CELL AND MOLECULAR ASPECTS OF THE DEVELOPING NERVOUS SYSTEM

Organizers: Theodore Slotkin and Ian Zagon

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Poster Sessions

Cell and Molecular Aspects of the Developing Nervous System

Neurodevelopment (joint)

CO 001 GENE TRANSFER INTO THE NERVOUS SYSTEM USING VIRAL VECTORS,

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Two means of gene transfer into the neonatal and adult rodent central nervous system are being explored using retrovirus and herpes virus-derived vectors. In the first system, cultured cells are genetically modified using replication-defective retroviral vectors and then grafted into the brain. Retrovirus vectors carrying coding sequences for mouse nerve growth factor (NGF) and bacterial beta-galactosidase have been used to infect dividing populations of primary astrocytes, immortalized astrocytes, and transformed PC12 pheochromocytoma cells. Astrocytes appear to be a good delivery vehicle for secreted compounds as they migrate in the brain and should cause no untoward effects. Pheochromocytoma cells become autocrine differentiated after infection with NGF-bearing vectors and can then be used as a source of dopamine to striatal neurons in Parkinsonian rodent models. In the second system, herpes virus-derived vectors are injected directly into the brain. Several mutants of herpes simplex type 1 (HSV1) have been evaluated as delivery vehicles. All are replication defective or replication compromised and contain the *lac Z* gene under viral promoters that express early during infection. Vectors with mutations in the immediate-early gene, ICP4, and an early gene, thymidine kinase, show no replication in the brain and mediate short-term expression of beta-galactosidase in only a few cells near the injection site. A vector with a mutation in the immediate-early gene, ICP0, has limited spread in the brain and short-term expression of beta-galactosidase is seen in glia and neurons in a halo around the injection site. Both gene transfer techniques provide a means of delivering neuroactive substances to focal areas of the brain.

CO 002 NEW PERSPECTIVES IN THE FIELD OF NEUROTROPHIC FACTORS.

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The recent molecular cloning of brain-derived neurotrophic factor (BDNF) (Leibrock et al., *Nature* **341**, 149-152, 1989) revealed that BDNF and Nerve Growth Factor (NGF) are members of a gene family. The primary structures of mature NGF and BDNF show striking similarities. In particular the 6 cysteine residues are strictly conserved suggesting that also the tertiary structure of the two molecules is very similar. However, the two molecules contain also structural differences sufficient to determine their distinctly different patterns of neuronal specificity. The common denominator of all BDNF-responsive neurons (so far known) is their projection to the central nervous system (CNS). Accordingly, BDNF is predominantly expressed in the CNS wherefrom the molecule was originally isolated and purified. NGF and BDNF will serve as examples to illustrate the concept of target-derived neurotrophic molecules synthesized in regionally differential limited quantities which regulate the extent of survival during embryonic development and the maintenance of specific functions of the responsive neurons. The characteristics of the physiological functions of NGF and BDNF will be compared with those of other molecules for which a neurotrophic action has been demonstrated in vitro which, however, do not fulfill the criteria of target-derived neurotrophic molecules. This will be discussed using ciliary neurotrophic factor (CNTF) as an example. Although CNTF was originally identified and partially purified as a target-derived neurotrophic molecule for parasympathetic ciliary neurons, the further analysis and in particular the recent molecular cloning (Stöckli et al., *Nature*, **342**, 920-923, 1989) demonstrated that CNTF, in spite of exhibiting neurotrophic activities on a relatively broad spectrum of neurons *in vitro*, does not fulfill the criteria of a target-derived neurotrophic molecule. In particular the developmental expression of CNTF precludes its role as a molecule regulating the extent of survival of CNTF-responsive neurons during embryonic development. Moreover, the fact that CNTF is a cytosolic molecule raises additional questions with respect to the "availability" of this molecule categorizing it either as a "lesion factor" or as a factor released by unconventional mechanisms as has been demonstrated for the release of IL-1 from activated macrophages.

Cell and Molecular Aspects of the Developing Nervous System

Extracellular Matrix and Neural Adhesion Molecules

CO 003 CELL INTERACTIONS IN NEURAL CREST CELL MIGRATION AND DIFFERENTIATION. Marianne Bronner-Fraser, Developmental Biology Center, University of California, Irvine, Ca. 92717

Neural crest cells migrate extensively along characteristic pathways in the embryo and give rise to numerous and diverse derivatives including neurons and glia of the peripheral nervous system, pigment cells, and cartilagenous elements of the face. The migratory pattern of neural crest cells in the trunk region is segmented such that neural crest cells migrate through the rostral half of each somitic sclerotome but not through the caudal half. This metameric pattern is later reflected in the segmental arrangement of neural crest-derived ganglia of the peripheral nervous system.

Present research concentrates on the role that interactions between neural crest cells and their surroundings play in influencing both cell migration and differentiation. Specifically, we examine: *i)* the functional significance of cell-cell and cell-extracellular matrix interactions in neural crest cell migration; and *ii)* the cell lineage decisions of individual neural crest cells. A combination of experimental approaches is used to study these questions, including analysis of neural crest cell behavior on defined extracellular matrix molecules *in vitro*, injection of function-blocking antibodies *in vivo*, and labelling individual neural crest cells in the embryo by intracellular injection of vital dye markers *in vivo* to follow the developmental potential of single cells.

Our results show that regionally distinct mechanisms are involved in neural crest cell migration. In cephalic regions, numerous extracellular matrix ligands are required for the normal migration of neural crest cells. This suggest that multiple or combinatorial cell interactions are important for development. In the trunk region, inhibitory interactions between neural crest cells and their surrounding environment may contribute to the metameric pattern of neural crest cell migration through the rostral half of each sclerotome. In terms of neural crest cell lineage, we have shown that many premigratory neural crest cells are multipotent, i.e. able to give rise to many and diverse derivatives. Therefore, rather than being "preprogrammed", neural crest cells are likely to respond to cues from their environment. These results suggest an important role of interactions between neural crest cells and their environment in cell migration and cell differentiation.

CO 004 NCAM AS A REGULATOR OF CELL-CELL INTERACTIONS, U. Rutishauser, Department of Genetics, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA. NCAM function can affect a wide variety of cell-cell interactions. These include specialized contacts that do not directly involve NCAM, such as neuromuscular junctions, gap junctions, signals that alter levels of neurotransmitter enzymes, and calcium-dependent adhesion junctions. In these cases, we have proposed that NCAM serves as a regulator of membrane-membrane apposition required for the initial formation and/or maintenance of the interaction. Membrane apposition is affected by changes in both the expression of NCAM and in the molecule's content of polysialic acid. The latter has the potential of allowing a cell to discriminate between interactions based on the degree of contact required. The NCAM on growing axons has a particularly high content of polysialic acid, suggesting that these membranes may use this carbohydrate to select environmental interactions that promote growth and guidance of nerves.

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CO 005 NEURAL CELL SURFACE RECOGNITION AND INTRACELLULAR CONSEQUENCES
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The transduction of cell surface recognition to intracellular events appears to be instrumental for a neural cell to decide whether to engage in stable or transient interactions with its environment. Evidence is accumulating that surface recognition can lead to adhesive or repulsive cell behaviour as a function of time of interaction. Cell behaviour is thus likely to depend on the integration of multiple signals that result from the activation of cell recognition molecules. The neural adhesion molecules L1 and N-CAM activate different intracellular signals depending on the cell type expressing them. Interaction of neural cells with the oligodendrocyte-derived J1-160 extracellular matrix molecule leads to cell type-specific adhesion or repulsion with time of interaction. Functional association of recognition molecules with ion pumps, such as the Na^+/K^+ ATPase, and ion channels have recently been found. Direct or indirect influences of recognition molecules on the cytoskeleton appear to play a decisive role in the stabilization of cell contacts between neural cells, in particular, pre- and postsynaptic membranes. These functional associations may not only be important during development, but also in the maintenance and plasticity of the adult nervous system.

Molecular Analyses of Channels and Receptors (joint)

CO 006 MOLECULAR BIOLOGICAL APPROACHES TO LONG-TERM MEMORY, Eric R. Kandel, Center for Neurobiology & Behavior, Columbia University & Howard Hughes Medical Institute, 722 West 168th Street, New York, New York 10032.

Short- and long-term memory seem to be a continuously graded process; yet inhibitors of protein or RNA synthesis block the long-term process without affecting the short-term process. Studies of sensitization in the gill-withdrawal reflex in Aplysia indicate that both the similarities and the differences between short- and long-term memory reflect intrinsic cellular mechanisms. What molecular mechanisms account for the similarities that gives memory its graded properties? What accounts for the difference in susceptibility to inhibitors of macromolecular synthesis? Using quantitative 2-D gels of phosphoproteins from sensory neurons, we find that the long-term synaptic changes are accompanied by increased phosphorylation of the same set of substrate proteins modified in the short-term. Whereas the phosphorylation accompanying the short-term process is transient and does not require new macromolecular synthesis, the phosphorylation following the long-term process persists for at least 24 hours and is dependent on translation and transcription. Thus, one of the functions of the genes and proteins required for long-term sensitization is a persistent phosphorylation of the same set of substrate proteins capable of eliciting the physiological effects seen in the short-term process. What are these genes and proteins? Analysis of ^{35}S-met incorporation into proteins on quantitative 2-D gels indicates that long-term training (or repeated exposure to 5-HT, the facilitating transmitter released by training) induces the transcription of early and late proteins. The early proteins have features similar to the immediate early proteins of mammalian cells in culture. Thus, in addition to mediating synaptic actions, modulatory transmitters important for learning, such as 5-HT, seem capable of regulating the transcriptional and translational machinery of the cell.

CO 007 THE STRUCTURE AND REGULATION OF THE NGF RECEPTORS, Eric M. Shooter, Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305-5401.

Two different types of NGF receptor are recognized on NGF-responsive neurons or PC12 cells by steady state binding, dissociation kinetics, trypsin sensitivity and Triton X-100 solubility. Crosslinking with water soluble agents identifies the rat low affinity receptor (LNGFR) as a protein of 83,000 molecular weight while crosslinking with lipid soluble agents identifies both this receptor and the high affinity receptor (HNGFR) of molecular weight 140,000. The antibody (MC192) against the rat LNGFR recognizes the crosslinked LNGFR but not the crosslinked HNGFR complex. The LNGFR comprises a single peptide chain with one membrane spanning domain and a core protein molecular weight of 42,000 with no obvious signal transduction mechanism. Comparisons of the sequences of rat, human and chick LNGFR as well as the construction of N-terminal deletion mutants identify the four cysteine-rich extracellular segments as important parts of the NGF binding domain. Elimination of the C-terminal cytoplasmic domain of the LNGFR has no effect on NGF binding. Even a construct which lacks the transmembrane spanning domain and is secreted still binds NGF. One model for the structure of the HNGFR is that it comprises the LNGFR and a second cytoplasmic protein responsible for the signal transduction and internalization. Evidence for this model is found in the shared peptides between LNGFR and HNGFR and in the restoration of a biological response in PC12 receptor minus cells after transfection with the cDNA for the LNGFR.

NGF regulates the expression of the LNGFR in PC12 cells and adult sensory neurons in vitro. In vivo trigeminal sensory neurons express low levels of LNGFR until their axons reach the maxillary process. After this time the increase in the levels of LNGFR on the sensory neurons parallels that of the increase in the levels of NGF in the target tissue. A second level of regulation is observed in Schwann cells. Release of contact between Schwann cells and axonal and myelin membranes induces LNGFR expression while reestablishment of contact represses receptor expression.

CO 008 VOLTAGE-DEPENDENT POTASSIUM CHANNELS: THE CONFLUENCE OF GENETIC AND BIOCHEMICAL APPROACHES. Bruce L Tempel, Richard A. Newitt, Lizbeth A. Adams, Khaled Houamed* and Wolfhard Almers*. GRECC, 182-B, VA Medical Center, Seattle, WA. 98108 and Depts. of Medicine, Pharmacology and *Physiology/Biophysics, University of Washington School of Medicine, Seattle, WA. 98195.

Potassium (K+) channels are a diverse family of membrane proteins, essential for controlling the excitability of many cell types. Voltage-dependent K+ channel genes were first isolated from the Shaker locus of Drosophila, using a genetic approach. Four different proteins are predicted to arise from the Shaker locus by alternative splicing. The size of the predicted proteins and the ability of each to form functional K+ channels with unique kinetics when expressed in Xenopus oocytes suggests that K+ channels may have a homomultimeric structure.

We have isolated homologous genes from the mouse. Each predicted protein is approximately 70% conserved relative to Shaker. However, in contrast to the Shaker gene, three closely related mouse K+ channel genes (MK1-MK3) are encoded by separate, intronless genes. Thus, a different strategy for K+ channel diversity is used in the case of MK1-MK3. The physiological characterization of these clones as well as their anatomical distribution will be discussed. In addition, we are mapping the chromosomal locations of several mouse K+ channel genes and will report on our ability to correlate K+ channel defects with neurological mutants of the mouse.

We have used the convulsent snake toxin, dendrotoxin, to purify K+ channel proteins from rat and bovine brain. The 65 Kd glycoprotein band which can be crosslinked to dendrotoxin is also recognized specifically by two different peptide-directed antibodies derived from the MK1 sequence. Thus, the dendrotoxin binding protein is immunologically related to the cloned MK1 gene. Protein sequencing of the 65 Kd protein as well as two smaller, co-purifying proteins is underway. Combining molecular and biochemical information, we are able to propose a model for a voltage-dependent K+ channel.

Cell Lineage

CO 009 THE USE OF MAMMALIAN CHIMERAS TO STUDY CELL LINEAGE RELATIONSHIPS DURING BRAIN DEVELOPMENT,

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Injection of dyes or application of genetically engineered viruses offer prospective approaches to the study of cell lineage relationships in the developing brain. The techniques of experimental mammalian embryology (specifically the aggregation of cells of two distinct genotypes at pre-implantation stages of mammalian embryogenesis) coupled with the availability of reliable independent cell markers allow a complementary, retrospective approach to be taken as well. For example, analysis of aggregation chimeras has provided data to support the contention that specific populations of neurons consist of numerical clones of cells that descend from a small number of progenitors defined during the earliest period of neurogenesis. To explore the spatial distribution of the various cell lineages in the three dimensional space of the CNS, we have used ß-glucuronidase as a cell marker, and plotted the location and genotype of each neuron in two regions of cerebral cortex of a $Gus^h/Gus^h \leftrightarrow Gus^b/Gus^b$ chimera. A χ^2 analysis of the variation of the Gus^b/Gus^b to Gus^h/Gus^h genotype ratio in the medial/lateral dimension revealed no significant organization to the distribution of the lineages. A similar analysis in the anterior/posterior dimension, however, revealed a highly significant organization. It appears that the neuronal lineages in cortex are arranged in "slabs" stacked in the A/P dimension. While finer subdivisions of the slabs seem possible, the overall pattern is reminiscent of other segmented structures along the neuraxis. Further, in the radial dimension, a χ^2 analysis suggests that the progenitors of the deep and superficial layers of the cortex are not identical. Overall, the data portray the ventricular zone as a complex spatial mosaic of different lineages with more highly directed spatial "instincts" than prospective experiments alone might lead one to expect. The development of transgenic mouse lines that carry the bacterial ß-galactosidase gene under the direction of the mouse Thy-1 promoter has provided an important cell autonomous marker that can be used to study the spatial intermingling of the cell lineages in the embryonic ventricular zone. Chimeras made between transgenic and wild-type embryos will permit testing of the hypotheses suggested from the analysis of the adult ß-glucuronidase chimeras.

CO 010 **DETERMINATION OF NEURONAL IDENTITY IN THE DEVELOPING MAMMALIAN BRAIN,** Susan K. McConnell, Dept. of Biological Sciences, Stanford Univ., Stanford, CA 94305.

The nervous system is populated by an astonishingly heterogeneous number of neurons, differing in their connections, neurotransmitters, morphologies, and locations. Recently we have begun to study the generation of this phenotypic diversity in the CNS of mammals. One prerequisite for exploring the state of commitment of an undifferentiated cell to its normal fate is that one can accurately predict the normal, mature fate of that cell. The mammalian cerebral cortex provides a surprisingly simple system for exploring the process of commitment to a specific phenotype during development. Neurons in the cerebral cortex are found in six layers, and each layer contains neurons with similar morphologies and axonal connections. Furthermore, neurons within a layer go through their terminal mitotic divisions at similar times during development. Thus, cell "birthday" (marked by labeling cells with [3H]thymidine during S-phase of their final division) provides an accurate tool for predicting the final position and connections of a newly-generated cortical neuron.

The relationship between a neuron's birthday and its adult fate have led us to explore two simple hypotheses that might explain this correlation. Newly-postmitotic neurons may already be committed to sitting in a certain layer and forming specific axonal projections quite early in their development, at the time of their final division; alternatively, each young neuron may be multipotent and be guided into one of many possible fates through instructive interactions with the local environment, which changes over time. To distinguish between these alternatives, we have exploited the classical definition of cell commitment: a cell is said to be committed if it continues to develop autonomously after transplantation into a new environment, differing in time or in position from the original environment. Thus we have challenged undifferentiated cortical neurons to change their normal fates upon transplantation into a host brains. Neurons that ought normally to migrate to layers 5 and 6 are labeled with [3H]thymidine, removed prior to migration, and transplanted into host brains in which upper-layer neurons are being generated. The transplanted neurons migrate out into this foreign territory, differentiate within the host cortical plate, and form axonal projections.

Our experiments have revealed that young cortical neurons become committed to their normal laminar fates around the time of their terminal mitotic division. If cells are labeled and transplanted shortly after labeling, when many cells are expected still to be in S-phase, transplanted neurons migrate to both the deep and upper layers; thus they represent a mixture of committed and multipotent cells. However, when cells are transplanted 24 hours after labeling, after all cells have gone through their terminal division in their original environment, transplanted neurons adopt positions exclusively in layers 5/6. Thus, shortly after their final division, all the neurons that migrate display a commitment to their normal fate. This evidence is consistent with the notion that neuronal stem cells are multipotent, and can be influenced by environmental cues at the time of mitosis to produce a variety of neuronal phenotypes. However, at the time of their final division, the progeny of these stem cells become committed to adopting a particular laminar position within the cortex, and to developing axonal projections typical of that layer.

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CO 011 CELL LINEAGE IN LEECH NEUROGENESIS, Gunther S. Stent, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

The body of the leech is built of 32 bilaterally symmetric segments. Each segment contains a serially iterated set of bilaterally paired, identifiable neurons: the approximately 200 CNS pairs located in the segmental ganglion and the few dozen PNS pairs located in the segmental body wall. Cell lineage tracer studies have shown that in the course of normal embryonic development more than half of the CNS neurons are derived from a bilateral pair of ectodermal cell lines called the n bandlets, the remainder being derived from three other bilaterally paired ectodermal cell lines called the o, p, and q bandlets. Each bandlet normally gives rise to cells only on its own side of the embryo. Although identified neurons arise from the precursor bandlets via determinate cell lineages, the cell division pattern does not place the neurons into their characteristic final positions. Rather, the definitive segmental pattern of neuronal positions is the product of stereotyped cell migrations.

One set of experiments examined the effects of depriving an embryo of one n bandlet on the differentiation (neurotransmitter phenotype and axonal outgrowth) and position of several identified neurons derived from the surviving n bandlet. It was found that the surviving n bandlet produces the same complement of identified neurons that it would have produced in a normal embryo. However some of these neurons had crossed the midline and occupied the normal positions of their absent contralateral homologs. These results imply that the neural precursor cells are committed to occupy a characteristic position on either body side and use extrinsic cues to find that position. Since it was found that identified neurons can take their characteristic positions in segments deprived of other (o, p and q) ectodermal bandlets, such cues are likely to be predominantly of nonectodermal origin.

The other set of experiments examined the role of the segmental mesoderm in allowing n-bandlet-derived cells to find the positions to which they are committed and in governing cell fate. In mesoderm-deprived leech embryos ectodermal morphogenesis, including the formation of segmental hemiganglia, is grossly disrupted. Furthermore, some n-bandlet-derived cells that originate on the deprived side of unilaterally mesoderm-deprived embryos cross the midline and take positions on the nondeprived side. The distribution and differentiation of identified neurons in embryos that had been unilaterally or bilaterally deprived of mesoderm implies that mesodermal tissues provide positional cues necessary for the organization of identified n-bandlet-derived neurons into normal patterns, but that neither interactions with mesoderm nor occupancy of appropriate positions is necessary for normal neurochemical differentiation. Thus ectodermal cells committed to give rise to specific neuronal types appear to use cues provided by the mesoderm to find their appropriate positions.

Learning/Plasticity (joint)

CO 012 CELLULAR MORPHOLOGICAL MECHANISMS OF THE MEMORY STORAGE PROCESS, William T. Greenough, James E. Black, Anita M. Sirevaag, Brenda J. Anderson, Krystyna R. Isaacs and Adriana A. Alcantara, Departments of Psychology and Cell and Structural Biology, Neuroscience Program and Beckman Institute, University of Illinois at Urbana-Champaign, IL 61801.

Recent studies have clearly implicated altered synaptic connectivity in the memory storage process. Prior reports had indicated that rats reared, or as adults housed, in complex, toy-filled social environments that provided many opportunities for learning had more highly branched cerebral and cerebellar cortical neurons with greater numbers of synapses per neuron, compared to rats housed individually or in small groups in standard cages. More recent reports have indicated that rats trained to solve multiple complex maze problems or to perform motor skill tasks acquire similar alterations in cerebral neuronal morphology. Current work emphasizes that these changes are *specifically related to learning, as opposed to the physical and associated neuronal activity that are necessary for learning to occur.* Synaptogenesis resulting from the induction of long-term potentiation (LTP) by electrical stimulation in the hippocampal slice *in vitro* was related specifically to those stimulus characteristics that produced LTP and not to the amount of stimulation *per se*. Synaptogenesis in the cerebellar cortex, which is also affected by associative conditioning, is dramatically altered by complex motor skill learning with comparatively little physical and neuronal activity and is not detectably affected by extensive physical and neuronal activity involving very little learning. These studies indicate that mammalian learning involves a complex of morphological events, including changes in both excitatory and inhibitory synapses as well as associated alterations in glial and vascular tissue. Supported by ONR, NIMH, and NSF.

CO 013 BEHAVIORAL AND EVOLUTIONARY ASPECTS OF THE *period* CLOCK GENE'S EXPRESSION Jeffrey C. Hall[1], Kathleen K. Siwicki[2], Jon W. Jacklet[3], William J. Schwartz[4], Bambos Kyriacou[5], and Michael Rosbash[1]
[1]Department of Biology, Brandeis University, Waltham MA; [2]Department of Biology, Swarthmore College, Swarthmore PA; [3]Department of Biological Sciences, SUNY Albany, Albany NY; [4]Department of Neurology, University of Massachusetts Medical School, Worcester MA; [5]Department of Genetics University of Leicester, Leicester UK.
The *period* gene, which was discovered in *Drosophila* based on circadian rhythm defects caused by mutations at this genetic locus, exhibits interspecific variation in terms of the amino acid coding information it contains and in regard to behavioral consequences of the gene products' expressions. These conclusions have resulted from "clone & sequence" data and from interspecific gene transfer experiments involving behavioral bioassays of the transduced *per* DNA. The relevant phenotypes are circadian locomotor rhythms and high-frequency oscillations in the males' courtship songs. Some of the pertinent molecular data have included determinations of highly conserved regions of the *per* genes among various fruit fly species. These results suggested that molecules similar to those encoded by the *Drosophila* clock gene might be detectable, in more widely ranging organisms, by an antibody generated using a "conserved region" peptide as the immunogen. Indeed, this antibody leads to staining of circadian pacemaker structures in neural tissues of many species, including molluscs and mammals. In at least one of these organisms, the level of the "*per*-like" substance fluctuates over the course of a given day. This molecular circadian rhythm is also a prominent feature of *per*'s expression in *Drosophila* itself, with respect to the mRNA and the protein encoded within this gene.

Intrinsic and Extrinsic Development Cues

CO 014 FUNCTIONAL INTERACTIONS BETWEEN GLIAL S-100$_B$ AND CNS 5-HT NEURONS
Efrain C. Azmitia[1], Daniel R. Marshak[2] and Patricia M. Whitaker-Azmitia[3]
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A neuronotrophic factor for CNS serotonergic neurons is S-100$_b$ and its release from glial cells is stimulated by 5-HT$_{1A}$ receptor agonists. A number of known CNS growth factors (NGF, EGF, Insulin and S-100 in serial dilution, 10 ug/ml to 1 ng/ml) were added daily to rat primary brainstem serotonergic cultures and growth of serotonergic neurons estimated by specific high-affinity uptake of ^{3}H-5-HT after 3 days of incubation in complete culture media (MEM + 7.5% FCS)(1). Only S-100 produced a stimulation (maximal 170% of control at 16 ng/ml) extending its known effects on chick cortical neurons (2). This stimulation was confirmed by morphometric analysis of 5-HT-immuno-reactive neurite length in cultures (147% of control at 3.2 ng/ml). Subsequent studies showed the activity to be predominately in the S-100$_b$ form compared to S-100$_a$ and not due its Ca^{++} binding property since calmodulin was without affect. Stimulation of a 5-HT$_{1A}$ but not a 5-HT$_{1B}$ or beta-adrenergic receptor in primary glial cultures releases a neuronotrophic factor into the media (5-HT-GCM) which was active on brainstem serotonergic neurons (3). Application of a polyclonal antibody raised against human S-100 (dilution 1/10,000) blocked both the neuronotrophic activity of 5-HT-GCM and of exogenously applied S-100$_b$. RIA measurements showed an increase in S-100$_b$ levels in 5-HT$_{1A}$-GCM. In vivo, a decrease in adult rat hippocampal levels of S-100$_b$ was found 3 weeks after selective (5,7-DHT) removal of 5-HT afferents. Our results suggest that 5-HT neurons can auto-regulate their own development by stimulation of S-100$_b$ from glial cells in culture and in vivo through a 5-HT$_{1A}$ receptor. S-100$_b$ and 5-HT may act as trophic factors in concert on a variety of target cells. NSF 8812892 (ECA) and NINCDS 2539102 (PMW-A).

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Thick Filament Assembly

C A015 DISTRIBUTION OF HEAVY CHAIN ISOFORMS IN NATIVE MYOSIN MOLECULES AND THICK FILAMENTS OF DEVELOPING CHICKEN SKELETAL MUSCLE, Everett Bandman, B. Kerwin, and L.D. Taylor, Department of Food Science and Technology, University of California, Davis, CA 95616.

Myosin, the major contractile protein of the thick filament, is a hexameric protein comprised of two heavy chains and 4 light chains. The heavy chain subunit is represented by a highly homologous family of isoforms which are differentially expressed during muscle development. We have produced a library of monoclonal antibodies which distinguish between the different isoforms. Using these antibodies, we investigated the distribution of myosin heavy chain isoforms in native myosin molecules, in native thick filaments, and in myofibrils *in situ* during chicken muscle development. In order to determine whether myosin could be composed of two different heavy chain isoforms we developed a double antibody sandwich ELISA. Purified monoclonal antibodies were bound to a microtiter plate and incubated with myosins purified from muscle at different stages of development. Bound myosin was then probed with biotinylated antibodies specific to epitopes present in the same isoform, or with antibodies specific to epitopes of different isoforms. Bound biotinylated antibodies were detected with streptavidin peroxidase. Our results demonstrate that very little myosin heavy chain heterodimer exists within cells expressing multiple isoforms. Studies of isolated thick filaments from muscle at these stages decorated with gold-conjugated monoclonal antibodies demonstrated that filaments could be heterogeneous in myosin content. Similar results were obtained with ultracryosections of developing muscle using gold-conjugated antibodies. However, the distribution of the different isoforms was not random in either isolated filaments or in myofibrils *in vivo*. The absence of heavy chain heterodimers in myosin synthesized *in vivo* suggests that either the mRNAs for different isoforms are spatially segregated within the cell, or that different heavy chain isoforms cannot form dimers. The latter possibility is being investigated using an *in vitro* assay for myosin assembly. Our observations on the non-random distribution of different isoforms within isolated thick filaments and myofibrils *in situ* is not compatible with a simple exchange process occurring *in vivo*. Our studies suggest that the assembly of myosin containing structures during muscle cell growth may be an ordered process occurring at specific sites within the muscle cell.

CA 016 SEQUENCE ANALYSIS OF cDNAs ENCODING AVIAN SKELETAL C-PROTEIN AND 86 KD PROTEIN: INTRACELLULAR MEMBERS OF THE IMMUNOGLOBULIN FAMILY. Steven Einheber, Kevin T. Vaughan, and Donald A. Fischman. Dept. of Cell Biology and Anatomy, Cornell University Medical College, New York, New York, 10021.

Thick filaments in vertebrate skeletal muscle are associated with a number of accessory proteins, many of which have no known function. In chicken muscle, these include: C-protein, myomesin, M-protein, M-CK, and 86kd protein. With the exception of M-CK, the primary sequence of these proteins is unknown. It has been suggested that C-protein may play a role in thick filament assembly, alignment, or muscle contraction. 86 kd protein has been shown to colocalize with C-protein in a series of periodic stripes in the crossbridge zone of the thick filament. We have begun a molecular genetic analysis of these proteins to learn more about their structure and function. cDNAs for C-protein and 86 kd protein have been isolated from a neonatal chicken pectoralis cDNA library. Sequence analysis of the C-protein clone reveals that it contains nine regions of internal homology. Six of these resemble immunoglobulin domains and three resemble fibronectin domains. Until recently, these sequence motifs have only been described in extracellular and cell-surface proteins. However, similar domains have now been reported in other proteins associated with the thick filament including smooth muscle myosin light chain kinase, twitchin and titin (Einheber and Fischman; Benian et al.; Trinick, unpublished results). In addition, preliminary sequence comparison of the 86 kd and C-protein clones indicate close homology in their carboxyl segments. Thus, the 86 kd protein may be another intracellular member of the C-2 set of the immunoglobulin family. (Supported by NIH AM32147 and MDA)

CO 017 ENDOGENOUS OPIOID SYSTEMS AND GROWTH REGULATION IN THE DEVELOPING NERVOUS SYSTEM, Ian S. Zagon, Department of Anatomy, The Pennsylvania State University College of Medicine, Hershey, PA 17033. Endogenous opioid systems (i.e., endogenous opioids and opioid receptors) regulate the growth of the nervous system. Recently, [Met^5]-enkephalin, a naturally occurring endogenous opioid derived from Proenkephalin A, has been identified as the most potent opioid peptide related to growth of the rodent brain. Neuropeptides related to μ, δ, κ, ϵ, and σ opioid receptors did not influence cell replication. This peptide serves as an inhibitory factor, and regulates cell proliferation; it is unclear whether other actions on cell migration and differentiation are influenced primarily, or secondarily. Subsequent studies revealed that [Met^5]-enkephalin interacts with a newly reported opioid receptor, zeta (ζ), to modulate growth. Using radiolabeled [Met^5]-enkephalin and homogenates of the developing rat cerebellum, specific and saturable binding was noted; the binding profile was consistent with a one-site model. Scatchard analysis revealed a binding affinity of 1.8 nM and binding capacity of 28 fmol/mg protein. Optimal binding required a cocktail of protease inhibitors. Binding isotherms were linear with protein, and dependent on time, pH, and temperature. Addition of Na^+, Mg^{++}, and guanyl nucleotides reduced binding. Displacement studies revealed that [Met^5]-enkephalin was the most potent competitor of [^{3}H]-[Met^5]-enkephalin, with ligands selective for other opioid receptor subtypes exhibiting no distinctive pattern of competition. Studies in humans also reveals the presence of the zeta receptor in early life, and its disappearance in adulthood. However, this receptor reappears in cases of neural neoplasia. These data suggest that the endogenous opioid systems play an important role in determining the ontogeny of neurons and glia, contribute to the formation and integrity of the nervous system, and are related to neural cancer and perhaps other abnormalities.

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Intracellular Communication (joint)

CO 018 EXPRESSION OF PROTEIN KINASE A GENES IN MOUSE BRAIN

G. Stanley McKnight, Gary G. Cadd, Leslay A. Correll, Kimberly V. Rogers and Christopher H. Clegg, Department of Pharmacology, University of Washington, Seattle, Washington, 98195. Cyclic AMP elicits most of its intracellular effects by activation of a small family of cAMP-dependent protein kinases. The holoenzyme form of these kinases contains 2 regulatory (R) and 2 catalytic (C) subunits and the C subunits are inactive until released from the holoenzyme by the binding of cAMP to the complex. The role of the cAMP-dependent kinases in secretion, gene induction, and ion channel regulation has been investigated by expressing mutated forms of the RI subunit (R_{AB})that confer a dominant inhibition of kinase activity. The ability of AtT20 pituitary corticotrophs to secrete ß-endorphin in response to CRF or isoproterenol is blocked by the R_{AB} mutation whereas VIP and phorbol esters continue to stimulate secretion in R_{AB} cells. Chloride channel activation by cAMP in T84 colon carcinoma cells is also blocked by expression of the R_{AB} protein although the chloride channels are still capable of responding to elevated Ca^{++}. The induction of specific genes by hormones can be prevented by the presence of the R_{AB} protein and in some cases, this mutant causes a significant decrease in the basal level of expression. These approaches allow us to demonstrate the direct involvement of the kinase in both basal and hormone induced responses.

Cloned cDNAs have been characterized which code for 4 regulatory subunit isoforms (RIα, RIß, RIIα, and RIIß) and 2 isoforms of the catalytic subunit (Cα and Cß). By using in situ hybridization techniques we have examined the levels of the various kinase subunit mRNAs in mouse brain and find a high degree of cell-type specific expression. In particular the expression of RIß is largely confined to neuronal cells in the brain and spinal cord whereas RIα is expressed constitutively in all tissues. A biochemical characterization of holoenzymes containing RIß demonstrate a 5-7 fold greater sensitivity to cAMP compared to holoenzymes containing RIα. The Cß isoform is also highly expressed and localized within the central nervous system. However, the activation of Cß containing holoenzymes is only slightly shifted to lower concentrations of cAMP. We suggest that expression of the ß isoforms may provide a mechanism for permanently altering the threshold at which the cAMP dependent kinase is activated in specific neuronal pathways.

CO 019 STIMULUS-TRANSCRIPTION COUPLING IN THE MAMMALIAN NERVOUS SYSTEM, James I. Morgan, June L. Sonnenberg, Frank J. Rauscher, III, Donna L. Cohen, James L. Hempstead and Tom Curran, Roche Institute of Molecular Biology, Roche Res. Center, Nutley, NJ 07110.

Stimulation of neurons in the central nervous system of mammals results in the rapid initiation of a series of temporally coordinated transcriptional events, that includes the induction of the *fos* and *jun* proto-oncogenes. In addition to c-fos and c-jun, a series of fos- and jun-related genes are induced in a temporally proscibed manner. The protein products of these fos- and jun-related genes form homomeric and heterodimeric protein complexes that bind to nucleotide sequences related to the transcription factor AP-1 binding site and the cyclic AMP-responsive element (CRE). Thus following stimulation the overall level of AP-1 DNA binding activity in neurons is increased for several hours, however, the composition of the transcription factor complexes changes with time after stimulation. These proteins have been proposed to function in signal transduction processes by coupling extracellular cues to long-term alterations in cellular phenotype by regulating gene expression. Stimulation of hippocampal neurons, particularly by glutamate receptor agonists, results in an activation of this cellular immediate-early gene response. In this particular context we have investigated preproenkephalin as a potential target of these genes as preproenkephalin mRNA accumulates in the hippocampus shortly after expression of Fos and Jun.. The preproenkephalin gene has AP-1 and CRE sites in its 5' regulatory sequence that are required for expression. Fos and Jun, bind cooperatively to the AP-1 site in vitro and in cotransfection assays c-fos and c-jun stimulate transcription of preproenkephalin synergistically. These observations suggest that preproenkephalin may be a target for cellular immediate-early genes in the hippocampus.

CO 020 RECEPTOR-MEDIATED CALCIUM ENTRY, Timothy J Rink, Smith Kline & French Research Limited, The Frythe, Welwyn, Herts, AL6 9AR, U.K.

There is recent evidence from measurements of cytosolic Ca, and electrophysiological measurement in cells, membrane patches and vesicles incorporated into bilayers, for a diversity of receptor-mediated calcium entry processes[1] (RMCE), defined as, any influx consequent on receptor occupation and not dependent on depolarisation that generates a biologically significant increase in cell Ca. We can sub-divide RMCE into: (1) that through 'ROCC's' receptor-operated calcium channels, where the ligand acts directly on a component of the channel complex. (2) 'SMOC's', second messenger-operated channels, where a chemical mediator generated by receptor occupancy activated the Ca entry, (3) intermediate between these may be Ca entry activated via G-proteins. We might also regard circumstances where chemical modulation, e.g. phosphorylation, activates voltage-gated Ca channels without an actual change in membrane potential as another class of receptor-mediated Ca entry. Yet another complexity is the possibility that internal Ca stores may be refilled by entry of Ca from the external medium through a "special" pathway across both the plasma membrane and organelle membrane, by-passing the cytosol. Work on smooth muscle cells, parotid gland cells, and human platelets, neutrophils and endothelium will be described, which has explored RMCE using a variety of techniques in cells loaded with fluorescent Ca-indicator dyes, including stopped-flow fluorimetry, single cell micro-spectrophotometry, digital imaging, and the combination of patch clamping with single cell fluorescence.

1. Hallam, T.J. and Rink, T.J. (1989). Trends in Pharm. Sci. 10, 8-10

Cell and Molecular Aspects of the Developing Nervous System

Cytoskeleton, Axons, and Growth Cones

CO 021 DEVELOPMENT OF THE NEURAL SPECTRIN MEMBRANE SKELETON, Steven R. Goodman, Department of Structural and Cellular Biology, University of South Alabama, Mobile, AL 36688. My colloquia presentation will deal with the mammalian brain spectrin isoforms termed brain spectrin(240/235), brain spectrin(240/235E), and brain spectrin(240/235A) [1]. Brain spectrin (240/235) is located in neuronal soma, axons, and presynaptic terminals; while brain spectrin(240/235E) (E stands for erythrocyte related) is found in neuronal soma, dendrites, and postsynaptic terminals [2,3]. A third isoform, brain spectrin(240/235A), is found in the soma and processes of astrocytes [4]. Functionally, brain spectrin(240/235) has been proposed to regulate the translocation and fusion of synapsin I containing synaptic vesicles [1]; and the neuronal spectrins bind to N-CAM 180 causing its accumulation at sites of cell-cell contact [5]. The structure of the 235 kDa ß subunits of the neuronal spectrin isoforms are beginning to be elucidated. I will report the cloning and sequencing of cDNA encoding the ß subunit of mouse brain spectrin(240/235E) and compare this sequence to the reported sequence of rbc spectrin. The use of specific DNA probes and Northern analysis to study the levels of mRNA encoding the ß subunits of the neuronal brain spectrin isoforms during mouse brain development will be described. Using isoform specific antibodies, we previously demonstrated that brain spectrin(240/235) is expressed in mouse fetal tissue and increases two fold during brain development, while brain spectrin(240/235E) is expressed beginning with the second postnatal weeks [6]. A model of the regulation of spectrin expression during mammalian brain ontogeny will be discussed.

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CO 022 INTERACTION OF MAP2 WITH THE NEURONAL CYTOSKELETON, Bernhard Brugg and Andrew Matus, Friedrich Miescher-Institut, P.O.Box 2543, 4002 Basel, Switzerland.

Microtubule-associated protein 2 (MAP2) is a 200 kDa neuronal protein that can bind to and cross-link microtubules in living cells. Little is known about how these interactions are regulated, but phosphorylation may be involved since it is known to influence the binding of MAP2 to tubulin polymers in vitro. We have studied this possibility by preparing purified MAP2 in different states of phosphorylation, injecting it into RAT-1 fibroblastic cells, where MAP2 is normally absent, and then following its distribution by immunofluorescence staining. The results show that MAP2 prepared as close to its native phosphorylation state as possible (10 mole Pi /mole) bound to cellular microtubules immediately after microinjection. Enzymically dephosphorylated MAP2 (2 mole/mole) did not initially bind to cellular microtubules but its distribution slowly changed so that 15 min after injection it was bound to microtubules. However, in cells grown in serum-free medium this redistribution did not occur, and MAP2 remained spread throughout the cytoplasm. Since protein kinase activity is high in cells grown in serum and low in those grown in serum-free medium, these results are consistent with the idea that phosphorylation of MAP2 is required for its binding to microtubules. However, further in vitro phosphorylation of MAP2 to 20 mole/mole via kinase activity that co-purifies with microtubules resulted in its not binding to cellular microtubules. We conclude that MAP2 contains various phosphorylation sites that differently influence its binding to microtubules in living cells.

CO 023 GROWTH CONE MEMBRANE PROTEINS: INSERTION AND CYTOSKELETAL INTERACTIONS, Karl H. Pfenninger, Steve Helmke, R. Owen Lockerbie, Malcolm R. Wood, Virginia Miller, Pascale Negre-Aminou, Lynn Frame and Becky de la Houssaye, Department of Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO 80262. A variety of biochemical studies on growth cone fragments isolated from fetal rat brain have resulted in new insights into mechanisms of plasmalemmal expansion and membrane-cytoskeleton interactions. Plasmalemmal expansion has been shown to be the result of a Ca^{2+}-dependent, depolarization-triggered membrane fusion event, and preliminary data on the insertion of sodium channels into plasmalemma suggest a molecular conversion from low to high affinity for saxitoxin at that time (1,2). Insertion-related molecular conversions are likely to be necessary also for proteins involved in membrane-cytoskeleton linkage. We found stabilization of the growth cone's cytoskeleton and its linkage to membrane proteins to require phosphorylation of many proteins including two likely linker proteins, c-src and pp46/GAP43 (3). Concerted regulation of these events may occur by a novel signal cascade that we have identified in growth cones and that involves Ca^{2+}-dependent phospholipase A_2 cleavage of polyphosphoinositide (4). We hypothesize that the released arachidonic acid stimulates protein kinase C and a lysopolyphosphoinositide may act as a fusogen and, perhaps, as a profilin-binding cytoskeletal regulator. Supported by: NIH grants NS24676 and NS24672; NSF grant BNS-88-12537.

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CO 024 EVOLUTION OF A GENE REGULATORY PATHWAY AND THE CONTROL OF AXON GROWTH. J. H. Pate Skene, Dept. of Neurobiology, Stanford University, Stanford, CA 94305-5401. One of the most abundant proteins in axonal growth cone membranes is an acidic phosphoprotein designated GAP-43 (a.k.a. F1, B-50, pp46, or neuromodulin). Synthesis and accumulation of this major growth cone component are developmentally regulated at the level of transcription of a single-copy gene, and expression of this and similarly regulated genes is strongly correlated with the ability of neurons to extend axons *in vivo* and *in vitro*. During development, nearly all neurons express high levels of GAP-43 mRNA and protein, but the gene is not expressed in differentiated cells outside the nervous system. Pluripotent embryocarcinoma cells fail to express detectable GAP-43 mRNA, suggesting that the neural-specific expression GAP-43 reflects positive induction of the gene during neural differentiation, rather than active repression during differentiation of other cells. As neurons mature, transcription of the GAP-43 gene declines 10 fold or more in most cells. This late down-regulation appears to reflect an active repression distinct from the positive signals that induce initial expression of the gene. Mature neurons can be induced to re-express high levels of GAP-43 mRNA by interrupting axons in the peripheral nervous system (PNS) of mammals and in certain central nervous system (CNS) tracts of fishes and amphibians. Pharmacological manipulations of these interrupted axons indicate that re-expression of GAP-43 mRNA is not mediated by altered propagation of electrical signals, nor does it require retrograde axonal transport of positive signals conveyed from the site of axon injury. Instead, axotomy appears to induce GAP-43 by interrupting the ongoing retrograde delivery of repressive signals conveyed along intact axons from synaptic terminals to neuron cell bodies. In contrast to their counterparts in fish and amphibians, many neurons in the adult mammalian CNS fail to induce GAP-43 when their axons are interrupted more than a few millimeters from their cell bodies, indicating that retrograde control of the GAP-43 gene has undergone a systematic modification during vertebrate evolution. The results show that an evolutionarily divergent, retrograde signalling pathway acts at the level of transcription to restrict expression of at least one major growth cone component in many mature neurons. The regulatory elements of the single GAP-43 gene can be expected to yield target sequences that render genes responsive to this growth-associated gene regulatory pathway.

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Intercellular Communication (joint)

CO 025 THE GLUTAMATE RECEPTOR GENE FAMILY

Heinemann, S., Bettler, B., Boulter,J., Deneris, E., Duvoisin,R., Hartley, M., Hermans-Borgmeyer, I., Hollmann, M., O' Shea-Greenfield, A., Papke, R. and Rogers, S. Molecular Neurobiology Laboratory, The Salk Institute P.O. Box 85800 San Diego, California 92138.

The glutamate receptor system is thought to be involved in the first steps of learning and memory acquisition and is perhaps the most important excitatory receptor system in the mammalian brain. We have used an expression cloning approach to identify a family of glutamate receptor genes. One gene that we have called GluR K1 codes for a functional glutamate receptor of the kainate subtype. The primary structure and the physiology of the GluR K1 glutamate receptor indicates that it is a member of the ligand-gated channel family, Hollmann,M., O' Shea-Greenfield, A., Rogers, S.W. and Heinemann, S., Cloning by functional expression of a member of the glutamate receptor family. Nature **342** 643-648 (1989).

Low stringency hybridization screening of brain cDNA libraries has identified four additional genes that code for proteins with sequence homology to the GluR K1 glutamate receptor. Two of these genes code for functional glutamate receptors. The expression of these five glutamate receptor genes has been mapped in the brain by in situ hybridization. The results suggest that the genes code for different glutamate receptors that are expressed in specific brain regions.

CO 026 BIOSYNTHESIS OF PEPTIDES FROM HIGHER MOLECULAR WEIGHT PRECURSORS: POST-TRANSLATIONAL PROCESSING ENZYMES AND SECRETION, Richard E. Mains & Betty A. Eipper, Dept. Neuroscience, The Johns Hopkins Univ.Sch.Med., Baltimore, MD 21205. The post-translational processing of peptide precursors into smaller products has been investigated in several primary cell culture systems and in cell lines transfected with cDNAs encoding foreign peptide precursors and processing enzymes. When cDNAs encoding human NPY were transfected into mouse pituitary corticotrope cells (AtT-20) and into rat somatomammotrope cells (GH_3 and GC), cleavage within the sequence -Tyr-Gly-Lys-Arg-Ser- occurred, followed by processing to yield NPY with a COOH-terminal -Tyr-NH_2. When the wildtype -Lys-Arg-(KR) pair of basic amino acids at the endoproteolytic cleavage site was replaced by -Arg-Arg- (RR), -Arg-Lys- (RK) or -Lys-Lys- (KK), different cleavage patterns were seen in the AtT-20 and GH cells. Although somatomammotrope cells do not normally cleave their endogenous hormones (growth hormone and prolactin), all of the proNPYs were cleaved to yield NPY ending with -Tyr-NH_2, with similar cleavage seen for all four pairs of basic amino acids. When the production of prolactin was dramatically increased and growth hormone production decreased by treatment of GH cells with insulin, estradiol and epidermal growth factor, the storage of wildtype and mutant NPY increased but the extent of cleavage was unaltered. By contrast, AtT-20 cells showed a striking hierarchy of cleavage site utilization, with the wild type KR and the mutant RR sites cleaved well, RK cleaved much less well, and KK showing negligible cleavage. The synthesis of high levels of the well-cleaved KR-proNPY or low levels of the poorly cleaved KK-proNPY inhibited cleavage of the endogenous pro-ACTH/endorphin. In the first weeks after birth, the synthetic rate and the extent of cleavage of pro-ACTH/endorphin to products by rat anterior pituitary corticotropes increased markedly, while cleavage of ACTH(1-39) to CLIP and ACTH(1-13)NH_2 decreased dramatically. The developmental pattern was enhanced in culture by physiological levels of glucocorticoids and reversed by the absence of glucocorticoids. The α-amidation of peptides is performed by a Cu^{++} and ascorbate-dependent enzyme (peptidyl-glycine α-amidating monooxygenase; PAM, EC 1.14.17.3). When the cDNA for the largest form of PAM and for PAM truncated after the NH_2-terminal catalytic domain were transfected into cultured cells, increased levels of PAM activity were measured using several different peptide substrates. When a vector encoding the 5' region of the PAM cDNA in the antisense orientation was transfected into AtT-20 cells, levels of PAM activity and the ability to α-amidate newly synthesized peptides were decreased. The alterations in PAM levels were largely confined to secretory granules. Support: DK-32948, DK-32949, DA-00266, DA-00097, DA-00098.

Developmental Disorders

CO 027 EMBRYONAL TUMORS OF THE PERIPHERAL NERVOUS SYSTEM: DEVELOPMENTAL AND CLINICAL IMPLICATIONS, M.A. Israel, Pediatric Branch, NCI/NIH, Bethesda, MD 20892

Some childhood malignancies are thought to arise in embryonic tissues. These tumors present unique opportunities to study the maturation of specific cellular lineages and to examine the possible role of alterations in the regulation of differentiation in tumor development. Several features of neuroblastoma, a tumor thought to arise in cells originating in the embryonic neural crest, suggest that it may be particularly useful in this regard. For example, neuroblastoma has been well-documented to regress spontaneously; and both spontaneous and treatment-induced maturation to a benign tumor, recognizable by the presence of well-differentiated neuronal features, has been described. In studies to examine the role of altered maturation in the development of this tumor, we identified a series of markers that characterize the various cell types of the peripheral nervous system which are recognizable in neuroblastoma tumor tissues. These include cells of the chromaffin, ganglionic, and schwannian lineage. We found that the expression of these markers was temporally regulated and that the patterns of expression that we observed marked recognizable stages in adrenal gland maturation. We then examined the expression of these markers in neuroblastoma tumor cell lines and tissues and found that these could be recognized as corresponding to specific stages of adrenal gland development. During the course of these studies, we found that while many neuroblastoma cell lines and tumors seem to grow in response to mitogenic stimulation by IGF-II, the proliferation of only a small subset seems to result from IGF-II-mediated autocrine growth stimulation. Interestingly, these malignant tissues express a variety of developmental markers that suggest they arise in cells that express IGF-II physiologically during the course of normal adrenal medullary development. Current experiments directed at understanding the cellular signals by which neural crest cell maturation is mediated may provide insights of therapeutic import, since neuroblastoma tumors corresponding to some stages of differentiation respond very differently to nonspecific cytotoxic therapies than tumors corresponding to other stages.

CO 028 CARDIOVASCULAR MALFORMATIONS AND THE DETERMINATION OF CELL LINEAGE IN THE CARDIAC NEURAL CREST, Margaret L. Kirby, Department of Anatomy, Medical College of Georgia, Augusta, Georgia 30912-2000

The cardiac neural crest provides neuronal elements and ectomesenchyme to the developing heart. The neuronal elements become the parasympathetic postganglionic cholinergic ganglia found in the cardiac plexuses and responsible for decreased chronotropic function of the heart in response to vagal stimulation. The ectomesenchymal cells are necessary for cardiac outflow tract septation and support the large arteries in the thorax and head. When the cardiac neural crest is removed, the heart develops without septation of the outflow tract, a condition called persistent truncus arteriosus (PTA). Even so, the cardiac parasympathetic innervation is morphologically and functionally normal. Using a combination of quail-to-chick chimeras and ablations, it was determined that the neural component of the cardiac neural crest is reconstituted from the nodose placodes when the cardiac neural crest is removed. Under normal circumstances the nodose placodes do not generate ectomesenchymal cells; however, after cardiac neural crest ablation, ectomesenchymal cells generated by the nodose placodes supported the thoracic vessels but were incapable of closing the outflow septa. When quail mesencephalic or trunk neural crest were transplanted into the cardiac region of chick embryo neural crest, neither was capable of replacing the ectomesenchymal cells derived from the cardiac neural crest, although again, these alternate areas of neural crest were competent to generate neural anlagen. Addition of mesencephalic neural crest to the cardiac region resulted in a high incidence of persistent truncus arteriosus, suggesting that ectomesenchyme derived from the mesencephalic neural crest interfered with ectomesenchyme derived from the cardiac neural crest. Trunk neural crest did not interfere with normal development of the heart. While mesencephalic neural crest seeded the cardiac ganglia with both neurons and supporting cells, this capability was limited in the trunk neural crest to the more mature regions. These studies indicate a predetermination of ectomesenchymal derivatives and a competition of neural anlagen to form neurons and supporting cells in the cardiac ganglia. Possible interactions involved in determination of cell lineages in the cardiac neural crest and nodose placodes will be discussed.

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CO 029 ROLE OF THE AMYLOID PRECURSOR PROTEIN IN DOWN SYNDROME AND ALZHEIMER DISEASE PATHOLOGY, Rachael L. Neve, Linda R. Dawes, Seth A. Fidel and Gerald A. Higgins, Department of Psychobiology, University of California, Irvine, CA 92717 and Department of Neurobiology and Anatomy, University of Rochester, Rochester, NY 14642

The isolation of the gene for the precursor of the Down syndrome and Alzheimer disease amyloid peptide, has made it possible to begin dissecting at a molecular level the processes whereby this normal protein may become altered in these diseases. The sequence of events which leads to the deposition of the small self-aggregating pathological amyloid peptide is not known. A key question concerns the causal relationship of amyloid to the neuropathology of Down syndrome and Alzheimer disease. The development of pathology in these disorders may be related to changes in the expression of the amyloid protein precursor (APP) mRNAs: we have used oligonucleotide probes that differentiate among the APP mRNAs to describe the expression of each APP transcript in the human brain. Our analyses show that one of the mRNAs is expressed selectively in the nervous system, that the messenger RNAs display different regional distributions in the adult human brain, and that the expression of these mRNAs is differentially affected in developing and aging Down syndrome brain, and in specific regions of Alzheimer disease brain.

To determine whether amyloid itself contributes to the neurodegenerative process or is simply a byproduct of that process, we transfected PC12 and fibroblast cell lines with portions of the gene for the human APP. Stable PC12 cell transfectants expressing the carboxyterminal 105 amino acids of the amyloid precursor protein gradually degenerated when induced to differentiate into neuronal cells with nerve growth factor. Conditioned medium from these cells was toxic to neurons in primary hippocampal cultures, and the toxic agent could be removed by immunoabsorption with an antibody directed against the amyloid peptide. Thus, a peptide derived from the amyloid precursor may be neurotoxic. We have shown that this neurotoxic conditioned medium is mitogenic for non-neuronal cells; neurons, which are terminally differentiated, may respond to this mitogenic agent by deteriorating. The neurotoxicity of the carboxyterminal 105 amino acids of APP is inversely correlated with neuronal density, and is ameliorated by the presence of glial cells. Altered processing or overexpression of APP in Down syndrome and Alzheimer disease may result in the generation of such a neurotoxic fragment.

CO 030 ALTERATIONS IN GENE EXPRESSION IN NORMAL, TRANSGENIC, AND ANEUPLOID MICE. Mary Lou Oster-Granite, Roger H. Reeves, and John D. Gearhart, Developmental Genetics Laboratory, Department of Physiology and Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205-2195

Modulation and modification of gene expression is requisite for normal development of the nervous system to occur. Most genes undergo a specific pattern of developmental expression in normal animals, and this pattern of expression is disrupted when too many or too few copies of a gene are present and expressed developmentally. Such a disruption occurs most frequently in mammals when chromosomal numbers are altered, a condition known as aneuploidy. In human beings, for example, the most common form of aneuploidy occurs as a result of triplication of genes present on chromosome 21, a condition known as Down Syndrome. In mice, many of these genes are present on chromosome 16. Mice with trisomy 16 exhibit many of the characteristic features observed in human beings with Down Syndrome. Thus, triplication of these specific genes and attendant imbalance of gene expression may cause these features to arise during development. In addition to studying trisomic mice, we also study transgenic mice, in which the expression of only one or a few of the genes is increased. In such transgenic and trisomic mice, we can examine the developmental expression of a variety of genes, not just those present in excess number or exhibiting excess levels of expression. We can study the various modes of expression that can occur in normal and abnormal development and the relationship of such expression to the developmental status of particular brain regions. For example, somatostatin, a peptide which acts as a neuromodulator and hormone in the adult central nervous system, is expressed transiently in particular cell populations in the nervous system during normal development. In trisomy 16 mice and in certain lines of preprosomatostatin transgenic mice, we attempt to correlate the location and distribution of cells expressing message with those immunoreactive for the functional products of preprosomatostatin, and finally, with the location and distribution of somatostatin receptors at selected pre- and postnatal stages. Still other genes, such as that encoding the amyloid precursor protein, are expressed in many, if not all cells, before organogenesis occurs. Differential splicing of this gene gives rise to a number of different peptides which vary in their location and distribution in cells in various tissues as the aging process occurs normally and abnormally, as in Alzheimer's disease. We attempt to determine whether the increased levels of message in trisomy 16 mice are due to an abundance of one form relative to others or to the cellular distribution and ontogenetic profile of the various forms. Finally, some genes, such as Growth Associated Protein 43, are expressed most abundantly during neuron development, axon regeneration, and synaptic plasticity. Peak expression of this gene in normal animals occurs postnatally, during the period when synaptogenesis is occurring most rapidly, and declines precipitously in normal adults. We attempt to compare and contrast the expression of such genes with the formation of neuron populations and the expression of specific neuronal markers, such as those expressed in the cholinergic basal forebrain, as development proceeds in normal, aneuploid, and transgenic mice.

Cell and Molecular Aspects of the Developing Nervous System

Extracellular Matrix; Neural Adhesion Molecules

CO 100 THE β-AMYLOID PRECURSOR PROTEIN MEDIATES NEURAL CELL ADHESION. Kieran C. Breen[1,2] & Brian H. Anderton[2], [1]Department of Neuroscience, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, England [2]Department of Pharmacology, University College, Belfield, Dublin 4, Ireland

Alzheimers disease has a characteristic brain histopathology which is comprised of specific lesions in the form of senile plaques and neurofibrillary tangles. The former are composed of inorganic aluminosilicate and a 42 amino acid polypeptide called the amyloid A4 or beta protein. This is produced from a larger beta-amyloid precursor molecule (β-APP). Although the exact function of the β-APP is as yet unknown, recent evidence suggests that a secreted form may play a role as a growth factor while the membrane-bound form may mediate cell adhesion. Using a differentiated neuroblastoma cell line as an experimental model, we have shown that antisera specific for the extracellular domain of the β-APP reduces cell-surface binding to a collagen substrate. Furthermore, the presence of the antisera inhibits the outgrowth of neurites from the cells. This suggests that β-APP may play a role in the mediation of neural cell-surface adhesion.

CO 101 A NOVEL ADHESIVE PROTEIN IN THE DEVELOPING PERIPHERAL NERVOUS SYSTEM, Maryellen M. Daston and Nancy Ratner, Department of Anatomy and Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45267. P30 has been described as an adhesive protein present in neurons of the young rat central nervous system, (Rauvala and Pihlaskari, 1987, J. Biol. Chem. 262:16625-16635.). We have shown that p30 is also present in the developing peripheral nervous system. P30 has been localized to neurons and Schwann cells of the sciatic nerve and dorsal root ganglion (DRG) by immunostaining of tissue sections with a polyclonal antibody raised against a synthetic peptide, (p30 N-terminal amino acids 1-13, Rauvala et al., 1988, J. Cell Biol. 107:2293-2305). Western blot analysis confirmed the presence of p30 in extracts of cultured DRG neurons and Schwann cells. The intensity of immunostaining peaks at postnatal day 8. P30 is present in early embryos, increases in concentration postnatally and persists at low levels in adulthood.

Several lines of evidence suggest that p30 expression in Schwann cells is regulated by contact with neurons: 1. Schwann cell immunoreactivity increases between embryonic day 15 and postnatal day 8 when remodeling of neuron-Schwann cell families is maximal, and decreases to undetectable levels in the adult. 2. There is a dramatic increase in p30 expression in Schwann cells of the distal stump after sciatic nerve transection prior to the regrowth of axons. 3. In co-cultures of Schwann cells and DRG neurons, Schwann cell immunoreactivity decreases within 3 hours after contact with neurites.

The pattern of p30 expression during development and regeneration, and its regulation by cell-cell contact suggests that p30 may play a role in the interaction between neurons and Schwann cells during morphogenesis of peripheral nerves.

This work was supported by a William Gradison Junior Faculty Award from the National Multiple Sclerosis Society and NIH grant #1RO1NS27227.

CO 102 THE BINDING OF AGRIN TO THE MUSCLE SURFACE IS CALCIUM DEPENDENT, Justin R. Fallon, Neurobiology Group, Worcester Fdn. for Exper. Biology, Shrewsbury, MA. Agrin is a molecule associated with the synaptic basal lamina at the neuromuscular junction that is likely to play a central role in directing the differentiation the postsynaptic apparatus during development and regeneration. We have developed an immunofluorescence technique to visualize the binding of agrin derived from Torpedo californica to the myotube surface. Binding is saturable, does not require the presence of serum or embryo extract, is observed at 4°C and is unaffected by the presence of metabolic energy inhibitors. No binding of agrin to the myotube surface is observed when Ca^{2+} is omitted from the incubation medium or specifically chelated with EGTA. Mg^{2+} or Mn^{2+} are not required for binding nor do they substitute for Ca^{2+}. The omission of Ca^{2+} does not appear to irreversibly alter either the ligand or receptor since pre-incubation of either the cells or the agrin with EGTA did not affect their subsequent binding in the restored presence of Ca^{2+}. Moreover, agrin is dissociated from its binding site by brief incubations in EGTA. These results suggest that both the formation and maintenance of agrin-receptor complexes is dependent upon the presence of extracellular Ca^{2+}.

CO 103 CHARACTERIZATION OF A DEVELOPMENTALLY REGULATED NEURAL ANTIGEN.
Margaret M. Kelly, Douglas Brees and Gregory J. Cole, Medical University of South Carolina, Charleston, SC 29425. The chicken antigen A2B11 is defined by monoclonal antibodies raised against embryonic (day 7) neural retinal cells. Preliminary characterization of this protein indicated an apparent molecular weight on gel electrophoresis of 260 kD, and an extreme susceptibility to proteolysis. In addition to retina, the antigen is also present in day 9 embryonic skin fibroblasts, optic tectum, brain, and heart, and absent in liver. However protein is not detected in late embryonic retina. Further analyses have demonstrated that the antigen is also developmentally regulated in brain tissue, but not in heart muscle. The protein does not bind heparin sepharose-conjugates nor the lectins ConA and WGA, does not contain a L2 (HNK-1) epitope, is not sulfated, and its size is not affected by trifluoromethanesulfonic acid treatment. The antigen is present in the conditioned media and in membrane and soluble fractions of total brain and glial enriched primary cultures, suggesting a possible association with the extracellular matrix. A cDNA expression library has been constructed from embryonic day nine brain RNA which will be screened with polyclonal antisera to initiate molecular characterization of the antigen's structure, regulation, and function.

CO 104 EXPRESSION OF AGRIN-RELATED MOLECULES BY CHICK MUSCLE CELLS IN CULTURE,
Erich Lieth and Justin R. Fallon, Neurobiology Group, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Agrin is an extracellular matrix protein enriched at synaptic specializations. When extracted from the ECM and incubated with muscle cultures, agrin clusters acetylcholine receptors (AchR) on muscle cell surfaces. The cellular sources of the agrin in the synaptic basal lamina have not been definitely established. We have previously shown that molecules antigenically related to agrin accumulate on the surfaces of aneural embryonic chick myotubes in vivo and in vitro. Here we show that these molecules are synthesized, at least in part, by muscle cells. In culture, the expression of these muscle-derived agrin related molecules (M-agrin) can be detected as early as 24 hours after plating, and they continue to accumulate for at least 2 weeks. When myotubes are cultured in the presence of spinal cord more than 70% of AchR clusters induced by nerve colocalize with M-agrin. These results indicate that muscle synthesizes at least a portion of the agrin in the synaptic basal lamina and that muscle-derived agrin plays a role in the formation of neuromuscular synapses.

CO 105 HOMING OF NEURAL CREST CELLS IN THE CHICKEN HINDGUT.
M.J.H. Peters-van der Sanden, J.H.C. Meijers, Th.M. Luider, A.W.M. van der Kamp, D. Tibboel, J.C. Molenaar. Depts. of Cell Biology and Pediatric Surgery, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

Vagal neural crest cells, the precursors for the enteric nervous system, migrate through the gut to form enteric ganglia. The aim of our study is to identify adhesion molecules involved in homing and differentiation of neural crest cells. Possible candidates are cell adhesion molecules carrying the HNK-1 epitope known to be involved in cell adhesion. In early embryonic, aneuronal, bowel we observed a layer of mesenchymal cells carrying the HNK-1 epitope, that disappeared as soon as the bowel became colonized with neural crest cells. This could be studied in a model system, in which aneuronal gut was cocultured with chicken or quail neural primordium on the chorioallantoic membrane. Migration of neural crest cells into the explanted gut could be traced with the quail nucleolar marker and the formation of enteric ganglia could be demonstrated with the neuronal marker E/C8. However this *in ovo* model system to study neural crest cell homing in a target tissue is complex. Therefore, we are developing an *in vitro* aggregation assay to identify adhesion molecules involved in homing. In such an *in vitro* assay the effect of interfering agents on the specific interaction between neural crest and target cells can be studied more easily. However, preliminary results fail to show a specific interaction between neural crest and target cells *in vitro*.

We surmize that the *in vitro* aggregation assay lacks the proper biological conditions for the specific interaction of neural crest and target cells to occur. The coculture system, although complex, appears to be the appropriate way to study neural crest cell homing.

CO 106 HUMAN NEURAL CELL ADHESION MOLECULE: cDNA SEQUENCE AND mRNA EXPRESSION, Robert A. Reid, Jane K. DeGuglielmo, John J. Hemperly, Becton-Dickinson and Company Research Center, P.O. Box 12016, Research Triangle Park, NC 27709 The neural cell adhesion molecule (N-CAM) is one of the best characterized proteins involved in cell-cell adhesion. Vertebrate N-CAM comprises at least three polypeptides which arise by alternate splicing of mRNA. We have isolated and sequenced cDNAs specific to the major forms of human N-CAM as well as to several variants. Probes based on these sequences were used to determine the expression of N-CAM transcripts in human cell cultures by Northern blot and polymerase chain reaction (PCR) analyses. Both techniques qualitatively delineate between N-CAM expressing and non-expressing lines. However, for N-CAM positive lines, all variant forms are detected by PCR in contrast to blot techniques, which show differences in the levels of the variants present. This difference between techniques is presumably due to the greater sensitivity of PCR compared to Northerns. We have begun to develop quantitative PCR techniques to more accurately define human N-CAM mRNAs, particularly those present in low amounts.

Cell Lineage

CO 200 EXPRESSION OF A RAT HOMEOBOX GENE IN NEURONAL AND GLIAL CELL LINES, Su Yun Chung, Jun Lei, Zi Yao Liu, and Dana Hilt, Uniformed Services University of the Health Sciences, Bethesda, MD and University of Maryland School of Medicine, Baltimore, MD.
We are investigating the role of the rat homeobox genes in the development of the mammalian central nervous system, concentrating on the spinal cord. As probe, we are using one of the rat homeobox genes, R5, which we have isolated and partially characterized (Falzon and Chung, Development 103, 601-610, 1988). The gene is expressed at high levels during development and in the adult in a tissue specific manner. We have studied the expression of this rat homeobox gene in a rat neural stem cell line capable of differentiating into a neuronal or a glial cell lineage. Northern blot analyses indicate that a 2.4 kb messenger RNA transcribed from R5 is present in the stem cells and in the neuronal and glial cell lineages. We have also prepared antisera against synthetic polypeptides for which the sequence was derived from R5. Protein blot analysis indicates that the antisera react with a 60 kD protein in the cell extract. Immunofluorescence experiments indicate that the homeobox gene product is nuclear in location. The system will provide a model for examining the differential regulation of homeobox gene expression in neuronal and glial cell types.

CO 201 PAX-3 EXPRESSION IN THE DEVELOPING MOUSE CNS Martyn D Goulding, Urban Deutsch and Peter Gruss.
A new murine paired-box containing gene has been isolated from an 8.5day mouse embryonic cDNA library. This gene encodes for a 478 amino acid protein that contains both a paired-box domain and a paired type homeodomain. Expression of Pax-3 is observed from day 8 and is localised in the dorsal half of the of the closing neural tube as well as in the adjacent somites. Expression of Pax-3 was restricted to the mitotic ventricular layer in the CNS with peak expression levels between day 9 and day 12. Expression in the somitic mesoderm was localised to the dermomyotome. Expression of Pax-3 was also observed in migrating neural crest cells that give rise to the dorsal root ganglia and to facial mesoectoderm. The expression pattern of Pax-3 is consitent with Pax-3 playing a role in the organization of the the vertebrate CNS.

Cell and Molecular Aspects of the Developing Nervous System

CO 202 P-BRAVO, A NOVEL IMMUNOGLOBULIN SUPERFAMILY MEMBER IN THE DEVELOPING NERVOUS SYSTEM, IS IDENTIFIED AND PARTIALLY CHARACTERIZED USING A NEW METHOD, Jon Faiz Kayyem, Janet M. Roman, Enrique J. de la Rosa, Uli Schwarz and William J. Dreyer, Division of Biology, California Institute of Technolgy, 156-29 Caltech, Pasadena, CA 91125. Cell-surface molecules play an essential role in guiding axons to their targets. To characterize these molecules, we have developed a method to generate monoclonal antibodies (MAbs) against cell surface molecules of defined molecular weight that are expressed during development of the chick nervous system. From MAbs generated against cell-surface molecules in the 120 to 150 kDa range, we have identified at least 14 different patterns of reactivity on embryonic tissue. A detailed study of the first of these staining patterns, Pattern Bravo (P-Bravo), reveals labeling of optic fibers in the retina but no staining on these same retinal axons in the tectum. The appearance of P-Bravo in vitro is modulated by environmental cues. Axons growing out from retinal explants on retinal basal lamina, their natural substrate, express P-Bravo, whereas such axons growing on collagen do not. Immunoaffinity purification of P-Bravo yields a major molecular mass doublet at 145/135 kDa and a broader band centered around 90 kDa, a 1D gel band pattern quite similar to that of the neural cell surface molecule L1. Also, as with L1, the higher molecular weight doublet has a higher mobility under non-reducing conditions than under reducing conditions (indicating internal disulphide bonds) and carries the HNK-1 epitope. Internal sequence data from a P-Bravo peptide showing similarity to the C2 immunoglobulin domain, as well as sequence data from the N-terminus and from other peptides (some with similarity to fibronectin type III repeats) show P-Bravo is related to L1, but is not NgCAM/G4, thought to be the chicken equivalent of L1. The N-terminal sequence of P-Bravo 145/135 is NH_2-LeuAspValProLeuAspSerLysLeuLeuGlu-?-LeuSer-?-Pro. The homology of P-Bravo to known adhesion molecules, as well as the topologically restricted pattern of expression, suggests P-Bravo is involved in axon guidance.

CO 203 MOLECULAR CLONING AND EXPRESSION FROM cDNA FOR HUMAN MACROPHAGE SCAVENGER RECEPTOR: ENDOCYTOSIS RECEPTOR FAMILY WITH COLLAGEN-LIKE TRIPLE HELICAL STRUCTURE, Tatsuhiko Kodama. The Third Department of Internal Medicine, University of Tokyo, Tokyo, Japan.
We have cloned and sequenced two types of cDNAs for human scavenger receptor from cDNA library of phorbor ester stimulated human macrophage-like cell line THP-1. These receptors mediate the receptor-mediated endocytosis of denatured or modified plasma proteins, lipoproteins and other negatively charged macro-molecules, and are related to the foam cell formation of atheromatous plaque. Human scavenger receptor consists of five domains; from N-terminus, cytoplasmic (50 amino acids), membrane spanning (26), N-linked sugar (leucine zipper-like, 196), collagen like triple herical (69), and C-terminus type specific domain (type I 110, type II 17). Two types of receptor mRNAs are probably generated by an alternative splicing of a single gene. Both type I and II receptors expressed on COS cell indicated fully functional receptor activity. The collagen-like domain may be related to the ligand specificity. The presence of extracellular matrix as a domain of endocytosis receptor indicate that these receptor may play a role on cell adhesion, movement, and information transmission during the contact.

CO 204 NEUROBLASTOMA CELL LINES AS MODELS FOR THE STUDY OF MIBG UPTAKE MECHANISMS.
P.G. Montaldo, M. Lanciotti, P. Cornaglia-Ferraris and M. Ponzoni. Children's Hospital, Pediatric Oncology Research Laboratory, L.go G. Gaslini 5, 16148 Genova, Italy.
Specific (type I) uptake of iodine-labeled metaiodobenzylguanidine (MIBG) by neural-lineage cells is responsible for successful imaging and [^{131}I]-MIBG treatment of neuroblastoma (NB) and is characteristic of relatively advanced stages of neural maturation. MIBG uptake is difficult to study "in vitro", because most NB cells lines are composed of immature elements which show low MIBG incorporation with non-saturation kinetics (non-specific uptake). To date, the only NB cell line reportedly showing MIBG uptake I is SK-N-SH; however, the marked heterogeneity of cell populations within this line limits its suitability as a model. We have studied the MIBG uptake in four NB cell lines (LAN-5, SK-N-BE (2)-C, GI-CA-N and GI-LI-N) by incubating the cells for 2 hours with various concentrations (5×10^{-9} - 5×10^{-6}) of [^{125}I]-MIBG in the presence or absence of specific uptake I inhibitors norepinephrine (NE) or imipramine (IMP). LAN-5 cells showed low and unsaturable [^{125}I]-MIBG uptake, which was unaffected by specific inhibitors. SK-N-BE (2)-C showed half-maximal [^{125}I]-MIBG uptake at $6.47 \pm 2 \times 10^{-8}$ M and saturation at 5×10^{-7} M. NE or IMP (10^{-4} M) inhibited by 90% MIBG incorporation (specific uptake). GI-CA-N cells incorporated MIBG at high but unsaturable levels; the addition of NE (10^{-4} M) had no significant effect on this uptake (although in some experiments NE strongly enhanced it), while IMP (10^{-4} M) inhibited it by 63%. GI-LI-N behaved like GI-CA-N, i.e., it showed high and unsaturable MIBG uptake, but was inhibited by 10^{-4} M NE and, to a major extent, by IMP (75% and 95%, respectively). Incomplete cell population homegeneity within the cell lines used may be partially responsible for the puzzling observations in this study; however, our data suggest that MIBG uptake is a complex biochemical phenomenon. The putative involvement of distinct receptors with different affinity for MIBG, saturation kinetics and selective pharmacological sensitivity is currently investigated.
Supported by AIRC and by Ricerca Corrente Gaslini 891701C.

Developmental Cues and Disorders

CO 300 DEVELOPMENTAL EXPRESSION AND TARGETING OF VOLTAGE-DEPENDENT Ca^{2+} CHANNELS TO DENDRITES IN HIPPOCAMPAL NEURONS, Kimon Angelides, James Littleton, Owen T. Jones, and Gabriel Schuster, Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas 77030. The targeting and segregation of surface components into discrete functional domains is an important feature of neuronal cell biology. The regulation of ion channel distribution is one of the most important factors in determining the electrical properties in various parts of the neuron. Voltage-dependent Ca^{2+} channels (VDCCs), in particular, orchestrate a variety of neuronal activities and are crucial for neurotransmitter release, the regulation of neuronal excitability, and their expression is implicated as a key event in the development of the neuron's integrative properties. In mature hippocampal neurons VDCCs are segregated and immobilized to the cell body and dendrites (Jones *et al.*, *Science* **244**:1189-1193, 1989). We have examined both the temporal and spatial events of VDCC expression during neuronal development to determine if VDCC expression is critical for the determination of neuronal polarity and whether VDCCs are targeted and committed to specific domains independent of the identity of the process (i.e., axon *vs.* dendrite). VDCC distribution was visualized on cultured embryonic hippocampal CA1 neurons using colloidal gold decorated biotin-ω-conotoxin a specific marker for L- and N-type VDCCs. After nine days in culture neurons begin to express VDCCs, although axonal ($GAP\text{-}43^+$) and dendritic ($MAP\text{-}2^+$) polarity is detected at day one. Using the transection paradigm of Goslin and Banker (*J. Cell Biol.* **108**:507-1516, 1989), where axons can be converted to dendrites, we examined whether the destination and targeting of VDCCs could be altered after switching the identity of the process. In 88% of the cases where VDCCs were expressed and the interconverted axon could be clearly re-identified, a process specified to become an axon and thus would not have normally expressed VDCCs, expressed VDCCs after interconversion to a $MAP\text{-}2^+$ dendrite. VDCC were not detected in those processes that were $MAP\text{-}2^-$. We conclude that **1)** expression of VDCCs is a relatively late event in neuronal development and does not appear to be critical for determination of final neuronal polarity, **2)** neurons exhibit plasticity in the targeting of VDCC's and **3)** that the identity and commitment of the process to an axon or dendrite determines ion channel distribution. Supported by the National Institute of Health.

CO 301 NEURONS IN THE DEVELOPING CHICKEN SPINAL CORD AND DORSAL ROOT GANGLION EXPRESS THE CALCIUM BINDING PROTEIN S100, Anita Bhattacharyya, Michael N. Lehman, Nancy Ratner, and Robert W. Brackenbury, Department of Anatomy and Cell Biology, University of Cincinnati School of Medicine, Cincinnati, OH 45267. S100 is a generally recognized marker for Schwann cells in adult peripheral nerve. We used polyclonal antisera recognizing S100 to stain 60um transverse sections of spinal cord and hindlimb from embryonic day 5 (E5) to embryonic day 18 (E18) chick to determine the stage in peripheral nerve development when S100 first appears in Schwann cells. Expression of S100 first became apparent in Schwann cells at E14, just before the onset of myelination. Staining was abolished by preincubation of the antisera with authentic S100 protein (a gift of Blake Moore). Surprisingly, prior to its expression by Schwann cells, S100 was detected in developing sensory neurons of the dorsal root ganglia and motor neurons of the ventral spinal cord. This staining was also sensitive to preincubation of the antibody with authentic S100 protein. Low intensity S100 immunoreactivity was first detected in DRG cells at E5. The large ventrolateral neurons in the DRG were intensely stained at E9 and while the intensity of the staining remained high, the number of stained neurons decreased through E18. In contrast, the small mediodorsal cells of the DRG were lightly stained throughout development. Staining of the motor neurons in the ventral spinal cord was apparent from E5 to E8 and peaked in intensity at E9; staining was undetectable by E18. While the function of S100 is not well understood in any system, the transient expression of S100 in some developing neurons is intriguing in light of the observation that purified S100β promotes neurite extension from E7 chick cerebral cortex neurons (Kligman,D., 1982, Brain Res., 250:93-100). Kligman and Marshak (1985, PNAS 82: 7136-7139) postulated that glial cells in the CNS secrete S100β which stimulates the process outgrowth in developing neurons. The present finding is consistent with the possibility that secreted S100 may act as a neurotrophic factor for developing PNS neurons. This work was supported by NIH grant #1RO1NS27227.

CO 302 EXPRESSION AND ALTERNATE SPLICING OF THE β-AMYLOID PRECURSOR PROTEIN IN EARLY MOUSE EMBRYOGENESIS, Shannon Fisher, John D. Gearhart, and Mary L. Oster-Granite, Developmental Genetics Lab, Department of Physiology, Johns Hopkins School of Medicine, Baltimore, MD 21205

The β-amyloid peptide, the main component of the amyloid plaques of Alzheimer's disease and Down syndrome, is part of a larger precursor molecule, the amyloid precursor protein (APP). The APP gene has been mapped to human chromosome 21 and to mouse chromosome 16. The human gene demonstrates tissue specific alternate splicing, and the splicing regulation is similar in the mouse. As a step toward understanding the role of APP in development, we have examined its expression in mice from ovulated oocytes to the late embryonic stage, using combined reverse transcription and the polymerase chain reaction. While the three major splicing forms are present at all stages, the ratios of the three are not constant; the relative amount of the shortest splicing form increases from the egg cylinder to the neurula stage. Screens of cDNA libraries from the same two stages indicate that abundance increases from 0.001% to 0.01% over this time period. Cellular localization of APP at these stages will help in understanding the role APP is playing in development.

CO 303 CRITICAL PERIOD FOR alpha-DIFLUOROMETHYLORNITHINE (DFMO) OTOTOXICITY IN THE DEVELOPING PIGMENTED RAT. Charles Henley, Jim Atkins, Glen Martin and Brenda Lonsbury-Martin, Department of Otorhinolaryngology, Baylor College of Medicine, Houston, TX 77030.

DFMO is a specific, irreversible inhibitor of ornithine decarboxylase (ODC), a key enzyme in polyamine biosynthesis. Development of the nervous system is partially under control of the ODC-polyamine system and inhibition of polyamine synthesis by DFMO during critical developmental periods produces deficits in cellular growth & maturation (Slotkin et al., Int J Develop Neurosci 1:7-16,1983). The purpose of this study was to (1) quantitate ODC in cochlear tissues of the rat during the period of development of the ear and (2) determine the effects of postnatal DFMO treatment on cochlear function. In order to quantitate ODC in cochlear tissues, rats (n=3) were sacrificed on postnatal days 3,5,10,12,16,20,25 & 60, their temporal bones removed, & cochlear tissues prepared for enzymatic analysis of ODC (decarboxylation of ornithine). Parallel incubations with DFMO (500 μM) were used to ensure that the observed decarboxylation was due to ODC. Cochlear ODC was significantly elevated throughout the maturational period of hearing. Activity increased rapidly during the first 10 days of life, peaking on day 10, followed by a rapid decline. The greatest activity was found in the organ of Corti (sensory cells). We then treated rats with DFMO (500 mg/kg/day) or saline (equivolume) for 10 days before (days 1-10), during (days 11-20) or after (days 21-30) the period of hypersensitivity to ototoxic drugs. Cochlear function was assessed by acoustic distortion product emissions (DPEs). DPEs are a functional measure of outer hair cell function. Only rats treated on postnatal days 1-10 demonstrated age & frequency dependent deficits in cochlear function. The developing rat appears to be hypersensitive to the effects of DFMO during the period when ODC activity is rapidly increasing. We are currently evaluating the effects of DFMO on cochlear polyamine metabolism to correlate biochemical effects with observed functional losses. These studies suggest that the polyamines are involved in the development of cochlear function.

CO 304 BASAL FOREBRAIN LESIONS AT BIRTH ALTER THE TIME COURSE OF CORTICAL MORPHOGENESIS AND RESULT IN PERSISTENT ABNORMALITIES IN CORTICAL MORPHOLOGY C.F. Hohmann and J.T. Coyle. Dept. of Psychiatry, The Johns Hopkins University, School of Medicine, Baltimore, MD 21205.

We previously reported that destruction of cortical cholinergic afferent from basal forebrain nuclei, at birth, resulted in a marked but transient reduction of cortical choline acetyltransferase [ChAT] activity during ontogeny. This was accompanied by disruption of cortical cytoarchitecture, ipsilateral to the lesion, in all areas with reductions of Acetylcholinesterase [AChE] histochemistry. Cortical disgenesis became appeared around postnatal day 4 [PND4] and attenuated as cholinergic markers recovered in cortex. However, some abnormalities in cortical cytoarchitecture persisted into adulthood.

We have now completed studies demonstrating that neonatal nBM lesions induce delayed neuronal differentiation and result in persistent alterations of cortical connections. For the study of cortical cytodifferentiation, we conducted a quantitative evaluations of Rapid Golgi impregnated brains on PND7 and 14 following the unilateral nBM lesion. We found significant reductions in dendritic elaboration and reduced cell body size at PND7 ipsilateral to the lesion. By PND14, differences in dendritic morphology had become attenuated but soma size still differed significantly from contralateral cortex. Qualitative abnormalities in dendritic morphology were also apparent at this age. Cortical connectivity was assessed by striatal and thalamic injections of Wheatgerm Agglutinin coupled Horseradish Peroxidase [WGA-HRP] into adult animals after neonatal nBM lesions. These experiments showed exuberant projections from cortical layer V to subcortical targets and abnormally distributed thalamocortical afferents.

In conclusion, we show here that transient cholinergic deafferentation during the neonatal period results in severe disruption of normal developmental patterns in cortex. These results are compatible with the hypothesis that ACh functions as a regulator of cortical plasticity.

CO 305 CHARACTERIZATION OF THE TWO SUBTYPES OF D-2 DOPAMINE RECEPTORS, Rita M. Huff, Mary Lajiness, and Chris L. Chio, Cell Biology, The Upjohn Co., Kalamazoo, MI 49001.

D-2 dopamine receptors are implicated in the pathology of schizophrenia. Recently clones for two molecular forms of these receptors have been isolated and termed D-2_o and D-2_{in} (1,2). The nucleotide sequences of these clones are identical except that D-2_{in} has an additional 87 nucleotides encoding an insert of 29 amino acids within putative intracytoplasmic loop 3. We have developed several stably transfected CHO cell lines which express densities of each of the two D-2 receptor subtypes ranging from 0.8 to 3.0 pmol/mg protein. The two receptor types have indistinguishable affinities for a variety of D-2 receptor antagonists. The D-2_{in} subtype appears to be the more abundant of the two forms of the receptor. Although the D-2_o and D-2_{in} receptor subtypes have both been cloned from rat tissues, Northern analysis of rat caudate nucleus or rat whole brain with selective oligonucleotide probes indicate that D-2_{in} is much more abundant (1). cDNA clones for only the D-2_{in} subtype have been obtained from human or bovine tissue sources (2,3). Northern analyses of various bovine tissues using selective oligonucleotide probes indicate that mRNA for the D-2_{in} receptor is found in caudate nucleus, substantia nigra and anterior pituitary. RNA for D-2_o is found in the caudate nucleus and substantia nigra, however, at much lower levels than RNA for D-2_{in}. The presence of mRNA for both D-2_{in} and D-2_o in substantia nigra suggests that dopaminergic neurons to the caudate may express both forms of D-2 receptors. No detectable RNA for D-2_o was found in the anterior pituitary.

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CO 306 SOMATOSTATIN TRANSGENIC MICE: NEUROBIOLOGIC CONSEQUENCES OF NEUROPEPTIDE OVEREXPRESSION, Stephen Kinsman, Timothy Moran, Roger Reeves, John Gearhart, and Mary Lou Oster-Granite, Departments of Neurology, Psychiatry, and Physiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

In order to provide a model for studying the neurobiologic consequences of somatostatin overexpression during development, we produced transgenic mice carrying extra copies of a 15kb mouse genomic fragment containing the preprosomatostatin gene and 9kb of 5' sequences. Three lines of somatostatin transgenic mice were produced. In the one line studied extensively, quantitative Northern blot analysis has shown 1.2-2.8 times as much preprosomatostatin message in newborn transgenic brain tissue relative to nontransgenic littermates. In behavioral studies, transgenic mice from this line show an increase in spontaneous motor activity of about 50% over control littermates. Currently, we are characterizing in detail the pattern of somatostatin overexpression in both developing and adult brains of these mice. To determine whether somatostatin overexpression results in any neurochemical imbalance, we are also performing studies of various neurochemical markers.

CO 307 EMBRYONIC GALANIN EXPRESSION IN NORMAL AND ANEUPLOID MICE. A.E. Mjaatvedt, C. Wiese, and M.L. Oster-Granite, Developmental Genetics Laboratory, Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205-2195.

Our long-term objective is to study the possible role of of the neuropeptide galanin during normal and abnormal development of the central nervous system. Galanin is a 29 amino acid peptide originally isolated from pig intestine. Elevated levels of galanin have been found in various tumors, indicating that it may play a role in proliferative and differentiative events. Galanin has also been implicated in the cholinergic deficits observed in basal forebrain nuclei of Alzheimer's disease patients. Since a similar cholinergic deficit is found in individuals with Down Syndrome, galanin may also be involved. To explore the role of galanin in neuronal development further, we used an animal model, the trisomy 16 (Ts16) mouse, that exhibits cholinergic deficits in basal forebrain nuclei during its development. This deficit is due, at least in part, to the premature withdrawal of these cells from the mitotic cycle. Our hypothesis is that galanin may be involved in both early differentiation events and in circuit formation during normal development of cholinergic neurons. To test this hypothesis we first examined the pattern of galanin expression at embryonic days 12 (E12) and 14 (E14) by *in situ* hybridization using a digoxigenin-labeled rat galanin cDNA probe. Galanin mRNA is expressed throughout the central nervous system at both days in normal mouse embryos; however, the levels of expression in most regions are lower at E12. When comparing the levels of antibody staining at both ages, there is a similar increase from E12 to E14. In addition, trisomic animals at each age show decreased immunoreactivity when compared to their normal littermates. This suggests that galanin may be involved proliferative events, although it is difficult at this point to determine if these changes in galanin immunoreactivity are related to specific cellular events or merely to overall maturity of neuronal populations. Correlation of the expression of galanin with cessation of proliferation and differentiation of these neurons in the basal forebrain may help us to determine whether this peptide provides a major influence of proliferative activity in these cells, and whether it later is involved in circuit formation.

CO 308 SYNAPSE ELIMINATION IN LURCHER CHIMERAS, Sylvia A. Rabacchi, Yannick Bailly, Nicole Delhaye-Bouchaud, Karl Herrup* and Jean Mariani, Institut des Neurosciences, CNRS URA 1199, Université P. et M. Curie, Paris, France, *EK Shriver Ctr, Waltham, Mass, USA.

The elimination of functional synapses is now recognized as an important step in the achievement of specific connections during development. For example, a developing Purkinje cell (PC) is transiently innervated by several climbing fiber afferents before the adult monoinnervation is established. However, the multiple innervation is maintained in various adult agranular cerebella, suggesting that the granule cells (i.e. the second afferent system of the PC's) play a fundamental role in the synapse elimination process. The granule cells could either act directly on the climbing fiber terminals (in this case the absolute number of granule cells is crucial), or, alternatively, their effect could be mediated by the PC (and the numerical ratio granule-Purkinje cells would be pivotal). In all the previously studied models both the absolute number of granule cells and the ratio granule-Purkinje cells are reduced.

In the present study, we used an experimental model that allows us to manipulate this ratio, i.e. aggregation chimeras between cerebellar mutant and wild type mice. We analysed the lurcher-wild type chimera, which exhibits a granule-Purkinje cell ratio higher than in the normal mouse, although the absolute number of granule cells is significantly reduced. The level of multiple innervation was assessed by in vivo intracellular recording from PC's: all the 45 cells recorded from 5 chimeras revealed to be innervated by a single climbing fiber. These results support the hypothesis that the role of the granule cells is exerted through their interaction with the PC's.

CO 309 bFGF STIMULATES MELANOGENESIS IN EMBRYONIC QUAIL DORSAL ROOT GANGLIA IN VITRO, Larry S. Sherman, Kate M. Stocker, Sean M. Rees, and Gary Ciment, Department of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, OR 97201.

Neural crest (NC) and some of the NC-derived cells of the dorsal root ganglia (DRG) of early avian embryos undergo pigmentation in culture. Dorsal root ganglia from older embryos (7 days or older), however, do not produce melanocytes under the same culture conditions. The loss of melanogenic capability by these older DRG cells can be reversed by culturing them in the presence of 12-0-tetradecanoylphorbol-13-acetate (TPA) (Ciment, et. al., Devel. Biol. 118:392-398, 1986).

This study demonstrates that basic fibroblast growth factor (bFGF) also stimulates melanogenesis in DRG and peripheral nerve (PN) cultures from 7-day quail embryos. bFGF (10ng/ml) acted additively with 10^{-8} or $3x10^{-9}$M TPA to cause pigmentation in 90-100% of DRG cultures. Furthermore, in the absence of TPA, bFGF caused pigmentation in 20-60% of DRG cultures. We obtained similar results with PN cultures, suggesting that the DRG cells that undergo melanogenesis in the presence of bFGF are of the Schwann cell lineage. Various other growth factors, including NGF, TGF-alpha, and EGF, had no effect on pigmentation in DRG cultured in the presence or absence of TPA. In contrast, TGF-beta (10ng/ml) significantly decreased pigmentation in DRG cultures in the presence of TPA or bFGF. These data suggest that bFGF and TGF-beta may play important roles in the survival and/or developmental decisions made by at least one subpopulation of NC cells.

CO 310 NEURAL CREST CELLS MIGRATE ALONG DEVELOPING BLOOD VESSELS IN JAPANESE QUAIL, S.G. Spence and T.J. Poole, Dept. of Anatomy and Cell Biology, S.U.N.Y. Health Science Center at Syracuse, N.Y. 13210

Within the trunk of the avian embryo the formation of the sympathetic ganglia is dependent on two pathways of neural crest migration. The first pathway is between two consecutive somites, where crest cells migrate ventrally in intersomitic space and rapidly reach the dorsal aorta. The second more predominant pathway is through the anterior portion of the somite, between the dermomyotome and the scleratome, eventually reaching the dorsal aorta to also contribute to the formation of the sympathetic plexus.

Here we examine the distribution of developing blood vessels within these pathways by double labeling with monoclonal antibodies HNK-1 (IgM, for neural crest) and QH-1 (IgG, for angioblasts) in whole mount and sectioned 30-35 somite embryos. In whole mount preparations, intersomitic arteries can be seen sprouting off the dorsal aorta into the intersomitic space 3-4 somites anterior to the last formed somite (-3 to -4). However, neural crest cells just begin to migrate intersomitically at -5 to -6. At these levels, a small population of neural crest cells can be seen migrating along the intersomitic artery to the dorsal aorta. At approximately -10 somites, within the anterior portion of the somite, a larger population of neural crest cells migrate dorsolaterally. Some of these crest cells will migrate along the somitic vein, between the dermomyotome and the scleratome, to the posterior cardinal vein and the dorsal aorta. Our data demonstrate that blood vessel development precedes neural crest migration and that trunk neural crest cells migrate on the surface or extracellular matrix of developing blood vessels. (Supported by NSF DCB8904400)

CO 311 NEUROPEPTIDE GENE EXPRESSION BY GLIA: PROENKEPHALIN WITHIN ASTROCYTES HAS A NOVEL INTRACELLULAR DISTRIBUTION **Barbara A. Spruce, Rory Curtis, Graham P. Wilkin, David M. Glover, Department of Biochemistry, Imperial College, London SW7 2AZ, U.K.** The existence of preproenkephalin A mRNA within cultured astrocytes has been reported previously by two groups. Using a novel series of monoclonal antibodies to the uncleaved mammalian enkephalin precursor, proenkephalin A, we have examined cryostat sections from postnatal rat brain regions, and dissociated cerebellar glial cultures, by indirect immunofluorescence. In cerebellar sections, we find that subpopulations of both grey and white matter astrocytes are surprisingly rich in proenkephalin, in the absence of mature enkephalin peptide fluorescence. We detect proenkephalin mRNA by in situ hybridisation. We also find strong glial fluorescence in all other brain regions so far examined, including hypothalamus, striatum, and cortex. We are able to reproduce the staining in dissociated glial culture. Proenkephalin expression within glia is not seen until the second postnatal week both in vivo and in vitro, and increases through to adulthood. In culture, the proenkephalin-expressing glia comprise a subpopulation of process-bearing astrocytes. The proenkephalin molecule has an unusual intracellular distribution, apparently in association with subsets of filamentous cytoskeletal elements, and in many cases is concentrated along one axis. We speculate that proenkephalin may have a special role within astroglial cells.

CO 312 DIFFERENTIATION SIGNALS FOR MONOAMINERGIC NEURONS, M. Staufenbiel, J. Hartikka, L. Merguin, M. Foguet, H. Lübbert, Preclinical Research, Sandoz Pharma AG, 4002 Basel, Switzerland. Differentiation of CNS neurons is thought to be induced and maintained by signals from outside the cell. To identify and study such signals we use primary cultures of dissociated embryonic rat brain cells and analyze differentiation markers (neurotransmitters and the enzymes synthezising them, their uptake systems, receptors). Our attention is focussed on cultures derived from the areas of substantia nigra and raphe nuclei isolated at day E14. About 2% of the cells are dopaminergic or serotonergic, respectively. Immediately after cultivating these neurons express differentiation specific markers while general neuronal markers are low or missing. The differentiated phenotype is rather stable for serotonergic neurons but dopaminergic neurons decrease under standard conditions. Other neuron types present show different kinetics of differentiation. We have studied the effect of cocultures and extracts from target areas as well as possible differentiation promoting molecules. Factors were identified which increase the number of surviving neurons or the degree of expression of the differentiation markers in dopaminergic neurons.

CO 313 DELAYING SYMPATHETIC INNERVATION PREVENTS THE β- TO α-MYOSIN HEAVY CHAIN SWITCH IN HEART, Doris A. Taylor, Leslie A. Leinwand and Howard J.Federoff, Dept Microbiology and Immunology and Depts of Medicine and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461. Sympathetic innervation of the heart occurs postnatally in rat and humans resulting in an altered physiologic state and response to pharmacologic agents. During this period of rapid growth and development, changes in cardiac gene expression occur including a change of myosin heavy chain (MHC) isoform expression (in rat from β-MHC to α-MHC). The role of innervation in these processes has not been ascertained. We have prevented sympathetic innervation to the heart for 30 days by treating rats with 6-hydroxy-dopamine (6-OH-DA) during the first 3 weeks of life. Total RNA isolated from the atria and ventricles of these animals and from controls was hybridized to gene-specific probes to determine the level of expression of α and β-MHC mRNA. In atria and ventricles from 30 day old control rats, α-MHC was predominant. In 6-OH-DA treated animals β-MHC was the predominant isoform expressed in ventricle but α-MHC remained the major isoform expressed in atria. When innervation was allowed to occur in a separate group of animals after treatment for 3 weeks with 6-OH-DA we found that at 60 days α-MHC was expressed in atria and ventricles at levels similar to untreated control animals. Thus, we have demonstrated that sympathetic innervation plays an important role in changes in gene expression in the heart during the postnatal period of growth and development.

CO 314 CELL-SPECIFIC CYCLIC AMP-MEDIATED INDUCTION OF THE PDGF RECEPTOR, Gerry Weinmaster and Greg Lemke, Molecular Neurobiology Laboratory, The Salk Institute for Biological Studies, San Diego, CA 92138.

Cyclic AMP (cAMP) cooperates with a wide variety of polypeptide growth factors to synergistically stimulate the proliferation of many vertebrate cell types. The cellular mechanisms underlying these cooperative interactions are for the most part unknown, however. We have identified one such mechanism by observing that (1) cultured rat Schwann cells proliferate in response to platelet-derived growth factor (PDGF) only if simultaneously cultured in the presence of agents that elevate intracellular cAMP, and (2) this unmasked PDGF response is accounted for by a dramatic cAMP-mediated induction of PDGF receptor mRNA and protein. cAMP-mediated induction of the PDGF receptor results in enhanced, ligand-dependent receptor autophosphorylation, and in enhanced PDGF activation of *c-fos* gene expression. In addition, this induction is unique to those cells, such as Schwann cells, for which cAMP is itself mitogenic. These results indicate that the synergistic proliferative effect obtained from the combination of cAMP and polypeptide growth factors may in large part result from the cAMP-mediated induction of growth factor receptors.

CO 315 AN INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN GENE IS EXPRESSED IN THE EMBRYONIC ECTODERM AND NEUROECTODERMAL DERIVATIVES IN EARLY AND MID-GESTATIONAL RAT EMBRYOS. Teresa L. Wood, Alexandra L. Brown, Mathew M. Rechler and John E. Pintar. Dept. Anatomy and Cell Biology, Columbia P & S, NY, NY 10032 and Molecular, Cellular and Nutritional Endocrinology Branch, NIH, Bethesda, MD 20892.
Genes encoding three distinct insulin-like growth factor binding proteins have recently been cloned; two predict an RGD sequence that indicates possible roles for these proteins as matrix constituents in addition to or other than mediating the actions of insulin-like growth factors. We have here used in situ hybridization to delineate the expression pattern for the rat insulin-like growth factor binding protein-2 (IGFBP-2) gene in early and mid-gestation rat embryos and have compared the expression pattern to that of insulin-like growth factor II (IGF-II). rIGFBP-2 mRNA has been detected as early as e7 in the post-implantation embryonic ectoderm and thus appears to be the first marker described for this lineage, while IGF-II is found in visceral endoderm and extraembryonic ectoderm at this age. During gastrulation, ectoderm-derived cells that will become both embryonic and extraembryonic mesoderm cells cease IGFBP-2 expression and instead begin to express IGF-II. IGFBP-2 expression is retained in ectodermal and certain neuroectodermal derivatives through mid-gestational ages. In rostral brain regions (especially the telencephalon) IGFBP-2 mRNA in general is located primarily in cells of the ventricular layer; expression decreases as cells leave this region and begin to differentiate, which suggests that the IGFBP-2 gene product may participate in neuroblast cell cycle regulation. In more caudal CNS regions, IGFBP-2 expression within neural progenitors decreases to non-detectable levels by e11. In contrast, high levels of expression characterize diverse neuroectoderm derivatives including the epithelium of the choroid plexus, the floor plate of the spinal cord, and the infundibular outpocketing of the diencephalon and suggest that IGFBP-2 may have different functions in distinct regions of the embryo. Supported by NS21970.

Cytoskeleton, Axons and Growth Cones

CO 400 COMPARATIVE STUDY OF MYELIN BASIC PROTEIN ISOFORMS IN DEVELOPING VERTEBRATE CENTRAL NERVOUS SYSTEM. ABSENCE OF 21.5Kd AND 20.2Kd MBPs IN CHICKEN MAY POINT TO THEIR IMPORTANCE IN MAMMALIAN MYELINOGENESIS, Claude C.A. Bernard, Nicole Kerlero de Rosbo, Shirley Tsang, Neuroimmunology Laboratory, Department of Psychology, La Trobe University, Bundoora, Victoria 3083, Australia.
Developmental appearance and accumulation pattern of myelin basic protein (MBP) isoforms were analyzed by quantitative immunoblotting in central nervous system of three mammalian (guinea pig, rabbit and calf) and one avian (chicken) species. In these four species, myelination onset occurred in the spinal cord well before birth. In addition to the 18.5Kd MBP observed in all species studied, a 21.5Kd and a 20.2Kd MBPs were detected in the three mammalian species but not in chicken. In calf and chicken, a 17.3Kd MBP was also observed. The 18.5Kd and 17.3Kd MBPs were the major MBP isoforms of chicken central nervous system where a faint MBP-related 14.5Kd protein could also be seen. The major difference between mammalian and avian MBP profiles was indeed the presence only in mammals of the 21.5Kd and the 20.2Kd MBPs. The developmental patterns of these two isoforms as well as their rate of accumulation as compared to the 18.5Kd MBP suggest that their role in mammalian myelination may be of greater importance at early rather than late stages of this process. Differences in quantity as well as type of MBP isoforms present may indicate that in diverging animal species, the process of myelination may follow a different pathway and possibly involve different MBP isoforms at different stages.

CO 401 ANALYSIS OF A CYTOSKELETAL ASSOCIATED COMPONENT OF THE GROWTH CONE. Eric Birgbauer, Jonathan H. Dinsmore, and Frank Solomon, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139.
A monoclonal antibody, 13H9, raised against in vitro cycled microtubule-associated proteins from brain recognizes the extraordinary microtubule organelle of the nucleated erythrocyte, the marginal band. This antigen is also a component of the growth cone of primary neurons in culture, where it appears to be associated with the cytoskeleton. In the growth cone, its immunofluorescence pattern is similar, though not identical, to the F-actin pattern, but complementary to, and distinct from, the axonal microtubules. This growth cone localization, however, is dependent on intact microtubules, since microtubule depolymerization causes the concomitant loss of the 13H9 antigen from the growth cone and delocalization down the axon. 13H9 recognizes an 80 kd protein, which appears to be a form of ezrin. In an Embryonic Carcinoma cell line which can be induced to differentiate into neurons, the 13H9 antigen is again localized to the growth cones of these neurites. Biochemical quantities of the differentiated neurons and their undifferentiated precursor can be obtained. Differential extraction of these cells and immunoblotting with a polyclonal serum against ezrin shows that the cytoskeletal association of ezrin changes upon differentiation.

CO 402 PIONEER NEURON GROWTH CONE MORPHOLOGY AND NAVIGATION *IN VIVO*, Janet S. Duerr, Timothy P. O'Connor, and David Bentley, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720. The first neurons to develop in the embryonic grasshopper limb bud extend their axons in a well-described stereotyped pathway from the periphery to the central nervous system. These pioneer neurons can be labeled with DiI and the behavior of their growth cones can be monitored with computer-enhanced video microscopy in a semi-intact preparation. During neurite extension, the growth cones traverse different regions of the limb expressing different guidance cues. By monitoring the behavior of individual growth cones over several hours of development, the overall morphology and rate of extension of individual growth cones has been observed to change in consistent ways. The relationship between axon advancement and the behavior of individual filopodia has been monitored. In most regions of the limb, the growth cone advances by infilling between several filopodia. However, the behavior of the growth cone when it initially contacts an immature guidepost neuron can be different. In some cases, the axon can form from the thickening of a single filopodium in contact with the guidepost neuron. This system will be used to examine components of the interaction of the growth cone with its natural substrate through observation of acute changes in growth cone morphology and behavior after the administration of agents known to perturb normal growth cone navigation in culture.

CO 403 INSULIN AND IGF-1 RECEPTORS IN NEURONAL GROWTH CONES FROM DEVELOPING RAT BRAIN, Robert S. Garofalo, Department of Anatomy and Cell Biology, State University of New York Health Science Center at Brooklyn, Brooklyn, NY 11203.
Receptors for insulin and insulin-like growth factor I (IGF-I) are expressed at high levels in developing rat brain. Both receptors are tetrameric complexes composed of two alpha subunits which contain the ligand binding domains, and two beta subunits which contain ligand-activated tyrosine kinase activity. In order for these receptors to have a direct role in the regulation of neuronal growth they must be present on neuronal cell membranes and possibly on the specialized membrane of the nerve growth cone. To address this, nerve growth cone particles (GCPs) prepared from E18 brain were examined for the presence of insulin and IGF-I receptors. Immunoblotting of salt-washed GCP membranes with several different antireceptor antibodies reveals the presence of immunologically distinct 92 kDa and 97 kDa beta subunits. Densitometric scanning of autoradiograms of immunoblots indicates that receptors are enriched approximately three-fold in GCP membranes relative to membranes prepared from total E18 brain homogenate. Insulin and IGF-I receptors in GCPs are functionally competent as indicated by their autophosphorylation on tyrosine in response to the appropriate ligands. The expression of insulin and IGF-I receptors in the specialized region of the neuronal plasma membrane termed the growth cone further supports a role for these hormones as trophic factors for neurons.

CO 404 THE EXPRESSION OF THE NEURONAL INTERMEDIATE FILAMENT PROTEIN PERIPHERIN IN THE DEVELOPING RAT EMBRYO. James D. Gorham and Edward B. Ziff, Department of Biochemistry and Kaplan Cancer Center, New York University Medical Center, New York, NY 10016.

The cDNA coding for the type III intermediate filament protein (IFP) peripherin was originally identified as a NGF inducible mRNA in PC12 cells. Expression of peripherin mRNA is largely restricted to peripheral and motor neurons. In the current studies we have examined the expression of peripherin mRNA, by *in situ* hybridization, and protein in the developing rat embryo. To facilitate protein distribution studies, we have generated antibodies against a trpE/peripherin *E. coli* fusion protein. We have determined that the expression of peripherin is initiated at E11.5, at a time when forming neurons are undergoing initial process outgrowth (neuritogenesis). Peripherin expression is not seen in dividing or migrating neural crest or neural tube cells. Peripherin protein is seen in the cell soma and processes of PC12 cells and of neural-crest derived neurons and motoneurons. We have also detected peripherin in olfactory neurons; these neurons are unusual in that they (1) undergo continuous regeneration and (2) do not express the "typical" neuronal IFPs (i.e. neurofilaments). In these neurons, peripherin is localized only to axons, and not to cell bodies and dendrites. We had originally identified peripherin as a "late" NGF-inducible gene. The finding that initial peripherin expression coincides with visible axonogenesis is consistent with its classification as a marker of terminal differentiation.

CO 405 CHARACTERISATION OF A cDNA REPRESENTING PART OF MAP1X, A GROWTH RELATED, NEURONE SPECIFIC MICROTUBULE ASSOCIATED PROTEIN, Lesley Harrison, Michael Cheetham and Rosaleen Calvert, Department of Neuroscience, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London, SE5 8AF. Monoclonal antibody G10 labels outgrowing but not mature axons in the rat nervous system. The G10 antigen is microtubule-associated protein MAP1X which is neurone-specific and declines in expression as development proceeds. cDNA representing part of MAP1X has been sequenced. There is no homology between this sequence and that of other known sequences of microtubule-associated proteins (MAP2 and tau). There is a single mRNA for MAP1X of >11kb. A polyclonal antibody raised against the fusion protein encoded by the cDNA shows that MAP1X has a wider distribution than the G10 epitope so this epitope is masked or absent in the adult rat and in some neuronal compartments during development.

CO 406 THY-1 AND GP39 EXPRESSION IN THE DEVELOPING AVIAN NERVOUS SYSTEM. Jeffrey, Peter L. Cunningham, Anne M. Dowsing, Bruce J. Sentry, John W. Tolhurst, Ornella and Henke, Robert. Children's Medical Research Foundation, P.O. Box 61, Camperdown, 2050 Australia. The localization and developmental expression of Thy-1 and GP39 in the avian nervous system has been investigated with specific monoclonal antibodies. Immunohistochemical localization studies have shown Thy-1 to have a generalized distribution on macroneurons e.g. Purkinge and retinal ganglion cells, interneurons do not show immunoreactivity. GP39 shows a pattern of localization at major synapses throughout nervous system and tends to be concentrated at sites where unlike membranes form appositions and synapses. The antigens have been purified by immunoaffinity chromatography. Protein sequence data obtained following CNBr digestion of Thy-1 when compared to both rodent and human Thy-1 confirm that the Thy-1 like glycoprotein from chicken is the avian homologue of mammalian Thy-1. The N-terminal and internal sequence of GP39 does not show strong identity to MRC-OX-2. The amino acid sequence data for Thy-1 and GP39 was used to produce degenerate primers which were then used in PCR reaction with reverse transcribed chick forebrain RNA. The fragments isolated and their use in library screening and developmental studies will be presented.

CO 407 CLONING AND NUCLEOTIDE SEQUENCE OF MOUSE BRAIN ß-SPECTRIN CDNA, Yupo Ma, Warren Zimmer, Steven Goodman, Department of Structural and Cellular Biology, College of Medicine, University of South Alabama, Mobile, AL 36688. Spectrin is a major cytoskeletal component of the brain and has at least 2 distinct neuronal isoforms: brain spectrin (240/235E) and brain spectrin (240/235). These two isoforms have been defined by localization with two isoform specific spectrin antibodies. We have isolated a 1185bp CDNA from a mouse brain library using an antibody specific for erythroid ß spectrin which has been shown previously to stain the cell body and the dendrite of neurons (brain spectrin (240/235E)). Sequence analysis revealed that this CDNA contains a single open reading frame of 999bp encoding a 333 amino acid sequence. The deduced amino acid sequence exhibited homology to spectrins, demonstrating the characteristic 106 amino acid repeat unit. The highest observed homology was found to human erythroid ß spectrin. This homology begins at the first 7 amino acids of the ß 15 repeat extending to the C-terminus exhibiting ~ 56% identity. An additional 62 amino acids were found at the C-terminus of the 235 KDa brain subunit not seen in human erythroid ß spectrin. The use of an erythroid ß spectrin antibody which stained neuronal soma and dendrites on immunohistochemistry to probe the library and the high homology of the deduced amino acid sequence with human erythroid ß spectrin support the suggestion that this sequence encodes the ß subunit of brain spectrin (240/235E).

CO 408 $pp60^{src}$-KINASE ACTIVITY IS ENRICHED IN A NEURITE PREPARATION FROM IN VITRO DIFFERENTIATED HUMAN NEUROBLASTOMA CELLS. Gabrielle Meyerson and Sven Påhlman, Dept.of Pathology, University of Uppsala,Uppsala,Sweden. The proto-oncogene product $pp60^{c-src}$ is a membrane associated tyrosine specific kinase. It appears to be expressed in all cell types but elevated levels have been found in e.g neurons. During TPA (12-O-tetradecanoylphorbol-13-acetate) induced neuronal differentiation of the human SH-SY5Y neuroblastoma cells the specific src-kinase activity is increased. This activation appeared during the same time period as the cells formed neurites with growth cones and neurosecretory granula. The SH-SY5Y cells express two forms of the src-proteins,the common form $pp60^{c-src}$ and the neuronal form ($pp60^{c-srcN}$). During the induced differentiation there is no change in the expression of these two proteins. In a neurite fraction from TPA-differentiated SH-SY5Y cells, $pp60^{src}$ ($pp60^{c-src}$/$pp60^{c-srcN}$) was 2.3 times higher than in the corresponding cellbody fraction. There was no difference in the localization of the two proteins. Immunoprecipitates of $pp60^{src}$ from the neurite fraction contained at least two more proteins (38kDa and 45kDa). The 45kDa protein could be immunoprecipitated with a synaptophysin antiserum. The 38kDa protein appeared to be covalently linked to the src-proteins via S-S bridges, because the $p38/pp60^{src}$ complex migrated electrophoretically as a broad 100-140kDa band under non-reducing conditions. This property and the V8-protease phosphopeptide pattern indicate that the 38kDa protein is a granula associated protein previously identified in chromaffine granule membrane (C.Grandori and H.Hanafusa,1988. J.Cell Biol.107:2125-2135).

CO 409 DISTRIBUTIONS OF GLYCOPROTEIN CLASTERS MARKED CYTOCHEMICALLY IN AXONAL MEMBRANES OF CULTURED SPINAL NEURONS, Dmitri A. Rusakov, Galina G.Skibo and Larisa M.Koval, Department of Biophysics, Dniepropetrovsk State University Research Institute of Biology, Dniepropetrovsk 320625,USSR. Topography of some axolemma glycoconjugates was studied in the monolayer developing culture preparations of dissociated spinal neurons (12 to 14-d-old mice) with the use of WGA,RCA,HPL,LBA lectin complexes with colloidal gold. Electron micrograms of investigated axonal membrane profiles (including the ones containing growth cones) were treated morphometrically. Two types of accidental functions corresponding properly to the observed distributions of marking particles were considered. Mathematical treatment of these functions gave the possibility to estimate the important quantitive parameters of a surface distribution of the marked membrane carbohydrate detrminant clasters. 3D-size distributions, interval spectra, indications and characteristics of some spatial regularity are derived for the above glycoconjugates within various ranges of their claster size. The unbiassed data obtained can predict some physical and chemical features of axonal membrane structure during development and differentiation.