P1-19

High Voltage Electron Microscopy is Useful for X-Ray Microanalysis

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X-ray microanalysis is a useful technique to qualify and quantify basic elements in biological specimens. We first quantified the end products of histochemical reactions such as Ag in radioautographs, Ce in acid phosphatase reaction and Au in colloidal gold immunostaining using semi-thin sections by high voltage electron microscopy at 300–400 KV. Then we analyzed various trace elements such as Zn, Ca, S, which originally existed in cytoplasmic matrix or cell organelles of various cells in various organs, or elements absorbed by administration into cells and tissues such as Al, using both conventional chemical fixation and cryo-fixation followed by cryo-sectioning, freeze-drying, or freeze-substitution using semi-thin sections similarly to radioautography. As the results, the P/B ratios of all the elements analyzed resulted in high P/B ratios at 300–400 KV. It was concluded that X-ray microanalysis using semi-thin sections by high voltage electron microscopy was very useful for quantifying trace elements in biological specimens.

P1-20

Special Cytochemistry in Cell Biology: A Novel Concept

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Cytochemistry is a science of localizing chemical components of cell organelles and cytoplasmic matrix on histological sections by using various techniques. We first aimed at studying cytochemistry by developing new techniques using various principles such as enzyme cytochemistry, microincineration, microspectrophotometry, radioautography, X-ray microanalysis and immunocytochemistry. We had concentrated on developing these methodologies, then applied them to various kinds of cells. These techniques were applied to various cells in various organ systems such as skeletal, muscular, digestive, respiratory, urinary, reproductive, endocrine, circulatory, nervous and sensory systems similarly to special histology. The results obtained from cytochemical studies applied to various cells should be designated as "special cytochemistry" forming a part of cell biology. This paper summarizes the results of our cytochemical studies on various cells carried out in our laboratory during these 47 years since 1955 designated as special cytochemistry in cell biology proposing a novel concept.

P2-01

Somatic Cell Genome Size for Six Species of Freshwater Copepods

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As part of our study of chromatin diminution during early cleavage stages in certain species of copepods, we have determined DNA levels for individual somatic cell nuclei from adult copepods fixed in 3:1 methanol-acetic acid, dispersed in 45% acetic acid, frozen on dry ice for coverslip removal and thawed in absolute ethanol before air drying. Simultaneous hydrolysis for 30 min in 5N HCl at 22° was followed by staining for 2 hrs in a 1% Schiff's reagent, three 5-min rinses in sulfite water and thorough washing in distilled water. To minimize non-specific light loss due to scatter, all tissues used for cytophotometry were mounted in matching refractive index liquids nD 1.524–1.540 before measurement at 560 nm of individual nuclei with a Vickers M86 scanning and integrating microdensitometer, using both chicken and trout RBC nuclei as internal reference standards of 2.5 and 5.0 pg DNA per nucleus, respectively, to estimate the DNA content of individual copepod nuclei. The 2C DNA level for Acanthocyclops robustus was 1.53 pg DNA+0.068, n=232; for Acanthocyclops vernalis, it was 1.55 pg DNA+0.062, n=206; for Cyclops strenuus, it was 1.72 pg DNA+0.014, n=924; for Mesocyclops edax, it was 3.02+0.021, n=1,331; for Mesocyclops longisetus, it was 1.78 pg DNA+0.012, n=808; and for Mesocyclops ruttneri, it was 1.45+0.096, n=170. These 2C somatic cell DNA values are like those found by Beerman (Chromosoma 60: 297, 1977) for other freshwater copepods, Cyclops furcifer (2.7 pg DNA), C. strenuus (1.8 pg DNA) and C. divulsus (3.6 pg DNA), but differ significantly from the large 2C genome sizes reported for marine copepods such as Calanus spp (12.8–24.3 pg DNA) and Pseudocalanus spp (4.3–13.6 pg DNA) (McLaren et al., Hydrobiologie 167: 275, 1988; Canad. J. Zool. 67: 565, 1989). We gratefully acknowledge support of these studies by NSF-DEB 0080921 (EMR) and NSF-INT 0000765 (AKG and GAW).

P2-02

Spatial Distribution Analysis of AT- and GC-Rich Regions in Nuclei Using Corrected Fluorescence Resonance Energy Transfer

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[Objective] We used microscopic fluorescence resonance energy transfer (FRET) measurements of cultured bovine aortic endothelial cell nuclei to investigate the spatial relation between AT-rich and GC-rich regions in the nuclear DNA. [Methods] Hoechst33258 (Ho) for AT base pairs and 7-aminoactinomycin D (7-AAD) for GC pairs were used as donor and acceptor, respectively. Digitized nuclear images of FRET between Ho and 7-AAD were corrected by Gordon's method with 3 fluorescence filter sets (Biophysical J 1998) to exclude the optical distortion, which included fluorescence cross talk and the dependence of FRET on the concentration of donor and acceptor. Quantitative analysis of these digital data by texture analysis was performed to compare the spatial distribution of AT-rich and GC-rich DNA in 100 nuclei in different phases of the cell cycle. [Results and Conclusions] The spatial heterogeneity of FRET before the correction seemed to be due to the closely spaced Ho in the three-dimensionally condensed AT-rich regions, rather than due to the distance between more closely spaced donors and acceptors in the nuclei. The corrected FRET images, from which were excluded the influence of donor and acceptor concentrations, showed the accurate distance between donor and acceptor. The corrected FRET images of 100 nuclei indicated increased number and larger size of the low FRET efficiency centers in the G2/M phases compared to G0/1 phases. In conclusion, texture analysis of FRET images quantitatively disclosed the spatial change and separation of AT- and GC-rich DNA during the cell cycle.

P2-03

Simultaneous Demonstration of B-DNA and Z-DNA Sequences within the Adult Bovine Ocular Lens

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The purpose of this molecular biological research project was to simultaneously demonstrate the presence of both right-handed double-stranded B-DNA and left-handed Z-DNA sequences in the adult normal ocular lens. Seventy-five adult eye globes were processed for the removal of normal (noncataractous) ocular lenses. Lenses were fixed for either 6, 12, 24 or 48 hr, in either Davidson's or Carnoy's