EPITHELIAL AND NEURONAL CELL POLARITY AND DIFFERENTIATION

Organizers: Enrique Rodriquez-Boulan and W. James Nelson

February 4 - 10, 1993; Tamarron, Colorado

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Morphogenesis of Polarity in Embryos

G 001 EPITHELIAL DIFFERENTIATION AND JUNCTION FORMATION IN THE MOUSE PREIMPLANTATION EMBRYO, Tom P. Fleming, Qamar Javed, Jane Collins, Greta Dunglison and Mark Hay, Department of Biology, University of Southampton, Southampton SO9 3TU, U.K.

Trophectoderm maturation during blastocyst formation in the mouse early embryo is used as a model for study of epithelial differentiation in vivo. Uvomorulin-mediated cell-cell adhesion and cell polarity first occur at compaction (8-cell stage) and, by 24 hours later (32-cell stage), the trophectoderm is functional in vectorial transport processes responsible for generating the blastocoele. Aspects of this differentiation programme can be stimulated by exogenous cues. Thus, for example, insulin treatment increases the number and size of endocytic vesicles in nascent trophectoderm. Our recent work has centred on the biogenesis and assembly of tight and desmosome junctions during trophectoderm differentiation. Apicolateral tight junction begin to form at compaction (focal sites) and appear complete (zonular) before cavitation. ZO-1 protein is first detectable (immunoblots) at the late 4-cell stage and assembles at the tight junction from compaction. Unlike ZO-1, cingulin is expressed by the maternal genome during oogenesis, but this protein pool does not appear to participate in tight junction formation. Synthesis of cingulin from the embryonic genome is enhanced significantly from compaction and assembly at the membrane usually begins at the 16-cell stage, suggesting that tight junction formation at the molecular level is progressive. Our data also suggest that biosynthesis and assembly of tight junction components is regulated by cell interactions that control tight junction tissue specificity. Desmosome formation coincides with blastocoele formation, from the 32-cell stage. Unlike tight junctions, nascent desmosomes appear complete in terms of their molecular composition; expression of desmosomal glycoproteins appears to control desmosome assembly.

G 002 EARLY EVENTS IN THE ESTABLISHMENT OF RENAL EPITHELIA, D. Herzlinger, Cornell University Medical College, New York, N.Y.

Reciprocal interactions between mesenchymal and epithelial cell types in the embryo guide organ morphogenesis. Such interactions have been well characterized in the developing metanephric kidney. The earliest kidney rudiment contains mesenchymal cells that promote the branching of a preformed epithelial structure, the ureteric bud. Once contacted by the branching ureteric bud epithelium, metanephric mesenchymal cells are induced to epithelialize and differentiate into diverse renal epithelial cell types. Lineage analysis studies utilizing the technique of retroviral mediated gene transfer demonstrate that the cells of the metanephric mesenchyme are multipotent but undergo commitment to distinct renal cell lineages during or immediately after receiving an inductive stimulus to epithelialize. (Herzlinger et al., 1991). We have immortalized cells from the earliest metanephric kidney rudiment (rat gestation day 12.5) and demonstrate that one clonal cell line established, RSTEM-1, fulfills the biochemical and functional criteria expected of a multipotent metanephric mesenchymal cell.

The RSTEM-1 cell line was immortalized by infection with a replication defective retrovirus (ts58a, Frederikson et al., 1988) that encodes for a temperature sensitive variant of SV40 Large T Antigen. RSTEM-1 exhibits a mesenchymal phenotype when grown at permissive temperature, as determined by the expression of mesenchymal specific intermediate filament and cell surface adhesion proteins and secretion of factors that promote ureteric bud branching. However, RSTEM-1 can be experimentally induced to epithelialize and after extended culture generates at least two terminally differentiated renal cell types; distal and proximal tubule epithelia. Thus, the RSTEM-1 cell line provides a novel system to probe the genetics of mesenchymal to epithelial transitions as well steps leading to the terminal differentiation of distinct epithelial cell types.

G 003 THE *WNT* GENE FAMILY AND PATTERN FORMATION IN *XENOPUS* EMBRYOS, Randall T. Moon, University of Washington School of Medicine, Seattle.

Wnts are a recently described family of secreted glycoproteins related to the *Drosophila* segment polarity gene, *wingless*, and to the proto-oncogene, *int*-1. *Wnts* are thought to function as developmental modulators, with signalling distances of only a few cell diameters. In *Xenopus*, at least six *Wnts*, including *Xwnts*-1, -3A, and -4, are expressed initially in the developing central nervous system, with some regions expressing multiple *Xwnts*. *Xwnt*-8 is expressed by mid-blastula stage, in ventral and lateral mesoderm. *Xwnt*-5A mRNAs are stored in the egg, and later are expressed throughout the embryo in both ectoderm and mesoderm, but with a pronounced enrichment in the head and tail. Recent studies in *Xenopus* have pursued the diverse roles of *Xwnts* in patterning mesoderm and the nervous system, the mechanisms by which *Xwnts* signal information between cells, and the cell physiological responses to *Xwnt* signals. With regard to *Xwnts* and mesoderm, data will be presented showing that maternal signals from the Nieuwkoop center positively regulate expression of the homeobox gene *goosecoid* in gastrula organizer cells, and that *goosecoid* negatively regulates *Xwnt*-8 such that it is excluded from organizer cells. Data support the conclusion that *Xwnt*-8 is instructive in the formation of ventral mesoderm, and attenuates the responsiveness of lateral mesodermal cells to dorsalizing signals from the organizer. With regards to *Xwnts* and the nervous system, data suggest that none of the identified *Xwnts* are important in neural induction, but that they play subsequent roles by mechanisms which include modulation of cell adhesion.

Mechanisms for Generating Polarity

G 004 CADHERINS AND CATENINS IN EPITHELIAL MORPHOGENESIS AND *XENOPUS* DEVELOPMENT, Pierre D. McCrea[1], William Brieher, Chung H. Lee, Elena Levine, and Barry M. Gumbiner. The Sloan-Kettering Institute, New York, NY and The University of California San Francisco, San Francisco, CA.

E-cadherin is a Ca^{2+}-dependent cell-cell adhesion molecule important for the development and maintenance of epithelial tissues. In early *Xenopus* embryos E-cadherin is first expressed at the time of gastrulation and its expression is limited to the ectoderm. Another cadherin, which we call C-cadherin, is expressed maternally in the oocyte and seems to be involved in the formation of the blastula epithelium prior to gastrulation. Experiments are being done to analyze the functions of these two cadherins during cleavage-stage development and gastrulation in *Xenopus*.

The mechanism by which E-cadherin expression becomes restricted to the ectodermal epithelial layer during gastrulation is being investigated using, as a model system, the induction of mesoderm in the *Xenopus* animal cap by the growth factor activin. Mesoderm induction is thought to involve the diversion of cells away from an ectodermal ground state. A suppression of ectodermal markers has been observed to accompany mesodermal induction. We have found that activin does suppress the expression of E-cadherin in the inner cells of the animal cap. However, the outermost cells express E-cadherin and form an ectodermal-like epithelial layer surrounding the induced mesodermal tissue, just as in intact embryos. Interestingly, E-cadherin expression is suppressed by activin in the outer layer of cells when it is isolated away from the inner cells. Therefore, some sort of interaction between inner and outer cells, either physical or chemical seems to maintain the expression of E-cadherin in the ectodermal layer during mesoderm induction.

The regulation of cadherin functional activity involves an interaction of the cadherin with cytoplasmic components, such as the actin cytoskeleton. Cadherin-mediated intercellular recognition events may also entail signal transduction mechanisms. To investigate the mechanisms of cadherin function in epithelia, we have begun to study proteins that bind tightly to the cytoplasmic tail of E-cadherin. Three proteins, named "catenins", form a stable protein complex with E-cadherin that can be isolated from epithelial cells. We have purified the beta-catenin and determined its primary amino acid sequence from a cloned cDNA. Beta-catenin is highly homologous to plakoglobin, a protein isolated from desmosomal cell junctions. However, beta-catenin and plakoglobin are distinct proteins and both are expressed in the same epithelial cells. Plakoglobin associates with both the desmosomal glycoprotein desmoglein-1 and the cadherin protein complex. Both beta-catenin and plakoglobin are highly related to the *Drosophila* segment polarity gene product *armadillo*, but both sequence homology and antibody cross-reactivity suggest that beta-catenin and *armadillo* are more closely related to one another. *Armadillo* was identified genetically in *Drosophila* embryos as a protein involved in the transmission of a developmental signals by *wingless*, a member of the wnt growth factor family. It is interesting, therefore, to consider the possibility that beta-catenin and cadherins participate in intercellular signaling events in vertebrate tissues.

We have begun to examine the function of beta-catenin in early development of the frog *Xenopus laevis*. We have observed that microinjection of anti-beta-catenin antibodies into cleaving embryos causes the formation of a secondary body axis. The phenotype is remarkably similar to the one caused by the injection of wnt mRNA. Further experiments are being done to determine the mechanism by which beta-catenin perturbation leads to this interesting developmental phenotype.

G 005 ROLE FOR THE MEMBRANE-CYTOSKELETON IN THE DEVELOPMENT OF EPITHELIAL CELL POLARITY, W. James Nelson, Helen McNeill, Peter Piepenhagen, James A. Marrs, Robert W. Mays, Debra Wollner, and Kathy Siemers, Stanford University School of Medicine, Stanford.

Cell-cell adhesion is at the top of a molecular cascade of protein interactions that leads to the remodelling of epithelial cell structure and function. The earliest events that initiate this cascade are poorly understood. Using high resolution differential interference contrast microscopy and retrospective immunohistochemistry, we have correlated changes in the organization of E-cadherin and the cortical cytoskeleton with immediate and early events upon cell-cell contact in MDCK epithelial cells. We show that formation of a stable contact is preceded by numerous, transient contacts. During this time and immediately following formation of a stable contact, there is no detectable change in the organization or relative amount of E-cadherin at the contact. After a lag period of ~10 minutes, there is a rapid acquisition of detergent insolubility of E-cadherin specifically at the stable contact, indicating association of E-cadherin with the cortical cytoskeleton. Significantly, the total amount of E-cadherin at the stable contact remains unchanged during this time. This suggests that the increase in size of the detergent insoluble pool of E-cadherin reflects recruitment of protein from a pre-existing pool at the contact site, and not from outside the contact. The increase in the detergent insoluble pool of E-cadherin, however, did not correlate with changes in the distributions of cortical cytoskeletal proteins, actin or fodrin. These results have implications for models of cell adhesion. We suggest that cell-cell adhesion occurs prior to interactions between E-cadherin and the detergent insoluble cortical cytoskeleton. The lag period and subsequent prompt nature of E-cadherin reorganization indicates a form of signalling is occuring. Subsequent interactions between E-cadherin and the cortical cytoskeleton may be important in strengthening the contact between cells, and in the long-term remodelling of the membrane in the development of cellular polarity. We have, therefore, begun to characterize in detail the interactions of different cytoplasmic (cytoskeletal) proteins with the cytoplasmic domain of E-cadherin; these proteins include the catenins, plakoglobin and fodrin. We suggest that interaction between E-cadherin and the cortical cytoskeleton drives assembly of the membrane cytoskeleton at the cell contacts, which results in the recruitment of specific membrane proteins to those sites (eg. Na/K-ATPase). This is supported by the isolation of protein complexes from MDCK cells that contain E-cadherin, Na,K-ATPase, ankyrin and fodrin. More directly, we have shown previously that expression of E-cadherin in fibroblasts results in the recruitment of fodrin and Na,K-ATPase to points of E-cadherin mediated cell adhesion. These results indicate that the development of cell surface polarity of the basal-lateral membrane in these cells is driven by localized assembly of the membrane-cytoskeleton, and the capture and subsequent retention of specific membrane proteins delivered from the Golgi complex.

Biogenetic Pathways

G 006 SORTING OF ION TRANSPORT PROTEINS IN POLARIZED CELLS, C.J. Gottardi, D.L. Roush, G. Pietrini, J. Ahn, M.J. Shiel and M.J.Caplan, Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven

The plasma membranes of polarized epithelial cells and neurons express distinct populations of ion transport proteins in their differentiated plasma membrane domains. In order to understand the mechanisms responsible for this polarity it will be necessary to elucidate the nature both of sorting signals and of the cellular machinery which recognizes and acts upon them. In our efforts to study sorting signals we have taken advantage of two closely related families of ion transport proteins whose members are concentrated in different epithelial plasmalemmal domains. The Na,K-ATPase occupies the basolateral membranes of most epithelia. The highly homologous H,K-ATPase occupies a storage compartment in gastric parietal cells. Stimulation of acid secretion results in the fusion of this compartment with the parietal cell apical membrane. Both pumps are composed of α and β-subunits. We have expressed the H,K-ATPase subunit polypeptides, as well as H,K-ATPase/Na,K-ATPase chimeras, in polarized epithelial cells derived from the renal proximal tubule ($LLC\text{-}PK_1$). We find that sorting information is encoded in both polypeptides. The H,K-ATPase α-subunit encodes a signal which ensures this protein's localization to the apical surface. The H,K-ATPase β-polypeptide manifests a strong coated pit localization sequence which, in this cell type specialized for apical endocytosis, appears to confer apical sorting. Finally, we have found that the COOH terminal portion of the α-subunits specify β-subunit preference. We are currently in the process of examining whether the signals responsible for epithelial sorting also play a role in the H,K pump's targeting to a regulated delivery pathway in its native parietal cell. The second class of transporters under study is involved in pre-synaptic neurotransmitter re-uptake. We have expressed the axonal Na,Cl-dependent GABA transporter (GAT) in MDCK cells. In keeping with the apparent correlation between axonal and apical sorting, we find that GAT is restricted to the apical cell surface. Another member of this family is normally expressed in the kidney and mediates betaine transport in response to hyperosmolarity. The betaine transporter (BAT) is 50% identical to GAT and yet is an endogenous component of the MDCK basolateral cell surface. We have prepared GAT/BAT chimeras and are currently analyzing their sorting behaviors in epithelia and in neurons. In order to study sorting machinery, we are in the process of developing a screen for the detection of Drosophila mutants which are unable to polarize their embryonic epithelia. We have generated transgenic flies which express either GPI-linked placental alkaline phosphatase (PLAP) or alkaline phosphatase linked to the tail of the VSV G protein (PLAPG). As would be expected from their behaviors in mammalian epithelia, we find that PLAP and PLAPG are concentrated in the apical and basolateral surfaces of a number of Drosophila tissues at several stages of development. (Supported by NIH GM-42136, a fellowship from the David and Lucille Packard Foudation and a NSF National Young Investigator Award)

G 007 TARGETING TO THE APICAL DOMAIN IN EPITHELIAL CELLS, A. Hubbard, M. Schell, M. Maurice, B. Stieger, V. Barr,O. Weisz and C. Machamer, Cell Biology and Anatomy Department, Johns Hopkins University School of Medicine, Baltimore,MD.

Our previous work has shown that in hepatocytes, all newly-synthesized plasma membrane (PM) proteins so far studied arrive first at the basolateral domain. Our working hypothesis is that apically-destined proteins are endocytosed from the basolateral surface and sorted to the apical domain via transcytosis. The apical molecules we originally studied are all single transmembrane proteins with short cytoplasmic tails. We have asked the following questions: 1) Do membrane proteins with different topologies travel a route similar to that of single transmembrane proteins? 2) What is the nature of the membrane vesicle carrying the apical PM proteins? 3) Where are the targeting signals on dipeptidyl peptidase IV (DPPIV), a type II apical protein?

1) A mechanism for the sorting of newly-synthesized glycophospha tidylinositol (GPI)-linked proteins has been proposed whereby they associate in lipid microdomains in the trans-Golgi network and then arrive at the apical domain directly. Such a mechanism poses a potential exception to the hepatocyte rule. We used pulse/chase techniques in conjunction with subcellular fractionation to compare the trafficking of 5'nucleotidase (5NT), an endogenous GPI-anchored protein of hepatocytes, with two single transmembrane proteins (DPPIV and the polymeric IgA-receptor(pIgA-R)). Using a technique to separate a highly-enriched fraction of Golgi-derived membranes from ER and PM, we found that both 5NT and the pIgA-R traverse the ER and Golgi with high efficiency. Using a method that resolves PM vesicles derived from the apical and basolateral domains, we found that 5NT first appeared at the basolateral domain as early as 30 minutes of chase. However, subsequent redistribution to the apical domain required more than 3.5 h of chase. This rate of transcytosis is much slower than that observed for DPPIV and may reflect the endocytosis of GPI-linked proteins via a non-clathrin-mediated pathway.

2) We have identified a compartment in the hepatocytes of bile duct ligated (BDL) rats that arises from the accumulation of transcytotic carriers around the bile canaliculus. These carriers contain the pIgA-R, solute endocytosed from the circulation and three newly-synthesized apical PM proteins. This compartment is dynamic: i.e., the vesicles and their contents have a finite half-life. Furthermore, pIgA-R and HRP are delivered to the bile canaliculus in the ligated animal, indicating that vesicular traffic has not stopped. Our working hypothesis is that one type of vesicle carries both transcytotic receptors and newly-synthesized apical proteins. We have isolated an enriched population of these vesicles and are characterizing their content of known proteins as well as searching for new components.

3) We have begun a systematic study of the sorting signals on rat hepatocyte DPPIV, a type II apical membrane protein. MDCK cells deliver endogenous apical and basolateral proteins directly to the appropriate domains. We determined that ~80% of the newly-synthesized liver DPPIV was delivered directly to the apical surface of transfected MDCK cells; however, about 20% was delivered first to the basolateral surface and reached the apical surface via slow transcytosis. A soluble form of DPPIV (solDPPIV) containing only the lumenal domain of the protein was efficiently transported and secreted by stably transfected MDCK cells. Surprisingly, 95% of the secreted solDPPIV was found in the apical medium. The high efficiency of apical secretion suggested that the transmembrane domain and cytoplasmic tail of DPPIV might contain competing basolateral targeting information. To test this hypothesis, we investigated the trafficking of a chimera in which these two domains were joined to lysozyme, an exogenous protein which appears to contain no sorting information. This chimera was delivered predominantly to the basolateral surface. Our results suggest that the lumenal domain of DPPIV carries dominant apical sorting information.

G 008 SORTING OF PLASMA MEMBRANE PROTEINS IN EPITHELIAL CELLS, Enrique Rodriguez-Boulan, Charlotte Anderson-Fissone, Doris Gundersen, Andre Le-Bivic, Annick Le Gall, Michael Lisanti, Ivan R. Nabi, Sharon K. Powell, and Chiara Zurzolo. Cornell University Medical School, New York, N.Y.

Our laboratory has been interested in identifying mechanisms that are responsible for the sorting of plasma membrane proteins into apical and basolateral routes and for their retention into the respective surface domains. We are currently using several different cell models to characterize these mechanisms, such as kidney (MDCK), intestinal (Caco2), thyroid (FRT) and retinal pigment (RPE-J) epithelial cell lines. Using these model systems we have noticed that sorting of apical and basolateral proteins occurs at the TGN or at the basolateral endosomes/ basolateral surface and that the sorting of several of these molecules is cell-type specific. The following are results from our laboratory in this area. 1) GPI-anchored proteins are apical in MDCK Caco 2 and LLC-PK1 but do not appear to have a preferred polarity in FRT cells. These results suggest that GPI acts as an apical targeting signal in several kidney and intestinal cell lines but not in FRT. 2) The polarized distribution of the neural cell adhesion molecule N-CAM is determined by its mode of anchoring (GPI >apical transmembrane->basal in MDCK cells) but also by interaction with the extracellular components: a transmembrane form of N-CAM is apical in RPE *in situ* but becomes basolateral in cultured RPE that underwent replication and in the RPE -J cell line. 3) Na,K-ATPase is sorted basolaterally in FRT cells but is unsorted in RPE-J cells. Basolateral polarity of Na, K-ATPase is induced by the transfection of E-cadherin into RPE-J cells. 4) An MDCK lysosomal membrane glycoprotein (LAMP-2) follows a novel route to the lysosome that involves passage through basal endosomes and perhaps the basolateral membrane before final delivery to their destination. We have identified signals that determine apical targeting (GPI) and basolateral targeting (cytoplasmic segments of p75-NGF receptor and N-CAM) and are currently characterizing them and attempting to identify sorter proteins that interact with these structures to mediate transfer into apical or basolateral routes.

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G 009 SORTING OF SURFACE PROTEINS IN THE TRANS GOLGI NETWORK OF MDCK CELLS, Kai Simons, Paul Dupree, Lukas Huber, Teymuras Kurzchalia and Robert Parton, European Molecular Biology Laboratory, Heidelberg, Germany.

Vesicular carriers that deliver their cargo from the trans Golgi network (TGN) to the apical and the basolateral domain have previously been isolated from MDCK cells. In order to dissect the putative sorting machinery, we have solubilized the Golgi-derived transport vesicles with the detergent CHAPS and shown that influenza virus HA formed a large complex together with several integral membrane proteins. This allowed the isolation of one protein of this complex, VIP21. Antibodies raised against this protein localized VIP21 to the trans Golgi network and to the apical and the basolateral domains. The protein was unexpectedly concentrated in caveolae. We propose that caveolae might also be involved in membrane recycling and that the mechanisms governing inclusion of proteins into these specialized plasma membrane invaginations may have similarities with the process of protein and lipid sorting at the TGN in MDCK cells.

Searching for rab proteins involved in the specific targeting of apical and basolateral proteins in polarized MDCK cells, we have found that rab8 is associated with TGN vesicles. By using 2D-gel electrophoresis and GTP-ligand blots, rab8 was found to be enriched in immunoisolated basolateral transport vesicles. Immunofluorescence using rab8 antibodies revealed a vesicular and lateral plasma membrane staining in MDCK cells.

Endocytic and Transcytotic Pathways

G 010. SPECIALIZED ENDOSOMAL PATHWAYS OF WATER CHANNEL AND PROTON PUMPING ATPASE RECYCLING IN KIDNEY EPITHELIAL CELLS, Dennis Brown, Renal Unit, Massachusetts General Hospital, and Dept. of Pathology, Harvard Medical School, Boston, MA.

The plasma membrane composition of virtually all eukaryotic cells is established, maintained and modified by the process of membrane recycling of specific protein and lipid components. In the kidney collecting duct, urinary concentration and urinary acidification are physiologically regulated at the cellular level by the shuttling of water channels and proton pumps between intracellular vesicles and the plasma membrane of highly-specialized cell types. In the principal cell, the antidiuretic hormone, vasopressin, induces the insertion of vesicles that contain proteinaceous water channels into the apical cell membrane, thus increasing the permeability to water of the epithelial layer. In the intercalated cell, hydrogen ion secretion into the urine is modulated by the recycling of vesicles carrying a proton pumping ATPase to and from the plasma membrane. In both cell types, "coated" carrier vesicles are involved in this process, but whereas clathrin coated vesicles are involved in the endocytotic phase of water channel recycling, the transporting vesicles in intercalated cells are coated with the cytoplasmic domains of the proton pumping ATPase. By a combination of morphological and functional techniques using FITC - dextran as an endosomal marker, we have shown that recycling endosomes from intercalated cells are acidifying vesicles but do not contain water channels, whereas principal cell vesicles that recycle water channels do not acidify their lumen in response to ATP. These non-acidic vesicles lack the 70kD catalytic subunit of the vacuolar proton ATPase, as well as the 31kD subunit. Furthermore, the 16kD proteolipid that forms the transmembrane proton pore was not detectable in purified principal cell endosomes. Surprisingly, the 56kD cytoplasmically-oriented B-subunit of the proton ATPase was abundant in these vesicles. Because these endosomes are directly derived via clathrin-mediated endocytosis, our results indicate that endocytotic clathrin coated vesicles are non-acidic compartments in principal cells. In contrast, recycling vesicles in intercalated cells contain large numbers of proton pumps, arranged in hexagonally-packed arrays on the vesicle membrane. These pumps are inserted into the apical plasma membrane of A-type (acid-secreting) intercalated cells, and the basolateral plasma membrane of B-type (bicarbonate-secreting) cells in the collecting duct. The polarized insertion into both domains is disrupted by microtubule depolymerizing agents.

G 011 MEMBRANE TRAFFIC AND TRANSCYTOSIS IN POLARIZED EPITHELIAL CELLS: SIGNALS, MECHANISMS, AND REGULATION. K. Mostov, G. Apodaca, B. Aroeti, C. Okamoto, W. Song, and *M. Bomsel, Departments of Anatomy, and Biochemistry and Biophysics, University of California, San Francisco, CA 94143-0452, USA, and *Unité de Pathogénic Microbienne Moléculaire, Institut Pasteur, Paris, France.

Polarized epithelial cells use two mechanisms to send proteins to the correct plasma membrane domain: direct delivery from the TGN to the final surface, and indirect delivery to one surface, followed by endocytosis and transcytosis to the opposite surface. We have used the polymeric immunoglobulin receptor (pIgR) as a model to study these processes. The membrane-proximal 17 residues of the cytoplasmic domain of the pIgR are an autonomous signal for delivery from the TGN to the basolateral surface. Transcytosis is regulated by two independent signals: phosphorylation of Ser^{664} on the cytoplasmic domain, and binding of ligand (IgA) to the extracellular domain. Activation of the heterotrimeric G protein, G_S, by cholera toxin stimulates transcytosis independently of these two signals. We have reconstituted budding of transcytotic vesicles from early endosomes in a perforated cell system. Both the α and $\beta\gamma$ subunits of G_S act to control sorting of pIgR into transcytotic vesicles. G_S is thus the first *identified component* of the machinery for polarized sorting of proteins in epithelial cells

G 012. CELL-FREE TRANSPORT OF VIRAL MEMBRANE GLYCOPROTEINS FROM THE GOLGI APPARATUS TO THE PLASMA MEMBRANE OF POLARIZED CELLS, Jean-Pierre Simon, Arie Mayer, Diego Gravotta, Ivan Ivanov, Milton Adesnik and David D. Sabatini, New York University School of Medicine, New York, NY 10016.

Polarized epithelial cells are capable of incorporating distinct sets of membrane proteins into their apical and basolateral plasma membrane domains. In particular, in virally infected MDCK cells, glycoproteins destined to opposite plasma membrane domains are sorted in the trans Golgi network (TGN), to be incorporated into different vesicles which are directly delivered to the cell surface domain where each protein accumulates. We have studied the process of vesicle formation in the TGN and of vesicle targetting to the cell surface using several different experimental systems. Firstly, we have used the toxin Streptolysin O to selectively permeabilize either the apical or basolateral membrane of MDCK cells which permits the examination of the effects of impermeant reagents on membrane protein transport to the intact surface. Studies with this system indicate that GTP-binding proteins are involved in the constitutive process that effects vesicular transport from the TGN to the plasma membrane and that they are charged with GTP early in this process, probably during vesicle formation. We have also achieved vesicle formation in an *in vitro* system using a Golgi fraction from VSV or Influenza virus-infected cells. In this system, vesicle generation is temperature, ATP, GTP, and cytosol dependent and a fraction of cytosolic proteins capable of supporting vesicle formation, which includes an NEM sensitive factor, has been purified about 100 fold by column chromatography. Cytosol obtained from yeast was as effective as rat liver or bovine brain cytosol in supporting vesicle formation. Delivery from the Golgi apparatus to the plasma membrane was studied employing a purified immobilized basolateral plasma membrane fraction from MDCK monolayers grown on cytodex beads. This process was abolished by alkylation of SH- groups and by the presence of GTP-γ-S, which implicates the requirement for GTP hydrolysis in one or more stages of the transport process. Mild proteolysis of the acceptor membranes abolished transport, suggesting the requirement for proteinaceous acceptor sites in the plasma membrane. Surprisingly, both VSV G and Influenza HA were transported with equal efficiencies to the basolateral acceptor membrane. However, at low concentrations, a partially purified microtubule protein fraction preferentially inhibited the transport of HA, although this effect was not abolished by microtubule depolymerizing agents.

Development of Neuronal Polarity

G 013 TRAFFIC OF SYNAPTIC VESICLES IN NEURONS AND ENDOCRINE CELLS. P. De Camilli, O. Mundigl, M. Matteoli, K. Takei and P. Cameron. Howard Hughes.Medical Institute and Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06510, USA

Neurons and endocrine cells are specialized for the regulated secretion of signal molecules. Neurons have at least two pathways of regulated secretion which involve two classes of secretory organelles: typical synaptic vesicles (SVs) and large dense-core vesicles. Large dense-core vesicles store and secrete peptide neurotransmitters and amines and may be seen as the neuronal counterpart of secretory granules of endocrine cells. SVs are highly specialized secretory organelles which store and secrete non-peptide neurotransmitters and play a dominant role in the fast, point-to-point signalling typical of the nervous system. Microvesicles which share a variety of biochemical and functional similarities with SVs (synaptic-like microvesicles, SLMVs) have recently been described in endocrine cells. SVs and SLMVs are closely related to vesicular carriers of the receptor-mediated recycling pathway. They undergo repeated cycles of exo-endocytosis which are thought to involve endosomal intermediates.

In adult neurons SVs are concentrated in axon endings. Likewise, SLMVs accumulate at the end of neuritic processes when endocrine cells undergo neuronal differentiation in vitro and extend axon-like neurites. To gain some insight into the mechanisms responsible for the selective accumulation in axon terminals of SVs and SLMVs, we have studied the traffic of a variety of SV proteins in developing hippocampal neurons in primary culture at different stages of differentiation. Additionally, we have studied the targeting of the SV protein synaptophysin when expressed by transfection in polarized epithelial cells (MDCK cells).

We have found that SV proteins are present in developing neurons already at stages which precede the establishment of neuronal polarity. As axons and dendrites form, SV proteins are found in both types of processes, although they become progressively more concentrated in the axon. Throughout these developmental stages SVs undergo an active exo-endocytotic recycling in both dendrites and axons. Vesicles which undergo exocytosis in minor processes of unpolarized neurons can be subsequently recovered in axons, thus indicating the occurrence of trancytosis. The nonpolarized distribution of SV proteins is observed even at stages when the transferrin receptor - a protein which in epithelial cells is present only at the basolateral surface - is already completely restricted to dendrites. This indicates that at least in immature neurons SV proteins are not selectively targeted to axons and that the accumulation in axons may be the result of a specific retention. In agreement with this finding we have found that synaptophysin, when transfected into MDCK cells, is targeted to both the basolateral and the apical membrane.

In order to begin elucidating the recycling pathways of SV in axons and dendrites we have studied the effect of brefeldin A on the localization of SV proteins in neurons and endocrine cells. We have found that the intracellular distribution of different SV proteins is differentially affected by brefeldin A and that this drug has a different effect on dendritic and axonal endosomes. These observations suggest the occurrence of a continuous sorting of SV proteins during recycling and support the hypothesis that SVs may be continuously regenerated by endosomal sorting. Additionally, they support the existence of two separate endosomal systems in axons and dendrites which may be related to the basolateral and apical endosomes of epithelial cells.

G 014 THE EXOCYTIC AND ENDOCYTIC PATHWAYS OF POLARIZED NEURONS, Carlos G. Dotti, Robert Parton, Toshihide Kobayashi, Lukas Huber, Paul Dupree, Brian Storrie, and Kai Simons. Cell Biology Program, European Molecular Biology Laboratory, D-6900 Heidelberg, Germany.

Infection of polarized hippocampal cells in culture with the RNA viruses Vesicular Stomatitis Virus (VSV) or Fowl Plague Virus (FPV) leads to the polarized delivery of the spike viral glycoproteins G and hemagglutinin to dendrites and axons respectively. Several aspects of this polarized delivery were investigated. The involvement of microtubules was first analyzed. Microtubules were disrupted using nocodazole and the localization of the viral glycoproteins analyzed by immunofluorescence microscopy. Nocodazole treatment inhibited the polarized delivery of VSV glycoproteins to dendrites and the axonal delivery of FPV hemagglutinin. Nocadazole removal re-established the polarized distribution to axons and dendrites of the viral glycoproteins. Secondly, the involvement of the small GTP binding proteins (sGTPbp) in directing axonal and dendritic glycoprotein traffic was studied. Biochemical analysis of microsomal fractions of hippocampal neurons before and after synaptogenesis revealed that different sGTPbp are expressed during development. One of the proteins which increased after synaptogenesis was identified as Rab 8. In polarized non-neuronal cells this protein is enriched in basolateral but not apical post-TGN vesicles. By immunofluorescence microscopy Rab 8 was found exclusively in the cell body and dendrites of mature hippocampal cells. Axons did not contain detectable Rab 8. Moreover, double labelling experiments after VSV infection utilizing anti-Rab 8 and anti VSV-G antibodies revealed partial colocalization of the two proteins. Thirdly, the existence of a barrier which would prevent the diffusion of the axonally inserted hemagglutinin protein into the somatodendritic territory was investigated by utilizing a liposome-plasma membrane fusion protocol. Liposomes containing fluorescently labelled phospholipids and the ganglioside GD1a were added to FPV-infected cells and fusion was induced by lowering the pH of the incubation medium.

After fusion, fluorescent signal was restricted to the axonal surface. The labelling started at the axonal origin leaving the cell body and dendrites completely unlabelled. Fluorescence recovery after photobleaching showed that fusion had occurred along the axonal surface. Fusion of symmetrically labelled liposomes in which the outer leaflet labelled lipids were quenched or fusion of inner-leaflet labelled liposomes also showed exclusive axonal labelling. These results suggest the existence of a functional barrier to movement of lipids located at the axonal hillock region and operating in both membrane leaflets which may explain how axonal and dendritic proteins are prevented from intermixing.

The axonal and dendritic endocytic pathways were analyzed by combining electron and video microscopy. Semi-thick sections and high activity HRP revealed a tubular network in the dendrites and at synaptic terminals but not along the axonal shaft. These structures constitute the early endosomal compartment. These structures in dendrites but not in axons were labelled after internalizing transferrin-HRP. The late endocytic structures were localized using late-endosome and lysosome-specific antibodies and found exclusively in the cell body. Video microscopy of living cells followed by electron microscopy revealed that multivesicular body-like structures and not tubular early endosomes are the structures responsible for the transport of ligands from the early endosomes to the degradative compartments in the cell body.

Mechanisms of Neuronal Polarity

G 015 ANKYRIN-BINDING PROTEINS RELATED TO NEURAL CELL ADHESION MOLECULES: CANDIDATES TO PROVIDE TRANSMEMBRANE AND INTERCELLULAR CONNECTIONS IN ADULT BRAIN, Vann Bennett and Jonathan Q. Davis, HHMI and Dept. of Biochemistry, Duke University Medical Center, Durham, NC 27710.

A family of ankyrin-binding glycoproteins has been identified in adult rat brain employing ankyrin and lectin affinity chromatography. GP186, which is the major form in forebrain, binds directly to ankyrin with high affinity and comprises 0.3% of total rat brain membrane protein. GP186 is co-expressed with the mature form of brain ankyrin in late post-natal development, and is co-localized with ankyrin on the plasma membranes of neurons and glial cells. Ig against GP186 also recognizes polypeptides of 155 and 140 kDa which have N-terminal sequences consistent with the possibility that all three polypeptides are derived by alternative splicing from the same pre-mRNA. GP155/140 bind to ankyrin with lower affinity than GP186, and exhibit distinct patterns of expression in development and in regional distribution. GP155 is enriched in nerve tracts and is localized to unmyelinated axons and Nodes of Ranvier. The primary structure of GP186 deduced from analysis of cDNAs contains 6 Ig-C2 repeats, 4 fibronenctin type 3 repeats, a region enriched in proline and threonine residues, a predicted membrane-spanning segment and a cytoplasmic domain of 109 residues. The sequence is closely related to chicken neurofascin, a neural cell adhesion molecule, with overall sequence identity of 70 per cent, and related to a lesser extent to the cell adhesion molecules L1, Ng-CAM, and Nr-CAM from vertebrate brain and neuroglian from Drosophila. The predicted cytoplasmic domains of these molecules are the most conserved in terms of sequence similarity. These sequences define a group of proteins, termed Ank-CAMs, which are predicted to bind to ankyrin and to have functions related to cell adhesion molecules. Ank-CAMs have the potential to mediate transbilayer connections between the cytoskeleton and the extracellular environment and may play a role in the establishment and/or maintenance of the terminally differentiated nervous system.

G 016 BIOGENESIS OF SECRETORY VESICLES IN ENDOCRINE CELLS. Regis B. Kelly, Dept of Biochemistry & Biophysics and Hormone Research Institute, University of California, San Francisco, CA 94143-0448.

Peptide-secreting endocrine cells sort proteins into their secretory granules by two steps, condensation and trimming. After exocytosis of their granule contents they efficiently recycle their membrane proteins back to the TGN via a conventional endocytotic pathway. Since some endocrine cells release neurotransmitter from their peptide-containing secretory granules, the proteins required for neurotransmitter uptake also recycle through the endocytotic pathway. In neurons and neuroendocrine cells neurotransmitter-uptake proteins are directed out of the endocytotic pathway into synaptic vesicles, organelles of homogeneous diameter from which other endocytotic markers such as the LDL receptor are excluded. It is speculated that three properties of synaptic vesicles, their homogeneous diameter, their sorting from the endocytotic pathway and their association with the plasma membrane, might be due to the expression of synaptic vesicle-specific proteins, such as synaptophysin and synaptobrevin, that are absent or rare in secretory granules.

Since synaptic vesicles are involved in paracrine communication between neurons, a reasonable assumption is that they are involved in paracrine communication between endocrine cells. Since paracrine communication requires that heterologous cell-cell contacts be precise, some mechanism must then exist in endocrine cells to assure that cell contacts are correct. We sought rat, pancreatic islet-specific, cadherins that might explain the segregation of exocrine cells from endocrine, and the segregation of α from β cells. N- and a new cadherin (X) are present in islets, but not exocrine tissue. In α-tissue culture cells the ratio of X to N is about 20 times greater than in β-tissue culture cells. Since X is closely related to retinal cadherin, neural-specific cadherins might regulate the development and morphology of endocrine tissue.

G 017 SYNAPTIC VESICLE PROTEINS AND MEMBRANE FLOW IN THE NERVE TERMINAL, Richard Scheller[1], [1]Howard Hughes Medical Institute, Beckman Center, Stanford, CA 94305-5428.

The molecular events that mediate synaptic transmission are not understood. As a first step in approaching this problem, we have characterized a number of cDNA clones encoding proteins specifically associated with synaptic vesicles. Studies of the marine ray and rat have defined the sequences of VAMP, low molecular weight GTP-binding proteins, p38 or synaptophysin, p65 or synaptotagmin and SV2. All of these proteins are members of small gene families that are differentially expressed throughout the nervous system. To study the function of these molecules, we are taking a variety of approaches. Mutant forms of the molecules are being transfected and microinjected into cells and the effect on localization of vesicles or exocytosis quantitated. Biochemical studies are being used to define protein-protein interactions which may be important in the functions of these molecules.

Cell Communication and Cytoskeleton Function

G 018 ASSOCIATION OF MYOSIN-I WITH GOLGI VESICLES, D. Burgess and K. Fath, Dept. of Biological Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15260.

The delivery of constituents to the apical membrane in polarized epithelia is thought to be facilitated by, but not absolutely require, intact microtubules. The immunolocalization of the mechanoenzyme myosin–I with cytoplasmic vesicles in intestinal enterocytes[1] led us to explore a role of myosin–I in vesicle trafficking in intestinal epithelia. We isolated cytoplasmic vesicles from undifferentiated enterocytes that express myosin–I on their cytoplasmic surface and bind actin filaments in an ATP–dependent manner[2]. We now report that these vesicles, which were isolated by methods established for Golgi purification, contain a 2–fold enrichment for galactosyltransferase (GalTase) specific activity, a marker enzyme for the *trans*–Golgi. Moreover, in that ~50% of the GalTase activity partitions with intact vesicles that were immunoisolated with myosin–I antibodies, suggests that myosin–I and GalTase are co–expressed on the same vesicle. Alkaline phosphatase, an apical plasma membrane–specific marker, was also associated with the vesicles. The presence of both enzymes suggests that these vesicles may represent carrier vesicles transporting membrane to the apical plasma membrane. By immunoblotting we found that the microtubule–based motor dynein was also bound to the vesicles. We are currently determining if single vesicles express both dynein and myosin–I, with the aim of exploring a role for both a microtubule– and an actin–based motility working in parallel in apical membrane targeting in intestinal epithelia.

[1]Drenckhahn & Dermietzel, (1988), *J.C.B.*, 107:1037.
[2]Fath & Burgess, (1991), *J.C.B.*, 115:39a.

G 019 THE MAKING OF A TIGHT JUNCTION, Marcelino Cereijido, María S. Balda, Lorenza González-Mariscal, Rubén G. Contreras, Juan M. Gallardo and M. del R. García-Villegas. Center for Research & Advanced Studies, México, D.F. México 07000.

MDCK cells plated at confluence establish tight junctions (TJs) in 12-15 hrs through a process that requires synthesis of proteins (1), calmodulin (2) a Ca^{2+}-dependent exocytic fusion of membrane components (3), formation of a ring of actin filaments in close contact with the lateral membrane (4), and the Ca^{2+}-activation of uvomorulin molecules located on the lateral membrane of neighboring cells (5). Newly plated monolayers incubated in the absence of this ion make no TJs. Yet if Ca^{2+} is added under these circumstances, TJs are made with a faster kinetics (6). Ca^{2+} is needed mainly at a site located on the outer side of the cell membrane (7) where it activates uvomorulin and triggers the participation of the cellular components mentioned above, via G-proteins associated to phospholipase C and protein kinase C (2).

No information is available on why some epithelia make TJs that confer a mere transepithelial resistance (TER) of 10 $\Omega.cm^2$ and others of 10.000. However, we detected a peptide in the urine of dogs that increases the electrical resistance of MDCK monolayers in a reversible and dosis-dependent manner, that may in principle account for the progressive increase in TER along the nephron. Thus as the glomerular filtrate proceeds from the glomerulus towards the collecting duct, it concentrates *ca* 170-fold. Therefore each segment of the nephron is in contact with a progressively higher concentration of the peptide. Since, as mentioned above, this peptide acts in a concentration-dependent manner, it may in principle account for the range of TER observed.

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G 020 DESMOSOME FUNCTION AND ASSEMBLY, Pamela Cowin and Susan Mechanic, New York Medical Center, 550 First Avenue, New York, NY 10016.

Desmocollins and desmoglein are major components of the adhesive core structure of desmosomes, an intercellular junction that anchors intermediate filaments to the membrane. The ectodomains of these molecules have extensive sequence similarity to the external domains of members of the cadherin family of cell adhesion molecules which themselves are concentrated at adherens junctions that associate with filamentous actin. We are interested in the question of how these rather similar adhesive molecules become sequestered into their neighboring yet biochemically distinct domains and specifically associate with different elements of the cytoskeleton. The most striking difference among the desmosomal glycoproteins and cadherins lies in their cytoplasmic sequences. Desmoglein and desmocollins diverge from each other as well as from the cadherins in this region and therefore comprise three distinct subtypes within this superfamily. Intriguingly, desmoglein and the alternatively spliced region that gives rise to desmocollin I show some similarity to the catenin-binding domain of the cadherins, a region that is known to regulate the adhesive properties of the cadherin ectodomain. The members of all three subtypes of the cadherin family interact with a commonplaque component plakoglobin. Plakoglobin was the first described member of a highly conserved family of proteins, which include the product of the *Armadillo* segment polarity gene, beta catenin and a putative substrate of *src* called p120. Several observations suggest that phosphorylation of members of the plakoglobin family may dynamically regulate the interactions of the adhesive glycoproteins in such a way as to affect their membrane distribution, stability and hence their adhesive function. Cadherins interact in the plane of the membrane and with molecules on neighboring cells in a highly specific, homophilic manner. The diversity of this family confer upon cells the ability to selectively adhere to each other during morphogenesis. In contrast to the cadherins the desmosomal glycoproteins form heterologous complexes during their biosynthesis. The desmosomal cadherins are diverse in the number and tissue expression patterns of their isoforms generated both from a multigene family as well as alternative splicing.

G 021 POST-TRANSLATIONAL PROCESSING OF CONNEXIN43: BOTH PHOSPHORYLATION AND LOSS OF SOLUBILITY IN TRITON X-100 ARE TEMPORALLY ASSOCIATED WITH ASSEMBLY INTO GAP JUNCTIONAL MACULAE, Linda S. Musil and Daniel A. Goodenough. Department of Anatomy and Cellular Biology, Harvard Medical School, Boston, Massachusetts, USA.

The formation and functional regulation of gap junctions is a complex and poorly understood phenomenon. The gap junction protein connexin43 (cx43) is translated as a 42kD protein (cx43-NP) that is efficiently phosphorylated to an M_r = 46kD species (cx43-P_2) in gap junctional communication-competent cells. In contrast, cell lines severely deficient in junctional communication (mouse S180 and L929 cells) synthesize cx43-NP but neither process it to the P_2 form nor accumulate cx43 in gap junctional maculae (Musil et al., 1990 *J. Cell Biol.* **111**:2077-2088). A combination of metabolic radiolabeling and immunoprecipitation of cx43 with affinity-purified anti-cx43(252-271) antibodies has shown that newly synthesized cx43-NP could be quantitatively solubilized from cellular membranes with isotonic solutions of 1% Triton X-100. Maturation of cx43 to the terminally phosphorylated P_2 form in communication- competent NRK cells was accompanied by acquisition of resistance to Triton solubilization; in contrast, cx43 was not processed to the P_2 form and remained Triton soluble in communication-deficient S180 and L929 cells. Immunohistochemical localization of cx43 with anti-cx43(252-271) in Triton-extracted NRK monolayers revealed that that cx43-P_2 (Triton-insoluble) was concentrated in gap junctional maculae whereas cx43-NP (Triton-soluble) was predominantly intracellular. Two lines of evidence indicated that phosphorylation of cx43 to the P_2 form and acquisition of Triton insolubility occurred after transport of cx43 to the plasma membrane. First, conversion of cx43-NP to Triton-insoluble cx43-P_2 was reversibly blocked by incubation of NRK cells at 20° C, a temperature at which transport of nascent proteins is halted in the trans Golgi. Second, cx43-NP was detected on the surface of intact NRK monolayers by cell surface biotinylation at 4° C; this biotinylated cx43-NP was processed to Triton-insoluble cx43-P_2 when the cultures were warmed to 37° C. Interestingly, surface biotinylation also revealed that cx43-NP was transported to the plasma membrane in S180 cells despite their lack of gap junctions. Taken together, these results demonstrate that assembly of cx43 into gap junctional maculae is temporally associated with acquisition of Triton X-100 insolubility and phosphorylation to the cx43-P_2 form, both of which occur (at least in part) after arrival of cx43 at the plasma membrane. Conversion of cx43 to the P_2 form is thus not required for transport of cx43 to the cell surface and must occur during subsequent assembly or functional processes.

Epithelial and Neuronal Cell Growth and Differentiation

G 022 THE DEVELOPMENT OF POLARITY BY HIPPOCAMPAL NEURONS IN CULTURE, Mark Jareb, Teresa Esch, Ann Marie Craig, and Gary Banker, Department of Neuroscience, University of Virginia School of Medicine, Charlottesville, Virginia 22908

In culture, embryonic rat hippocampal neurons acquire their characteristic form in a stereotyped way. Initially the cells form several short, apparently identical processes that cannot be clearly categorized as either axons or dendrites (referred to as developmental stage 2). After 12 to 24 hrs, one of these processes begins to elongate rapidly, becoming the cell's axon (developmental stage 3). Only some days later do the remaining processes begin to elongate and acquire the taper and branching pattern characteristic of dendrites (developmental stage 4). These morphological changes are paralleled by a change in the polarity orientation of microtubules. At stage 2 and 3, the microtubules in all processes are uniformly oriented with plus-ends distal. At stage 4, dendritic microtubules are of mixed polarity, while axonal microtubules retain a uniform orientation.

To trace the development of membrane polarity we used immunostaining to follow changes in the distribution of three axonal proteins--the integral membrane protein L1, a cell adhesion molecule, synaptophysin, a transmembrane protein of synaptic vesicles, and GAP-43, a protein that associates with membranes via palmitylation--and of the GluR1 receptor, an integral protein of the somatodendritic membrane. At stage 2, there was no evidence of a selective localization of any of these proteins. By stage 3, the three axonal markers were preferentially concentrated in the axon, although a low level of expression persisted in other processes. In contrast, the GluR1 receptor was uniformly distributed; it did not become selectively localized to the somatodendritic domain until stage 4. Residual dendritic expression of axonal proteins also declined at this stage. Killish et al. (Neuron 7:927-936, 1991) have previously reported that the GABA-A receptor, another somatodendritic protein, is also uniformly distributed at stage 3 of development. Thus the polarized distribution of membrane proteins appears to arise in two distinct phases that parallel the morphological stages of development. If these results can be generalized, they would also suggest that axonal proteins become selectively distributed before those of the somatodendritic domain.

We also examined the effects of brefeldin A (BFA), a drug that interrupts traffic along the exocytic pathway and disrupts transcytosis, on these aspects of neuronal morphogenesis. When BFA (1 ug/ml) was added to stage 3 cells, it blocked axonal elongation within 1 hour. Within a few hours, axons began to retract, while other processes were unaffected; in some cases axonal retraction progressed until polarity was lost. When BFA was added to cells at stage 2 of development, axons failed to develop. All of the effects of BFA were completely reversible. The effects of BFA were much more profound than those of protein synthesis inhibitors. In the presence of cycloheximide (10 ug/ml), axons became thinner, but continued to elongate for 4 to 6 hours.

From these observations we would argue that mechanisms for the directed transport of membrane proteins to the axon are already present at stage 3 of development, and that these mechanisms cannot depend on differences in the polarity orientation of microtubules. Directed traffic along the exocytic pathway may be particularly important for the initial emergence of the axon, the first event in the expression of neuronal polarity.

This research was supported by NIH grant NS17112, by NIH training grant HD07323 (M.J.), by a graduate research fellowship from the NSF (T.E.) and by an NIH postdoctoral fellowship (NS09248 to A.M.C.).

G 023 OVER-EXPRESSION AND TARGETING OF TRANSFORMING GROWTH FACTOR ALPHA (TGFα) IN POLARIZED EPITHELIAL CELLS. Peter J. Dempsey, Ramona Graves-Deal, Mary Katherine Meise and Robert J. Coffey. Departments of Medicine and Cell Biology, Vanderbilt University, Nashville, TN 37232-2279.

Transforming growth factor alpha (TGFα) is a member of an expanding family of epidermal growth factor (EGF)-related ligands which share both structural homology with EGF and the ability to bind the epidermal growth factor receptor (EGFR). Other EGF family members include amphiregulin (AR), heparin-binding (HB)-EGF and possibly cripto. The human pro-TGFα precursor is synthesized as a 160 amino acid integral membrane glycoprotein which contains an N-terminal hydrophilic signal sequence and undergoes complex processing. In CHO and fibroblastic cell lines transfected with pro-TGFα, the pro-TGFα precursor is synthesized and transported to the cell surface where it is then cleaved by elastase-like enzymes to release bioactive TGFα. The mature 50 amino acid, 5.6 kDa polypeptide is the predominant TGFα species released from cells. It is the mature molecule that shares 35% sequence homology with EGF, binds to the same receptor and has similar (although not identical) biological effects. A commonly cited action of both TGFα and EGF is that they act as potent mitogens in a number of epithelial cell systems. In addition, TGFα stimulates epithelial cell migration, promotes angiogenesis, induces bone resorption, inhibits gastric acid secretion and can protect gastric mucosa against ethanol and aspirin-induced damage. The mutated membrane-anchored form of pro-TGFα is also biologically active and is able to subserve other functions such as cell-cell adhesion.

TGFα is expressed within epithelial cells throughout the gastro-intestinal tract. We have demonstrated recently a gradient of increasing TGFα expression from the crypt to villus in the small intestine. Although the exact function(s) of TGFα in intestinal epithelium are not known, its mechanisms of action are presumably via binding to the EGFR. Previous studies have indicated that EGFRs are expressed only on the basolateral surface of polarized epithelial cells, including intestinal epithelium. This implies that for endogenous TGFα to act in an autocrine/paracrine manner within the fully differentiated and polarized intestinal epithelium of the villus, it must be presented to the basolateral surface. However, little is known about the biosynthesis, sorting or secretion of TGFα in polarized epithelial cells. It is not known whether the proTGFα precursor and therefore the transmembrane form of TGFα are targeted to any specific membrane domains. This may have important biological significance for both the delivery and mechanisms of TGFα action in epithelial cells and tissues.

To investigate the expression of pro-TGFα in polarized epithelial cells, we have overexpressed human pro-TGFα cDNA in nontransformed MDCK II cells under the control of the steroid inducible MMTV promoter/enhancer. The MDCK II cell line provides an excellent in vitro model of polarizing epithelium in which the EGFR is located only on the basolateral surface. MDCK II cells stably transfected with TGFα showed low basal TGFα expression but extremely high TGFα levels were produced upon induction with dexamethasone. These MMTV-TGFα transfectants have been used to study the biosynthesis and sorting of pro-TGFα as well as the secretion of soluble TGFα in polarized MDCK cells. The status of the EGFR with respect to both synthesis and level of tyrosine phosphorylation has also been examined. These studies have been complemented by the analysis of MDCK II cells stably transfected with the mutated membrane-anchored form of pro-TGFα under the control of SV40 promoter. The effects of TGFα over-expression in MDCK II cells with respect to cell growth and transformation will also be discussed.

Control of Epithelial Gene Expression and Differentiation

G 024 REGULATION OF GENE EXPRESSION IN KERATINO-CYTES IN VIVO AND IN VITRO. Fuchs, E., Byrne, C., Leask, A., Aneskievich, B., Faus, I. and Hsu, H. Howard Hughes Medical Institute, Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637.

Keratins are the major structural proteins of keratinocytes. They can be subdivided into type I and type II subgroups, which are coexpressed as specific pairs that form obligatory heteropolymers. Since keratins are differentially expressed during differentiation and development in stratified squamous epithelia, they provide useful biochemical markers to study how these complex differentiation programs are controlled. A knowledge of the sequences and factors involved in regulating keratin genes should lead to insights into how particular epithelial differentiation fates are controlled during embryonic development. Finally, the promoters-/enhancers to these genes have provided valuable tools to target expression of regulatory and structural genes to specific epithelial cells of transgenic mice, a feature which has important medical as well as biological implications. In this talk, I will review what is presently known about how genes are regulated within the keratinocyte. Our major focus is on the genes encoding keratins 5 and 14, which account for ~25% of the total cell protein of a keratinocyte. We have used transgenic mice and gene transfection of cultured keratinocytes to identify the sequences involved in keratinocyte and differentiation specificity. We have also characterized many of the transcription factors involved in this process.

G 025 USE OF TRANSGENIC MICE TO STUDY GUT STEM CELL BIOLOGY AND AXIAL PATTERN FORMATION. Jeffrey I. Gordon[1], Steven M. Cohn[2], Deborah C. Rubin, Theodore C. Simon[1], Kevin A. Roth[3] and Steve Kim[4], [1]Departments of Molecular Biology and Pharmacology, [2]Medicine, [3]Pathology, and [4]Surgery, Washington University School of Medicine, St. Louis, Missouri.

The mouse gut epithelium represents a continuous developmental system. Its four principal cell lineages - enterocytes, goblet, enteroendocrine, and Paneth cell - are derived from a single multipotent stem cell functionally anchored near the base of crypts. The descendants of this stem cell undergo amplification, lineage allocation and differentiation during a well-organized migration in vertical coherent bands from monoclonal crypts to their associated polyclonal villi (or the colonic homolog of villi known as the surface epithelial cuffs). Migration/differentiation and subsequent exfoliation are rapid and perpetual processes. Cephalocaudal differences in the differentiation programs of each lineage are established from the time of initial cytodifferentiation of the gut epithelium in late gestation and maintained throughout adulthood, despite perpetual renewal of this cellular population. The intestinal epithelium thus provides a unique mammalian model for studying the biological features of stem cells (e.g. their ability to undergo asymmetric division, their enormous proliferative capacity, their capacity for functional anchorage), exploring how stem cell hierarchies are established and maintained, examining the relationship between passage through the cell cycle and lineage allocation, and understanding the mechanisms that provide stem cells with a positional address along the cephalocaudal axis. We have used transgenic mice to map cis-acting regulatory elements in members of a family of fatty acid binding protein genes (*Fabp*) that control their (i) expression during lineage allocation and differentiation, (ii) geographic patterns of expression along the cephalocaudalaxis, and (iii) activation during late fetal life. *Fabp*/reporter transgenes have been utilized to define stem cell hierarchies within and between crypts in developing and aging mice and to examine what effects re-entry into the cell cycle has on cellular differentiation programs.

G 026 EVIDENCE FOR STEM CELLS AND LINEAGES IN EMBRYONIC AND ADULT LIVER, A TISSUE ASSUMING A QUIESCENT STATE. S.H. Sigal, S. Brill, P. Holst, I. Zvibel, A. Fiorino, A. Ochs, M. Agelli, and L. M. Reid, Albert Einstein College of Medicine, Bronx, New York

The structural and functional unit of the liver is the acinus organized between two vascular beds: six sets of portal triads around the periphery and a central vein at the center. The liver parenchyma consist of cell plates arrayed between the portal triads and the central vein. Based on evidence summarized in a recent review (1), we have hypothesized that the liver, at all ages, contains stem cells, antigenically related to oval cells, and derived from cells located at or near the Canals of Hering at each of the portal triads. The stem cells are hypothesized to produce daughter cells undergoing a unidirectional, terminal differentiation process ending near the central vein. The plates of parenchymal cells are proposed to be lineages of maturing liver cells with age-dependent size, ploidy, growth and differentiative potential. Thus, the well documented heterogeneity of gene expression in the liver is interpreted to be lineage-dependent phenomena of "early", "intermediate", and "late" genes. The stem cells are hypothesized to be partially transformed to become "oval cells", small cells with oval-shaped nuclei induced to proliferate extensively in animals treated with appropriate oncogens (chemicals, viruses, radiation), or completely transformed to become hepatomas or cholangiocarcinomas. Studies to date characterizing the phenotype of oval cell antigen positive cells, OCAP cells, derived from normal livers, indicate extensive overlap in their phenotypes with that of oval cells and with hepatomas (1). Thus, many so-called "tumor markers" are likely to be normal "early" genes.

We have been developing methodologies for identifying, isolating and culturing the hepatic precursor cells. Of the monoclonal antibodies (MABs) to antigens characteristic of oval cells, we have found ones such as 374.3 that, with fluorescence activated cell sorting (FACS), enrich for cells expressing albumin and alpha-fetoprotein. Interestingly, all MABs to oval cell antigens also identify myeloid and erythroid precursor populations necessitating panning and other protocols to eliminate these hemopoietic contaminants. The enriched hepatic precursor cell population isolated from animals from embryonic day 13 to adult is being characterized using immunohistochemistry, flow cytometry, and molecular hybridization assays for many fetal and adult liver markers. In addition, matrix, basal medium, hormonal and feeder layer requirements have been identified permitting high density and colony growth in culture for three weeks or more of OCAP cells. The strictest requirement for OCAP cell growth in culture proved to be age- and tissue-specific stromal feeder layers. Preliminary studies of *in vivo* and *in vitro* bioassays are underway to determine the fate(s) of these cells but is suggestive that the cells are capable of maturing into adult parenchymal cells.

Our long term goals are to fully develop the hepatic precursor model systems as representatives of liver development and differentiation, systems that should permit one to study aspects of gene expression that cannot be analyzed with existing liver cultures and that will be true controls for liver tumor cells. The data are discussed as evidence that quiescent tissues are variant forms of stem cell and lineage systems and the implications of this for studies on growth regulation, regeneration, gene expression, carcinogenesis, virology, aging, and gene therapy.

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Disease States

G 027 EPITHELIAL CELL PATHOPHYSIOLOGY IN POLYCYSTIC KIDNEY DISEASE (PKD). Ellis D. Avner, William E. Sweeney, Jr, University of Washington and Children's Hospital and Medical Center, Seattle, WA

Recent data demonstrate that renal tubular cyst formation and progressive enlargement in PKD are mediated by epithelial hyperplasia and increased transepithelial solute flux. Studies from our and other laboratories suggest that EGF-like peptides may be sequestered in renal cysts, and, therefore, could mediate autocrine/paracrine mitogenesis in cystic tubular epithelium if there is abnormal apical membrane expression of the EGF receptor (EGF-R). We, therefore, studied the expression of EGF-R at progressive stages of collecting tubule cystogenesis in the CPK mouse, a murine model of autosomal recessive polycystic kidney disease. In addition, we studied the effects of EGF on in vitro collecting tubule cystogenesis and EGF-R membrane localization utilizing our previously described method of serum free organ culture of renal explants from CPK mice.

Standard Western and immunohistologic analysis were performed on control and CPK tissue at postnatal days 0, 7, 14, and 21 utilizing a mouse monoclonal antibody raise against the EGF-R of A-431 cells. In addition, cyst formation (morphometry) and epithelial hyperplasia (autoradiography) were immunohistologically compared to the plasma membrane distribution of EGF-R in lectin isolated collecting tubules from control and 7 day old cystic explants following 120 hours of incubation in completely defined basal, or EGF (20 ng/ml) supplemented medium with and without tyrosine kinase inhibition (genistein 5.5×10^{-4} M).

At all stages of disease progression, EGF-R expression was significantly greater than age matched controls ($p<.001$). EGF-F was present in epithelial basal lateral cell membranes and control collecting tubules at all stages. In contrast, EGF-R was expressed apically as well as basolaterally in cystic CPK collecting tubules. In vitro, following culture under basal conditions, CPK collecting tubular cysts regressed in association decreased hyperplasia and redistribution of EGF-R solely to basolateral membranes ($p < 0.001$ vs. CPK preculture; NS vs control). Following culture with EGF, collecting tubule cyst formation was stimulated in CPK explants in direct relation to increased hyperplasia and localization of EGF-R to apical as well basolateral surfaces in collecting tubule epithelium ($p<.001$ vs control or CPK basal medium; NS vs CPK preculture). Tyrosine kinase inhibition completely blocked all EGF induced hyperplasia and cyst formation. Both in vivo and in vitro, no alterations were seen in localization of 8 apical and basal renal tubular cell plasma membrane markers under any experimental conditions studied.

We conclude that increased EGF-R expression is present in CPK cystic kidneys. In addition, EGF-R is mislocated to apical cell surfaces of cystic collecting tubules in this murine PKD model, and tightly linked to epithelial hyperplasia and cyst formation during in vitro modulation of CPK cystogenesis by EGF. In concert with EGF-like factors sequestered in tubular cyst fluid, such alterations in EGF-R expression may mediate cyst formation and progressive enlargement in PKD.

G 028 MODELING OF RETINOPATHIES IN CULTURED RETINAL PIGMENT EPITHELIUM, Dean Bok[1,2,3], William O'Day[1], Marcia Lloyd[1],and Paul Bernstein[3], [1]Department of Anatomy and Cell Biology, [2]Jules Stein Eye Institute and [3]Brain Research Institute, University of California, Los Angeles.

Human and bovine retinal pigment epithelium grown on permeable supports develope transepthelial resistances in excess of 500 Ohms.cm^2 and transepithelial potentials of 3-5 mV. Infection of these cultures with influenza and vesicular stomatitis viruses results in apical and basolateral budding respectively. The cultures can also be used to study the polarized uptake, processing and release of retinoids thereby allowing analysis of the entire retinoid cycle, beginning with the receptor-mediated, basolateral uptake of all-trans retinol and the apical release of 11-cis-retinal. Furthermore, when the apical culture medium contains interphotoreceptor retinoid binding protein (IRBP), the apical release of 11-cis-retinal is maximal whereas the substitution of other proteins, including cellular retinal binding protein (CRALBP) results in a four-fold reduction in release. The results suggest that both the basolateral and apical membranes contain specific mechanisms for retinoid uptake and release and that the cultured cells contain all of the metabolic machinery for retinoid processing preparatory for delivery to the retinal photoreceptors. The capability of cultured RPE for retinoid processing allows us to test one of the theories regarding the mechanism for retinal degeneration in Refsum's disease, an autosomal recessive condition whereby phytanic acid derived from the ingestion of plants cannot be metabolized. The accumulation of this branched fatty acid is thought to interfere with retinoid processing by forming a retinyl ester which cannot serve as a substrate for retinoid isomerase. If this were the case, the RPE would not be able to provide photoreceptors with 11-cis-retinal, the chromophore for the visual opsins. Cultured RPE exposed to 0.2 mM phytanic acid shows the morphological hallmarks of Refsum's disease but it is fully capable of retinoid esterification and isomerization in spite of the accumulation of large amounts of retinyl phytanate and phytanic acid in the cells. Thus, the theory that phytanic acid inhibits retinoid processing appears to be unfounded.

Development of Polarity and Sorting Mechanisms

G 100 LATERAL LOCALIZATION OF Na,K-ATPase IN E-CADHERIN (L-CAM) TRANSFECTED RAT RETINAL PIGMENT EPITHELIAL CELLS (RPE)

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Retinal pigment epithelia (RPE) exhibits reversed apical polarity of the Na,K-ATPase similar to choroid plexus epithelia but opposite to most other epithelia (e.g. kidney, where the enzyme is basolateral). Also, contrary to other *epithelia* RPE does not express the adhesion molecule E-cadherin but does express the neuronal adhesion molecule N-CAM (D. Gundersen *et al*, J.C.B., In Press). A hypothesis for the basolateral localization of Na,K-ATPase in E-cadherin expressing epithelia (e.g. kidney and intestine) is that Na,K-ATPase interacts with cytoskeletal E-cadherin associated elements which stabilize it to the basolateral surface (W.J. Nelson *et al*, JCB, 110:349-357, 1990; H. McNeill *et al*, Cell, 62:309-316, 1990). To test this hypothesis we transfected a recently developed polarized RPE cell line (RPE-J), (I.R. Nabi *et al*, J. Cell Sci., In Press) with E-cadherin and analyzed the distribution of Na,K-ATPase and E-cadherin. The RPE-J cell line exhibits a lateral distribution of N-CAM and a non polarized distribution of Na,K-ATPase, similar to the distribution in primary cultures of RPE cells. After E-cadherin transfection the Na,K-ATPase is strikingly polarized to the lateral surface and colocalize with E-cadherin, as seen by laser scanning confocal microscopy. These results indicate that E-cadherin plays a crucial role in the basolateral targeting of Na, K-ATPase. Supported by N.I.H. grant 525871 (to E.R.B.)

G 101 IS THERE A ROLE FOR THE EXPRESSION OF MUC-1, AN INTEGRAL MEMBRANE MUCIN, DURING EPITHELIAL SHEET FORMATION IN THE MOUSE EMBRYO?

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Muc-1 is a large integral membrane glycoprotein that is expressed in the apical domain of simple secretory epithelia. Its cytoplasmic tail, which is highly conserved among different mammalian species, interacts with the actin filaments. The large extracellular domain is highly glycosylated and appears as a rod-like structure due to the presence of many prolines and O-linked glycans Previous studies have shown that the expression of Muc-1 mRNA and protein correlate with epithelial sheet formation and branching morphogenesis in many different organs (Braga et al. Development, 115: 427, 1992). In order to clarify whether the spatial and temporal correlation is relevant for these processes, an *in vitro* system is needed in which Muc-1 expression can be altered in a branching epithelium. We have been using the *in vitro* differentiation of lung buds to approach this question. Lung buds were dissected from 11.5 day mouse embryos and cultured for a period of 4 days. Very low levels of Muc-1 mRNA are detected by quantitative RT-PCR at days 1 and 2. This RNA level increases rapidly by the end of the culture, following the increase in the cytokeratin 8 mRNA levels. The quantitation of cytokeratin 8 RNA expression was used for monitoring the epithelial proliferation. The amount of Muc-1 protein in the epithelial cells cultured *in vitro* is higher when compared with the Muc-1 expression *in vivo*. In addition to the quantitative difference, the pattern of expression in the lung buds is changed after culture. While the Muc-1 protein is observed only at the apical surface *in vivo*, a small amount of this protein is also found in the cytoplasm and basolateral domain of the epithelial cells *in vitro*. An attenuation of the Muc-1 expression during embryonic lung development *in vitro* is currently being performed using antisense oligos, to determine whether a decrease in Muc-1 expression alters the formation of lumens by lung epithelia.

G 102 EXPRESSION OF CHICKEN VINCULIN COMPLEMENTS THE ADHESION-DEFECTIVE PHENOTYPE OF A MUTANT MOUSE F9 EMBRYONAL CARCINOMA CELL, Robert M. Ezzell[1], Michael Samuels[2], Timothy J. Cardozo[1], David R. Critchley[3], Jean-Luc. Coll[2], and Eileen D. Adamson[2], [1]Surgery Research Laboratory, Mass. General Hospital, Charlestown, MA 02129; [2]La Jolla Cancer Research Fdn. La Jolla, CA 92037; [3]Dept. Biochemistry, University of Leicester, LE1 7RH, U.K.

Mouse F9 embryonic carcinoma cells have been widely used to examine the role of cell interactions in development and epithelium formation. Recently, a F9 cell variant (called 5.51) has been studied which does not adhere to substrates, compact in suspension cultures, or form an epithelium in response to retinoic acid. We have previously shown that neither uvomorulin (E-cadherin) nor integrins are responsible for the mutant phenotype (Calogero et al., Dev. Biol., 146, 499, 1991). In this study, several cytoskeletal proteins were assayed and only vinculin was found to be absent in 5.51 cells. 5.51 cells have active surface motility characterized by filopodia, but no lamellipodia. A chicken vinculin expression vector was transfected into the 5.51 cells and clones were selected that were adherent to the substrate. Two clones, 5.51Vin3 and Vin4, were analyzed by video and laser confocal microscopy as well as by biochemical and molecular biological techniques. Both clones adhered well to substrates and both exhibited F-actin stress fibers with vinculin localized at stress fiber tips in focal contacts. This was in marked contrast to 5.51 parental cells, which had no stress fibers and no vinculin. Transfection of the vinculin gene into 5.51 cells also resulted in expression of normal levels of uvomorulin in one clone. 5.51Vin3, which expresses ten-fold higher amounts of vinculin, had more stress fibers and vinculin staining in comparison to 5.51Vin4. Interestingly, 5.51Vin3 cells did not spread as well as 5.51Vin4, suggesting that overexpression of vinculin effects lamellipodia extension. The mutant and complemented F9 cell lines will be useful models to examine the complex interactions between cytoskeletal and cell adhesion proteins.

G 103 ISLET CELL REGENERATION IN INS-IFN-G TRANSGENIC MICE. Danling Gu and Nora E Sarvetnick, Department of Neuropharmacology, The Scripps Research Institute, La Jolla, California, 92037 Islets are derived from the epithelial cells in embryonic development. We describe an islet cell regenerating process which closely resembles the embryonic islet morphogenesis in adult transgenic mice carrying interferon-g (IFN-g) gene linked to the regulatory region of the insulin gene. The islet cell regeneration occurs after the lymphocytic infiltration and the islet destruction. Concurrent to the islet loss, there is a increase in the population of pancreatic ducts by cell proliferation. The duct wall thickens at various loci forming buds along its length. The buds contain differentiated endocrine cells and "bud" off the duct wall as described in the islet formation in embryos. However, in addition to the normal budding as in embryonic islet neogenesis, the buds frequently protrude into the duct lumen. A phenomenon we call reversed budding. We tested the idea whether pancreatic lymphocytic inflammation is prerequisite for islet regeneration by back-cross twice ins-IFN-g transgenic mice with SCID mice. The resulting ins-IFN-g/SCID transgenic mice exhibit remarkable duct cell proliferation and differentiation of pancreatic endocrine cells identical to those observed in ins-IFN-g transgenic mice. Thus, the absence of functional lymphocytes and their induced islet loss does not affect the regeneration of islet cells in ins-IFN-g transgenic mice. We also observed pancreatic lymphocyte infiltration but no islet cell regeneration in ins-IL-10 transgenic mice. IL-10 is known to inhibit macrophage function. These results suggest that (1) IFN-g can initiate a pathway leading to the regeneration of islet cells and (2) macrophages (not lymphocytes) may be activated by IFN-g to produce growth factor(s) which stimulate duct cells to proliferate and differentiate to endocrine cells.

G 104 MEMBRANE-CYTOSKELETON, B-CADHERIN AND APICAL NA^+, K^+-ATPASE POLARITY IN THE CHOROID PLEXUS EPITHELIUM. James A. Marrs and W. James Nelson. Stanford University School of Medicine, Department of Molecular and Cellular Physiology, Stanford CA.

In order to create a transepithelial Na^+ gradient, the sodium pump is restricted to specific membrane domains in epithelial cells. Na^+, K^+-ATPase is apically polarized in the choroid plexus epithelium where it secretes sodium, thus producing cerebral spinal fluid. The membrane-cytoskeleton has been previously shown to be instrumental in generating and maintaining polarity of the sodium pump in other epithelia. Fodrin and ankyrin colocalized with Na^+, K^+-ATPase at the apical plasma membrane in the chicken choroid plexus epithelium. Na^+, K^+-ATPase, fodrin and ankyrin were found in a membrane-cytoskeletal multiprotein complex by using sucrose gradients, and nondenaturing gel analysis of these sucrose gradient fractions. Fodrin was also localized to the lateral plasma membrane in chicken choroid plexus epithelium, but fodrin was found only apically localized in the rat choroid plexus epithelium. The Ca^{++}-dependent cell adhesion molecule, B-cadherin is expressed in the chick choroid plexus, is localized to the lateral plasma membrane, and colocalized with ankyrin, fodrin and adducin. B-cadherin, ankyrin and fodrin were found in a membrane-cytoskeletal complex by sucrose gradient and nondenaturing gel analysis, accounting for the lateral localization of fodrin and ankyrin in these cells. These novel findings show that in contrast to observations from other cell types, Na^+, K^+-ATPase does not colocalize with cadherin molecules. Our data point to cell type specific mechanisms for generating polarity of proteins.

G 105 MAC-2 (CARBOHYDRATE BINDING PROTEIN 35): AN S-LECTIN ASSOCIATED WITH INTESTINAL EPITHELIAL DIFFERENTIATION AND NEOPLASTIC PROGRESSION, Arthur M. Mercurio, Margaret M. Lotz, and Charles. W. Andrews, Jr., Laboratory of Cancer Biology, Deaconess Hospital, Harvard Medical School, Boston, MA 02115

The Mac-2 lectin, also known as carbohydrate binding protein 35 (CBP 35), is a soluble, 32-35kDa phosphoprotein that binds N-acetyllactosamine and other galactose containing glycoconjugates. Despite the wealth of data that has accumulated on Mac-2, its biological functions remain elusive. Based on the precedent that lectins function as cell adhesion or recognition molecules, considerable emphasis had been placed on this possible role for Mac-2. However, recent data, including the observation that Mac-2 is primarily an intracellular protein, argue against such an adhesive function. An emerging hypothesis is that Mac-2 may be associated with cell growth and differentiation but more definitive data are needed to support this possibility. Progress in elucidating this function of Mac-2 has been hampered, in part, by a lack of data on its expression *in vivo*. This situation prompted us to examine Mac-2 expression in human tissue specimens by immunohistochemistry. The results obtained were unexpected based on previous *in vitro* studies. We conclude from these studies that Mac-2 is largely an epithelial specific lectin with predominant expression seen in intestinal epithelium. In this epithelium, the proliferating crypt cells do not express Mac-2 but its expression correlates with terminal differentiation along the crypt to surface axis. Of particular interest is the observation that Mac-2 expression is concentrated in the nuclei of these cells. Because of the putative association of Mac-2 with growth control, we studied its expression in pre-neoplastic and neoplastic colonic tissue. We found that the progression from normal colonic mucosa to adenoma to adenocarcinoma is characterized by a reduction in Mac-2 mRNA and protein levels and a loss of its nuclear localization. This difference in Mac-2 nuclear localization between normal mucosa and tumor does not appear to result from mutation in its cDNA. These observations suggest that Mac-2 may be involved in intestinal epithelial differentiation and that its exclusion from the nucleus may be related to the neoplastic progression of colon cancer.

G 106 E-CADHERIN AND CATENINS IN THE REGULATION OF CELL ADHESION

I. S. Näthke[1], L. Hinck[1,2,3], J. Papkoff[2,3], W. J. Nelson[1]; [1]Dept. Molecular and Cellular Physiology, Stanford University, Stanford, CA; [2]Cancer Biology Program, Stanford University, Stanford, CA; [3]Cancer and Developmental Biology, Syntex Research, Palo Alto, CA.

Cadherins are well characterized calcium-dependent cell adhesion molecules that play a crucial role during vertebrate development. Cadherins interact with a set of cytoplasmic proteins and this interaction is necessary for cadherin-mediated cell adhesion. These proteins include α–, β–, and γ–catenin and plakoglobin. One role of catenins is the attachment of cadherins to the cytoskeleton. We have developed monospecific antibodies against α– and β–catenin as well as plakoglobin and different domains of the E-cadherin molecule. These probes were used to characterize the catenins in MDCK cells, which provide an excellent model system for analyzing different stages of epithelial development. The antibodies were used to immunoprecipitate and immunolocalize catenins and cadherins at different times after cell contact was initiated.

In our experiments both the E-cadherin precursor and β–catenin are detected in a Triton-insoluble fraction immediately after a brief pulse label, even before cell contact is initiated. This indicates that both these molecules acquire insolubility before reaching the cell surface. In E-cadherin immunoprecipitates, the associated β-catenin is turned over at the same rate as E-cadherin (2-4 hours). In β-catenin immunoprecipitates, the associated E-cadherin is turned over with the same apparent half life of 2-4 hours, whereas β-catenin is much more stable ($t1/2 > 8$ hours). This suggests that there are at least two different pools of β-catenin. One pool is E-cadherin–associated and turns over with E-cadherin. A second pool is independent of E-cadherin and has a substantially longer half life.

Pulse chase analysis of α-catenin in association with E-cadherin shows that labeled α-catenin is detected long after labeled E-cadherin is turned over. This suggests that labeled α-catenin associates with unlabeled E-cadherin and indicates that α-catenin is able to exchange from one E-cadherin molecule to another.

Our experiments reveal the novel result that E-cadherin rapidly becomes insoluble and that α- and β-catenin are regulated independently.

G 107 BASOLATERAL ANTIGENS ARE EXPRESSED IN A SUB-DOMAIN OF THE APICAL PLASMAMEMBRANE OF FRT AND MDCK CELLS, Lucio Nitsch, Rosa Negri and Corrado Garbi, Dpt. Biologia e Patologia Cellulare e Molecolare, University of Napoli 'Federico II', Second Medical School, Napoli, Italy.

The apical domain of epithelial cells undergoes dramatic changes in its molecular composition and morphological organization following the interaction with ECM components such as collagen. In an attempt to detect molecules on the apical plasmamembrane, that are responsible for the interaction with the ECM, we have identified an apical sub-domain where basolateral antigens are present.

FRT (rat thyroid) and MDCK (dog kidney) epithelial cells have been cultured to confluency on filters, in bicameral systems. Collagen solutions have been added to the apical domain and allowed to gel. A dramatic drop in transepithelial resistance was observed within two hours after addition of collagen. Changes were observed by EM in the organization of the apical domain of cells in contact with the collagen gel. Those cells also showed a rearrangement of the apical actin. The distribution of several membrane antigens was determined by immunofluorescence in confluent monolayers, before and after the interaction with the collagen gel. It was found that, in the absence of any treatment, several basolateral proteins were expressed in a single spot on the apical domain of a limited number of cells (<10%). These proteins included: α1 and β1 integrin subunits, Na,K-ATPase β subunit and 35/40 kD antigen. In that same spot were also localized some antigens recognized by HPA lectin.

We are currently investigating the early changes in the overall distribution of these antigens induced by the interaction with the collagen gel. We also wish to determine whether any of these proteins play a functional role in cell-collagen interaction.

G 108 THE ROLE OF THE CYTOPLASMIC TAIL DOMAIN IN ACTIN INTERACTIONS AND IN THE POLARIZED LOCALIZATION OF A CELL ASSOCIATED MUCIN, MUC1, L. Pemberton, J. Taylor-Papadimitriou and S.J. Gendler. Imperial Cancer Research Fund, PO Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.

MUC1 is a cell-associated mucin glycoprotein synthesized by secretory epithelial cells and located exclusively in the apical domain of cells. The protein consists of a large extracellular domain, a hydrophobic transmembrane region and a cytoplasmic tail. Using antisera to the cytoplasmic tail we have shown that this region of the molecule is highly conserved between humans and other mammalian species. This conservation points to an important function for the cytoplasmic tail. The tail of MUC1 has been shown to interact with components of the actin cytoskeleton. To test whether this interaction is responsible for the apical distribution of the mucin and to define more precisely the sequences involved in the actin interaction, deletion mutants of the cytoplasmic domain have been constructed and expressed in a pancreatic cell line, Panc-1. The expression pattern of these mutants has shown that the entire tail is not necessary for apical localization, as a mutant expressing only 18 out of 69 amino acids is expressed apically. This mutant does not interact with the actin cytoskeleton and can be completely extracted from the cell using 0.5% NP40 detergent. A mutant expressing 45 amino acids of the tail is able to interact either directly or indirectly with the actin cytoskeleton and can not be extracted with detergent. These results suggest that the domain involved in the actin interactions lies between amino acids 18 and 45 of the cytoplasmic tail. This region is very highly conserved among a variety of species and includes a potential PKC site. Bacterial fusion proteins corresponding to the cytoplasmic tail region have been made and we hope to use these to identify putative MUC1/actin binding proteins from whole cell lysates. To define the apical localization sequences, chimeric proteins of MUC1 and CD2 are being constructed and expressed in epithelial cells.

G 109 MOLECULAR CLONING AND CHARACTERIZATION OF THE HUMAN E-CADHERIN cDNA SEQUENCE. David L. Rimm and Jon S. Morrow, Dept. of Pathology, Yale University Medical School, New Haven, CT 06510

E-cadherin (also known as Uvomorulin, L-CAM, cell CAM120/80) is a 120 kD transmembrane glycoprotein that has been shown to mediate Ca^{++} sensitive homotypic epithelial cell adhesion and has be implicated in the establishment of cell polarity. Cloning and sequencing of human liver and colon cDNAs show high levels of homology (84% at the protein level and 80% at the DNA level) with the mouse and chicken molecules. Although Northern blots of colonic mRNA show a single band at approximately 5000 bp we are exploring the possibility of the existence of multiple isoforms. The liver sequence shows a 26 aa processed sequence, significantly smaller than the 156 aa sequence in mouse and 159 aa sequence from chicken. The shorter sequence shows a good Kozak concensus start sequence and no upstream open reading frames in the 180bp of the cDNA clone preceding the start. Of the 26 processed aa, 11 are identical to the mouse and chicken clones in this region. Preliminary sequence a cDNA from human colon shows a longer processed sequence with a low degree of homology to the mouse and chicken processed N-terminal domain, however this region of the cDNA is not yet completely characterized. The remainder of the human sequence is highly homologous to mouse and chicken sequences. The SHAVS sequence shown to be necessary for homotypic interaction is identical in sequence and location to that seen in mouse and chicken. Human E-cadherin shows the same 3 internal repeat structures seen in mouse with 79-86% similarity to the mouse sequence and 22-36% similarity between repeats. The 4 cysteine residues in the region immediately external to the transmembrane binding domain are identically conserved as are the 3 consensus sequences for N-glycosylation in that region in spite of the fact that this region is the least similar overall (69%). The region with the highest similarity is the C terminal region of the molecule with the 24 transmembrane amino acids showing 100% identity and the cytoplasmic domain showing 95% similarity. These results raise the possibility that nascent E-cadherin may undergo tissue specific processing prior to its assembly at the membrane.

G 110 TRANSFECTION OF E-CADHERIN INTO TWO INVASIVE HUMAN BREAST CANCER CELL LINES DOES NOT ALTER MORPHOLOGY OR INVASIVE PHENOTYPE. Connie L. Sommers, Edward P. Gelmann and Stephen W. Byers, Department of Anatomy and Cell Biology and the Lombardi Cancer Research Center, Georgetown University, Washington, D.C. 20007

We and others have described loss of expression of E-cadherin in some human breast carcinoma cell lines (bccl's). E-cadherin distribution in epithelioid bccl's was restricted to the basolateral plasma membrane, concentrated at points of cell-cell contact. The epithelioid bccl's were weakly invasive when tested in modified Boyden chamber or Matrigel outgrowth assays. All highly invasive bccl's had complete loss of E-cadherin expression. These cells also have a fibroblastoid phenotype, express vimentin and have down regulated keratins. To examine the effect of E-cadherin expression on invasiveness in these bccl's, we transfected the mouse E-cadherin cDNA into the invasive bccl's HS578T and BT549. For comparison we also transfected weakly invasive, cadherin-positive MCF-7 breast cancer cells and cadherin-negative mouse L929 fibroblasts. E-cadherin protein was detected in the transfected clones by immunofluorescence microscopy and immunoprecipitation. E-cadherin-transfected invasive cells did not assume a more epithelioid morphology and still exhibited an invasive phenotype in Matrigel. Immunostaining of live HS578T and BT549 cells showed that transfected E-cadherin was present on the cell surface but that its distribution was nonpolarized. Triton treatment of transfected HS578T and BT549 cells prior to immunofluorescence microscopy resulted in loss of E-cadherin immunostaining. In contrast, Triton-insoluble E-cadherin was present at cell-cell borders in the transfected MCF-7 and L929 cells. These results suggest that in the invasive bccl's HS578T and BT549, the transfected E-cadherin is not appropriately linked to the actin cytoskeleton.

G 111 EPITHELIAL MORPHOGENESIS IN *DROSOPHILA*, Ulrich Tepass, Eileen Gruszynski, Arne Lekven and Volker Hartenstein, Department of Biology, UCLA, Los Angeles, CA 90024

The *Drosophila* embryo contains a variety of epithelia of different structure and developmental origin which might serve as useful model systems to study epithelial morphogenesis. Based on structural (mainly cellular junctions) and ontogenetic criteria, three basic types of epithelia can be distinguished: (1) Ectodermally derived epithelia (epidermis, trachea, salivary glands etc.) have a *zonula adhaerens* that is established before organogenesis. Pleated septate junction, spot- and hemi-adherens junctions are assembled late in development in these tissues. (2) The mesodermally derived dorsal vessel (heart) forms a simple endothelial tube and differentiates an adherens ("endothelial") junction and hemi-adherens junctions. (3) The endodermally derived midgut epithelium forms by a conversion from mesenchym to epithelium. This process is not accompanied by the formation of a particular junction. Later in development hemi-adherens junctions and smooth septate junctions differentiate in the midgut epithelium. We have studied several genes which are required for epithelial morphogenesis: *crumbs* (*crb*) and *stardust* (*sdt*): Both genes act in the same pathway and are needed to stabilize epithelial cell structure of ectodermally derived epithelia. *crb* encodes a transmembrane protein that is expressed on the apical cell surface and contains 30 EGF-like domains and 4 domains related to the G domain of Laminin A. *bazooka* (*baz*): *baz* is required for the formation of many epithelia in *Drosophila* including the blastoderm epithelium, ectodermally derived epithelia and the midgut epithelium. *faint sausage* (*fas*): In *fas* mutant embryos all epithelia show strong morphogenetic defects and an incomplete differentiation. *shotgun* (*shg*): *shg* is required in regions of epithelia which undergo morphogenetic movements like delamination of single cells (e.g. ventral neurogenic regions) or invagination of cell groups (e.g. Malpighian tubules). *shg* function enables epithelial cells in these territories to compensate for this "morphogenetic stress" and to reestablish proper epithelial morphology. The poster will present a description of epithelia in the *Drosophila* embryo in wild type and mutant condition.

G 112 CHARACTERIZATION OF THE *DROSOPHILA DISHEVELLED* PROTEIN.

Frank van Leeuwen, John Klingensmith and Roel Nusse. Department of Developmental Biology, Howard Hughes Medical Institute, Beckman Center, Stanford CA 94305.

The segment polarity gene *wingless (wg)* encodes a secreted protein which is essential to establish segmental pattern in *Drosophila* . The *wg* gene product is expressed in a narrow band of cells in each segment. The removal of *wg* however affects a much larger domain, comprising the posterior half of each segment. Three other segment polarity mutants, *porcupine*, *dishevelled* and *armadillo* show embryonic phenotypes that are identical to the extreme *wg* phenotype and mediate the effects of *wg* in adjacent cells, suggesting that these genes may be involved in the *wg* signalling pathway. Mutations in *dishevelled* and *armadillo* behave in a cell autonomous way and their products are thus thought to be involved in receiving or interpreting the *wg* signal. The *armadillo* protein has been shown to be related to the vertebrate proteins *plakoglobin* and *ß-catenin*, both components of epithelial cell-cell junctions. The *dishevelled* gene has been cloned and encodes a novel protein. We have generated antibodies against a bacterial *dsh-trpE* fusion protein. Here we report on the results obtained in characterizing the *dsh* protein using cell lines derived from imaginal discs and speculate about its involvement in wg signalling.

G 113 THE GENE *crumbs* AND ITS FUNCTION IN MAINTENANCE OF EPITHELIAL CELL POLARITY IN *Drosophila melanogaster*

Andreas Wodarz, Uwe Hinz and Elisabeth Knust, Institut für Entwicklungsbiologie, Universität Köln, Gyrhofstr. 17, 5000 Köln 41, Germany

The protein encoded by the gene *crumbs* is required for maintenance of epithelial cell polarity in *Drosophila melanogaster*. CRUMBS is a large transmembrane protein of 2147 aa with 30 EGF-like repeats and four laminin A G-domain repeats in its extracellular part. The protein is localized exclusively in the apical membrane of ectodermally derived epithelial cells. Fly embryos lacking functional CRUMBS protein show depolarization and degeneration of epithelia, in some tissues frequently followed by cell death.

In order to address the question whether overexpression or ectopic expression of CRUMBS affects embryonic development, we made use of the yeast GAL4-system. Transgenic flies were generated that carried a *crumbs* minigene downstream of GAL4-sensitive upstream activating sequences (UAS). Activation of the minigene was achieved by crossing these flies to a couple of fly strains that carried GAL4-transgenes driven by different tissue-specific enhancers. The effects of CRUMBS-overexpression were clearly dosage-dependent. Whereas low levels of additional CRUMBS did not cause any obvious abnormalities, high levels of overexpression led to severe disorganization of epithelia. In highly overexpressing cells CRUMBS was no longer restricted to the apical membrane but could be detected on the basolateral membrane as well. Whether this mislocalization of CRUMBS is the cause for the observed defects is currently under investigation.

G 114 CONDITIONALLY-IMMORTAL RENAL MESENCHYME SECRETES HEPATOCYTE GROWTH FACTOR/SCATTER FACTOR (HGF/SF).

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The adult mammalian kidney forms as a result of an interaction between the metanephric mesenchyme and the ureteric bud. HGF/SF is produced by a variety of cells of mesenchyme origin and causes the in-vitro branching of Madine Darby Canine Kidney (MDCK) epithelial cysts. We hypothesised that the renal mesenchyme may release this factor which would cause the branching of the adjacent ureteric bud to form the ureter and collecting ducts.

We directly-derived conditionally-immortal embryonic day 11.5 renal mesenchyme cells from the $H2k^b$-tsA58 transgenic mouse. In this strain the SV40 T Antigen is inducible by the addition of interferon-γ and cells can be indefinitely propagated at the permissive temperature (33°C); at the phsyiological temperature (38-39°C) the T Antigen is thermolabile and the cells are no longer immortal. These cells express Wilms' tumour transcripts at the permissive temperature, confirming their identityt as nehprogenic progenitors.

Serum-free conditioned media (CM) was harvested from these cells and scattering activity was assessed using monolayers of MDCK cells as a target. Activity was observed in CM from renal mesenchyme cells under both permissive and non-permissive conditions, and was 1/8 of the titre of CM from clone D4-Ras NIH3T3 cells, which served as a positive control. The bioactivity of the CM from both the renal mesenchyme and fibroblasts was completely blocked by pre-incubation of the CM with an anti-mouse HGF/SF antibody raised in the rabbit. A non-specific rabbit anti-mouse antibody had no blocking effect.

These results suggest that renal mesenchyme precursor cells secrete HGF/SF in vitro. These same cytokine may cause the branching of the ureteric bud which occurs during metanephrogenesis in vivo.

G 115 CHIP28 WATER CHANNELS--BIPOLAR DISTRIBUTION WITHIN POLARIZED EPITHELIA,

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CHIP28 is an abundant integral membrane protein in red cells and kidney and was recently demonstrated to be the molecular water channel of these cells (Preston et al, 1992. *Science* 256, 385-387). Immunohistochemical and immunoelectron microscopy were performed using affinity-purified polyclonal rabbit IgG raised to human red cell CHIP28; one antibody reacted with a synthetic peptide corresponding to the N-terminus of CHIP28 protein, and the other reacted with the 4 kDa C-terminal cytoplasmic domain. The quantity of CHIP28 within renal cortical brush border membrane vesicles was determined by dilutional immunoblot to be approximately 3.8 % of the total membrane protein. CHIP28 was identified by immunomicroscopy in both the apical and basolateral membranes of proximal tubules and descending thin limbs, nephron segments known to contain constitutively active water channels. Gold labeled anti-CHIP28 was distributed throughout the brush border microvilli but was not identified in endocytic vesicles or other intracellular structures. CHIP28 immunostain-ing was not detected in ascending thin limbs or distal tubules, segments known to be highly impermeable to water. Likewise, CHIP28 was not identified in collecting ducts which are known to contain vasopressin-regulated water channels. CHIP28 immunostaining was also prominent in several other polarized epithelia including intestinal and muscle capillary endothelium, peritoneum, pulmonary epithelium, choroid plexus and in ciliary body of the anterior eye chamber. CHIP28 water channels are therefore unlike most membrane transporters which exist only at the apical or at the basolateral membranes of polarized epithelia. It is likely that the bipolar distribution of CHIP28 explains how passive transcellular passage of water occurs across these epithelia in the direction of salt gradients.

G 116 CHRONIC TPA TREATMENT MODULATES BOTH EXPRESSION AND LOCALIZATION OF GAMMA-GLUTAMYL TRANSPEPTIDASE (gGT) IN A RENAL EPITHELIAL CELL, Kurt Amsler and Jen-Li Chen, Department of Physiology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854

Subconfluent populations of LLC-PK_1 cells expressed minimal activity of the brush border membrane enzyme marker activity, gGT. Progressive expression of gGT activity occurred after populations attained a confluent cell density and entered growth arrest. Expressed enzyme activity was membrane-bound and inhibited by the gGT inhibitor, acivicin. Surface gGT activity, localized almost exclusively to the apical membrane, accounted for only about 35% of total gGT activity, measured in cell homogenates. Upon attaining confluence there was a roughly parallel increase in total and surface gGT activity. Using enzyme histochemistry to localize surface gGT activity, the progressive increase in surface gGT activity observed after confluence was correlated with an increasing proportion of cells which stained positive for the enzyme activity. Older cell populations exhibited a greater proportion of strongly staining cells, suggesting that expression of "mature" levels of apical membrane gGT activity in a single cell required several days. Cell populations propogated in culture medium containing the potent tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (0.1 uM; TPA), exhibited much lower levels of total gGT activity than untreated cell populations throughout the experimental period. In TPA-treated cell populations there was an almost complete absence of surface gGT activity, measured both by enzymatic quantitation and enzyme histochemistry. These data indicate that in LLC-PK_1 cells: 1) gGT is membrane-bound and present both in an intracellular compartment and at the apical membrane; 2) gGT is expressed progressively on a cell-by-cell basis after the population attains confluence; 3) once expression is initiated, gGT requires several days to attain full expression in a single cell; and 4) gGT expression both in the intracellular compartment and, particularly, at the apical surface is inhibited by chronic treatment with TPA.

G 117 EFFECT OF BILE DUCT LIGATION ON THE SUBCELLULAR DISTRIBUTION OF POLYMERIC IMMUNOGLOBULIN A - RECEPTOR FORMS IN RAT HEPATOCYTES, R. Fuchs, I. Stefaner, E. Sztul*, and J. Schmid, Dept. Gen. Exp. Pathology, Univ. Vienna, Austria and * Dept. Mol. Biol., Princeton Univ., USA.

In rat hepatocytes, the polymeric IgA-receptor (pIgA-R) mediates the transcellular movement of polymeric immunoglobulin A (pIgA) from the sinusoidal to the canalicular membrane, where it is cleaved to 80 kDa secretory component (SC), releasing pIgA with SC into bile. Short time cholestasis induced by bile duct ligation (BDL) has been shown to reduce biliary secretion of pIgA, whereas release of pIgA-R continues at near normal levels. To gain more insight into the mechanism leading to uncoupling of the pathways of pIgA and its receptor, we investigated the effect of two hour BDL on the subcellular distribution of pIgA-R forms. Livers from normal and BDL rats were fractionated into total microsomes, endosome enriched Golgi fractions and sinusoidal plasma membranes. Proteins were separated by SDS-PAGE and antigens were detected by immunoblotting using an antiserum against the ectodomain of the pIgA-R and a very sensitive ECL detection system. Three intracellular forms of the pIgA-R were analyzed and quantitated: a 116 kDa terminally glycosylated form, a phosphorylated 120 kDa receptor, and a still uncharacterized 124 kDa form. Total microsomes from normal and cholestatic rats exhibited no significant differences in the amount and distribution of pIgA-R forms. However, endosome enriched fractions from BDL animals had decreased levels of 116 kDa (50% of control), 120 kDa (55% of control) and 124 kDa (11% of control) forms when compared to control rats. In contrast, sinusoidal plasma membranes purified from BDL rats contained elevated amounts of 116 kDa (125% of control), 120 kDa (137% of control), and 124 kDa (300% of control) receptor forms. BDL had no influence on the subcellular distribution of the asialoglycoprotein receptor. Thus, short time cholestasis leads to selective redistribution of pIgA-receptors from intracellular compartments (early endosomes, transcytotic endosomes?) to the sinusoidal plasma membrane. The pronounced effect of BDL on redistribution of the 124 kDa form concomitant with drastically reduced biliary pIgA-secretion suggests a role of the 124 kDa receptor in mediating efficient transcytosis of pIgA under physiological conditions. Supported by Austrian Science Foundation P 8435-MED.

G 118 THE H,K-ATPase ENCODES MULTIPLE APICAL SORTING SIGNALS, C.J. Gottardi and M.J. Caplan, Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06510.

Gastric parietal cells are polarized and maintain two structurally *and functionally* similar ATPases on distinct apical and basolateral membrane domains: the Na,K-ATPase is restricted to the basolateral membrane, while the H,K-ATPase is enriched in a pre-apical tubulovesicular compartment. We are examining the molecular signals which serve to establish and/or maintain the differential distribution of these two proteins. We have expressed full length and chimeric H,K-ATPase/Na,K-ATPase cDNAs in polarized renal proximal tubular epithelial cells (LLC-PK_1) and have examined the distributions of these proteins by confocal microscopy. We find that both α and β subunits of the H,K-ATPase localize to the apical brush border. A chimera encoding the NH_2-terminal half of the H,K-ATPase and the carboxy-terminal half of the Na,K-ATPase is localized exclusively to the apical membrane, suggesting that an apical sorting signal lies within the NH_2-half of the H,K-ATPase. When the H,K-β subunit is expressed without its α, the protein localizes to the apical membrane and to vesicles of the endocytic pathway. Interestingly, the H,K-β possesses a signal which has been shown to mediate the entry of other proteins into endosomes. We hypothesize that this putative endocytosis signal may also serve to target the H,K-β to the endocytically active apical domain of LLC-PK_1 cells. We therefore suggest that not only do endocytosis signals serve to target a protein to a cell surface, but that endocytosis signals may specify targetting to a cell's functionally endocytic, rather than topographic domain. (Supported by NIH GM-42136 and a the David and Lucille Packard Foundation)

G 119 EGF RECEPTOR EXPRESSION IN CANINE (MDCK II) AND MONKEY (BSC-1) RENAL EPITHELIAL CELLS, Michael E. Hobert and Cathleen R. Carlin, Department of Physiology and Biophysics, School of Medicine, Case Western Reserve University, Cleveland, OH 44106

Proliferation of renal tubular epithelial cells is regulated by growth factors including epidermal growth factor (EGF). The effects of EGF are mediated by binding to specific membrane receptors with intrinsic ligand-induced tyrosine kinase activity. The purpose of this study was to determine the membrane-domain localization and biogenesis of EGF receptors (EGFRs) using filter-grown renal epithelial cells. MDCK II (Madin-Darby canine kidney, strain II) cells originate from the collecting duct of the renal tubule and have been shown to grow as polarized epithelial monolayers expressing both apical and basolateral membrane specific markers. When grown on porous membrane filters these cells form tight junctions with less than 1% ^{3}H-inulin leakage and transbilayer resistance measurements of approximately 100 Ω/cm^2. The African green monkey kidney epithelial cell line (BSC-1) is also of renal tubule origin, but is less well characterized. Transport studies indicate that the BSC-1 cell line may also exhibit a polarized phenotype. We have conducted ^{125}I-EGF binding assays that demonstrate the polarized distribution of EGFR in both the MDCK II and BSC-1 cells grown on membrane filters. MDCK II cells show a 1:10 (apical:basolateral) ratio of EGFRs, in contrast to BSC-1 cells which exhibit a 6:1 (apical:basolateral) ratio. Domain specific biotinylation of pulse-labeled BSC-1 cells has revealed that the EGFR is delivered to both apical and basolateral membranes. However, newly synthesized receptors directed to the apical surface arrive in a wave between 1.5 and 2 hours after synthesis, whereas receptors destined for the basolateral membrane arrive gradually during a 3 hour period. In BSC-1 cells, degradation of receptors from both the apical and basolateral membrane appears to occur at equivalent rates. Similar experiments are currently being conducted to determine biogenesis and sorting of EGFR in MDCK II cells. We are also examining the effect of domain-specific ligand stimulation on EGFR-mediated signaling. The difference in apical versus basolateral EGFR distribution in these two renal epithelial cell lines offers a unique opportunity to determine whether other components essential for EGF-induced mitogenesis are expressed in a domain-restricted fashion.

G 120 PHOSPHORYLATION OF LACTOSE-BINDING LECTIN L-29 IN POLARIZED EPITHELIAL CELLS

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Lactose-binding lectin L-29 represents about 1% of total soluble cell protein in several polarized epithelial cell lines including T84 and MDCK. Isoelectrofocusing of lectin labeled *in vivo* with ^{32}P shows that in resting cells, 15%-20% of L-29 is phosphorylated. To identify the phosphorylation site(s), we have used the following techniques: phosphoaminoacid analysis; **HPLC** of peptides generated by sequential cleavage with collagenase, α-chymotrypsin and ASP-N; **peptide sequencing**; **mass spectroscopy.**

The results show that L-29 is phosphorylated exclusively on serine. About 90% of the phosphate is located at ser-6 and the remaining 10% at ser-12 . Ser-6 lies within a consensus sequence for casein kinase II which is known to phosphorylate many proteins involved in growth regulation, suggesting that intracellular L-29 could also function in that way.

In addition, a mass spectrum of the N-terminal phosphopeptide shows the presence of further modification on phosphoserine-6, tentatively identified as adenosine. We are currently characterizing this modification.

Supported by TRDRP to University of California.

G 121 TARGETING OF NEUTRAL ENDOPEPTIDASE (NEP) IN LLC-PK1 CELLS, **Christian Lanctôt and Philippe Crine, Department of Biochemistry, University of Montreal, P.O. Box 6128, Montreal, Quebec, Canada, H3C 3J7**

LLC-PK1 cells were transfected with a vector carrying the rabbit NEP cDNA under the control of the cytomegalovirus IE gene promoter. Clones expressing high levels of the protein were isolated and characterized. Selective biotinylation and radioimmunolabeling were used to determine that 85% to 95% of NEP was localized in the apical domain of filter-grown LLC-PK1 cells. Forty percent of newly made NEP transited by the basolateral membrane before reaching the apical membrane through the transcytotic pathway. Moreover, a soluble form of NEP was found to be secreted in equal amounts from both sides of the monolayer, when expressed in LLC-PK1 cells. This behaviour contrasts with the one observed in MDCK cells, where both the transmembrane and secreted form of NEP were shown to be directly targeted to the apical membrane.

G 122 MECHANISMS OF SORTING IN AN INTESTINAL EPITHELIAL CELL LINE Caco-2.

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Intestinal epithelial cells are a good model to study cell polarity *in vitro*. Using the Caco-2 cell line we have investigated the biogenetic pathways of several apical and basolateral plasma membrane proteins. Sucrase-isomaltase (SI), alkaline phosphatase (PLAP) and CEA180 follow preferentially a direct route from the Golgi complex to the apical membrane while aminopeptidase (APN) and dipeptidyl peptidase IV (DPPIV) mostly travel by an indirect route (via the basolateral membrane). We are now dissecting these direct and indirect pathways taken by apical proteins. By differential detergent extraction we have found that several GPI-anchored proteins (PLAP, CEA180 and GP97) aggregate into membrane microdomains during their transport to the Golgi complex. There was no evidence for such a phenomenom for other apical markers (DPPIV, APN and CEA110) or basolateral proteins (transferrin receptor and Ag525). We have isolated these membrane microdomains and we are in the process of characterizing them. We also purified exocytic vesicles from perforated Caco-2 cells and we are analyzing the contents of apical and basolateral vesicles to better understand the sorting mechanisms of intestinal cells.

G 123 APICAL SECRETION AND ENDOCYTOSIS OF L-29, AN ENDOGENOUS LECTIN IN MDCK CELLS.

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L-29 (also known as Mac-2, CBP-35 and εBP) is a cytosolic protein present in columnar epithelia and activated macrophages. This lectin has affinity for β-galactoside containing glycoconjugates and we have demonstrated that in MDCK cells it is secreted predominantly into the apical medium. About 10 % of newly synthezeized lectin is found in the apical medium at 12 h, and after that timethere is a very slow increase of the lectin in the apical medium. The same relationship between intracellular and apical L-29 was shown by meassuring the L-29 in cells and media by an ELISA. The secretion is not inhibited by brefeldin A or monensin but it is inhibited to about 50 % in a 3 h chase by nocodazole.

To study the fate of secreted L-29 we have meassured its binding to the MDCK cell surface and subsequent internalization.. ^{125}I-labeled recombinant human L-29 (rL-29) was bound to the apical or basal side of filter-grown MDCK cells and the amount bound, internalized and transcytosed was meassured. Of the apically bound lectin, 24% was internalized during a 60 min. chase and of this amount 59% was transcytosed. Of the basal bound lectin about 35% was endocytosed and 38% of the internalized lectin was transcytosed. Endocytosis was confirmed using FITC-labeled rL-29. To learn more about the molecular mechanism for the endocytosis we examined the nature of the cellular ligand and the part of L-29 was responsible for binding to the cells. The binding and endocytosis of L-29 appear to be mediated by cell surface galactosides because it was inhibited by lactose. Cell surface coimmunoprecipitation suggested identified one 95 kDa glycoprotein as the predominant ligand for L-29. There are reports indicating that the proline-glycine rich N-terminal of the lectin may aggregate the lectin and increase the binding affinity to glycoconjugates. However, the FITC-labeled carbohydrate binding C-terminal domain was internalized to a similar extent, suggesting that polyvalent binding to the cell surface is not necessary for binding and internaliztion.

G 124 **ATP DEPLETION: A NOVEL METHOD TO STUDY JUNCTIONAL PROPERTIES IN EPITHELIAL TISSUES.**
Lazaro Mandel, Alan Garfinkel, Steve Monke, Guido Zampighi, and Robert Bacallao, Division of Physiology, Department of Cell Biology, Duke University, Durham, N.C; Department of Physiological Scienceand Department of Anatomy and Cell Biology, UCLA, Los Angeles, CA; Division of Nephrology and Hypertension, Department of Molecular, Cellular and Structural Biology; Northwestern University, Chicago, IL.
The cellular injury caused by depletion of intracellular ATP stores was studied in the Madin-Darby Canine Kidney (MDCK) and JTC cell lines. In prior studies, it was shown that ATP depletion uncouples the gate and fence functions of the tight junction (Mandel et al., Nature, submitted). In this communication, the changes in the actin cytoskeleton and junctional complexes are studied using confocal fluorescence and electron microscopy in combination with computer aided three dimensional reconstruction. ATP depletion disrupts the junctional complexes in epithelial cells and the time course of disruption correlates with changes in the actin cytoskeleton. The actin cytoskeleton appears to depolymerize after 20-30 min of ATP depletion, initially affecting the cortical actin network running along the apical-basal axis of the cell. At about this time, internalization of Na,K-ATPase and E-cadherin are also observed, suggesting that cell-cell contacts may be affected. The next actin structure to be disrupted are the stress fibers. Finally, after 60 min of ATP depletion, the actin ring at the apical portion of the cell is compromised and correlates with ultrastructural changes in tight junction strands, and the loss of the molecular fence function. During the process of actin network dissolution, polymerized actin aggregates form in the cytoplasm. The changes in the junctional complexes and the potential to reverse the ATP depletion suggest that this may be a new method to study junctional complex formation and its relationship to the actin cytoskeletal network.

G 125 Alterations of Integrins in Transformed MDCK Cells Lacking Apical Polarity. K.S. Matlin[1,2], A. Zuk[1,2], G. Zinkl[1,2], D.M. Kendall[2], and C.A. Schoenenberger[2,3] 1)Renal Unit, Massachusetts General Hospital, Boston, MA; 2) Department of Anatomy and Cellular Biology, Harvard Medical School, Boston, MA; and 3)Maurice Müller Institute, Basel, Switzerland.
We have previously shown that MDCK cells transformed with the K-*ras* oncogene lose apical polarity while retaining the ability to form tight junctions and restrict basolateral proteins to regions of cell-cell contact (J. Cell Biol.*112*::873-889). Because the transformed cells also show defects in cell adhesion, we have begun to examine the integrin family of adhesion receptors to determine if their alteration can be correlated with the transformed phenotype. Normal MDCK cells express a complex assortment of integrins including at least three α subunits associated with β1, one associated with β3, and one associated with β4. Two of the α subunits which form complexes with β1 have been tentatively identified as α2 and α3 based on SDS-gel mobilities with and without reduction and reactivity with specific antibodies. The α6 subunit associates with β4. The β1 subunit is synthesized in excess of the α subunits such that endoH-sensitive β1 can be continuously detected following pulse-labeling and chase incubations. All of the β1 integrins appear to be targeted directly to the basolateral domain immediately after synthesis and can be localized there by immuno-fluorescence. In transformed MDCK cells, the relative amount of the β1 subunit declines, and complexes of β1 and an unidentified α subunit are no longer detectable. These changes in the integrin complement of the transformed cells may partially account for the transformed phenotype and the loss of apical polarity.

G 126 Na^+,K^+-ATPASE THAT REDISTRIBUTES TO THE APICAL MEMBRANE DURING ATP DEPLETION REMAINS FUNCTIONAL. Bruce A. Molitoris, Departments of Medicine and of Cellular and Structural Biology, Univ. of Colorado Sch. of Med., Denver, CO 80220
We have previously demonstrated using immunocytochemical, histochemical and biochemical techniques that ischemia in vivo and ATP depletion in vitro result in dissociation of Na^+,K^+-ATPase from the spectrin cytoskeleton and redistribution of Na^+,K^+-ATPase to the apical domain in renal proximal tubule cells. To directly evaluate whether apical Na^+,K^+-ATPase retained Na^+ pumping activity a rapidly reversible model of ATP depletion was developed in confluent $LLCPK_1$ cells grown on semi-permeable membranes. Tight junction integrity, monitored by electrical resistance and mannitol flux studies, was lost during ATP depletion and reestablished during 2 hr of ATP repletion. Under physiologic conditions and following 2 hr of ATP depletion and 2 hr ATP repletion total cellular Na^+,K^+-ATPase activity and total surface membrane ^3H-ouabain binding remained constant but apical ^3H-ouabain binding increased (7 ± 3 vs 26 ± 5 fmoles/filter, $p<0.01$). Remodeling of apical Na^+,K^+-ATPase occurred during ATP repletion with baseline levels being achieved following 5 hours of ATP repletion. Apically applied ouabain was used to selectively inhibit apical Na^+,K^+-ATPase. It had no effect on apical to basolateral (A → BL) Na^+ flux under physiologic conditions (2.5 ± 0.9 vs 2.6 ± 1.2 meq/filter/hr), but it increased the A → BL Na^+ flux in ATP depleted/repleted monolayers (0.8 ± 0.2 vs 1.7 ± 0.5 meq/filter/hr, $p<0.01$) implying apical Na^+,K^+-ATPase retained Na^+ pumping activity. Taken together, these data imply ATP depletion induces loss of surface membrane polarity resulting in redistribution of functional proteins to the alternate domain. This, in turn, may play a pivital role in cellular dysfunction following cellular injury.

G 127 POLARIZED SECRETION OF HUMAN CORTICOSTEROID BINDING GLOBULIN BY MDCK AND BEWO CELLS, Neal A. Musto, Department of Anatomy and Cell Biology, Georgetown University Medical Center, School of Medicine, Washington, DC 20007
Polarized epithelial cells are able to faithfully direct certain secretory protein components to either their apical or basolateral environments. The mechanism by which these cells accomplish this is still not entirely understood. It is hypothesized that a membrane associated "sorting receptor" recognizes an intrinsic signal contained within the sorted protein. This interaction directs the secretory protein into the appropriate domain-specific vesicle for transport to either the apical or basolateral face. The nature of this sorting signal and the recognition receptor have not been established.
In an effort to understand this phenomenon, a study was undertaken to ascertain whether human corticosteroid binding globulin (hCBG) contains intrinsic signals capable of directing its secretion to a particular side of polarized epithelial cells. To this end, MDCK and BeWo cells were transfected with an expression vector containing the cDNA for hCBG. The stable transfectants were examined for their ability to secrete hCBG with polarity. The results of these studies have revealed that hCBG is selectively secreted into the apical environment by both MDCK and BeWo cells. Furthermore, this polarized secretion is unaffected by either; 1) agents that inhibit N-linked oligosaccharide processing or 2) lysomotrophic drugs, which alter the intravesicular pH.
It is concluded that hCBG possesses an intrinsic signal for apical secretion, that can be recognized by two polarized cell types of differing origins. This signal does not appear to be present in the N-linked oligosaccharide moieties of hCBG nor is it affected by an elevation of the intravesicular pH within the *trans*-Golgi network. The use of hCBG transfected MDCK and BeWo cells constitute a useful model system for the investigation of the signals involved in the sorting of secreted proteins.

G 128 STREPTOLYSIN O PERMEABILISED MDCK CELLS PROVIDE A SINGLE *in vitro* SYSTEM TO STUDY THE MULTIPLE PATHWAYS OF POLARISED MEMBRANE TRANSPORT
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Many in vitro systems have been developed that faithfully reconstitute a given step of vesicular transport. Such systems, that have reconstituted both the exocytic and endocytic events, have provided significant insights into the general mechanism of the membrane transport. However, most such systems do not allow to study the process of protein sorting since that necessitates studying more than one process in the same system. To overcome this limitation and since our major interest lies in studying the process of epithelial polarised sorting, we have developed a single system that allows access to multiple steps of transport in one system. We use viral glycoproteins (HA of influenza or G of vesicular stomatits viruses) as the marker proteins. Filter grown MDCK cells are infected with VSV or influenza virus and the viral glycoprotein is allowed to accumulate either in the endoplasmic reticulum or in the *trans*-Golgi network. The apical surface in VSV infected and basolateral surface in influenza infected cells is then selectively permeabilised with the bacterial toxin streptolysin O and the cytosol is washed off. Under these conditions 3 different steps of the biosynthetic pathway can be measured as follows:

1) the ER to Golgi transport by acqusition of Endo H resistense by HA;
2) the TGN to apical transport by trypsin sensitivity of the apically delivered HA ; and
3) the TGN to basolateral transport by surface immunoprecipitation of the transported VSV G.

We show that in our assay conditions all the three transport steps are dependent on temperature, addition of the exogenous cytosol and supply of energy. Currently we are using this system to identify factors that are involved specifically in the apical and basolateral transport.

G 129 The Packaging of Soluble and Membrane-Bound Forms of the Pancreatic Protein GP-2 in Secretory Granules Is Cell-Type Specific.
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GP-2 is the major zymogen granule membrane protein. It is targeted both to secretory granules and directly to the apical plasma membrane of pancreatic acinar cells. Using GP-2 as a model polypeptide, we have been studying the molecular mechanisms responsible for the incorporation of proteins into secretory granules of exocrine cells, which are epithelial cells whose secretory granules undergo regulated exocytosis at the apical membrane. When introduced into the pancreatic acinar cell line AR42J by transfection of its cDNA, GP-2 was found to be present in secretory granules as determined by immunofluorescence and immunoelectron microscopy. Incubation of the cells with the secretagogue CCK resulted in the stimulated release of a soluble form of GP-2 believed to be derived from the membrane-bound molecule. To investigate whether granule packaging information resides in the protein portion of the molecule, a secretory form of GP-2 ($GP\text{-}2^-$) was generated by deleting, in the corresponding cDNA, the sequence specifying attachment of the lipid anchor of this glycosyl phosphatidyl inositol-linked membrane protein. In permanent transformants of AR42J cells, $GP\text{-}2^-$ was localized to secretory granules and underwent regulated secretion after incubation of the cells with secretagogues. These results suggest that GP-2 is packaged in secretory granules in much the same manner as soluble content proteins -- by co-condensation of its protein moiety with other granule constituents. In contrast to the results obtained in AR42J cells, we have observed that native GP-2, after expression in the endocrine pituitary line AtT20 or rin5F insulinoma cells, does not enter the endogenous hormone-containing granules. When expressed in AtT20 cells, $GP\text{-}2^-$ also did not localize to secretory granules and, in pulse-chase radiolabeling experiments, was secreted exclusively via the constitutive pathway. These results indicate that there exist cell-specific mechanisms for the sorting of some secretory granule membrane proteins. The basis for this distinction may lie in specific interactions between granule proteins like GP-2 and endogenous components present only in exocrine cells. These types of interactions may serve to insure that the membrane and condensing content proteins are segregated in concert during granule formation in the trans Golgi network.
Supported by the American Heart Association, New York City affiliate, and the N.I.H.

G 130 RETINAL-DERIVED FACTORS REGULATE THE DEVELOPMENT OF TIGHT JUNCTIONS IN THE PIGMENT EPITHELIUM,
Lawrence Rizzolo and Zhi-Qiang Li, Dept. of Cell Biology, Emory Univ., Atlanta, GA 30322

The retinal pigment epithelium (RPE) lies at the interface between the neural retina and the choriocapillaris where it forms a blood-retinal barrier. Barrier function requires a polarized distribution of plasma membrane proteins and "tight" tight junctions. During chicken embryogenesis, these features develop gradually. Although terminal junctional complexes are established by embryonic day 4 (E4), the distribution of the Na,K-ATPase is not polarized in all cells of the epithelium until E11. Similarly, the tight junctions of early embryos were leaky, but became tight by hatching (E21). We used primary cell culture to examine the molecular basis of this gradual induction of polarized function. RPE harvested from E7, and cultured on filters, formed monolayers coupled by junctional complexes. The distribution of the Na,K-ATPase was non-polarized and the tight junctions were leaky with a transepithelial electrical resistance (TER) of 20-30 Ω-cm^2. Neural retinas were isolated from E14 embryos and incubated at 37°C in base medium for 6 h. The conditioned medium was added to the apical chamber of freshly cultured RPE. Although the Na,K-ATPase remained non-polarized, the TER gradually increased 2-3 X over 7 days. If the RPE was cultured for 7 days before the addition of conditioned medium, the TER failed to increase. The retinal-derived factors were heat and alkali labile and smaller than 3 kDa. Additionally, they were acid stable and insensitive to cathepsin D. Pharmacological studies suggest one of the active factors is dopamine or serotonin. These studies provide the first direct evidence of retinal-derived factors that affect the polarized functions of the RPE.

G 131 SORTING OF H,K-ATPase, A TRANSMEMBRANE ION PUMP, TO THE REGULATED PATHWAY,
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The H,K-ATPase normally resides in an intercellular tubulo-vesicular compartment of the gastric parietal cell. This regulated storage compartment also contains intrinsic factor, a soluble protein. Stimulation of gastric acid secretion leads to fusion of the tubulo-vesicular elements with the apical plasmalemma and concomitant release of intrinsic factor. The H,K-ATPase is, thus, one of a small group of membrane proteins known to be associated with a regulated delivery pathway. We are interested in determining whether the signals and mechanisms involved in sorting to regulated pathways are similiar for membrane versus secreted proteins. We are also interested in determining the domains of the H,K-ATPase important for targeting this unusual membrane protein to regulated storage compartments. As no *in vitro* model for the gastric parietal cell has been well established, we will examine these questions by expressing the H,K-ATPase α and β subunits concurrently or individually in cell lines containing endogenous regulated secretory pathways. In addition, comparison of the H,K-ATPase with proteins which normally undergo constitutive delivery, such as the Na,K-ATPase, will allow identification of domains important for directing the H,K-ATPase to regulated storage compartments. We find that the H,K-ATPase β subunit is able to reach the cell surface when expressed by transfection in rat pancreatic islet β cells (RIN) and in rat myoblasts (L6). This implies that information for sorting into regulated storage compartments is not contained within the β subunit. Similiarly, co-expression of the α and β subunits in L6 myoblasts does not result in a concomitant increase in H,K-ATPase contained within storage compartments. These findings indicate that the mechanisms involved in sorting membrane versus secreted proteins into regulated delivery compartments may not be universal.
(Supported by NIH GM 42136 and a fellowship from the David and Lucille Packard Foundation)

G 132 Aminopeptidase N carries a signal for apical sorting on its catalytic head group.

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Aminopeptidase N is localised to the microvillar (apical) membrane of the small intestine. It is anchored to the membrane by a segment close to its N-terminal end and has its main part, including its active site on the extracellular side. It has been shown by negative staining of purified aminopeptidase N inserted into lipid vesicles, that the ectoplasmic part consists of a 5 nm stalk and a catalytic head group. By fusion of the signal peptide of influensa virus hemagglutinin with various ectoplasmic parts of aminopeptidase N, stable expression in MDCK cells of secretory forms of the enzyme was achieved. Only clones with normal folding indicated by preserved enzymatic activity were chosen for further studies. The secretion to the apical/ basolateral domains was studied by culture on polycarbonate filters and the distribution monitored by the enzymatic activity released into the medium. We have shown that a soluble form of the complete ectodomain was found to be targeted mainly to the apical side of MDCK cells. A soluble form of the catalytic head group (amino acid 65-967) of aminopeptidase N was expressed and was found to be targeted mainly to the apical side of MDCK cells. This demonstrates that the apical sorting signal on aminopeptidase N is situated on the catalytic head group.

G 133 BREFELDIN A BLOCKS MULTIPLE PATHWAYS OUT OF THE TRANS GOLGI NETWORK.

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Brefeldin A (BFA) blocks the endoplasmatic reticulum (ER) to Golgi and intra-Golgi vesicular transport of membrane and secretory proteins and causes a reflux of Golgi markers to the ER (Lippincott-Schwartz et al, Cell 56:801, 1989). The mechanism of BFA' s action appears to be interfering with the budding of the transport vesicles responsible for anterograde bulk membrane transport (Orci et al, Cell 64:1183-1195, 1991). This inhibition is mediated by the rapid release of one of the major coat protein subunits (COPs) of Golgi non-clathrin coated vesicles, beta-COP (Donaldson et al, J.Cell Biol. 111:2295-2306, 1990). We report here that addition of BFA to polarized epithelial Madin Darby Kidney (MDCK) cells partially inhibits exit from the trans Golgi network (TGN) and delivery of influenza hemagglutinin (HA), vesicular stomatitis virus (VSV) G protein and a lysosomal membrane glycoprotein AC17 to, respectively the apical membrane, the basolateral membrane and the basolateral membrane/early endosome. Furthermore a missorting of HA and AC 17, increasing with higher concentrations of BFA is observed. Since these three proteins are believed to exit the TGN via distinct types of coated vesicles and since we show that BFA instantly releases beta-COP from the TGN of MDCK cells, our results clearly implicate COP coated vesicles and other BFA sensitive elements in the transport between the TGN and the cell surface. Supported by NIH grant GM 34107 to ERB.

G 134 BASOLATERAL TARGETING OF GPI-ANCHORED PROTEINS AND GLYCOSPHINGOLIPIDS IN A POLARIZED THYROID EPITHELIAL CELL LINE.

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The distribution of several apical (DPPIV) and basolateral markers (ßNaK-ATPase, uvomorulin, transferrin receptor, 35-40 Kd Ag, ZO1) in Fisher rat thyroid (FRT) cells is identical to that displayed by these antigens in the model epithelial cell lines MDCK and Caco2 (Zurzolo et al,EMBO, 11:2337, 1992). By a biotin targeting assay, we demonstrated that both apical and basolateral transmembrane proteins were directly targeted to the plasma membrane of FRT cells, indicating that the targeting phenotype of FRT cells was similar to that of MDCK cells. It has been shown that in different kidney and intestinal epithelial cells, both endogenous and exogenous glycosylphosphatidylinositol (GPI)-anchored proteins are apically polarized, suggesting a possible role of GPI as an apical targeting signal (Lisanti and Rodriguez-Boulan,TIBS,15:113, 1990). The hypothesis has been proposed that GPI-proteins, and possibly other apical proteins may be introduced into the apical route via clustering with apically targeted glycosphingolipids (van Meer and Simons, J. Cell. Biochem, 36:51, 1988). Unexpectedly we found that the large majority of endogenous GPI anchored proteins of FRT cells are preferentially localized on the basolateral domain, while some of them are apical and some are not polarized. A chimeric GPI-anchored protein (gD1-DAF) formed by the ectodomain of the Herpes glycoprotein gD1 and the DAF signal for GPI-addition was basolaterally targeted in transfected FRT cells, whereas the same fusion protein had been shown to be apically distributed in MDCK cells. Analysis of sphingoglycolipid sorting in FRT cells showed that it was reversed relative to MDCK cells: there was a two- fold greater basolateral targeting of glucosyl-ceramide as compared to SPH. We are led to conclude that clusters of GPI-proteins and glycolipids may be sorted together to the basolateral membrane of FRT cells and suggest that (at least in this cell line) clustering with glycolipids may not have a role in the sorting of trasmembrane apical proteins.Supported by grants from NIH GM 34107 and GM 41771 to ERB.

Neuronal Polarity and the Cytoskeleton

G 200 PROTEIN DOMAINS IMPORTANT FOR TARGETTING OF THE SYNAPTIC VESICLE PROTEIN, VAMP. Nicole Calakos, William S. Trimble, and Richard H. Scheller. Department of Molecular and Cellular Physiology, HHMI, Stanford University School of Medicine, Stanford, CA, 94305.

VAMP is an integral membrane protein of synaptic vesicles. The mechanism by which synaptic vesicle proteins are identified and sorted to synaptic vesicles remains unclear. However, because of VAMP's relatively small size, 120 aa, it is amenable to analyses mutating its primary sequence to identify domains involved in targetting to the synaptic vesicle.

Initially, a series of deletions of the VAMP cDNA were constructed and expressed in a variety of neuronal (NG108,B103), neuroendocrine (PC12), and non-neuronal cell lines (CHO, COS). As assayed by immunocytochemistry, the amino-terminal 20 amino acids are not essential for localizing VAMP to the tips of NGF-induced PC12 cell processes. Deletion of the carboxy-terminal membrane anchor appeared to potentially be toxic when expressed in several cell lines as judged by the appearance of cells transiently expressing this construct and the inability to obtain clones stably expressing this construct. In order to further explore the role of the transmembrane domain, a set of genes encoding chimeric proteins were constructed which reciprocally exchange the membrane domains of VAMP and the asialoglycoprotein receptor (ASGR), both Type II membrane proteins. Initially the constructs were transiently expressed in COS cells to determine their localization in a non-neuronal cell which lacks the machinery for regulated exocytosis. Currently being explored is their localization in neuroendocrine cell lines which express VAMP endogenously and concentrate synaptic vesicles at the ends of processes. These experiments will help to understand the significance of the transmembrane domain as either merely a hydrophobic anchor or as an important domain in directing VAMP to synaptic vesicles.

G 201 **NEUROTRANSMITTER RELEASE FROM PC12 CELLS INVOLVES P65.** *Lisa A. Elferink, Michael R. Peterson and Richard H. Scheller.* Howard Hughes Medical Institute, Department of Molecular and Cellular Physiology, Stanford University Medical School, Stanford, CA. 94305

The release of neurotransmitters from the nerve terminal is a highly coordinated multistep process, requiring the targeting of synaptic vesicles to the active zone of the presynaptic membrane, the fusion of these membrane compartments and subsequent recycling of the synaptic vesicles within the nerve terminal. The identification of several proteins specifically associated with the membranes of synaptic vesicles, suggests that synaptic vesicle proteins mediate various aspects of neurotransmitter release. To examine the potential roles of synaptic vesicle proteins in regulated secretion, microinjection studies have been used on the rat pheochromocytoma PC12 cell line, which contain endogenous forms of synaptic vesicle proteins and secrete catecholamines and acetycholine in a Ca^{2+} dependent fashion. Exocytosis in microinjected PC12 cells was monitored using Dopamine-B-Hydroxylase, an integral membrane protein of large catecholamine containing vesicles, which becomes exposed on the surface of PC12 cells during Ca^{2+} regulated secretion. Microinjection of antibodies specific to VAMP, Rab3A, synaptophysin and SV2, did not interfere with either the targeting of synaptic vesicles to the nerve terminal or Ca^{2+} regulated exocytosis. In contrast, microinjection of antibodies directed against p65(synaptotagmin), an abundant synaptic vesicle protein implicated in vesicle docking or fusion, disrupted Ca^{2+} dependent secretion as assayed by diminished staining of Dopamine-B-Hydroxylase on the cell surface. Microinjection of bacterially expressed, soluble forms of p65 devoid of their transmembrane domain, also interfered with Ca^{2+} dependent secretion. These results uneqivocally confirm that p65 is a component of the machinery involved in the regulated release of neurotransmitters.

G 202 DEVELOPMENT OF HYPOTHALAMIC NEURONS INMONOLAYER CULTURES DERIVED FROM POSTNATAL MALE AND FEMALE RATS IDENTIFIED BY IMMUNOCYTOCHEMISTRY IN CONJUNCTION WITH MAP-2, GAP-43 AND ESTROGEN-RECEPTOR ANTIBODIES. Mitsuhiro Kawata, Kazunari Yuri, and Noriyuki Morita, Department of Anatomy, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602, Japan

Immunocytochemical studies have shown that estrogen induces a significant changes of numbers of immunoreactive nerve fibers containing neurotensin, CGRP, Met-enkephalin, CCK-8, and tyrosine hydroxylase in the hypothalamus, and the relationship between the existence of estrogen receptor and gene expression of these biochemical messengers with the process formation have been discussed, particularly in the context of male/female differences. In the present experiment, we developed monolayer cultures from male and female rat hypothalami and investigated the differences of process formation. Cultures derived from postnatal (P0) *Wistar* rats of both sexes were found to contain neurotensin-, CGRP-, Met-enkephalin-, CCK-, and tyrosine hydroxylase-immunoreactive neurons, in addition to other neuropeptides, such as oxytocin and vasopressin. Some of these neurons showed estrogen receptor-immunoreactivity in their cell nucleus. From morphological characterization by using MAP-2 and GAP-43 antibodies, these neurons were classified as many multipolar cells and few bipolar cells. The extension of neurites of cells from male hypothalamus was much greater than those from female ones, regardless of the treatment of estrogen into the culture medium, suggesting that male hypothalamic neurons of P0 stage have a peculiar process formation which has already been established before birth.

G 203 THE CYTOPLASMIC DOMAIN OF THE TRANS-MEMBRANE FORMS OF N-CAM CONTAINS BASOLATERAL TARGETING INFORMATION, Annick H. LeGall, Sharon K. Powell, Ayyappan Rajasekaran, Enrique Rodriquez-Boulan, Department of Cell Biology and Anatomy, Cornell Univ. Medical College, New York, NY, 10021

Transmembrane and GPI-anchored isoforms of the neural cell adhesion molecule, N-CAM, are targeted to opposite domains when transfected into the polarized epithelial cell line, MDCK. While the GPI anchored form (ssd) is targeted directly from the TGN to the apical surface, the two transmembrane forms (sd and ld), are directly delivered to the basolateral surface (S.K. Powell et al, *Nature* 353:76, 1991). Because all three isoforms share a common ectodomain but are differentially targeted, localization must be determined either by regions of membrane association and/or in the cytoplasmic domain. To address this question, the cytoplasmic domain of the 140 kD isoform of N-CAM was deleted. Deletion of the cytoplasmic tail resulted in the non-polar distribution of N-CAM when transfected in MDCK as assessed by immunofluorescence and cell surface biotinylation, suggesting that targeting information is not localized in the membrane spanning region portion of N-CAM, but in the cytoplasmic domain. To further characterize the putative basolateral sorting determinant, deletion mutants of the cytoplasmic domain have been constructed, and are currently under analysis. Supported by an NSF predoctoral fellowship RCD-9253029 to AHL, and grants from the Spinal Cord Research Foundation #1152 to SKP, and NIH GM 34107 and GM 41771 to ERB.

G 204 THE EFFECTS OF BREFELDIN A ON THE SYNAPTIC VESICLE RECYCLING PATHWAY IN CULTURED HIPPOCAMPAL NEURONS, Olaf Mundigl[1,3], Michela Matteoli[1,3], Reinhard Jahn[2,3], Pietro De Camilli[1,3], Department of Cell Biology[1], Department of Pharmacology[2], Howard Hughes Medical Institute[3], Yale University School of Medicine, New Haven, CT 06510

Neurons store and release non peptide neurotransmitters through highly specialized secretory organelles, the synaptic vesicles (SVs). Following vesicle fusion with the axolemma, synaptic vesicle membranes are retrieved from the presynaptic terminal by endocytosis and reused for the formation of new SVs.

Whether the recycling pathway of SV membranes involves an endosomal compartment in the nerve terminal has not been completely resolved yet. However, in the neuroendocrine cell line PC12 it has been demonstrated that a substantial fraction of synaptophysin, a major SV membrane protein, colocalizes with transferrin receptor, a marker for early endosomes. This finding indicates that SVs might be continuously regenerated from early endosomes.

As a further step toward elucidating the recycling pathway of synaptic vesicles, we have studied the effects of the fungal metabolite Brefeldin A (BFA) on the distribution of SV-proteins. BFA causes early endosomes to tubulate and merge into a tubular endosomal reticulum, while recycling between the endosomal network and the plasmamembrane is not affected. We have found that the intracellular distribution of different SV-proteins is differentially affected by BFA. These studies have led us to identify the existence of two distinct endomembrane systems positive for SV-proteins in polarized hippocampal neurons grown in vitro. The first membrane pool was BFA-resistant and includes axonal membranes and a fraction of the somatodendritic SV-protein containing membranes. A second membrane pool was tubulated by BFA and was represented by the endosomal membranes of the somatodendritic domain. In this membrane system a complete overlap of synaptophysin and transferrin receptor or internalized transferrin was found. Transferrin receptors are only present in the somatodendritic region. Since early endosomes of the somatodendritic domain contain both recycling SV-components and proteins of the receptor-mediated recycling pathway, we conclude that an extensive sorting of SV-proteins must take place in order to maintain the unique membrane composition of the SSVs. Our results speak against an origin of preassembled SV membranes at the level of the Golgi complex. The BFA resistance of the axonal endomembrane system indicates that endosomes in the presynaptic terminal may differ in properties from those found in the somatodendritic region.

G 205 LOCALIZATION OF OVEREXPRESSED aFGF IN SENSORY AND MOTOR NEURONS IN CULTURES OF RAT DORSAL ROOT GANGLIA AND VENTRAL ROOTS, Cornelia Oellig, Lesley Taylor, Peter Liljeström, Yihai Cao, and Ralf Pettersson, Ludwig Institute for Cancer Research, Karolinska Institute, Stockholm, Sweden

Acidic fibroblast growth factor (aFGF) is a member of a family of at least seven structually related polypeptide growth factors, which are characterized by their high affinity to heparin. aFGF as well as basic FGF, the second prototype of this group, have been demonstrated to display pleiotropic biological activities including mitogenesis, angiogenesis, chemotaxis, and induction of differentiation in a variety of different *in vivo* and *in vitro* systems. bFGF is widely distributed in vertebral non neural and neural tissues, whereas aFGF has been found mainly in distinct regions of the nervous system. The preferential localization to the nervous system suggests that aFGF may have a more selective role for neural tissue than does bFGF. Significant levels of aFGF expression have been found in rat dorsal root ganglia (DRG) and the ventral and dorsal roots (Elde et al.,1991, Neuron,7:349-364). The aFGF expression was clearly restricted to the neuronal cells, in particular the axons of the motor and sensory neurons of these regions were shown to contain high amounts of aFGF protein aligning the plasma membrane. In order to elucidate this peculiar membranous pattern of aFGF immunreactivity associated with the axons, we developed an *in vitro* system based on the overexpression of aFGF in the neurons of DRG-cultures, to study the expression and transport of the protein in the extending axons and their mechanism of association with the axonal membranes. We used the Semliki Forest virus expression system for overexpressing the human aFGF protein in embryonic DRG-cultures from rat. We find high expression of the recombinant protein in the neuronal cell body as well as varying amounts in the axons, dependent on the time point after infection as well as on the age of the neuronal cultures. Extensive studies of the infection time and developmental stage dependent localization of the protein in the neuronal cells have to be done to determine the optimal conditions for biochemical investigations of the membran-protein interactions. Furthermore comparative biochemical studies of aFGF purified from disected tissue of the rat DRG, the dorsal white column, and cauda equina, other neural regions of significant aFGF expression, are being performed.

G 206 NEURONAL AND EPITHELIAL POLARITY : THE GABA AND BETAINE TRANSPORTERS ARE SORTED TO OPPOSITE MEMBRANES OF POLARIZED EPITHELIAL CELLS, Grazia Pietrini[1,3], Young J. Suh[2], Lambert Edelmann[2], Gary Rudnick[2] and Michael J. Caplan[1], Departments of Cellular and Molecular Biology[1] and Pharmacology[2],Yale University School of Medicine, New Haven,CT 06510 ; CNR Center for Cytopharmacology[3],Via Vanvitelli 32, Milan, Italy

The surface membranes of neurons and epithelial cells are divided into distinct domains characterized by markedely different protein compositions. Viral glycoproteins which are sorted to the epithelial apical and basolateral surfaces have been shown to accumulate in the axonal and dendritic domains, respectively, of infected neurons. Thus, the signals and mechanisms responsible for apical and basolateral sorting in epithelial cells may result in axonal and dendritic targetting in neurons, respectively. We have examined the sorting behavior of two members of the same transporter gene family: the neuronal Na,Cl-dependent GABA and the epithelial Na,Cl-dependent betaine transporters. In cultured hippocampal neurons we find the transporter to be present in the axolemma of GABA-ergic neurons. Uptake of betaine has been shown to occur from the basolateral membranes of MDCK cells. Our functional and immunological analysis of the GABA transporter transfected in MDCK cells show that the transporter behaves as an apical protein while the over-expressed betaine transporter, as well as the endogenous molecule, behaves as a basolateral protein.These results suggest that axonal sorting information leads to apical targetting in polarized epithelial cells and are consistent with the hypothesis of similar sorting mechanisms employed by both cell types. Moreover, two structurally and functionally similar proteins appear to contain distinct sorting information. Studies on chimaeric products between the two transporters are in progress.

G 207 **INTERCELLULAR CA^{2+} SIGNALING MEDIATED BY IP_3 IN NON-EXCITABLE CELLS.** Michael J. Sanderson, Andrew C. Charles, Scott Boitano and Ellen R. Dirksen. Departments of Anatomy and Cell Biology and Neurology, School of Medicine, University of California, Los Angeles, CA 90024.

Intercellular communication is important in the function of epithelial cells but the nature of the signals that pass between cells is not fully established. Calcium is known to be an important second messenger and two types of Ca^{2+} signaling occur in non-excitable cells - propagating intercellular Ca^{2+} waves and non-propagating oscillations in $[Ca^{2+}]_i$. Mechanical stimulation of a single airway epithelial or brain glial cell in culture initiates an increase in $[Ca^{2+}]_i$ in the stimulated cell that propagates, in a cell-by-cell manner, through adjacent cells as a Ca^{2+} wave. The propagation of the Ca^{2+} wave between cells is blocked by gap junction inhibitors and requires the presence of connexin43. In Ca^{2+}-free conditions, the stimulated cell does not show an increase in $[Ca^{2+}]_i$, but a Ca^{2+} wave is still propagated indicating that an elevated $[Ca^{2+}]_i$ is not required to initiate a Ca^{2+} wave and that Ca^{2+} waves release intracellular Ca^{2+}. The Ca^{2+} wave is abolished by thapsigargin, a Ca^{2+}-ATPase inhibitor, reduced in magnitude by dantrolene, an inhibitor of Ca^{2+}-induced Ca^{2+} release and initiated by microinjection of IP_3. Selective loading of cells by high frequency electroporation with heparin, an IP_3 receptor antagonist, inhibited the propagation of a Ca^{2+} wave even though $[Ca^{2+}]_i$ in adjacent heparin-free cells was elevated. The failure of increases in $[Ca^{2+}]_i$ to initiate Ca^{2+} waves is emphasized by the occurrence of asynchronous Ca^{2+} oscillations in these cells. A Ca^{2+} wave can be initiated to propagate through cells displaying Ca^{2+} oscillations. These results indicate that IP_3 but not Ca^{2+} acts as the intercellular messenger to propagate Ca^{2+} waves. We suggest that Ca^{2+} oscillations influence the activity of individual cells whereas intercellular Ca^{2+} waves provide a mechanism to initiate and coordinated multicellular activity. A color video tape of these results will be shown. Supported by Smokeless Tobacco Research Council, Inc., the Tobacco Related Disease Research Program of the University of California, the American Academy of Neurology (ACC) and a Veterans Administration Career Development Award (ACC).

G 208 SORTING OF SYNAPTOPHYSIN DURING NEURONAL AND NEUROENDOCRINE DEVELOPMENT, Bertram Wiedenmann, Gudrun Ahnert-Hilger, Andrée Tixier-Vidal, Department of Internal Medicine, Steglitz Medical Center, Free University of Berlin, Berlin, Germany and Department of Cellular and Molecular Neuroendocrinology, Collège de France, Paris, France

Neurons are characterized by two separately regulated secretory pathways. These secretory processes are mediated by two distinct vesicle types, i.e. the large dense core vesicles (LDCV) and the small synaptic vesicles (SSV), which in turn release their vesicular content at the apical surface, both in neuroendocrine and neuronal cells.

Our laboratory has focused on the characterization of integral membrane proteins of SSV and LDCV using poly- and monoclonal antibodies against the major integral membrane protein of SSV, synaptophysin (Wiedenmann and Franke, 1985, Cell 41, 1017 - 1028) and cytochrome b561 of LDCV (Ahnert-Hilger et al., submitted). Regulated secretory vesicles are regarded as markers for cell differentiation and oncogenesis. We show now that the biogenesis of these two vesicle types is regulated differently during neuronal and neuroendocrine development. In the case of neurons, these studies are done on differentiating primary cell cultures at the light-microscopic and electron-microscopic level (Kagotani et al., 1991, J Histochem Cytochem 39, 1507 - 18; Tixier-Vidal et al., 1992, Neuroscience 4, 967 - 78). Evidence is presented that the small GTP-binding protein rab 6p appears to be differentially associated with synaptophysin-containing vesicles during neuronal development (Tixier-Vidal et al., submitted). As a model for neuroendocrine cells, we used the pancreatic cell line AR42J, which can be differentiated upon treatment with dexamethasone (Rosewicz et al., 1992, Eur J Cell Biol, in press).

Current investigations in our laboratories focus on the requirements for secretion of synaptophysin-containing vesicles in pancreatic neuroendocrine cells, which, similar to neurons, contain the classical neurotransmitters GABA, glutamate and glycine (Rosewicz et al., 1992).

G 209 **Polarized distribution of an actin filament nucleator in PC-12 cells.**

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Nerve growth factor induces the redistribution of a 43 kD actin-binding protein from the microtubule organizing center to the growth cones of emerging neurites in PC-12 cells. The focal distribution of this actin nucleator on the plasma membrane after NGF stimulation suggests that it contributes to the specification of the site of growth cone-neurite emergence. By computer-assisted video microscopy, this 43 kD protein associates with the barbed ends of rhodamine-phalloidin-labelled actin filaments in vitro. In addition, it stimulates the formation of actin filaments from actin monomers as visualized by fluorescence video microscopy of rhodamine-labelled actin monomers. It decreases the critical concentration for actin filament formation and increases the speed of filament formation. Thus, its presence at the site of growth cone emergence suggests that an early event in the establishment of neuronal polarity is the localized polymerization of actin.

Supported by the Council for Tobacco Research.

G 210 ASSOCIATION OF MYOSIN-I WITH GOLGI VESICLES, K. Fath and D. Burgess, Dept. of Biological Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15260.

The delivery of constituents to the apical membrane in polarized epithelia is thought to be facilitated by, but not absolutely require, intact microtubules. The immunolocalization of the mechanoenzyme myosin-I with cytoplasmic vesicles in intestinal enterocytes (Drenckhahn & Dermietzel, (1988), *J.C.B.*, 107:1037) led us to explore a role of myosin-I in vesicle trafficking in intestinal epithelia. We isolated cytoplasmic vesicles from undifferentiated enterocytes that express myosin-I on their cytoplasmic surface and bind actin filaments in an ATP-dependent manner (Fath & Burgess, (1991), *J.C.B.*, 115:39a). We now report that these vesicles, which were isolated by methods established for Golgi purification, contain a 2-fold enrichment for galactosyltransferase (GalTase) specific activity, a marker enzyme for the *trans*-Golgi. Moreover, in that ~50% of the GalTase activity partitions with intact vesicles that were immunoisolated with myosin-I antibodies, suggests that myosin-I and GalTase are co-expressed on the same vesicle. Alkaline phosphatase, an apical plasma membrane-specific marker, was also associated with the vesicles. The presence of both enzymes suggests that these vesicles may represent carrier vesicles transporting membrane to the apical plasma membrane. By immunoblotting we found that the microtubule-based motor dynein was also bound to the vesicles. We are currently determining if single vesicles express both dynein and myosin-I, with the aim of exploring a role for both a microtubule- and an actin-based motility working in parallel in apical membrane targeting in intestinal epithelia.

G 211 BIOGENESIS OF CYTOSKELETON-MEMBRANE INTERACTION OCCURS IN THE ENDOPLASMIC RETICULUM . *Gomez S.and **Morgans C.*Laboratoire de Biologie de la Differenciation Cellulaire Faculté de Siences de Luminy13288 Marseille France**Department of Biological Sciences Stanford UniversityCA 94305

In the erythroid system, it is well established that band 3 (a major transmembrane anion exchanger) interacts with ankyrin (a cytoplasmic protein) providing band 3 a linkage to the cytoskeleton. However, it is not known in which cellular compartment this interaction occurs. This study adressess this question in murine erythroleukemic (MEL) cells, as well as the role that this association plays in the processing and the distribution of band 3. MEL cells were pulse-labeled with L-(35S) Met. Immunoprecipitation of the newly synthesized proteins using anti-band 3 antibodies brought down a 215 kDa protein in addition to band 3. We used a second round of immunoprecitation with an anti-ankyrin antibody to assess that the 215 kDa protein present in the immunoprecipitate was immunologically related to ankyrin. The passage of the band 3-ankyrin complex through the endoplasmic reticulum (ER) or the first Golgi compartment, was assayed by its sensitivity to digestion with endoglycosidase H (endoH). Digestion of the living cells with chymotrypsin was used to discard the putative presence in the immunoprecipitates of labeled ankyrin complexed with plasma membrane band 3. In addition, we used concavaline A (ConA) lectin to enriched ER and plasma membrane fractions in pulse-chase experiments. Co-immunoprecipited band 3-ankyrin complexes were detected in fractions flowing through the lectin in unchased samples, while in samples chased for 4 hours, they were retained and could be eluted with α methyl mannoside . These results strongly suggest that the association between these two proteins occurs in the ER or the first Golgi compartment. Because this early association may affect the targeting of band 3, we investigated the fate of band 3 when expressed in the absence of ankyrin. Neither band 3 nor ankyrin are dectected by immunoprecipitation in the embryonic human kidney cell line 293. When such cells were transfected with a cDNA encoding band 3, the expressed protein was sensitive to endoH, indicating it does not transit through the golgi complex. In contrast, when the cells were co-transfected with cDNAs encoding band 3 and a band 3 binding fragment of ankyrin, a fraction of the expressed band 3 became resistant to endoH. These data imply that ankyrin not only anchors band 3 to the cytoskeleton but may be also responsible for its exit out of the ER. Furthermore, they support a model in which a band 3 complex is inserted as a "cassette" into the cytoskeletal network.

G 212 THE MEMBRANE SKELETON OF THE RETINAL PIGMENT EPITHELIAL CELLS. Virva Huotari, Raija Sormunen, Veli-Pekka Lehto and Sinikka Eskelinen, Biocenter and Department of Pathology, University of Oulu, Kajaanintie 52 D, SF-90220 Oulu, Finland

The retinal pigment epithelium (RPE) of the eye consists of a monolayer of cells separating the outer segments of the visual cells from the choroid layer. Like other transporting epithelia, RPE rests on a basement membrane and is asymmetric in both structure and function. The apical surface faces the sensory cell layer. An interesting feature of RPE is the apical localization of Na, K-ATPase pump, in contrast to other transporting epithelia where these pumps are basolateral. In kidney epithelial cells the membrane skeleton, composed of fodrin and actin, is linked via ankyrin to Na,K-ATPase and is located along the lateral walls. The reports on the localization of the membrane skeleton in RPE cells are contradictory: there is evidence for the apical as well as for the basolateral localization of fodrin. We decided to investigate the distribution of Na,K-ATPase and fodrin in chicken RPE both in vivo and in vitro to solve this discrepancy. In tissue sections from the eyes of 10-day old chicken embryos, Na,K-ATPase was primarily located on the apical surfaces of RPE cells. Fodrin staining was seen both along the apical surfaces and lateral walls. For in vitro studies, the retinal pigment epithelial cells were digested from the tissues with proteolytic enzymes and placed in culture dishes. In primary cultures the cells started to divide and preserved their epithelial morphology and intracellular pigment granules. Na,K-ATPase and fodrin were found predominantly along the lateral walls. Only a weak staining was seen at the apical surface of the cells. Thus, segregation of the proteins into specialized domains in primary cultures differs from that in intact tissues suggesting that RPE-cells in culture change their polarization. This also shows that cell polarization is not maintained by morphogenetic "memory" but, instead, is critically dependent on external factors.

G 213 TWO HOMOLOGS OF *FRIZZLED*, A CYTOSKELETAL POLARITY GENE IN *DROSOPHILA*, ARE WIDELY EXPRESSED IN MAMMALIAN TISSUES, Robert A. Nissenson, Samuel D.H. Chan, Mary Fowlkes, Mark Y.C. Liu, and Gordon J. Strewler, Endocrine Unit, VA Medical Center and University of California, San Francisco, CA 94121.

The *frizzled* (*fz*) locus in *Drosophila* encodes a protein with 7 transmembrane spanning segments (TMs), a feature shared by members of the G-protein-coupled receptor superfamily. Mutations at this locus disrupt transmission of cytoskeletal polarity signals during epidermal development, resulting in disordered hair and bristle patterns. We now report the cloning from a UMR-106 rat osteosarcoma cell library of a cDNA (fz-1) encoding a 641-residue protein (Fz-1) with 46% homology with *Drosophila Fz*. We have also cloned a cDNA (fz-2) encoding a 570-amino acid protein (Fz-2) that is 80% homologous with Fz-1, differing mostly in its extracellular domains. Both Fz proteins display 7-TM profiles, but Fz-2 has a truncated N-terminus compared to Fz-1. Southern blots of rat and mouse genomic DNA indicated that Fz-1 and -2 are products of distinct genes. Northern blots revealed two fz-2 mRNAs (2.5-kb and 4.5-kb) and a single fz-1 mRNA (4.7-kb) in rat tissues. The fz-1 and fz-2 genes are widely expressed, with the highest levels of mRNA in rat kidney, liver, heart, uterus, and ovary. Greater levels of fz-1 and fz-2 mRNA were found in neonatal than in corresponding adult tissues. Treatment of UMR-106 cells with the bone-active agents parathyroid hormone (bPTH[1-34], 10nM), EGF (100ng/ml), or $1,25(OH)_2D_3$ (10nM) produced increases in fz-1 and -2 mRNA levels. Serum-deprived rat vascular smooth cells expressed high levels of fz-1 and fz-2 mRNA; fz-1 mRNA was undetectable and fz-2 mRNA barely detectable 6 hours after the addition of 10% FCS. In summary, we have isolated cDNAs encoding two widely expressed mammalian homologs of *Fz*, a protein involved in intercellular communication of cytoskeletal polarity information during epidermal development in *Drosophila*. Expression of mammalian fz genes is regulated by agents that alter cell growth and function in vitro. Mammalian fz may constitute a gene family important for transduction and intercellular transmission of polarity cues during tissue morphogenesis and/or in differentiated tissues in higher organisms.

G 214 Apical carrier vesicles in influenza virus-infected MDCK cells bind to microtubules in a nucleotide-dependent fashion. F. Schmitz and D. Drenckhahn, Institute of Anatomy, University of Würzburg, Germany

We have previously shown that colchicine-induced disruption of microtubules in the intestinal epithelium caused mistargeting of apical membrane proteins to the basolateral plasma membrane finally leading to the formation of basolateral brush border. We wondered whether the polarized transport to different plasma domains might be mediated by association of Golgi-derived carrier vesicles with microtubule-dependent motor proteins. In influenza virus-infected MDCK cells carrier vesicles containing the apical membrane protein hemagglutinin were shown to bind to microtubules in vitro only in the presence of cytosol. Cytosol from polarized epithelial cells (kidney) was more effective in mediating vesicle binding than cytosol isolated from non polarized epithelial cells (thymus). No binding was observed in the absence of cytosol. Binding of apically targeted vesicles to microtubules was nucleotide-dependent. In the presence of cytosol binding of vesicles was significantly increased by AMPPNP but was inhibited by both ATP and GTP. Most interestingly, strong binding of hemagglutinin-containing vesicles to microtubules was induced by AlF_4^- even in the absence of cytosol. AlF_4^--induced rigor complexes could be released by both ATP and GTP. Preliminary results of immunoprecipitation experiments using affinity-purified antibodies against bovine brain kinesin indicate that a kinesin-like motor protein is associated with hemagglutinin-containing carrier vesicles.

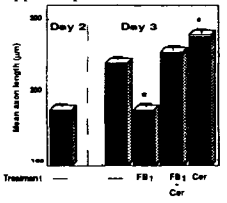
G 215 ASSOCIATION OF THE AMILORIDE-SENSITIVE SODIUM CHANNEL WITH THE SUBMEMBRANE CYTOSKELETON IN RENAL EPITHELIAL CELLS, Peter R. Smith*, Eun-Hye Joe†, Kimon J. Angelides†, and Dale J. Benos‡, *Department of Biology, Syracuse University, Syracuse, NY 13244, †Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030, and ‡Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, AL 35294.

Amiloride-sensitive Na^+ channels are localized to the apical microvilli in sodium-reabsorbing renal epithelial cells, such as the distal tubules and collecting ducts. Maintenance of the polarized distribution of the Na^+ channels to the apical membrane is essential for transepithelial Na^+ transport. To elucidate the mechanisms that maintain the polarized distribution of the Na^+ channels to the apical membrane, we searched for specific proteins associating with the channel. Triton X-100 extraction of A6 renal epithelial cells reveals the Na^+ channels are associated with the detergent insoluble and assembled cytoskeleton. Indirect immunofluorescence microscopy shows that the Na^+ channels are segregated to the apical microvillar membrane and colocalize with analogues of ankyrin and fodrin in A6 cells. We show by immunoblotting that ankyrin and fodrin analogues remain associated with the Na^+ channels after isolation and purification from bovine renal papillary collecting ducts. ^{125}I-labeled native human erythrocyte ankyrin can be precipitated by anti-Na^+ channel antibodies only in the presence of the purified bovine Na^+ channel complex. Direct binding of ^{125}I-labeled human erythrocyte ankyrin reveals ankyrin binds only to the 150 kDa subunit of the channel complex. Fluorescence photobleach recovery measurements of lateral diffusion indicate Na^+ channels are immobile [$D_L < 10^{-12}$ cm^2/s] or have very limited lateral mobilities [$D_L = (4.3\pm2) \times 10^{-11}$ cm^2/s] in A6 cells, thereby corroborating an interaction between Na^+ channels and the submembrane cytoskeleton. We conclude that the amiloride-sensitive Na^+ channel is linked to ankyrin and fodrin and that this interaction may sequester Na^+ channels to the apical microvilli and maintain their polarized distribution in renal epithelial cells. Supported by NIH grants DK37206 (DJB) and NS24606 (KJA).

Late Abstract

SPHINGOLIPID SYNTHESIS, SORTING AND TRANSPORT IN CULTURED HIPPOCAMPAL NEURONS. Anthony H. Futerman, Rotem Harel, Koret Hirschberg, Anat Sofer and John Stone, Department of Membrane Research and Biophysics, Weizmann Institute of Science, Rehovot 76100, Israel.

The apical domain of a number of epithelial cells is enriched in sphingolipids (SLs) compared to the basolateral domain. The observation that some proteins that are targeted to the apical domain of MDCK cells are targeted to axons of hippocampal neurons, and that a protein targeted to the basolateral domain of MDCK cells is targeted to the dendrites of hippocampal neurons, has led to the proposal that SLs may similarly be preferentially targeted to, and enriched in axons (Dotti and Simons, Nature, **349**, 158), and may be involved in the sorting of axonal proteins. No data exists on SL sorting in neurons, and we have therefore initiated studies to examine the synthesis, sorting and transport of SLs in hippocampal neurons.



[4,5-^{3}H]sphinganine is metabolized to a variety of complex glyco-SLs by hippocampal neurons during the first 6 days in culture, but low amounts of sphingomyelin are formed. Inhibition of SL synthesis by Fumonisin B_1 (FB_1) (an inhibitor of ceramide synthase) severely retards axonal outgrowth between days 2 and 3 in culture (see Fig.). Addition of C_6-NBD-ceramide together with FB_1 reverses this effect, and moreover, addition of C_6-NBD-ceramide by itself causes a significant increase in axon length. Data will also be presented on the effects of FB_1 and C_6-NBD-ceramide on dendritic outgrowth. We assume that SLs are essential components of the vesicles that deliver newly-synthesized material to the growing axon and that inhibition of synthesis reduces or completely arrests axonal outgrowth since vesicle transport to the axon is disrupted. Experiments testing this model will be presented.

We have also begun to analyze the distribution of SLs in neurons using anti-ganglioside antibodies. Preliminary data suggests that at least two gangliosides are non-uniformly distributed on neurons. Finally, SL internalization is being examined using various fluorescent SL analogs that enter the cell via an energy and temperature dependent mechanism.