soluble fraction from infected cells, whereas preimmune immunoglobulin G did not. These results are consistent with there being an important role for the DNA-binding protein in adenovirus-DNA replication as suggested by Van der Vliet et al. (1977). Further studies are required to characterize the products of DNA synthesis in vitro, and the host-cell enzymes and virus proteins involved in this process.


The Effect of Oxidised Nicotinamide-Adenine Dinucleotide on Ribonucleic Acid Synthesis in Isolated Nuclei from Baby-Hamster Kidney Cells (BHK-21/C13)

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Several studies on mammalian cell metabolism have established that there is a relationship between the intracellular concentration of NAD$^+$ and the growth state of the cell (Wintzerith et al., 1961; Ferris & Clark, 1971; Schwartz et al., 1974). This relationship led to the idea, originally proposed by Morton (1958), that NAD$^+$ may control cellular growth. The way in which this could occur is not readily understandable in terms of the classic role of NAD$^+$ as a cofactor in biological redox reactions. There are, however, reactions in which NAD$^+$ participates not as a cofactor but as a substrate. In particular, it is now appreciated that the ADP-ribose moiety of NAD$^+$ may be covalently bound to proteins (Sugimura, 1973; Hilz & Stone, 1976; Shall et al., 1977).

In mammalian cells the enzyme that catalyses this reaction (ADP-ribosylation) is localized in the nucleus, the site of NAD$^+$ biosynthesis. The mammalian enzyme [poly(ADP-ribose) polymerase] can also catalyse the addition of further ADP-ribose units (by a 2'-1' glycosidic linkage), forming the homopolymer poly(ADP-ribose). Poly(ADP-ribose) polymerase modifies all classes of nuclear protein in vitro (Rickwood et al., 1977). Thus it seems unlikely that this modification reaction will have a unique role in cellular metabolism. It does, however, suggest that this modification reaction may be the link between the intracellular NAD$^+$ concentration and the growth state of the cell.

If we accept this hypothesis, then it seems likely that ADP-ribosylation of nuclear proteins will be involved in the three major cellular growth processes, i.e. DNA, RNA and protein synthesis. In the case of DNA and RNA synthesis it is possible to test this involvement directly. Isolated nuclei from mammalian cells are capable of synthesizing DNA and RNA in vitro (Kidwell & Mueller, 1969; Zylber & Penman, 1971). A possible experimental approach therefore would be to examine the effect of NAD$^+$ on DNA and RNA synthesis in isolated nuclei.

The effect of NAD$^+$ on DNA synthesis in isolated nuclei has been extensively studied (Burzio & Koide, 1970; Roberts et al., 1973; Burzio et al., 1975; Suhadolnik et al., 1977). On the other hand there are few reports dealing specifically with the involvement of ADP-ribosylation in RNA synthesis. Müller & Zahn (1976) reported that the activity of the endogenous RNA polymerase in isolated nuclei from quail oviduct is inhibited by the addition of NAD$^+$. They also provided evidence for a covalent linkage between
ADP-ribose and RNA polymerase I. In the present communication we describe the effect of ADP-ribosylation of nuclear proteins on RNA synthesis in isolated nuclei from BHK (baby-hamster kidney) cells.

Previous work (Furneaux & Pearson, 1977) has established that NAD$^+$ added to nuclei isolated from BHK cells is rapidly incorporated into acid-insoluble material. This acid-insoluble material was shown to be composed of ADP-ribose. To examine the effect of NAD$^+$ on RNA synthesis in isolated nuclei it is necessary to analyse its metabolic fate in more detail. Extraction of the acid-soluble fraction, after incubation of NAD$^+$ with isolated nuclei, and analysis by PEI-poly(ethyleneimine)-cellulose t.l.c. revealed no other acid-soluble metabolites.

Analysis of the acid-insoluble material by Cs$_2$SO$_4$-density-gradient centrifugation (containing 6M-urea and 0.4M-guanidinium chloride to disrupt non-covalent binding) revealed the association of radioactive residues and nuclear proteins. Hydrolysis with NaOH removed the ADP-ribose residues from nuclear protein. These results strongly suggest that the ADP-ribose residues are covalently bound to nuclear proteins.

RNA synthesis in isolated nuclei was followed by the incorporation of $[^3H]UTP$ into acid-insoluble material. We have found that the effect of NAD$^+$ on RNA synthesis in these nuclei depends on the ionic strength of the incubation medium. Under conditions of low ionic strength, NAD$^+$ inhibits RNA synthesis, whereas at high ionic strength it stimulates RNA synthesis. Addition of nicotinamide (a powerful inhibitor of ADP-ribosylation) prevents the inhibition. This demonstrates the effects induced by NAD$^+$ are due to ADP-ribosylation of nuclear proteins and not to any non-specific changes in incubation conditions. With our method of isolation, nuclei from BHK cells were found to contain two RNA polymerase activities (Cooper & Keir, 1975). These correspond to the RNA polymerase I and II isolated by other workers. In general, RNA polymerase I is most active under conditions of low ionic strength, whereas RNA polymerase II is most active under conditions of high ionic strength. Thus it seems likely that ADP-ribosylation of nuclear proteins inhibits RNA polymerase I activity and stimulates RNA polymerase II activity. We have studied the inhibition in more detail. To distinguish between the two enzymes, a-amanitin, a specific inhibitor of RNA polymerase II (Kedinger et al., 1971) was added to the incubation

![Graph](image-url)

Fig. 1. Effect of preincubating nuclei with NAD$^+$ on the subsequent activity of partially purified RNA polymerase I

■, Activity of RNA polymerase I isolated from control nuclei; □, activity of RNA polymerase I isolated from nuclei previously incubated with NAD$^+$. 1978
medium. Under low-ionic-strength conditions and in the presence of α-amanitin (1μg/ml) the inhibition of RNA synthesis by NAD+ was increased. This inhibition may be due either to the ADP-ribosylation of the DNA-binding proteins that control the transcription of the DNA template or to the direct modification of the RNA polymerase itself. To distinguish between these two possibilities, nuclei were incubated in the presence and absence of NAD+. The RNA polymerases were then extracted from the control and NAD+-treated nuclei and assayed with exogenous DNA template in the presence of α-amanitin. The result shown in Fig. 1 suggests that the inhibition is due to the ADP-ribosylation of RNA polymerase I.

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Oestrogen-Induced Changes in the Population of Uterine Polyribosomal Polyadenylated RNA

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Complementary DNA, prepared from polyribosomal polyadenylated uterine RNA, has been used to study oestrogen-induced changes in the mRNA population of rat uterus. Uterine polysomes were prepared as previously described (Merryweather & Knowler, 1977) and polysomal RNA was extracted as follows: polysomal pellets were suspended in 2ml of 0.1M-buffer (0.1M-NaCl, 0.001M-EDTA, 0.01M-Tris/HCl, pH7.5, and 0.2% sodium dodecyl sulphate) containing 200μg of proteinase K [Boehringer Corp. (London), London W.5, U.K.] and incubated at 37°C for 30min. Then 2ml of phenol/chloroform/3-methylbutan-1-ol (1:1:0.1, by vol.) was added and vortexed briefly. RNA was extracted on a mechanical shaker for 10min at room temperature. The phases were separated by centrifugation at 20000g for 6min at 4°C. The phenol and interphase were subjected to two further extractions with 0.1M-Tris/HCl buffer, pH9.0, containing 1mg of bentonite/ml and 1% (w/v) sodium dodecyl sulphate. The combined aqueous phases were centrifuged at 20000g for 15min to remove bentonite and subsequently precipitated with 2vol. of ethanol at −20°C overnight. The polysomal RNA was washed twice with ethanol and dried in a gentle stream of N2. Polysomal RNA (0.9–1mg) was then chromatographed on 1.5–2ml bed volume of poly(U)-Sepharose 4B [Pharmacia (G.B.), London W.5, U.K.] and poly(A)+ RNA