Cytochemical Investigations of Deoxyribonucleic Acid-mediated Control of Ribonucleoprotein Metabolism

The interdependence of various types of macromolecular synthesis in the cell has been demonstrated by the use of various antimetabolites. Synthesis of some ribonucleoproteins (RNP) in the nucleus is dependent on continued formation of deoxyribonucleic acid (DNA)[1-3]. One of these ribonucleoproteins is present in the nucleolus in the form of spherules called 'nucleolini'[3]. When synthesis of DNA was inhibited by 5-fluorodeoxyuridine (FUDR), the nucleolini were at first enlarged and later disappeared. The pars amorpha of the nucleolus was not reduced during this process[3]. Further cytochemical observations and biochemical investigations of HeLa cells, grown as previously described[3], and treated with 2×10^{-5} M FUDR are presented here.

Cells were stained by the toluidine blue–molybdate method[4]. Nuclear and cytoplasmic fractions were prepared by the citric acid procedure[5]. Nucleic acids were estimated by a modified Schmidt–Tannhauser procedure[6], Burton's modification of the Dische reaction[7] and the orcinol reaction[8]. Protein was determined by the method of Lowry *et al.*[9]. The results presented in Table 1 show that, during a period of 48 h, synthesis of DNA, nuclear RNA and protein was inhibited by FUDR. Examination of stained preparations made at intervals during the 48-h period indicated that the nucleolini and the chromosomal, perichromosomal and granular cytoplasmic RNP[4] gradually disappeared. The cytoplasmic changes occurred later than those in the nucleus. Cytochemical investigations have indicated that the nucleolini, chromosomal and so-called 'perichromosomal' RNP appear to exist as hybrids with DNA[10]. The changes were not due to conversion of FUDR to fluorouracil, since they could be prevented by incorporation of equimolar thymidine, but not by uridine in the medium[11].

Table 1. Effects of Fluorodeoxyuridine (FUDR) on Nucleic Acids and Protein per Culture during 48-h Incubation*

Cultures	Percentage of initial amount per culture (nuclear fraction) DNA	RNA	Protein
Untreated	407	390	506
Treated with FUDR (2×10^5 M)	95	86	159
Difference ± *S.E.*	312 ± 61	304 ± 53	347 ± 12

* Mean of four experiments.

Table 2. Effects of Actinomycin on Nucleic Acids and Protein per Culture during 24-h Incubation

Cultures	Percentage of initial amount per culture DNA	RNA	Protein
Untreated	197	224	208
Treated with actinomycin (0·01 μg/ml.)	190	104	153
Difference ± *S.E.*	7 ± 14	120 ± 27	55 ± 25

* Mean of four experiments.

The results of similar investigations of HeLa cells treated with actinomycin *D* (0·01 μg/ml.) for 24 h are presented in Table 2. Treatment with actinomycin produced a pronounced inhibition of synthesis of RNA, some reduction of protein formation and no significant effect on DNA metabolism. Cytochemical investigations indicated that, after 24 h, the nucleolini and pars amorpha of the nucleolus had almost completely disappeared and that the granular cytoplasmic RNP was decreased. Sequential observations revealed that the nucleolar changes preceded the decrease in cytoplasmic RNP. Chromosomal and perichromosomal RNP were unaffected.

In summary, the results with FUDR show that inhibition of synthesis of DNA results in a failure of synthesis of DNA–RNA hybrids in the chromatin and nucleolini; granular RNP in the cytoplasm is also reduced. Inactivation of DNA-primed RNA synthesis by actinomycin[12], on the other hand, inhibits the formation of RNA in all parts of the nucleolus, and later results in the reduction of granular RNP in the cytoplasm. The work of Perry indicates that low concentrations of actinomycin, such as those used here, may selectively inhibit the formation of rapidly labelling high molecular weight ribosomal-precursor RNA in the nucleolus, and later lead to a decrease in ribosomal RNP[13]. This would suggest that at least one of the ribonucleoproteins of the nucleolus is a precursor of the granular cytoplasmic RNP which is, therefore, probably of the ribosomal or polyribosomal type. Since the RNP of the nucleolini and not the pars amorpha was affected in the FUDR system, in which the granular cytoplasmic RNP was also reduced, the RNP of the nucleolini may be of the ribosomal precursor type. On the other hand, as previously suggested[3,6,14-16], it may be of the messenger type, since the latter is necessary for the formation of ergosomes or polyribosomes[17] and only large aggregates of ribosomes could possibly be visualized by cytochemical techniques for light microscopy.

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Anomalous Ionization of Methyl Linolenate by Metastable Argon Atoms: Possible Linolenic Acid Participation in Photosynthetic Reactions

During the past few years there has been an increasing number of reports pointing to the possible specific role of α-linolenic (*cis*-9,12,15-octadecatrienoic) acid in photosynthetic reactions of green plants. In algal cells grown in the dark α-linolenic acid is virtually absent; if the cells are exposed to light, the concentration of linolenic acid increases in parallel with that of chlorophyll[1]. The same process has been observed during the greening of etiolated *Citrullus vulgaris* seedlings[2].

Approximately 85 per cent of the cellular α-linolenate is accounted for by the chloroplast fraction[3] as an acyl component of galactolipids of the chlorophyll–lipoprotein membranes[4]. According to Bloch *et al.*, the presence of linolenic acid in *Euglena gracilis Z* cells seems to be necessary for the Hill reaction and the evolution of oxygen[3].

The present-day concept of photosynthesis involves the absorption of light quanta by a chlorophyll molecule followed by the conversion of the latter to a triplet excited state. The excitation energy is transferred by a mechanism as yet obscure to a photochemical reaction centre to effect photolysis of water and simultaneous oxygen evolution[5]. It has been suggested that the linolenic