

CELL BIOLOGY

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ABSTRACTS

Observations on the Tertiary Structure of Ribosomes Released from Membranes of Rough Endoplasmic Reticulum. JOHN H. ELDER and D. JAMES MORRÉ, Purdue University, West Lafayette, Indiana 47907.—Much knowledge concerning the tertiary structure of ribosomes has been deduced from morphological and biochemical studies in which ribosomes have been examined by electron microscopy either from thin sections or in negatively stained preparations or from studies where ribosomes have been dissociated into subunits and component proteins and reassembled as well as from studies of attachment of mRNA and the growing polypeptide chain. Generally, ribosomes consist of a major and minor subunit with a groove or channel to accommodate the mRNA plus a channel in the large subunit to direct the growing peptide chain out of the ribosome and into the membrane lumen or soluble cytoplasm. In the present study, image enhancement of negatively stained ribosomes from polyribosomes released from rough endoplasmic reticulum of rat liver has been used to provide a 3-dimensional view of one class of eukaryotic ribosomes. Work supported in part by a grant from the National Science Foundation.

Complex Gangliosides Bind Fibronectin. DAVID C. EVERS and D. JAMES MORRÉ, Purdue University, West Lafayette, Indiana 47907.—Surface properties of cells that permit tumors to metastasize are poorly understood. In normal cells, fibronectin, a large glycoprotein, may be employed as a linker molecule permitting cells to attach to a collagen-rich stroma. The present study identifies several complex gangliosides that bind fibronectin and may serve as the cellular binding site for fibronectins. Findings will be correlated with results of previous studies from our laboratory where ganglioside levels have been determined and compared in metastatic and non-metastatic hepatocellular carcinomas of the rat. Work supported in part by a grant from the American Cancer Society.

Electrophoretic Study of the Deoxyribonuclease Activity of Human Urine. D. K. HANSEN, W. CHAEKAL and M. E. HODES, Department of Medical Genetics, Indiana University School of Medicine, Indianapolis, Indiana 46223.—The deoxyribonuclease activity present in human urine was analyzed by several common electrophoretic techniques. Multiple bands of activity can be resolved following electrophoresis in polyacrylamide gels, incubation of the gel with an agarose overlay containing DNA, precipitation of the undigested DNA and staining of the DNA with methyl green or toluidine blue. After isoelectric focusing, bands of activity with wide differences in pH were visualized. The focusing pattern was not affected by treatment with neuraminidase. Common disc electrophoretic systems were used to study these activities. Smearing of some bands made analysis of anodally migrating activity difficult. The cathodally migrating bands from over 100 urine samples have been analyzed. The relative mobilities of the resultant one

or two bands generally seen are quite reproducible. Occasionally a third band is visible closer to the origin. Treatment of the urine with neuraminidase prior to electrophoresis has no effect on the band number or mobility. In addition, analysis of low (to 50 pg) quantities of bovine pancreas DNase in the pH 4.5 cathodal system did not reveal the variation in mobility with amount of enzyme applied that had previously been demonstrated for RNase A and human urine RNase. It is thought that this invariance of mobility is due to the lack of anion binding by the relatively acidic (pI 4.7) DNase I as opposed to the extreme anion binding by the very cationic (pI ca. 9.5) RNase.

Characterization of A Fast Transported Calcium—Binding Protein in Mammalian Nerve. ZAFAR IQBAL, Departments of Physiology, Biochemistry and Medical Biophysics, Indiana University School of Medicine, Indianapolis, Indiana 46223.

—Axoplasmic transport, a process required to carry down cellular components essential for the maintenance of proper form and function of nerve, was found dependent on Ca^{2+} . Following an injection of $^{45}\text{Ca}^{2+}$ into the L-7 dorsal root ganglion of cat, $^{45}\text{Ca}^{2+}$ radioactivity was found to be associated with a calcium-binding protein (CaBP) of about 15,000 dalton and both were transported in sciatic nerve at a fast rate close to 410 mm/day (Iqbal and Ochs, 1978; J. Neurochem. 31:409-418). This CaBP now has been isolated from dog sciatic nerve and purified by a combination of separation techniques such as gel filtration on Sephadex G-100 and G-25, ion exchange chromatography on DEAE-cellulose and preparative isoelectrofocusing. The purified CaBP shows similarities to calmodulin, a calcium-dependent regulatory protein in regards to its mobility on SDS containing polyacrylamide gels, and analytical isoelectro-focusing gels and activation of cyclic nucleotide phosphodiesterase. A fast transport of CaBP in the nerve and its similarities to calmodulin suggests its involvement in the axoplasmic transport of materials in nerve fibers. In the transport filament model proposed for fast axoplasmic transport, energy supplied by the hydrolysis of ATP by Ca-Mg-ATPase is required to move the transport filament to which various components are bound, down the microtubules. The activity of Ca-Mg-ATPase and the assembly-disassembly of microtubules has been shown to be regulated by calmodulin. Additionally, calmodulin regulates a number of enzymes including those involved in the metabolism of carbohydrates required to maintain the proper supply of ATP needed for the transport system to operate. Supported by NIH RO1 8706-11, American Cancer Society IN-46T and the Indiana Academy of Science.

The Role of Beta-alanine in Pigmentation Polymorphisms. M. E. JACOBS, Department of Biology, Goshen College, Goshen, Indiana 46526.—The influence of beta-alanine in integumentary pigmentation is investigated. In addition to its presence in tanned insect cuticles, beta-alanine is present in the tanned mycelial walls of the fungus, *Morchella esculenta* and in human hair. Its possible role in pigmentation of such structures is discussed. Beta-alanine forms yellow-red tanning pigments when incubated with N-acetyldopamine or leucyltyrosine oxidized by tyrosinase, or with unsaturated phospholipids, such as lecithin, oxidized by heat.

Antioxidants, Fatty Acids and Oxidant Stress, and the Control of Cell Proliferation in Culture. JAMES S. MILLER, Division of Natural Sciences, Goshen College, Goshen, Indiana 46526 and SAMUEL O. IKHAREBHA, VICTOR C. GAVINO, GEORGE E. MILO, and DAVID G. CORNWELL, Ohio State University, Columbus, Ohio 43212.
—Previous work showed that several polyunsaturated fatty acids inhibit the proliferation of smooth muscle cells and fibroblasts in tissue culture. α -tocopherol

(Vitamin E) and other antioxidants had the opposite effect, enhancing proliferation of smooth muscle cells.

In the present study we show that polyunsaturated fatty acids produce oxidant stress in smooth muscle cells, as measured by the thiobarbituric acid (TBA) assay for lipid peroxidation. α -tocopherol was an inhibitor of this lipid peroxidation produced by polyunsaturated fatty acids.

α -tocopheryl quinone (Vitamin E-quinone), a metabolite of α -tocopherol, was better than α -tocopherol as a stimulator of the proliferation of both smooth muscle cells and fibroblasts in culture. α -tocopherylquinone was also better than α -tocopherol in inhibiting the formation of lipid peroxides in cells incubated with polyunsaturated fatty acids. These results demonstrate that α -tocopherylquinone is an antioxidant *in vivo*.

Several other quinones also were studied: menadione, phyloquinone, and ubiquinone-10. Menadione was a potent inhibitor of smooth muscle cell proliferation in culture; the other two quinones had no effect. The different results with these quinones may be due to specific structural differences.

Effects of Cytochalasins on Selected Species of *Aspergillus* and *Mucor*. KENNETH C. MILLER and STANLEY N. GROVE, Goshen College, Goshen, Indiana 46526.—The effects of cytochalasins are surveyed on a variety of *Aspergillus* species. The intent is to clarify the relationship between sensitivity to the inhibitors and potential pathogenicity. Likewise, several *Mucor* species are similarly surveyed to test the generalization that fungi which are capable of causing infections of deep organs in higher animals are characterized by an ability to develop in the yeast form. The sensitivity to the cytochalasins of dimorphic species is compared with that of non-dimorphic species. Our preliminary findings suggest that some pathogenic or dimorphic forms are less sensitive to cytochalasins than closely related non-pathogenic or non-dimorphic forms. Supported in part by a grant from the National Institute of Allergy and Infectious Diseases.

Effect of Vitamin A on the Heptaic Golgi Apparatus Architecture. DOROTHY M. MORRÉ, D. JAMES MORRÉ and MARTHA WALTER, Department of Foods and Nutrition and Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Indiana 47907.—The role of the Golgi apparatus in glycosylation was shown first by autoradiography with goblet cells, which secrete large quantities of mucin, a mucoprotein of high sugar content. The sulfation process, that is the transfer of sulfate from an activated donor to an appropriate receptor, requires the activity of sulfotransferases and may occur in the Golgi apparatus as well. Vitamin A is required not only for activation of sulphatide but also for other processes of mucopolysaccharide synthesis. In this study, we investigated the effects of vitamin A on the architecture of the Golgi apparatus. Male weanling CDF rats were fed diets containing either no, adequate (4,000 IU/kg diet) or excess (100 times adequate) amounts of vitamin A as retinyl acetate. Animals were killed at 2 to 8 weeks postweanling and portions of the liver were prepared for electron microscopy. Quantitative and qualitative determinations were made from montages of randomly selected whole parenchymal cells. The extent of individual Golgi apparatus stacks (dictyosomes) paralleled vitamin A intake. Relative to controls, there was a reduction in total membrane surface occupied by Golgi apparatus membranes in cells of vitamin A deficient livers while there was an increase in excess. Secretory vesicles and vesicular profiles appeared unaffected by vitamin A

extremes. Generally, Golgi apparatus of livers of vitamin A deficient animals appeared smaller with more fenestrated cisternae while livers of excess animals had more extensive sheet-like cisternae with more well-developed complex cisternae on the mature face in the GERL region. The overall appearance of the Golgi apparatus architecture was influenced by dietary vitamin A levels. Supported by grants from the NIH CA 18801 and the American Cancer Society.

Distribution of Acid Phosphatase in Transplanted Hepatomas of the Rat. DOROTHY M. MORRÉ and ANN L. ROSENTHAL, Department of Foods and Nutrition, Purdue University, West Lafayette, Indiana 47907.—Acid phosphatase is considered to be a marker enzyme for lysosomes even though it is present in other parts of the cell (e.g. Golgi apparatus). In our work, we have combined determinations of acid phosphatase using β -glycerophosphate as a substrate with simple cell fractionation procedures. Male weanling inbred CDF rats were subcutaneously injected with hepatocellular carcinoma cells of the rat derived from solid tumors induced in syngeneic donors by 2-acetylaminofluorene. Livers were homogenized and fractionated into nuclear, mitochondrial-lysosomal and microsomal fractions by differential centrifugation. Total activity of acid phosphatase was measured as activity present when Triton X-100 was added to the incubation medium whereas free activity was the activity present with no added detergent. Fraction composition was confirmed by morphometric measurements determined from electron micrographs. Both total and latent acid phosphatase activities in all hepatoma fractions were significantly less than corresponding fractions from control livers. Based on latent activity, the lysosome populations would be about $\frac{1}{2}$ that of normal liver.

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Exocytosis: Routes and Kinetics of Delivery of Secretory and Membrane Proteins. D. JAMES MORRÉ, EDWARD M. CROZE, CHERYL FRANZ and C. M. EPPLER, Purdue University, West Lafayette, Indiana 47907.—Vectorial movement (flow) of membranes is an integral part of the activities of most eukaryotic cells. Involved are various endomembrane components including endoplasmic reticulum (ER), Golgi apparatus (GA) and plasma membrane (PM) as well as vesicles and tubules associated with each of the major structures. Studies that correlate movement of both membrane constituents and constituents of secretory products simultaneously in the same experimental system have been few. Our studies with rodent liver, correlate exocytosis of membrane markers, mouse H-2 histocompatibility antigens and nucleoside diphosphate phosphatase, with exocytosis of secretory lipoproteins and albumin. A correlative approach utilizes metabolically labeled constituents, highly purified cell fractions, and specific immunoprecipitation analyses. The order of labeling is ER, GA and PM; turnover kinetics suggest transfer of both membrane and secretory product from ER to GA with accumulation in PM or the extracellular environment. Work supported by grants from the National Institutes of Health and the National Science Foundation.

Comparative Cytochemical Studies of Onion Root Tip with Specific and Non-specific Substrates for Nucleoside Diphosphatase(s). MICHAEL J. STEWART and CHARLES W. GOFF, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.—Nucleoside diphosphatase (NDPase) has been used as a marker enzyme for the Golgi apparatus in a number of plant and animal systems.

Previous studies from our laboratory have shown (1) NDPase to be localized in a number of subcellular sites and (2) that there are two classes of NDPase isoenzymes—one which hydrolyzes only nucleoside diphosphate substrates and another class which not only hydrolyzes nucleoside diphosphates but also certain nucleoside monophosphates and thiamine pyrophosphate (TPP). Cytochemical studies at neutral pH were undertaken to determine whether different subcellular localization patterns could be observed using different nucleotides and TPP, which are hydrolyzed by one or both classes of onion root tip NDPases.

IDPase reactivity occurred in the Golgi apparatus, endoplasmic reticulum, nuclear envelope, plasmalemma, cell wall, and tonoplast of certain tissues of the onion root tip. ADPase reactivity was found in the ER, nuclear envelope, tonoplast, and cell wall of various root tissues although the intensity of staining was less than that seen for IDPase. Very little ADPase reactivity was found in the Golgi apparatus and very little, if any, reactivity was found on the plasmalemma. TPPase reactivity was similar to that seen using ADP except that there was very intense reaction product in the definitive cell wall surrounding lateral cap, epidermal, and cortical cells. Little, if any, reaction product appeared in the Golgi apparatus in tips incubated with TPP. AMPase reactivity was found in the ER, cell wall, and vacuoles of certain tissues and the staining was much less intense relative to IDPase reactivity. No reaction product was found in the Golgi apparatus. Preliminary results from gel filtration studies indicate that peaks of enzyme activity may be shared by enzymes which hydrolyze IDP, other nucleotides, and TPP.

Our results lend additional support to previous findings which suggest that there are two classes of NDPase isoenzymes: one which may be referred to as specific NDPases, the other as non-specific phosphatases. The findings from our present investigation suggest the potentiality for discriminating between these two classes of NDPases with the ultimate goal of clarifying the marker enzyme question for the onion root tip.

Cytochalasin A (0.1-1 ug/ml) Rapidly Halts Hyphal Tip Growth in *Rhizoctonia solani*. JAMES A. SWEIGARD AND STANLEY N. GROVE, Goshen College, Goshen, Indiana 46526.—Within two minutes of inhibitor application growth slows, Spitzenkorper integrity is disrupted and hyphal tips become bulbous. Our initial observations suggest that the phase-light area of the Spitzenkorper immediately retracts from the hyphal apex upon treatment but that growth continues for a short time. A possible mechanism for this inhibition is the disruption of essential microfilaments in the tip region. Supported in part by a grant of the National Institute of Allergy and Infectious Diseases.

Kinetics of Appearance of Lipoprotein Particles in Cisternae of Subsurface Smooth Endoplasmic Reticulum of Isolated Rat Livers Perfused with Free Fatty Acid. MARTHA TWADDLE, RALPH A. JERSILD AND JAMES MORRÉ, Indiana University School of Medicine, Indianapolis, Indiana 46223, and Purdue University, West Lafayette, Indiana 47907.—Vesicles containing single lipoprotein particles are found in the pericapillary cytoplasm of rat hepatocytes in the proximity of particle containing rough-smooth endoplasmic reticulum transition elements. These lipoprotein-containing smooth endoplasmic reticulum elements provide an alternative source to Golgi apparatus as a supplier of very low density lipoprotein particles to the circulation. To test this possibility, the kinetics of appearance of lipoprotein particles in these elements was determined for isolated rat livers per-

fused with free fatty acid. The results show that the smooth endoplasmic reticulum elements of the pericapillary cytoplasm acquire lipoprotein particles in advance of elements of the conventional Golgi apparatus and that the particles may be secreted directly to the circulation in a manner completely bypassing the Golgi apparatus.

Persistent Nucleoli in Various Meristems of *Phaseolus aureus* (mung bean).

MARTIN A. VAUGHAN, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809 and J. P. BRASELTON, Department of Botany, Ohio University, Athens, Ohio 45701.—The nucleolus, a non-membrane-bound structure characteristic of interphase nuclei of eukaryotic cells, is the site of ribosomal ribonucleic acid (rRNA) synthesis. Nucleoli generally do not arise from pre-existing nucleoli by division, but undergo cyclic changes during the mitotic cycle.

Persistence of the nucleolus during mitosis in vascular plants is not an unusual phenomenon and in many groups persistence of the nucleolus to metaphase and beyond is common. In addition to their natural occurrence, persistent nucleoli also may be induced by experimental conditions, such as exposure to cobalt salts, 5-fluoro-deoxyuridine (FUDR), ethidium bromide, relatively low temperatures, and centrifugation.

Most observations of persistent nucleoli in higher plants were described from cell divisions in root tips of recently germinated seedlings; and a few cases were reported in root tips of mature plants. Persistent nucleoli at metaphase in primary root, secondary root, and shoot apical meristems of the mung bean were observed by light microscopy of hematoxylin-stained paraffin sections 4, 8, and 14 days after germination. The percentage of metaphases containing persistent nucleoli were recorded in each meristem to establish whether persistence of nucleoli at metaphase is associated with the age or the type of meristem.

There was a significantly lower percent of persistent nucleoli at metaphase in shoot apical meristems as compared to primary or secondary root meristems at each of the ages tested. There was no significant difference in percent of persistent nucleoli at metaphase in the primary or secondary root meristems.

The variation in nucleolar behavior of mung bean shoot and root meristems may be explained by differences in the length of the nucleolar dispersion cycle or differences in the length of the mitotic prophase corresponding to the dispersive phase of the nucleolar cycle.

The Effect of Sympathectomy on the Structure of the Rat Pineal Gland.

HENRY C. WOMACK and MOHINDER S. JARIAL Department of Physiology and Health Science and Muncie Center for Medical Education, Ball State University, Muncie, Indiana 47306.—Female albino rats at the age of 4 months were bilaterally sympathectomized under ether anesthesia by surgical excision of a small segment of the sympathetic trunk just below the superior cervical ganglion to disrupt the light pathway to the pineal gland. Control animals were subjected to a sham operation. The appearance of ptosis in the experimental animals provided confirmation of complete sectioning of the sympathetic trunk.

Following surgery the animals were maintained under conditions of 14 hours light and 10 hours darkness with food and water *ad libitum*. After 7 days the animals were sacrificed under either anesthesia by intravascular perfusion with 2% glutaraldehyde. The pineal was removed and fixed in a mixture of glutaraldehyde and osmic acid in phosphate buffer for electron microscopy and in Bouin's fluid for light microscopy.

Electron microscopy revealed small membrane bound granules in the vicinity of endoplasmic reticulum and Golgi apparatus. In the experimental animals the number of such granules was enhanced as compared to the controls. There was no difference in the number of lipid droplets in the experimental and control animals. Epon 1 micron thick sections stained with azur II, showed medium to large size granules which in thin sections stained with uranyl acetate and lead citrate, revealed an outer concentric ring. Such granules were present in relatively larger numbers in the experimental animals than in the controls. Occasionally these granules were seen in the lumina of capillaries. It appears that these large granules in rats represent concretions or brain sand which in humans have been called *corpora arenacea*. Numerous nerve terminals were observed in the perivascular space and some in association with pinealocytes. In the experimental animals the nerve terminals contained empty vesicles while in the controls vesicles were granulated.

The results of this study suggest that in the absence of light input to the pineal gland as a result of the lesion of preganglionic sympathetic fibers, the postganglionic neurons and nerve terminals fail to synthesize neurotransmitters such as norepinephrine and serotonin. It appears that the secretory activity of the pinealocytes is not affected by sympathectomy, but the secretion products are not released for use by the animal. In the control animals secretion products that are being synthesized are also being utilized. It is suggested that some of the secretory material like melatonin produced by the pinealocytes may be accumulated in the form of hard granules or concretions which have been observed in large numbers in the experimental animals.

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