STIMULATION OF NUCLEAR DNA AND RNA SYNTHESIS BY CYTOPLASMIC EXTRACTS IN VITRO

Lorin R. Thompson and Brian J. McCarthy

Departments of Genetics and Microbiology

University of Washington, Seattle, Washington 98105

Received December 26, 1967

Cytoplasmic control over nucleic acid biosynthesis has been demonstrated by nuclear transplantation (Gurdon, 1967) and in heterokaryons resulting from cell fusion (Harris, 1967). In these systems, cytoplasmic factors can stimulate RNA and/or DNA synthesis in nuclei which do not normally synthesize either. This paper presents evidence for similar cytoplasmic effects on DNA and RNA synthesis in isolated nuclei in vitro.

MATERIALS AND METHODS

Preparation of nuclei: Mouse liver nuclei were prepared from 5-8 week old Swiss-Webster mice by a method based on that of Widnell and Tata (1964).

Hen erythrocytes were isolated by the method of Harris (1967) and lysed in distilled water. The nuclei were isolated by a method similar to that described by Shearer and McCarthy (1967). The nuclei were finally suspended in 0.05 M Tris pH 7.6, 0.003 M $MgCl_2$, 0.25 M sucrose (TMS). Microscopic examination of nuclear preparations showed them to be free of cytoplasmic contamination. DNA concentration in the nuclei preparations was determined by the diphenylamine method.

Cytoplasmic preparations: L-cells were grown as monolayer cultures in Eagle's minimum essential medium with 10% calf serum. Taper liver tumor (TLT) cells (Taper, et al., 1966) were grown in ascites form intraperitoneally in 5-8 week old Swiss-Webster mice. Cytoplasm was prepared by adding an equal volume of ice-cold 0.2 M Tris pH 8.0, 0.05 M $MgCl_2$, 0.03 M β-mercaptoethanol,

to minced mouse liver or packed TLT, or L-cells. The cells were homogenized with one stroke in a glass homogenizer with a teflon plunger. The homogenate was then centrifuged at 1200 x g for 10 minutes and the crude supernatant free of nuclei was used as cytoplasm (Church and McCarthy, 1967). Cytoplasmic protein concentrations were determined by the method of Lowry.

DNA synthesis was measured by the incorporation of (^{3}H) TTP into TCA insoluble material in the presence of the other three deoxyribonucleotide triphosphates (0.03 M each). RNA synthesis was measured by the incorporation of (^{3}H) UTP into TCA insoluble material in the presence of the other ribonucleotide triphosphates (0.03 M).

0.15 ml of nuclei containing 75-200 µg of endogenous DNA was added to an equal volume of cytoplasm or buffer which contained deoxyribo- or ribotriphosphates and incubated at 37°C. 50λ aliquots were taken at intervals and precipitated with 1 ml 10% ice-cold TCA. The TCA precipitates were centrifuged for 15 minutes at 1200 x g. The pellets were resuspended in 1 ml 0.2 M Tris pH 8.0 and 20 µl of 9 N NaOH and reprecipitated with 2 ml ice-cold TCA. The final precipitate was trapped on Whatman GF/C filters and washed with 15 ml ice-cold TCA. Filters were dried and counted in a liquid scintillation counter.

RESULTS

DNA synthesis: Mouse liver nuclei incorporated (^{3}H) TTP into acid precipitable DNA slowly for at least 45 minutes. The addition of mouse liver cytoplasm caused a slightly elevated background but did not stimulate the rate of synthesis. The addition of ascites cell cytoplasm to mouse liver nuclei caused a 15-20 fold increase in the rate of synthesis as shown in Figure 1.

When isolated hen erythrocyte nuclei, which do not synthesize DNA in vivo, were incubated alone or in the presence of mouse liver cytoplasm, very little TTP incorporation took place. The addition of L-cell cytoplasm to the hen erythrocyte nuclei caused an increase of approximately 10-fold in the total incorporation (Figure 2). When L-cell, TLT or mouse liver cytoplasm was incubated without nuclei, less than 0.015 µµmole TTP was incorporated.

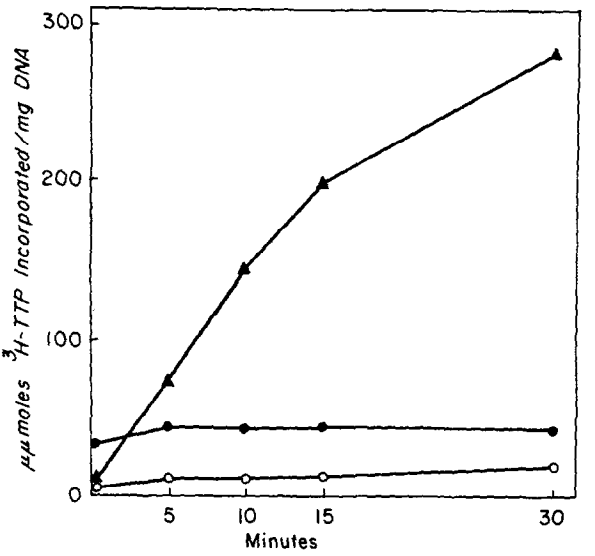

FIGURE 1 - DNA synthesis by isolated mouse liver nuclei. Total volume .29 ml. ^{3}H-TTP (5 μc/incubation mixture). O - nuclei alone, ● nuclei + mouse liver cytoplasm, ▲ nuclei + TLT cytoplasm. Samples with nuclei alone contained buffer instead of cytoplasm and had BSA carrier added before TCA precipitation to equalize the protein concentration of each sample at 280 μg.

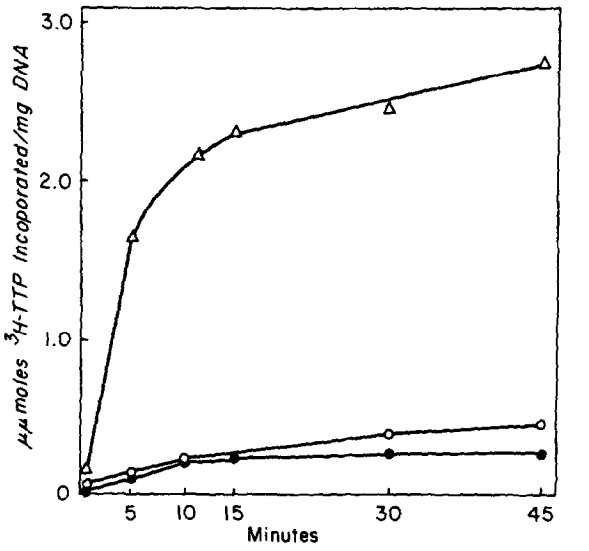

FIGURE 2 - DNA synthesis by isolated hen erythrocyte nuclei. O hen erythrocyte nuclei alone. ● nuclei + mouse liver cytoplasm. △ nuclei + L-cell cytoplasm 214 μg protein per sample.

RNA synthesis: Isolated mouse liver nuclei incorporated (^{3}H) UTP into TCA-insoluble material for periods of 10-12 minutes. By this time the action of ribonucleases in the nuclei and cytoplasm decreased the apparent rate of synthesis. Again, mouse liver cytoplasm failed to stimulate synthesis, but ascites cytoplasm produced a 2-fold stimulation in the rate of synthesis and total incorporation as shown in Figure 3. With hen erythrocyte nuclei RNA synthesis was very low. The addition of mouse liver cytoplasm caused little stimulation, whereas the addition of L-cell cytoplasm caused a dramatic increase in incorporation rate (Figure 4). When L-cell, TLT or mouse liver cytoplasm was incubated without nuclei less than 0.02 µµmole UTP was incorporated.

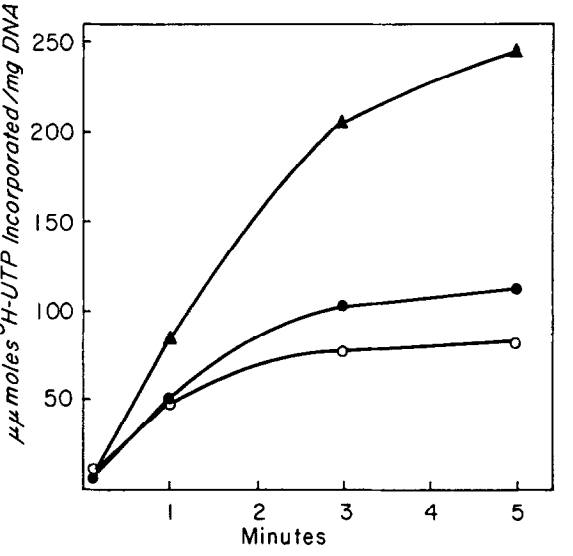

FIGURE 3 - RNA synthesis in isolated mouse liver nuclei. Total volume .29 ml ^{3}H-UTP (5 µc/incubation mixture). O nuclei alone, ● nuclei + mouse liver cytoplasm, ▲ nuclei + ascites cytoplasm. 600 µg protein per sample.

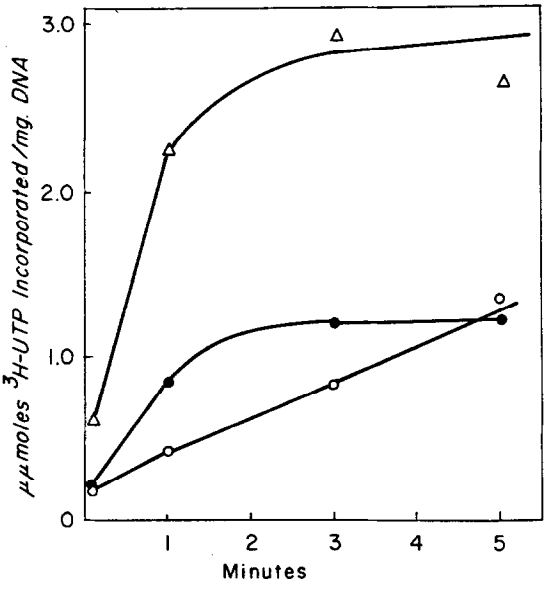

FIGURE 4 - RNA synthesis in isolated hen erythrocyte nuclei. O hen nuclei alone, ● nuclei + mouse liver cytoplasm, Δ nuclei + L-cell cytoplasm, 340 μg protein per sample.

DISCUSSION

Although trivial explanations have not been entirely eliminated, it seems likely that these *in vitro* effects on nucleic acid synthesis are manifestations of the influence of cytoplasm upon nuclei operative *in vivo*. *Xenopus* brain nuclei transplanted into early embryonic cells undertake increased DNA synthesis (Gurdon, 1967) and similar effects have been observed in Stentor (DeTerra, 1967). A mitotic inducing factor, which may be concerned with the synthesis of DNA, has been observed in embryos of *Rana pipiens* (Briggs and Cassens, 1966). Inactive nuclei of avian or mammalian origin synthesize both RNA and DNA when the cells are fused with other cells in which active nucleic acid synthesis occurs (Harris, 1967). In all these cases it appears that the cytoplasm can exert a positive inducing effect on the inactive nuclei. Inhibition of nuclear DNA and RNA synthesis by surrounding foreign cytoplasm is not observed (Harris, 1967).

The same positive control has been observed in the present investigation with nuclei in vitro. In all cases the addition of mouse liver cytoplasm has little effect. The addition of ascites or L-cell cytoplasm to nuclei causes an immediate increase in the rate of synthesis and the total incorporation observed. All these cytoplasmic preparations exhibit considerable variability in their stimulatory capacity. Such variations appear to be a function of the state of the cells at the time of harvesting. The stimulatory factors are found in the cytoplasm of very abnormal cells having rapid rates of growth. The activity seems to be correlated with the rate of nucleic acid biosynthesis in the cells from which the cytoplasm is derived. Thus, although normal liver cytoplasm is quite inactive, stimulation of RNA synthesis in mouse liver nuclei is observed with the cytoplasm of 18 hour regenerating mouse liver.

The stimulating factor or factors is neither species nor order specific. This is apparent from the fact that HeLa cell cytoplasm can substitute for mouse L-cell cytoplasm in the stimulation of RNA synthesis in mouse liver nuclei (unpublished results). It is also shown by the stimulation of synthesis in hen erythrocyte nuclei by the cytoplasm derived from mouse TLT or L-cells. Again, this result is analogous to those obtained with cellular heterokaryons (Harris, 1967).

The partial purification of the factor which stimulates DNA synthesis will be presented elsewhere. The factor has been shown to be a heat stable molecule which can be distinguished from cytoplasmic DNA polymerase. Preliminary experiments with the factor which stimulates RNA synthesis show it also to be heat stable. However, RNA stimulating activity is lost upon freezing, while DNA stimulating activity withstands repeated freezing and thawing. Experiments are in progress to purify these factors and characterize their mode of action.

REFERENCES

Briggs, R., and Cassens, G., Proc. Natl. Acad. Sci. U.S., 55, 1103 (1966).
Church, R. B. and McCarthy, B. J., Proc. Natl. Acad. Sci. U.S., 58, 1548 (1967).
DeTerra, N., Proc. Natl. Acad. Sci. U.S., 57, 607 (1967).
Gurdon, J. B., Proc. Natl. Acad. Sci. U.S., 58, 545 (1967).
Harris, H., J. Cell Sci., 2, 23 (1967).
Shearer, R. W. and McCarthy, B. J., Biochem., 6, 283 (1967).
Taper, H., Woolley, G., Teller, M. and Lardis, M., Cancer Res., 26, 143 (1966).
Widnell, C. C. and Tata, J. R., Biochem. J., 92, 313 (1964).