE ACETYL-TRANSFERASE PROMOTER. E.E.Baetge and C.M.Sampson. CNS Research, Bristol Myers-Squibb Company, Wallingford, CT. 06492. To begin studies aimed at delineating DNA elements responsible for targeting gene expression to cholinergic neurons we have isolated genomic clones corresponding to the porcine choline acetyl-transferase(ChAT) gene. Approximately 4.2 kb of genomic DNA corresponding to the 5'end of the porcine ChAT gene was characterized by Southern analysis and DNA sequencing. 600 bp of a 2.7 kb Sst I fragment was sequenced revealing the ATG start codon and 20 AA's before diverging at a 5' splice donor sequence. Proceeding 37 bp from the ATG start into the untranslated region, the sequence again diverges at a 3'splice junction. The intron at this junction spans approximately 2,225 bp where it rejoins the next exon at nucleotide 38 in the cDNA. This exon covers a distance of 630 bp as determined by primer extension analysis with two independent oligonucleotide primers. 20-25 bp upstream from the presumed start of transcription, a perfect "TATAAA" consensus sequence is found. We have characterized 1000 bp of DNA upstream of the TATAAA consensus by sequence analysis and have employed convenient restriction sites to couple the presumptive ChAT promoter regions to a β-galactosidase reporter gene. These constructs express β-galactosidase when transiently transfected into PC12 cells and the β-galactosidase levels can be upregulated in the presence of NGF.

CQ 303 **EXPRESSION OF THCAT FUSION GENES IN MAMMALIAN CELLS.** Joanne M. Carroll and Tong H. Joh, Laboratory of Molecular Neurobiology. Cornell University Medical College, White Plains, N.Y. 10605

Tyrosine hydroxylase catalyzes the rate limiting step in catecholamine biosynthesis. The level of TH mRNA, protein and enzymatic activity are responsive to hormonal and neural signals. To investigate the role of transcriptional activation in TH induction, a genomic clone was isolated and functional analysis of cis-acting elements is in progress. Sequences residing in the 5' upstream region of the TH gene were responsive to activation of signal transduction pathways namely, elevation of cyclic AMP by forskolin and activation of protein kinase C by the phorbol ester, TPA. Fusion gene constructs containing as little as 151 bp upstream of the transcription start site, a region containing a putative CRE, are sufficient to confer the responsivity to second messengers in several neuronal and nonneuronal cell lines. Constructs containing 500 bp of upstream sequence exhibited enhanced expression compared to the 151 bp one suggesting that important elements reside within this region. We are currently examining longer constructs for additional enhancer elements especially those responsible for restricting the expression to neuronal cells.

CQ 304 CLONING OF cDNA ENCODING α_1- SUBUNIT OF NEURONAL VOLTAGE- SENSITIVE CALCIUM CHANNEL AND REGULATION OF ITS EXPRESSION BY cAMP, Hemin Chin*, Hyung-Lae Kim*, Ernst Freese* and Marshall Nirenberg†, *Lab of Molecular Biology, NINDS, and †Lab of Biochemical Genetics, NHLBI, NIH, Bethesda, MD 20892

To isolate cDNA's corresponding to neuronal voltage-sensitive calcium channels, we prepared oligonucleotide probes based on the sequence of rabbit skeletal muscle calcium channel (Tanabe et al., Nature 328:313, 1987). Four oligonucleotides, corresponding to transmembrane domains highly conserved among voltage-sensitive ion channels, were used to screen an oligo d(T)-primed, size-selected rat brain λgt11 cDNA library. Some priming occurred from the poly(A+) tail and some from an internal oligo-A region of mRNA. We have obtained and sequenced three overlapping cDNA clones that encompass the entire coding region of L-type calcium channel. The primary structure deduced from rat brain cDNA shares a high degree of similarity to those of rabbit skeletal muscle and cardiac calcium channels in transmembrane domains, but diverges in non-transmembrane domains. On Northern blot hybridization analysis, the cDNA probe hybridized to a brain poly(A+) RNA species of approximately 9.5 kb in size. This RNA transcript was not detected in growing neuronal NG108-15 cells, but was induced when the cells were treated with agents known to increase intracellular cAMP shifting to a differentiated state. Thus, expression of neuronal L-type calcium channel gene appears to be regulated by cAMP at the transcriptional level.

CQ 305 REGULATION OF Na^+ CHANNEL GENE EXPRESSION IN THE RT4 FAMILY OF NEURAL TUMOR-DERIVED CELL LINES, **Laurel M. Donahue and Noboru Sueoka, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder CO 80309-0347.**

We study the branching in vitro of a neural progenitor "stem cell" into both "neuronal-like" and "glial-like" derivatives. The family of cell lines we use is RT4, which was isolated from an ethylnitrosourea-induced rat peripheral neurotumor. The multi-potential putative "stem cell" line (RT4-AC) gives rise reproducibly to three morphologically distinct derivative cell types and expresses both neuronal and glial properties such as voltage-dependent Na^+ and K^+ channels, S100β protein, and GFAP. Upon conversion to the derivative cell types the "neuronal" and "glial" properties segregate. We have been studying the regulation of Na^+ channel gene expression to examine the segregation of this marker. The voltage-dependent Na^+ channel consists of three proteins in rat brain (α, β_1, β_2). There are four different cDNA clones isolated from rat brain encoding α-proteins and they are designated R_I, R_{II} (two variants) and R_{III}. Using a cDNA probe that is from a highly conserved domain of all known Na^+ channel genes (gift from Dr. Gail Mandel), we have been able to detect a strongly hybridizing ~9.0 Kb RNA on Northern blots using poly (A^+) RNA from the RT4 "neuronal-like" derivatives. In contrast, barely detectable hybridization is observed using poly (A^+) RNA from the "glial-like" derivative and the multipotential "stem cell" line. RNase protection assays have revealed that while both R_I and R_{II} are expressed at low levels in all of the RT4 cell lines, the highest levels of expression are observed in the "stem cell" line and the "glial-like" derivative. R_{III} is expressed at extremely low levels in all of the cell lines. The total amount of RNA detected in RNase protection assays of the "neuronal-like" derivatives appears to be well below that observed by northern blot analysis and suggests that the RT4 "neuronal-like" derivatives are expressing other Na^+ channel genes which are not expressed at high levels in rat brain. These putative "new" Na^+ channel genes may be peripheral nervous system specific or may derive from skeletal muscle. We are pursuing these possibilities.

CQ 306 NGF INDUCTION OF GENE EXPRESSION IN RAT PC12 CELLS - A MOLECULAR ANALYSIS OF THE ORNITHINE DECARBOXYLASE (ODC) GENE, Stuart C. Feinstein, Susanne R. Muller, Shayne Y. Huff and James Chang, Neuroscience Research Institute and Department of Biological Sciences, University of California, Santa Barbara, CA 93106

In an effort to understand the molecular mechanisms by which NGF alters specific gene expression and the NGF signal transduction pathway, we have been examining the NGF mediated induction of ODC transcription in PC12 cells. ODC transcription is induced within one hour of NGF administration (Greenberg et al., J Biol Chem 260:14101). By 4 hours of NGF treatment, the levels of ODC mRNA increase approximately 10 fold (Feinstein et al. PNAS 82:5761). In the present study, we have used a mouse ODC cDNA (Gupta et al., J Biol Chem 260:2941) to isolate rat genomic clones harboring ODC sequences. Analysis of these clones, in conjunction with genomic DNA blotting experiments, suggests that there are three ODC genes (termed ODC1, ODC2 and ODC3; some subset may be pseudogenes). We next isolated a full length ODC cDNA from a PC12 library. The 5' most 163 base pairs of the PC12 ODC cDNA (all non-coding) was used as a probe and found to hybridize to only ODC1, but not to ODC2 or ODC3. We conclude that ODC1 is a transcriptionally active ODC gene. RNA blotting data confirm this interpretation and also demonstrate that ODC1 is an NGF inducible gene. Primer extension experiments define the start sites of ODC1 transcription (one major and several minor), and show that the same initiation sites are used in the same relative ratios for both basal and NGF induced transcription. Therefore, the NGF induction involves increased efficiency in the utilization of start sites as opposed to utilization of alternative sites. Sequence analysis upstream of the start site shows that it is a region tightly packed with potential sites for known specific DNA binding proteins, including a TATA box, many SP1 sites, a cAMP response element, possible serum response elements and a possible AP1/jun binding site. Functional promoter assays are underway to define the NGF response element(s), as well as to examine the roles of the many other putative binding sites. This work was supported by a grant from the Muscular Dystrophy Association.

CQ 307 DIFFERENTIAL EXPRESSION OF A HUMAN AMYLOID BETA PROTEIN-CAT FUSION GENE IN TRANSGENIC MICE, N. Fox[1], K. Ward[2], E. M. Johnstone[2], J. Schrementi[1] and S. P. Little[2], Molecular Biology[1] and Molecular Genetics Research[2], Eli Lilly and Company, Indianapolis, Indiana 46285.

Differential regulation of the amyloid beta protein gene may play a role in the pathogenesis of Alzheimer's disease. To investigate the regulation of beta gene expression in vivo, a 2.9 kb fragment of the human beta gene promoter-5' flanking region was fused with the chloramphenicol acetyltransferase (CAT) reporter gene and used to produce transgenic mice. Four germline transgenic lines were generated and analyzed for CAT expression in various tissues. Expression of the transgene was most prevalant in the brain in each line, the highest levels being present in the olfactory bulbs. Low levels of CAT activity were detected in the stomach, uterus, heart, muscle and skin of some mice. These studies suggest that the human amyloid beta gene may contain cis-regulatory sequences which are preferentially activated by trans-acting factors in the brain.

CQ 308 ONE PAROXYZMAL DISCHARGE STIMULATES TEMPORALLY DISTINCT CHANGES IN NEURONAL NGF AND IMMEDIATE-EARLY GENE EXPRESSION. Christine M. Gall, Julie C. Lauterborn, and Paul J. Isackson, Departments of Anatomy and Neurobiology and Biological Chemistry, University of California, Irvine, CA 92717. Previous work in our laboratories has demonstrated that recurrent limbic seizure activity stimulates a large increase in NGF mRNA content of the hippocampal dentate gyrus granule cells. The present study was conducted to determine if one paroxyzmal discharge (PD) is sufficient to induce increased expression of NGF mRNA by the granule cells and the temporal relationship of this event to changes in the expression of the immediate-early genes c-*fos* and TIS 8 (also known as NGF-IA). Adult Sprague Dawley rats were anesthetized with ketamine/xylazine and the perforant path was stimulated at 10Hz until a PD was recorded from hippocampus (~ 70 pulses). Paroxyzmal discharges thus initiated continued 30-45 sec. following the termination of stimulation. Rats were overdosed with sodium pentobarbital and perfused with 4% paraformaldehyde 12, 33, and 63 min. following the onset of stimulation; sections through hippocampus were processed for the in situ hybridization localization and quantification of mRNA's encoding NGF, Fos and TIS 8 using both film and emulsion autoradiographic techniques. Hybridization to both Fos and TIS 8 mRNA was increased 5-fold by 12 min. post-stimulation, increased further to over 20-fold normal levels by 33 min., and declined by the 63 min. time point. In contrast, NGF mRNA content was not significantly increased at 12 min., was increased 2.7-fold above control levels by 33 min., and was further elevated at the 63 min. time point. These data demonstrate that one PD is sufficient to increase NGF expression and that the time course of this increase is different than that of the immediate-early genes. The earlier expression of the immediate-early genes is consistent with the hypothesis that immediate-early gene products mediate changes in neuronal NGF transcription in response to physiological activity. Supported by NS26748 to C.M.G. and NS24747 to P.J.I.

CQ 309 REGULATION OF S100β GENE EXPRESSION IN THE RAT NEUROTUMOR FAMILY RT4, **Nobuko Hagiwara and Noboru Sueoka, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder,CO 80309-0347**

There have been several *in vivo* studies which suggest that common progenitor cells of both neurons and glia exist in the mammlian central nervous system. How these multipotential stem cells differentiate into two different types of cells is an important question. One approach we can apply to study this problem is to use an *in vitro* system as a model. RT4 is a family of cell lines derived from a rat peripheral neurotumor and consists of four morphologically distinctive cell types. RT4-AC ("multipotential" stem cell type) expresses neuronal markers (voltage dependent Na^+ and K^+ channels) as well as glial markers (GFAP and S100β protein). RT4-B and E ("neuronal-like" cell types) express neuronal markers but not glial markers; whereas, RT4-D ("glial-like" cell type) expresses glial markers but not neuronal markers. In culture, RT4-AC reproducibly gives rise to the other three cell types. We are interested in how segregation of gene expression in these cell lines is regulated at the molecular level . One of the glial marker genes, S100β, was chosen to study which regulatory sequences are responsible for glial specific expression. We have constructed S100CAT expression vectors, which contain S100β 5' untranscribed sequences linked to the bacterial chloramphenicol acetyl transferase gene as a reporter gene. From serial deletion analysis, we have found a cis-acting negative element which supresses S100β expression in the neuronal-like derivatives. In addition, we have found an enhancer-like sequence element in the first intron of the S100β gene. To obtain glial specific expression, the 5' cis-acting negative element is sufficient; however, to achieve a high level of tissue specific expression, down stream sequences are required. We are now working to further specify these glial specific cis-acting elements and are trying to isolate the proteins which presumably bind to these elements.

CQ 310 DIFFERENT EXPRESSION OF KROX-24, JUN, JUN B, JUN D, FOS B AND FOS PROTEINS IN ADULT CNS: DEPENDENCE ON NEUROBIOLOGICAL EVENTS.

Thomas Herdegen, John Leah and Rodrigo Bravo*, Inst. Physiology, Univ. of 6900 Heidelberg, Germany; *Squibb Inst. Med. Research, Dept. Mol. Biol., Princeton, NJ 08543. Expression of JUN, JUN B, JUN D, KROX-24, FOS B and FOS was studied in CNS of the adult rat by immunocytochemistry. The basal immunoreactivity (IR) of the proteins differed from each other as well as the IR following transynaptic neuronal activation and block of axonal transport. Basal JUN D-IR was in motoneurons, preganglionic para- and sympathetic neurons and many neurons throughout the CNS, basal KROX-24-IR was visible in the termination area of peripheral nerves and in many neurons rostral to the brainstem; basal JUN, JUN B, FOS B and FOS-IR was predominantly restricted to some areas in brain as cortical layers, colliculus or hippocampus. Noxious (but not non-noxious) transynaptic activation of spinal neurons was followed by induction of all proteins, thereby the temporal and spatial IR-pattern were comparable except for KROX-24 which was more easily induced and less stable. All IR were positively related to intensity of stimulus. Repetition of noxious stimulation provoked a delay of disappearence of JUN B, JUN D and FOS B as well as induction in additional cells in spinal cord compared to a single stimulus. Block of axonal transport and transection of nerve axons induced selectively expression of JUN and JUN D in the corresponding cell body. Inhibition of regeneration evoked a constitutive lifelong expression of JUN and JUN D in surviving neurons, whereas successful regeneration abolished these IR.

CQ 311 **TRANSCRIPTIONAL CONTROL OF PROOPIOMELANOCORTIN GENE EXPRESSION IN XENOPUS LAEVIS**, Judith A. Hewitt and Y. Peng Loh, Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, Bethesda, MD 20892.
The proopiomelanocortin (POMC) system is one of the first peptidergic systems to be expressed in early neural development, as evidenced by immunocytochemistry and in situ hybridization studies on murine and Xenopus laevis embryos in this laboratory. In order to examine the factors controlling the temporal and spatial pattern of gene activation, the Xenopus POMC gene was isolated from a genomic library using a PCR generated probe directed against 300 bp of upstream sequence. Restriction mapping showed that the isolate contains approximately 2 kb of additional sequence upstream of the start of transcription. A series of deletions of the upstream region were prepared for sequence and transcriptional control analysis, using the CAT reporter gene. These constructs will be transfected into a mouse pituitary tumor cell line (AtT20) and injected into Xenopus oocytes to look for cAMP regulatory elements. These experiments also address the species specificity of transcription factor activity. Mapping of the regulatory elements will further our pursuit of developmental mechanisms controlling POMC gene expression.

CQ 312 FUNCTIONAL ANALYSIS OF MAMMALIAN PROMOTER- AND ENHANCER-BINDING TRANSCRIPTION FACTORS. Stephen P. Jackson, Dirk Bohmann, Albert Courey, Richard Turner and Robert Tjian, HHMI, Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720
To understand the mechanism and regulation of transcriptional initiation in eukaryotes, we have isolated and characterized a number of mammalian sequence-specific transcription factors, including the Sp1 and AP-1 proteins. The isolation of cDNA clones encoding these factors has allowed us to define regions that are responsible for sequence-specific DNA binding and/or transcriptional activation. Functional dissection of Sp1 has revealed that DNA binding is imparted by three "zinc finger" motifs in the C-terminal portion of the protein, and that two glutamine-rich regions of Sp1 serve as transcriptional activation domains. Interestingly, a wide variety of other known and putative transcription factors also contain glutamine-rich regions, and at least one of these can also serve as transcriptional activation domain. AP-1 consists of a heterogeneous set of proteins that include the c-Jun and c-Fos proto-oncogene products. We have shown that homodimeric Jun and heterodimeric Jun-Fos complexes are capable of binding to AP-1 DNA recognition sequences, whereas Fos lacks the ability to form stable homodimers and bind to DNA. Mutagenesis of c-Jun and c-Fos has revealed that dimerization is mediated by "leucine repeat" motifs of these proteins, and that DNA binding is imparted by conserved basic regions immediately N-terminal to the leucine repeat structures. A transcriptional activation domain of the c-Jun protein has been mapped to a C-terminal region that is rich in proline and acidic amino acid residues. Interestingly, we have demonstrated by in vitro transcription assays that c-Jun is a much less potent transcriptional activator than its oncogenic viral counterpart, v-Jun. Analysis of Jun mutants indicates that this difference is due to a regulatory region present only in the c-Jun protein. This suggests that during retroviral transduction, a constitutively active Jun protein has been generated by deleting a negatively acting domain.

CQ 313 MODULATION OF RAT ENKEPHALIN/CAT CHIMERIC GENE EXPRESSION IN QUAIL NEURORETINA (QNR/D) CELLS, Jay Joshi and David Trisler, Lab. Biochemical Genetics, NHLBI, NIH, Bethesda, MD 20892. Enkephalins are opioid pentapeptides that function as neuromodulators. We sought to identify potential regulators of the proenkephalin gene (ENK) in neuronal cells using transient transfection assays. A chimeric ENK/CAT gene(pREJCAT-1) was constructed by inserting a rat ENK fragment(-2750/+53bp relative to the transcription start site) into a promoterless pSV0CAT derived plasmid bearing a bacterial chloramphenicol acetyltransferase (CAT) reporter gene. A neuronal clonal cell line (QNR/D) derived from a RSV-transformed quail embryo neuroretina culture (Pessac et al. Nature 302:616,1983) was transfected with this fusion gene and treated with various agents for 24 hr. Transiently expressed CAT activity in cells treated with forskolin (F,20μM), prostaglandin E_1 (PGE_1,10uM)+ theophylline (Th,1mM) and retinoic acid (RA,10μM) increased 12-,6-, and 4-fold, respectively. PMA, NGF, and calcium ionophore A23187 elicited no effect. In preliminary studies with rat C6 glioma cells, PGE_1+Th and RA had no effect whereas NGF (50ng/ml) modestly increased CAT activity indicating a possible neuronal-glial specificity of ENK regulation. Endogenous QNR/D pro-ENK mRNA levels detected by Northern hybridization [rat cDNA probe] also increased with F. PGE_1, Th and F exert their effect via cAMP-signal transduction and may increase gene expression by mediating interactions between protein factors ENKTF-1, AP-1, and AP-4 with their respective DNA binding sites. RA, a morphogen not previously reported to regulate the ENK gene, may enhance transcription via protein factor AP-2 binding to its putative cis-acting element(-72 to-78bp) in rat ENK. Our results indicate that the transacting factors are well conserved in quail suggesting the common transcription mechanism between the two species. We conclude that QNR/D cells, putative amacrine neurons, express ENK mRNA and are useful to investigate transcriptional regulation of the ENK gene in a functional subset of neurons in retina and also provide a new cell model to study neuronal gene regulation in general.

CQ 314 PROTEIN KINASE C INVOLVEMENT IN THE NGF-INDUCTION OF THE METALLOPROTEINASE TRANSIN IN PC12 CELLS, Cynthia M. Machida and Gary Ciment, Department of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, OR. 97201
In previous work, we found that nerve growth factor (NGF) induced the mRNA transcript encoding the metalloproteinase transin in PC12 cells. We report here that staurosporine -- a potent inhibitor of protein kinase C -- blocked this NGF induction of transin mRNA expression. In contrast, various protein kinase C activators augmented the NGF-induction of transin expression, but did not cause induction in the absence of NGF. These same effects were also seen in transient transfection assays with a plasmid containing a 750 base pair region of the 5' flanking region of the transin gene fused to a reporter gene, indicating that this region of the transin promoter contains DNA sequences responsible for these effects of NGF and protein kinase C. This region of the transin promoter is known to contain an AP1 consensus sequence which is believed to bind the proto-oncogene products Fos and Jun. To determine the possible involvement of these proto-oncogenes in the NGF signalling pathway, we then examined the effects of protein kinase C inhibitors and activators on the NGF-induction of c-fos and c-jun mRNA levels. We found that staurosporine blocked the NGF induction of both c-fos and c-jun expression, whereas various protein kinase C activators augmented the NGF-induced levels of these proto-oncogene transcripts. These data suggest that activation of protein kinase C is necessary, but not sufficient, for the NGF induction of transin. These data are also consistent with the notion that activation of protein kinase C is part of the signalling pathway by which NGF induces transin mRNA expression in PC12 cells, and that Fos and Jun may serve as intermediate signals in this pathway.

CQ 315 Multiple signal transduction pathways regulate c-fos expression in Swiss 3T3 cells: The role of cyclic AMP Huseyin Mehmet and Enrique Rozengurt, Imperial Cancer Research Fund, P.O. 123, London WC2A 3PX, U.K.
The promoter region of the c-fos gene contains several enhancer elements which bind sequence-specific protein factors and thereby control transcription of the gene. Using in vitro assays, a number of DNA sequences have been identified which control c-fos transcription in a signal-specific manner. The TPA-responsive element (TRE) is required for c-fos induction by phorbol esters (presumably acting through a protein kinase C-dependent pathway), whereas the cyclic AMP-responsive element (CRE) controls the transcription of c-fos induced by cyclic AMP. However, we have previously shown that physiological concentrations of cyclic AMP do not increase c-fos mRNA or protein levels in quiescent Swiss 3T3 cells. Here, we report that cyclic AMP will potentiate c-fos expression induced by distinct intracellular signals which act either through protein kinase C-dependent or -independent pathways. We propose that CRE can act in synergy with other enhancer elements, including TRE, to regulate c-fos transcription in vivo.

CQ 316 CLONING AND CHARACTERIZATION OF THE GLUTAMINE SYNTHETASE GENE, John F. Mill and Hemant J. Purohit, Lab. Molec. Biol., NINDS, NIH, Bethesda MD 20892. Glutamine synthetase (GS) catalyzes the synthesis of glutamine from glutamate and ammonia. Though expressed in a variety of somatic tissues, GS plays a central role in nitrogen metabolism and ammonia detoxification in the central nervous system. GS expression is under complex regulation by glucocorticoids, cAMP, insulin, and thyroid hormone, and is expressed differentially between astrocytes and neurons.

We used our previously described full length cDNA clone to isolate the GS gene from a rat genomic library constructed in Charon 4A. Sequence analysis has been used to determine the intron/exon structure of the gene and 2 Kb of the 5' flanking region. Several areas of potential regulatory interest have been identified in the 5' flanking region, including binding site consensus sequences for SP1, AP2, and silencer regions.

A series of deletion mutants were made by PCR amplification of the regions of interest, and cloned into the CAT expression vector pSV_0CAT. These constructs were then transfected into primary cultures of astrocytes and a number of cell lines. We have identified areas of the promoter which confer cell-type specificity of expression, and among the lines positive for expression we have found quantitative differences between the HepG2 liver carcinoma, C6 glioblastoma cell lines, and primary astrocytes.

CQ 317 TRANSCRIPTION FACTORS FOR MYELIN PROTEOLIPID PROTEIN (PLP) IN INDUCED C6 GLIOBLASTOMA CELLS. Klaus-Armin Nave and Greg Lemke, Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92138.
The elaboration of the myelin sheath by mature oligodendrocytes requires the coordinated expression of a set of myelin-specific genes, most prominently the genes encoding the structural proteins PLP and Myelin Basic Protein (MBP). We found that the transcription of the PLP gene can be studied in rat C6 glioblastoma cells when induced by high cell density or increased levels of intracellular cAMP. The 1.2 kb PLP promotor, when fused to the CAT reporter gene, is active in transiently transfected C6 glioma cells as well as in heterologous NIH3T3 fibroblasts. However, in gel mobility shift assays using various PLP promotor fragments, nuclear extracts from brain, glioma and 3T3 cells reveal overlapping but distinct sets of DNA binding proteins. By DNAase I footprint analysis we identified several binding sites on the PLP promotor for proteins that appear specific to induced C6 glioma cells. The protected regions show sequence homology with corresponding positions in the promotor of the MBP gene, suggesting that some transcription factors are shared between coordinately expressed myelin-specific genes.

CQ 318 DEVELOPMENTAL REGULATION OF GLUCOCORTICOID INDUCIBILITY OF A GLUTAMINE SYNTHETASE-CAT FUSION PLASMID IN TRANSFECTED INTACT CHICKEN RETINAL EXPLANT CULTURES. H. Pu and A.P. Young, Division of Pharmacology, College of Pharmacy and Biotechnology Center, Ohio State University, Columbus, OH. 43210. The glucocorticoid inducibility of glutamine synthetase(GS) gene transcription increases during chicken retinal development. However, glucocorticoid receptor levels are not altered during the period when the GS gene becomes hormonally responsive(i.e. after embryonic day 7, E7). We questioned whether methods of gene transfer and analysis utilized to study transcriptional control in cell lines might be applied to the intact retina in an effort to better understand the underlying mechanism by which inducibility of the GS gene is developmentally regulated.

After cloning and determining the structure of the chicken GS gene, plasmids were constructed containing either 1.3 or 2.5 kb of GS 5' flanking DNA and the initial 31 nt of exon 1 adjacent to the CAT reporter gene. Electroporation of E10 retina with either plasmid results in a measurable basal level of CAT activity. However, glucocorticoids induce an approximate 5-fold increase in CAT activity only after transfection of E10 retina with the larger of the two fusion plasmids. Therefore, glucocorticoid response elements are localized between 1.3 kb and 2.5 kb upstream of the GS transcription start site. Moreover, glucocorticoids do not produce increases in CAT activity after electroporation of E5 retina with the larger fusion plasmid. Thus, a GS-CAT chimeric plasmid exhibits the appropriate pattern of expression in transfected intact retina. These data establish that gene transfer methodology can be exploited to study transcriptional control mechanisms in the developing chicken retina.

CQ 319 *c-fos* EXPRESSION IN HYPOTHALAMUS DURING PREGNANCY, Joan Y. Summy-Long, Garret L. Yount and Jeffrey D. White, Dept. Pharmacology, Hershey Med. Center, Hershey PA and Div. Endocrinology, SUNY Stony Brook, Stony Brook, NY
The cellular proto-oncogene *c-fos* is thought to act as part of the AP1 transcription factor to mediate cellular responses to a variety of cell surface stimuli. Within the nervous system, increased *c-fos* expression and *c-fos* protein levels have been demonstrated in response to seizure, chronic peripheral nerve stimulation and ischemic damage. In the hypothalamus, *c-fos* expression is augmented in magnocellular neurons that synthesize either vasopressin or oxytocin during osmotic stimulation or chronic dehydration. Each of these paradigms share the common mechanism that increased *c-fos* expression follows repetitive activation of the neurons. Pregnancy and lactation are conditions when oxytocinergic neurons are activated, late in gestation by a continuously increased firing rate and in lactation by high frequency bursting, but under differing steroid milieus. In this study we examined the levels of *c-fos* mRNA in hypothalamic tissue extracts from female rats at 7,14,18 and 20 days of pregnancy using nuclease protection analysis and found these levels to be progressively elevated above baseline. In preliminary studies, we have evaluated *c-fos* expression during lactation and have determined that hypothlamic *c-fos* mRNA levels are increased following suckling but rapidly decline following removal of the suckling stimulus. In situ hybridization-immunohistochemistry studies are currently in progress to determine if increased *c-fos* expression is localized to oxytocinergic neurons. These studies indicate that increased *c-fos* expression in neurons does not require high frequency stimulation and can be induced in physiological states associated with moderate increases in physiological activity and neuropeptide gene expression. NSF BNS-8909205 (JYS-L); NIMH 42074, 00801 (JDW)

CQ 320 TRANSCRIPTIONAL REGULATION OF PERIPHERIN GENE EXPRESSION DURING NGF-MEDIATED NEURONAL DIFFERENTIATION, Mary Ann Thompson, Deirdre Lawe, and Edward B. Ziff, Department of Biochemistry,New York University Medical Center, New York, NY 10016.

We are studying the induction of neuronal differentiation by nerve growth factor (NGF) as a model for how an external stimulus causes a cell to enter a particular pathway of differentiation. In vitro, NGF treatment of PC12 cells, a cell line derived from a rat pheochromocytoma, results in acquisition of a neuronal phenotype by the cells. Differential screening of cDNA libraries from PC12 cells which were untreated or treated with NGF for two weeks identified 4 mRNAs which are increased in PC12 cells between 4 hours and 2 days after NGF exposure, corresponding to the time at which the cells develop neuronal characteristics (Leonard, Ziff, and Greene, Mol. Cell. Biol. 1:3156, 1987). This time course is in contrast to the rapid induction of immediate early genes such as c-fos. We have focused on the transcriptional regulation of one of these genes: peripherin, a neuronal-specific type III intermediate filament protein. Nuclear run-on assays show an increased rate of transcription of the peripherin gene after 12 hours of NGF treatment (E. Gizang-Ginsberg, unpublished data).

Genomic cloning and sequencing of the peripherin gene have shown that the promoter has a canonical TATAA box and several potential CCAAT elements and GC box elements. A hybrid construct which contains 3.85 kb of peripherin 5' flanking sequence coupled to the CAT reporter gene is induced 6 to 10 fold by NGF. Deletion mapping of this region reveals a distal positive regulatory element greater than 1kb upstream from the transcriptional start site, a negative modulating element between -305 and -110, and proximal positive promoter elements. NGF responsiveness is retained when only 110 bp of promoter sequence remains. Tissue-specific expression requires sequences other than those present in 3.85 kb of 5' flanking sequence. The results of fine mapping of the NGF-responsive element will be presented, as well as an analysis of factors binding to these functional elements.

CQ 321 STIMULUS-SPECIFIC EXPRESSION OF AN INSULIN-RELATED GENE FAMILY IN THE LIGHT GREEN CELLS OF THE *LYMNAEA STAGNALIS* CENTRAL NERVOUS SYSTEM,

Harm van Heerikhuizen[1], Guus Smit[2], Pascal Favrel[1,2] and Wijnand Geraerts[2], Departments of Biochemistry[1] and Endocrinology[2], Vrije Universiteit, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

The central nervous system of the freshwater pulmonate *Lymnaea stagnalis* consists of ~15,000 large, identifiable neurons, many of which are peptidergic in nature. Within the cerebral ganglia four groups of in total ~200 cells are located which are involved in the peptidergic neuroendocrine regulation of growth and energy metabolism, the 'light green cells' (LGCs). These LGCs are shown to express at least 4 members of a 6-membered gene family coding for proteins that are strongly related to insulin (MIPs, molluscan insulin-related peptides). All 6 genes of the MIP gene family have been fully characterized and appear to have an intron/exon structure similar to the one found in vertebrate insulin genes: exon 1 encoding the major part of the 5'-untranslated region, exon 2 encoding the rest of the leader sequence, the signal peptide, the B-chain and a few amino acids of the C-peptide, whereas exon 3 encodes the remainder of the C-peptide, the A-chain and the 3'-untranslated region. We show that various environmental conditions, e.g. different feeding behaviour, result in a strongly altered steady state concentration of the different MIP-RNAs, suggesting that the MIP genes are subjected to a stimulus-specific transcriptional regulation. We have set out to locate the cis-acting elements involved in transcription regulation of the MIPs (both the basal transcription, the cell-type specific transcription and the stimulus-specific transcription) by constructing MIP-promoter/luciferase gene fusions. These constructs are currently being injected into *Lymnaea* neurons (both LGCs and other neurons that normally do not express MIPs) in *in vitro* incubated complete central nervous systems. If luciferase production in one or a couple of injected cells can be reproducibly measured, we will start assaying similar constructs having (partially) deleted promoters to pinpoint the regions of interest.

CQ 322 BI-PHASIC RESPONSE OF HIPPOCAMPAL DYNORPHIN EXPRESSION FOLLOWING SEIZURE, Jeffrey D. White, Garret L. Yount, Julie C. Lauterborn and Christine M. Gall, Div. Endo. SUNY Stony Brook, Stony Brook, NY and Dept. Anatomy and Neurobiology, UC Irvine, Irvine CA.

The opioid peptide dynorphin (DYN) is expressed at high levels in dentate gyrus granule cells in the rat hippocampus. Following hilus-lesion (HL) induced recurrent limbic seizures, all granule cells also express enkephalin (ENK) at high levels while basal expression is quite low. Thus, granule cells are capable of simultaneous expression of two opioid peptide genes. In the present study we investigated the directionality and magnitude of changes in DYN expression following HL-induced seizures to examine the regulation of expression of co-localized transmitters. Unilateral HL were placed sterotaxically in ketamine-xylazine anesthetized male Sprague-Dawley rats. This procedure reliably produces bilateral electroencephalographic seizures within 90 min of lesion placement that recur for up to 10 hr thereafter. At selected times after HL, animals were killed and dentate gyrus subfields were dissected from hippocampus. Total RNA was isolated from these samples and the amount of preproDYN mRNA present in each individual sample was measured by nuclease protection analysis. PreproDYN mRNA levels were found to increase above control values by 6 hr post-HL but by 24 hr were depressed below control values and remained below control values for up to 96 hr post-HL. Preliminary in situ hybridization analyses confirmed these changes in preproDYN expression occurred in granule cells. These findings starkly contrast with those for ENK expression and suggest ENK and DYN may serve different roles in hippocampal physiology and that the control mechanisms for regulating opioid peptide gene expression are not universal. NIMH 42074, 00801 (JDW); NIH NS 26748 (CMG)

CQ 323 GONADOTROPIN RELEASING HORMONE GENE EXPRESSION IN NEURONAL CELL LINES DERIVED FROM TRANSGENIC MICE.

David B. Whyte, Jolene J. Windle, Pamela L. Mellon, The Salk Institute, La Jolla, CA 92037.

Regulation of the gonadotropin releasing hormone (GnRH) gene has been difficult to study due to its highly specific expression in only a very small number of hypothalamic neurons. We have derived immortal cell lines from tumors of these neurons induced in transgenic mice by a hybrid gene expressing SV40 T antigen under the control of the GnRH 5' regulatory region. Although transformed, these cells retain many of the characteristics of hypothalamic GnRH-secreting neurons. To investigate the regulation of GnRH gene expression, we have transfected these cells with a hybrid gene containing the GnRH promoter on the CAT reporter gene. Expression of this promoter is tightly restricted to the GnRH neuronal cell lines; no expression is seen after transfection into glial cells, fibroblasts, or PC12 cells. Transfection with 5' truncated GnRH promoter contructions has established that elements between 1100 and 800 base pairs upstream of the transcription start site are essential for expression. Regions of DNAse I protection by nuclear proteins from these cells are found in this region centered on partially palindromic sequences. An oligonucleotide representing one of these palindromes binds a factor which is apparently specific to nuclear extracts of the GnRH neuronal cell lines.

CQ 324 HORMONAL REGULATION OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE AND THE ADRENERGIC PHENOTYPE, **Dona Lee Wong, Carolyn L. Bildstein, and Stephanie A. White,** Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305.

Adrenal medullary phenylethanolamine N-methyltransferase (PNMT) appears to be hormonally regulated in distinct ways during ontogeny and in the mature rat. Developmentally, steroids are capable of inducing the synthesis of PNMT, elevating the enzyme beyond normal values. ACTH treatment significantly increases PNMT when administered to pups between the ages of P0-P6 and P4-P10. A slight, albeit less significant rise in PNMT occurs in pups administered this hormone between P2-P8. In contrast, in the mature animal, glucocorticoids do not control the synthesis of the enzyme but alter the rate of enzyme degradation. If steroids are depleted through hypophysectomy, PNMT activity and PNMT protein are reduced five-fold. Both enzyme activity and protein are restorable by ACTH treatment. However, restoration of the enzyme in the adult never exceeds normal values. In addition, PNMT mRNA while showing a slight depression following glucocorticoid depletion, is not elevated by glucocorticoid replenishment. Current efforts are directed at defining in greater detail the critical period during adrenergic ontogeny where glucocorticoids are inductive and capable of stimulating the synthesis of PNMT and the switchpoint, where in the mature animal, glucocorticoids become permissive and capable of only restoring the enzyme to basal levels.

Signal Transduction

CQ 400 INTRACELLULAR CA^{2+} STIMULATES THE ACTIVITY OF DIHYDROPYRIDINE-SENSITIVE CALCIUM CHANNELS THROUGH A CA^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE

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Dihydropyridine-sensitive Ca^{2+} channels in the plasma membrane of mammalian neurons regulate Ca^{2+} influx associated with neurite outgrowth and synaptic plasticity. In the absence of dihydropyridines, these voltage-activated channels respond most frequently with short bursts of very brief openings, <1 ms on average, when the membrane is depolarized more positive than -20 mV. In the presence of dihydropyridine agonists like BAY K 8644, the channels respond to depolarization with much longer openings, >10 ms on average, at a significantly lower threshold of activation near -45 mV, the approximate "resting" potential of many spontaneously active neurons. Consequently, Ca^{2+} influx increases dramatically. We now report that the channels can be stabilized in this long-opening mode of activity without any dihydropyridines by a Ca^{2+} and ATP-dependent process which can be reconstituted in cell-free patches with a purified Ca^{2+}/calmodulin-dependent kinase. Thus, the long-opening mode appears to be an endogenous conformation of the channel that is stabilized by phosphorylation and/or binding of dihydropyridines. Further accumulation of intracellular Ca^{2+} above 10 μM rapidly inactivates the dihydropyridine-sensitive channels by a process that requires cAMP-dependent phosphorylation to reverse (Armstrong '89 *TINS* **12**:117). Whether intracellular Ca^{2+} transients enhance or inhibit channel activity in a particular set of circumstances will be determined by the magnitude and location of the transient, and by the asymmetric regulation of Ca^{2+}-dependent kinases and phosphatases.

CQ 401 PROTO-ONCOGENE TRANSCRIPTION INDUCTION BY MUSCARINIC RECEPTOR ACTIVATION IN NEUROBLASTOMA CELLS, Noel J. Buckley and Gillian A. Gajtkowski, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.

Nuclear phosphoproteins such as c-fos and NGFI-A can be induced by a wide range of extracellular signals and have been proposed to act as a link between membrane activation and gene transcription in the nucleus. Neurotransmitter receptor activation leads to acute changes in membrane properties and second messenger metabolism, but can also lead to longer term effects such as neurotransmitter gene activation, receptor down regulation and activation of mitosis. In order to examine the link between receptor activation and gene activation we have stimulated muscarinic receptors on the surface of neuroblastomas that express endogenous or recombinant muscarinic receptor genes. After 15, 30, 60 and 120 mins of stimulation, RNA was harvested and subjected to Northern blot analysis using oligonucleotides corresponding to N-terminal domains of c-fos and NGFI-A. Activation of m3 receptors on SH.SY5Y neuroblastoma (coupled to PI metabolism) leads to a stimulation of c-fos and NGFI-A transcription whilst activation of m4 receptors on NG108-15 neuroblastoma X glioma cells (coupled to inhibition of adenyly cyclase) has no effect. No induction of c-fos or NGFI-A was seen in unstimulated cells. These effects are seen in both dividing and differentiated cells and are abolished by muscarinic receptor antagonists and attenuated by PKC inhibitors. We have also examined the effects of receptor activation on c-fos and NGFI-A induction in NG108-15 and CHO-KI fibroblasts transfected with recombinant muscarinic receptor genes. These data support the notion that activation of neurotransmitter receptors coupled to PI metabolism and PKC activation can induce transcription of nuclear transcription factors. This may provide a mechanism to link receptor activation to long term changes in the nervous system.

CQ 402 DEVELOPMENT AND REGULATION OF $5HT_{1c}$ AND $5HT_2$ RECEPTORS IN RAT BRAIN. Roland D. Ciaranello, MarkW. Hamblin and Bryan L. Roth, Department of Psychiatry, Stanford University School of Medicine, Stanford, CA 94305 $5HT_{1c}$ and $5HT_2$ receptors are down-regulated by certain antidepressants. Treatment with mianserin, a serotonergic antagonist, results in a decline in the density of both receptor subtypes by 60-80%. This dramatic response to antagonist treatment prompted us to investigate whether there were consequent changes in receptor-specific mRNA as a result of drug treatment. Our results indicate that rats treated with mianserin for 21 days show a 70% decline in brain $5HT_{1c}$ receptors; *in situ* hybridization with cRNA probes localize this receptor primarily to the choroid plexus. $5HT_{1c}$ mRNA, measured by quantitative Northern analysis or solution hybridization shows a dynamic response of receptor mRNA to chronic blockade: initially, there is a transient, nonsignificant decline in mRNA levels, which rapidly rebound to >150% of basal levels. Despite this, the receptor levels remain profoundly reduced. These results suggest the existence of a signaling mechanism between the receptor and the cell nucleus.

We will also describe our studies on the developmental expression of $5HT_{1c}$ and $5HT_2$ receptor mRNA in rats. $5HT_{1c}$ mRNA can be observed by E17 of gestation, and undergoes a rapid increase of over 6-fold between days P5 and P17. In contrast, $5HT_2$ mRNA can be barely detected by E17, undergoes a modest increase around P2 and achieves adult levels around P7; it's levels do not increase further.

CQ 403 DIFFERENCES IN CROSSTALK BETWEEN ADENYLATE CYCLASE AND PHOSPHATIDYLINOSITOL (PI) SIGNALLING SYSTEMS IN BRAIN AND MODEL CELL SYSTEMS. Dermot M.F. Cooper and Christine L. Boyajian. Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

Evidence is accumulating on the various levels of interaction between individual signal transduction systems within cells. Much of this evidence arises from *in vitro* studies; unambiguous proof of interactions in intact cells is more difficult to obtain. We have been concentrating on the interplay between Ca^{2+}-mobilizing and cAMP-generating systems at the level of signal generation. In most brain regions, Ca^{2+} concentrations corresponding to those achieved upon membrane depolarization or activation of the PI system, stimulate adenylate cyclase activity via calmodulin. This permits synergistic stimulation of neurotransmitter synthesis and release in response to a single stimulus. However, in a number of peripheral systems and cultured cell lines (including platelets, NCB-20 and GH3 cells) these concentrations profoundly inhibit (~45%) plasma membrane adenylate cyclase activity. This effect is independent of calmodulin, although highly cooperative for Ca^{2+} ions. It requires a stimulated activity state of adenylate cyclase, and is not sensitive to the actions of pertussis toxin. In intact NCB-20 cells, bradykinin, which stimulates PI hydrolysis and Ca^{2+}-mobilization, causes a significant inhibition of cAMP production, which is not due in any part to stimulation of cAMP phosphodiesterase activity. Blockade of the mobilization of Ca^{2+}, by the intracellular chelator, MAPTAN, diminishes the effects of bradykinin. These data indicate that Ca^{2+} plays diverse roles in modulating the responsiveness of cAMP-generating systems, which may vary between cell types to accommodate specific physiological roles.

CQ 404 STABLE EXPRESSION OF *SUBSTANCE K RECEPTOR* IN CHO:K1 CELLS: MOBILIZATION OF INTRACELLULAR CALCIUM AND STIMULATION OF MITOGENESIS

Harald R. Eistetter, Alessandro M. Capponi#, Marie-Francoise Schulz, Eric Kawashima and Ann Mills, Glaxo Institute for Molecular Biology, 46 route des Acacias, and #Division of Endocrinology, Department of Medicine, University Hospital, CH-1211 Geneva, Switzerland.

A mammalian expression vector encoding bovine *substance K receptor* (***SK-R***) under the transcriptional control of the SV40 early promoter has been constructed. Chinese Hamster Ovary (CHO:K1) cells were co-transfected with a plasmid conferring neomycin resistance (pSVtkneo), and stable transfectants were selected. As assessed by binding of radiolabeled ligand (*neurokinin A* [*NKA*]) to whole cells, recombinants (***CHO/SK-R***) were found to express from about 15,000 to 25,000 receptors/cell, the calculated dissociation constants being in the range of 1 to 100 nM. *NKA* induced a concentration-dependent and biphasic rise of cytosolic free calcium concentration ([Ca2+]*i*) in cells bearing recombinant receptors, as determined with the fluorescent probe *fura-2*. A significant increase in [Ca2+]*i* could already be observed at *NKA* concentrations as low as 2.5 nM, the maximal response occuring at 1 uM. Moreover, as measured by incorporation of tritiated thymidine into cellular DNA, ***CHO/SK-R*** cells were found to proliferate when exposed to the ligand. Within a 24 hours interval up to 45% increases in *de novo* DNA synthesis were observed. The effects described were neither found in non-transfected CHO:K1 cells, nor in cells that had been transfected with the selection plasmid only. Our results indicate that *substance K receptors* may act like growth factor receptors in certain cells.

CQ 405 ISOLATION AND CHARACTERIZATION OF GENOMIC AND CDNA CLONES ENCODING A DROSOPHILA SEROTONIN RECEPTOR WHICH ACTIVATES ADENYLATE CYCLASE. R. Hen, P. Witz, Borrelli E., L. Maroteaux and N. Amlaiky*. LGME/CNRS - U184/INSERM - Institut de Chimie Biologique, (*Institut de Pharmacologie, URA DO 589 CNRS), Faculté de Médecine, 11 rue Humann, 67085 STRASBOURG Cédex - France.

A new serotonin receptor gene was isolated employing low stringency hybridization with oligonucleotides corresponding to a consensus sequence present in G protein-coupled receptors. The deduced protein which is 453 amino acids in length, contains seven putative transmembrane domains characteristic of G protein-coupled receptors. Sequence comparisons reveal highest homology with the human 5HT1A receptor. Probes derived from this receptor cDNA hybridize to a single mRNA species, 5kbp in length which is found predominantly in adult heads. In order to generate stable cell lines expressing this Drosophila serotonin receptor we introduced into mouse NIH_3T_3 cells an expression vector containing the receptor cDNA together with the gene encoding resistance to the antibiotic neomycine. In these transformed cells serotonin induced an increase in cAMP levels that was concentration-dependent and saturable. The concentration of serotonin required for half maximal stimulation was 10^{-7}M and stimulation was inhibited by ergot alkaloïds such as LSD and dihydroergocryptine. We will discuss the relation between this new serotonin receptor and other vertebrate and invertebrate serotonin receptors which stimulate adenylate cyclase. In addition, we are investigating a possible involvement of this receptor in the control of circadian rythm.

CQ 406 PURIFICATION OF THE BRADYKININ RECEPTOR USING AFFINITY AND LECTIN CHROMATOGRAPHY, Kurt Jarnagin, Barb Daine-Matsuoka, Sunil Bhakta, Department of Molecular Biology, Syntex Research, Palo Alto, CA 94304

The bradykinin receptor in guinea pig ileal membranes can be solubilized with CHAPS. The solubilized receptor is found to have an apparent Kd of 2.5nM. Binding of [^{3}H] bradykinin to these solubilized receptors is inhibited by bradykinin, and the Thi^{5,8}D-Phe^7 BK antagonist but not by des-Arg^9-BK. The binding activity is not inhibited by a cocktail of protease inhibitors which completely inhibit the proteolysis of bradykinin, thus the binding site is not a proteolytic enzyme. The binding activity can be purified at least 5 fold by chromatography on wheat germ lectin sepharose or concanavalin A sepharose. Approximately 50% of the binding activity elutes with the column break through on lectin columns. The binding activity can be purified at least 5000 fold by chromatography on a bradykinin affinity column. The molecular weight of the most prominent band after affinity chromatography is approximately 150,000 kd.

CQ 407 GABA$_A$-RECEPTOR; FUNCTIONAL EXPRESSION AND SITES OF GENE TRANSCRIPTION IN RAT BRAIN, P. Malherbe*, T. Giller*, J.G. Richards*. E. Persohn*, E. Sigel§, G. Trube* and H. Möhler+, *F. Hoffmann-La Roche Ltd, Basel, §Institute of Pharmacology, University of Bern, +Institute of Pharmacology, University of Zürich, Switzerland

The synaptic action of γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the mammalian CNS, is initiated mainly by its interaction with a specific GABA$_A$/benzodiazepine receptor complex. The structural requirements for fully functional GABA$_A$ receptors in mammalian brain are not yet known. Various subunit cDNAs were cloned from rat brain (α_1, α_2, α_3, α_5, α_6; β_1, β_2, β_3; γ_2), which could then be expressed in either Xenopus oocytes or mammalian cells. GABA$_A$ receptors, expressed from α, β plus γ_2 subunits, display bi-directional modulation by benzodiazepine ligands, i.e. enhancement by diazepam and inhibition by DMCM.
In situ hybridization histochemistry in rat brain allowed us to visualize different patterns of subunit gene expression. Distinct neuronal populations, resulting from the assembly of particular subunits, could be identified in many brain areas. Pronounced differences in hybridization intensities can be seen with both α- and β-subunit variants, thus reflecting a heterogeneity in the subunit composition of GABA$_A$ receptors *in vivo*. In the near future, investigations of antibodies against oligopeptides specific for subunit variants might reveal the precise cellular distribution of these receptor components in the CNS.

CQ 408 CLONING AND CHARACTERIZATION OF A PEPTIDE-LIKE RECEPTOR IN DROSOPHILA. Luc Maroteaux and Dominique Monnier. Institut de Chimie Biologique, CNRS, Faculté de Médecine, 11, rue Humann, 67085 Strasbourg Cedex, France.

There is increasing evidence that biologically active peptides originally isolated from vertebrates are also present in invertebrates. Fruit fly has a number of attractive characteristics which suggest that they could be used to study neuropeptides functions: Immunoreactivity with antiserum to substanceK as well as isolation of a gene coding for a related FMRFamide neuropeptide and a gene coding for a peptide showing an obvious similarity to the mamalian cholecystokinin (CCK), have been recently reported in Drosophila melanogaster. Using oligonucleotides corresponding to the conserved part of the sixth and seventh transmembrane domain of the known G-protein coupled receptors we performed PCR reaction on D.melanogaster genomic DNA. From this experiment, we cloned a fragment related to the mamalian substanceK, and we used it as probe to screen D.melanogaster head cDNA library. Out of 500 000 plaques we found 5 positives. Sequencing of the longest clone reveal a fairly strong homology to the mamalian substance P receptor. In Situ hybridization experiments will be presented as well as expression into Cos7 cells and ligand binding properties.

CQ 409 CHANGING THE PARADIGM FOR THE ACQUISITION OF NUCLEOTIDE SEQUENCE DATA, McLeod, M. J., Gilna, P., Burks, C., Cinkosky, M.J., Tomlinson, L.J., Hayden, J.E., Benton, D*, and Ryals, J*. **GenBank**, Los Alamos National Laboratory, Los Alamos, NM 87545 and (*) IntelliGenetics, Inc., Mountain View, CA. 94040. The present exponential increase in the volume of nucleotide sequence data being generated when combined with the projected data from large scale genome sequencing projects mandates a major shift of emphasis in the policies for acquisition of this data by the nucleotide databanks. We document the developments at GenBank to ensure that such data will be collected and made available to the public. Policies governing support of author-initiated submission of data, links to large-scale sequencing projects, automated data submission, increased access by, and availability of the data to the public will be presented.

CQ 410 THE EFFECT OF INSULIN AND IGF-I ON GROWTH AND DIFFERENTIATION IN NEUROBLASTOMA CELLS IS DEPENDENT ON THYE STAGE OF NEURONAL DIFFERENTIATION. Sven Påhlman, Gabrielle Meyerson, Irja Johansson. Dept of Pathology, University of Uppsala, Uppsala, Sweden. The human SH-SY5Y neuroblastoma cells differentiate into neuron-like cells when treated with 12-O-tetradecanoyl-phorbol-13-acetate (TPA). This differentiation, which is induced in fetal calf serum containing medium, is accompanied by growth inhibition. Using a defined, serum-free, medium we have previously shown that insulin, insulin-like growth factor (IGF) I and IGF-II are mitogens for untreated SH-SY5Y cells, and that the corresponding receptors are expressed in these cells. After TPA treatment the cells do not replicate when stimulated with either of these three factors. However, the insulin receptor and the IGF-I receptor still are expressed by these cells and the receptors can mediate early pre-replicative responses to IGF-I and insulin, such as receptor autophosphorylation, actin reorganization and c-fos induction. Our hypothesis has been that these receptors changes during neuronal differentiation from acting in a mitogenic pathway to take part in non-mitogenic signal transduction, important for the development or function of the differentiating neuroblastoma cell. Here we show that insulin and IGF-I, respectively, strongly potentiate neurite outgrowth of TPA-treated SH-SY5Y cells cultured in serum-free medium. At the biochemical level this is supported by an insulin and IGF-I induced potentiation of the expression of the axonal growth cone associated protein, GAP 43, meassured at the mRNA level.

CQ 411 BRAIN SPECTRIN INTERACTS WITH SMALL SYNAPTIC VESICLES, Aleksander F. Sikorski, Ian S. Zagon and Steven R. Goodman, Department of Structural and Cellular Biology, University of South Alabama, College of Medicine, Mobile, AL 36608. In the current study we have investigated the interaction of brain spectrin with 50 nm diameter synaptic vesicles. Small synaptic vesicles, radioiodinated with ^{125}I-Bolton-Hunter reagent were incubated with brain spectrin covalently immobilized on cellulosic membranes (Memtest, 0.65 um) in a Bio-Dot filtration apparatus. Binding was found to be rapid (equilibrium is reached in 1 min), salt dependent (optimum at 50-70 mM NaCl), and pH dependent (optima at 6.0 and 7.4 -7.8). Scatchard analysis revealed a biphasic binding isotherm suggesting that spectrin binds to at least two distinct classes of noninteracting sites: high affinity (K_D~30 ug/ml) and low affinity (K_D~127 ug/ml). Synaptic vesicles extracted with high salt solution displayed very little binding capacity towards brain spectrin. In an overlay experiment where vesicle protein was separated by SDS PAGE, transferred onto nitrocellulose filter, and incubated with ^{125}I-spectrin, the bands binding the most spectrin were synapsins Ia, Ib, IIa and IIb. Isolated synapsin I was found to competitively inhibit the binding of ^{125}I-synaptic vesicles to brain spectrin with an apparent K_i of 95-120 nM synapsin I. The above findings are consistent with the cytoskeletal-mediated release hypothesis in which the spectrin-synapsin interaction is proposed to be critical in the regulation of synaptic vesicle translocation and fusion.

CQ 412 IDENTIFICATION OF THREE GAMMA-LIKE SUBUNITS OF THE GABA RECEPTOR, Donna Wilson-Shaw and James M. Sikela, Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian brain, and the $GABA_A$ receptor is the site of action of a number of important pharmacological agents, including GABA, benzodiazepines, barbiturates, some convulsants, and possibly ethanol. A number of subunits of the $GABA_A$ receptor have been cloned and the receptor appears to be a member of a ligand gated ion channel superfamily that includes the acetylcholine, glycine, and glutamate receptors. Two common features of the cloned GABA subunits are 1) the presence of four proposed membrane spanning regions (MSRs), which when compared between subunits often show stretches of conserved amino acid sequences, and 2) the presence of a large cytoplasmic loop between the third and fourth MSRs, the sequence of which appears to be highly variable among subunits. We have utilized this information together with the polymerase chain reaction (PCR) to develop a strategy to isolate other, as yet unidentified members of the GABA receptor family. We report here the partial sequences of three cDNAs that appear to encode members of the gamma class of GABA receptor subunits. One of these is identical at the amino acid level to the rat gamma-2 subunit, and therefore is probably the mouse homolog of this subunit. However, since only two gamma subunits have been reported in the literature at least one of the remaining two cDNAs appears to represent a new member of the gamma subunit class of the GABA receptor.

CQ 413 RAT ASTROCYTES EXPRESS mRNA FOR TYPE II AND TYPE III ISOFORMS OF THE BRAIN VOLTAGE-GATED Na^+ CHANNEL. Paul J. Yarowsky*, C. Erik Olson, Robert D. Koos, and Bruce K. Krueger. Depts. of Pharmacology & Experimental Therapeutics* and Physiology, University of Maryland School of Medicine, Baltimore, MD 21201

Astrocytes, like neurons, contain voltage-gated Na^+, K^+, and Ca^{++} channels, thus providing them with the capacity for action potential generation. In order to determine if astrocytes express any of the three known types of rat brain Na^+ channel, we have used reverse transcription-polymerase chain reaction (RT-PCR) to probe for mRNA's coding for the Type I, II and III isoforms. A single pair of oligonucleotide primers (22 and 20 bases) was designed that would hybridize to two sequences nearly identical for all three isoforms. These primers bracketed similar-sized regions of the large intracellular loop between domains I and II of all three channels (508, 511, and 514 bases, respectively). The intervening sequences for each channel contained unique restriction enzyme sites. Using RNA from cultured neonatal rat astrocytes (>14 days *in vitro*), two intense bands were observed at approximately 500 bp after fractionation of the RT-PCR product by PAGE. Treatment with *HindIII*, which cleaves the Type III channel sequence at two sites, eliminated one (the lower) of the bands and produced three fragments of the expected sizes (179, 145, & 190 bp). Treatment with *PstI*, for which there is one site in the sequences of both Type II and III channels, eliminated both bands and produced two closely-spaced bands at approximately 400 bp and one band at approximately 100-120 bp (predicted fragments: Type II - 400 & 111 bp; Type III - 403 & 111 bp). *EcoRI*, which should produce bands at 277 and 231 bp when the Type I channel PCR product is present, was without effect. The same primer pair effectively promoted the amplification of an *EcoRI*-sensitive 500 bp product from whole rat brain. Thus, transcripts for Type II and Type III, but not Type I, Na^+ channel isoforms were detected in astrocyte RNA preparations. These results indicate that astrocytes contain a specific subset of the Na^+ channel isoforms present in whole brain.