CELL BIOLOGY

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ABSTRACTS

Changes in Nucleic Acid and Protein Content in Nuclei of Human Cervical Cells. Pradeep K. Bhattacharya, Department of Biology, Indiana University Northwest, Gary, Indiaa 46408 and Aristotel J. Pappelis, Department of Botany, Southern Illinois University, Carbondale, Illinois 62901.——Nuclei in five classes of cervical cells observed in Pap smears were studied using quantitative epifluorescence microscopy. The five classes of cells were: parabasal (Pb) cells; intermediate cells with round (I-R), oval (I-O), and rod-pyknotic (I-RP) nuclei; and pyknotic (P) cells. Six nuclear traits were measured: total nucleic acid, DNA, RNA, total protein, histone, and non-histone protein. The six nuclear indices increased as Pb cells became I-R cells (cell enlargement and maturation), and then decreased as I-R cells degenerated through the following senescense sequence: I-O, I-RP, and P. We infer that these changes continue and result in anucleate, superficial cells. Pb cells are probably in early stages of DNA synthesis (S-phase of the cell cycle) since the mean for DNA increased as they became I-R cells. The following types of cells comprised the Pap smears studies: Pb, 7.0%; I-R, 19.0%; I-O, 55.0%; I-RP, 8.0%; P, 9.0%; superficial cells with nuclei devoid of nucleic acids, 1.0% and anucleate cells, 1.0% We conclude that cervical exfoliative cytology provides a model system for the study of human cell development, maturation, senescence, and death in addition to its use in detecting early through late stages of cervical cancer. The high correlation between the nuclear indices studies suggests that several quantitative nuclear parameters other than DNA may be useful for cancer detection.

Genetic Differentiation among Populations of *Collinsia verna*. Judith K. Greenlee, Department of Chemistry, University of Notre Dame, Notre Dame, Indiana 46556.——*Collinsia verna* (blue-eyed Mary) is an understory herb found on floodplains in the northern midwest, with populations sizes being very small and populations often separated by several miles. Since such isolation seemingly precludes gene flow among varius populations, the possibility of genetic differentiation leading to incipient speciation exists. Recently, *C. verna* populations were found to differ in several parameters including chiasmata frequencies, morphological characters and isozymic frequencies (Greenlee and Rai, 1983). The data from the above study showed considerable genetic divergence among populations. It was decided to extend these studies to include two additional parameters—the nuclear DNA amounts among various populations and other species, and the comparative karyotypes of *C. verna* and *C. heterophylla*.

The dogma in *Collinsia* cytology in the past has been that there are 7 pairs of metacentric chromosomes, which are approximately equal in size. The comparative karyotypes show that this is not the case for either species. Change in centromere position

indicative of pericentric inversions was seen in two pairs of chromosomes. Interspecific hybrids showed desynapsis and heteromorphic bivalents.

Populations of *C. verna* were examined by Feulgen cytophotometry to determine the extent of their variation and two other *Collinsia* species were measured for comparative purposes. Nearly a four fold difference in DNA amount was found among these populations, which were collected from a 300 mile area of the midwest.

While the pattern of determining factors is not entirely clear, the nearly four fold difference in DNA values combined with the karyotype evidence, and the parameteric differences previously found clearly show that genetic differentiation has occurred among these *Collinsia verna* populations.

Ontogenesis of a Calcium-binding Protein Specific to Brain. ZAFAR IQBAL, All-India Institute of Medical Sciences, New Delhi and Northwestern University Medical School, Chicago, Illinois 60611.——Calcium plays a significant role in the regulation of a number of key cellular processes. It is now recognized that most of the actions of calcium are mediated through calcium-binding protein(s). In developing chick embryo brain we have identified a calcium-binding protein which is organ specific and shows a developmental pattern identical to that observed for two key enzymes involved in nerve-conduction and transmission of impulses; viz., acetylcholinesterase and Na-K Atpase (Iqbal et al, J. Neurochem). 15:1217-1222; Iqbal & Talwar, J. Neurochem. 18:1261-1267). Employing acrylamide gel electrophoresis and immunochemical procedures, a detectable amount of protein was found on day 6 and the contents of the protein increased with age attaining a plateau on day 13, a period which coincides with the maturation of the electrical activity in brain. Whether this protein, through its calcium-binding property is involved directly in the manifestation of the functional activity of the brain is yet to be determined.

Real Time Visualization of Nerve Function Might Be Possible Using Magnetic Dyes. EDWARD A. KIMBLE/ITT Educational Services, Fort Wayne, Indiana 46825.——In most optical and ultrasound applications relating to nerve measurement, clear high speed measurements cannot be made due to high background and symmetry related noise problems. A method is suggested for using magnetic dyes to break the symmetry of the normally circular nerve fiber and to provide a mechanism for overcoming background signal effects. In this technique, a competition would be established between orientation of radio-opaque dye molecules by an external magnetic field and orientation by nerve related electric fields. Directional scattering would then be used to detect relaxation of electric fields. This technique could be applicable to x-ray, ultrasound, or Raman spectroscopic techniques. A prototype detector system for testing this concept and imaging x-ray scattering will be presented. Other approaches to the general problem will be discussed.

Effects of Selected Antifungal Agents on Hyphal Tip Growth in *Rhizoctonia solani*. GEORGE A. MECKLENBURG, KEVIN W. MILLER and STANLEY N. GROVE, Goshen College, Goshen, Indiana 46526.——In our continuing search for suitable probes to test the current model for hyphal tip growth we treated growing hyphal tips on slide cultures with growth media containing selected chemical agents. Within four minutes after application of 1.0 mM diamide tip growth slows to 40.0% of pretreatment growth rate and the phase contrast bright portion of the Spitzenkorper (apical body) retracts from its usual location adjacent to the apical wall. The usual organization of the cytoplasm becomes disrupted as evidenced by the abrupt loss of the typical orientation of mitochondria. Since diamide is reported to cause increased polymerization of actin our results may be explained as due to the limitations placed on the dynamic cytoskeleton. A pool of unpolymerized actin would seem necessary to allow the constant cytoskeletal

modification required to conform to the growing cytoplasm.

Eugenol (0.16 mM), an uncoupling agent in mitochondrial respiration, causes the Spitzenkorper to lose its phase dark component and leave the usual apical location with 25 sec. after treatment. This response is followed within 2 minutes by cessation of growth but some cytoplasmic motion persists for up to 15 minutes.

In tips treated with 5-cinnamic acid (0.16mM), which blocks Golgi mediated secretion, the growth rate drops to 25.0% within 2 minutes and then recovers slightly to about 30.0% after 5 minutes. The Spitzenkorper disappears at about 75 seconds and is not evident during the period of lowest growth rate but reappears just prior to the recovery at 5 minutes.

Naftifine (0.15 mM), which is thought to effect lipid metabolism, causes no measurable change in the growth rate for up to 12 minutes after treatment. These observations support the suggestion that the cytoskeleton and secretory phenomena are key elements in tip growing cells. It appears that eugenol and possibly other respiratory inhibitors will be useful in examining energy dependent elements while short effects on lipid metabolism may not be critical to tip growth.

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Micromanipulation of Exocytotic Aggregates from *Paramecium multimicronuleatum*. Christopher C. Schroeder, Thomas A Cole and Willis H. Johnson, Department of Biology, Wabash College, Crawfordsville, Indiana 47933.——Axenic, starved *Paramecium multimicronucleatum* cells rapidly take up fluorescent latex beads (1.5 micron diameter). After about one hour the beads begin to be eliminated as monodisperse units or as spherical aggregates of 30-40 beads. In order to determine whether the aggregates are bounded by a membrane, a micromanipulator for picking up these aggregates and transferring them one-by-one to fresh medium has been developed. When an isolated aggregate is damaged with a glass needle, the individual beads disperse rapidly. The stability of aggregates and synthetic lipid vesicles (liposomes) to the action of various agents has been compared. These results and an evaluation of the evidence concerning the question of whether the aggregates are membrane bound will be presented.

Mitochondrial Membrane Potential in Cytochalasin Treated Hyphal Tips of *Rhizoctonia solani*. Marvin D. Slabaugh and Stanley N. Grove, Goshen College, Goshen, Indiana 46526.——Within two minutes following application of cytochalasin A (CA) to growing hyphal tips the characteristic Spitzenkorper is disrupted, tip growth slows, and the tip becomes bulbous. To determine whether these responses to CA are due to inhibition of respiratory activity we used the cyanin dye 3,3 ′ diethyloxacarbocyanine [$DiOC_2(3)$] which is a membrane potential-dependent fluorescent probe for levels of mitochondrial activity. When growing hyphal tips on slide cultures are exposed to the dye dissolved in liquid growth medium and examined by epifluorescence microscopy at 450-490 nm excitation the mitochondria stain intensely while only two levels of stain are present in the cytoplasm. Upon the addition of CA in liquid growth medium to the stained hyphae the typical morphological changes occur and the stain remains in the mitochondria for up to 15 minutes. However, when the proton ionophore 2,4-dinitrophenol (DNP) which dissipates the mitochondrial trans-membrane potential is added in liquid growth medium the mitochondria lose their stain rapidly (by 30 sec) leaving a low level of cytoplasmic stain. These findings suggest that the actions of CA do not include inhibition in respiratory enzyme activities sufficient to alter the mitochondrial trans-membrane potential. Supported in part by a grant from Research Corporation.

Measurements of Cytosolic Calcium and Sodium During Acetyl-choline Stimulation of Parotid Salivary Glands. ROBERT J. STARK, Department of Zoology, DePauw University, Greencastle, Indiana 46135.——Conventional, calcium- and sodium-selective microelectrodes were used to examine the ionic mechanisms regulating salivary secretion in response to acetylcholine (ACh) stimulation. The concentration of free cytosolic calcium ([Ca]i) and sodium ([Na]i) in unstimulated cells was determined to be 0.44 ± 0.04 uM (n = 47) and 9.9 ± 1.2 mM (n = 29) respectively. By measuring the induced changes in intracellular electrode potentials (Em, E_{Ca}, & E_{Na}), ACh at 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, & 10^{-5} M was observed to hyperpolarize Em by 0.0 ± 0.4, 1.4 ± 0.2, 4.9 ± 0.2, 8.4 ± 0.3, & 8.4 ± 0.5 mV; increase [Ca]i by 0.20 ± 0.02, 0.61 ± 0.04, 0.53 ± 0.02, 0.30 ± 0.05, & 0.14 ± 0.03; and increase [Na]i by 1.4 ± 0.1, 1.8 ± 0.1, 2.1 ± 0.3, 1.6 ± 0.3, & 1.7 ± 0.3 mM respectively. Even though ACh always induced an increase in [Ca]i, [Na]i and membrane hyperpolarization, the magnitude of the responses were dose-dependent with lower ACh concentrations (10^{-9} to 10^{-7}M) inducing dose-dependent increases in these parameters, while the cells appear to buffer the response to higher ACh concentrations by lmiting or reducing the magnitude of the increases in [Ca]i and [Na]i. The patterns of [Ca]i and [Na]i regulation during ACh stimulation appear to be similar indicating a possible linkage, while both are different from the induced membrane hyperpolarizations suggesting that stimulation of these exocrine glands with ACh may trigger at least two independent ionic events. (Supported by PHS Grant AM26246 and an Indiana Academy of Science Research Grant)

Silver Staining of Persistent Nucleoli in Mung Bean as a Valid Indicator of Synthetic Activity. 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.——In some animal and many plant cells the nucleolus remains more or less intact through metaphase of mitosis or later and are termed persistent nucleoli (PN). In several mammalian cell lines PN have been stained positively with silver nitrate. It has been proposed that the selectivity of this stain for the nucleolus and nucleolus organizing regions of chromosomes is due to interaction of the silver with certain proteins associated with the functionally active nucleous; such staining has therefore been used as an indicator of functionally active nucleoli.

The purpose of this study was: 1) to determine if and to what extent PN of a plant, the mung bean, stain with silver nitrate, 2) and to compare the ultrastructure of PN of mung bean to the ultrastructure of synthetically active interphase nucleoli.

Primary root tips of mung bean were prepared for light microscopy, then stained with silver nitrate or processed for transmission electron microscopy. Positively silver stained PN occurred in 76.0% of the metaphase figures scored in the mung bean, which others claim is suggestive that these PN are synthetically active. Ultrastructurally PN in mung bean at metaphase consist predominently of loose fibrils and a few scattered granules as opposed to the distinct fibrillar and granular regions typically of synthetically active interphase nucleoli.

Although silver staining has been used as an indicator of synthetically active nucleoli, ultrastructural evidence in the mung bean indicates that PN are structurally different than synthetically active interphase nucleoli. These data suggest that silver staining should not be used as an indicator of the synthetic activity of nucleoli until and unless definitive evidence for this correlation is developed.