PROTAMINE ENHANCEMENT OF RNA UPTAKE BY CULTURED CHICK CELLS

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In a recent report (Gartler 1960) it has been clearly demonstrated that mammalian cells can ingest and retain deoxyribonucleic acid (DNA) in highly polymerized form. Incorporation by L cells is markedly enhanced if the DNA is presented as protein-DNA particles from 0.5 to 50 microns in diameter (Bensch and King 1961). These and earlier reports of nucleic acid incorporation by animal cells deal exclusively with DNA.

We have found that in primary cultures of chick embryo cells phenol-extracted ribonucleic acid (RNA) (Gierer and Schramm 1956) from Escherichia coli advances the initiation of protein synthesis 12 to 16 hours. This effect was abolished by repeated freezing and thawing of the RNA before its addition to the culture. It was also not observed on pretreatment of the RNA with ribonuclease or on substitution of combinations of nucleosides for RNA.

The use of P^{32}-labelled RNA revealed that only 1 to 2% of some 200-300 µg of RNA presented to 10^7 cells were incorporated. "Incorporated RNA" is defined as trichloracetic acid (TCA)-insoluble P^{32} that is not separable from the cells by washing or on continued incubation of the washed cells in medium containing P^{31} orthophosphate.

The kinetics of RNA incorporation are presented in Figure 1. Curve 1 represents the degradation of the input RNA by ribonucleases of the medium bathing the cells. Curve 1A represents the incorporation of RNA by the cells.

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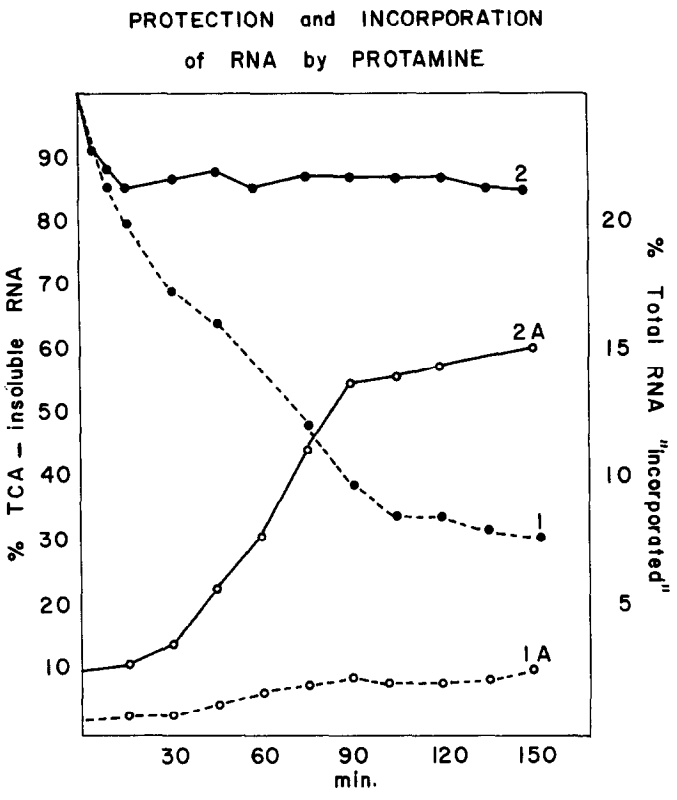

Figure 1.

The addition of protamine sulfate (10-50 ug/ml of salmine obtained from Nutritional Biochemicals Corporation) to cells in a monolayer protected the RNA from degradation to acid-soluble products (curve 2). Furthermore, the protamine stimulated the uptake of RNA by the cells (curve 2A). In its presence 8 to 20 times as much RNA was incorporated by cells in a 2 to 3-hour exposure. The concentrations of protamine and RNA employed did not interact to produce visible turbidity or particles detectable with oil-immersion-darkfield optics. Visible aggregates were formed when ten times as much of each was added.

Other polybasic materials - streptomycin, spermine, and spermidine - in comparable concentrations were just as effective as protamine in protecting RNA against degradation by both "serum ribonucleases"

and purified pancreatic ribonuclease. However, these other compounds either did not stimulate or actually depressed slightly the incorporation of RNA by the cells. Even preparations showing visible aggregation failed to stimulate RNA uptake.

The effectiveness of protamine in stimulating incorporation was not reduced by high concentrations of spermine or spermidine.

The kinetics of uptake of RNA reveal a period of 15 to 30 minutes before significant incorporation can be demonstrated, then for approximately one hour the rate of incorporation is constant and maximal. In the protamine-stimulated cells little further uptake can be demonstrated, while the cells exposed to RNA alone continue a slow incorporation for several hours.

Protamine is insoluble in 10% TCA. Limited hydrolysis by trypsin or by carboxypeptidase produced TCA-soluble fragments which retained the ability to protect RNA against degradation and to stimulate its uptake. Prolonged treatment with trypsin effected the release of free amino acids and small peptides and the progressive loss of stimulatory and finally of protective activity.

DNA prepared by the method of Kirby (1959) was taken up to about the same extent as RNA. Protamine protected it from degradation by enzymes of the cell culture medium as well as from the action of purified deoxyribonuclease, and stimulated its uptake even more dramatically than that of RNA.

The nature of the protein synthesized under the influence of bacterial RNA, the fraction of bacterial RNA responsible for this activity, localization of the incorporated RNA by the use of tritiated uracil and autoradiography, and the effect upon both phenomena of exposure to bacterial DNA are under investigation.

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