STIMULATION OF DNA SYNTHESIS IN ISOLATED HELA NUCLEI BY CALF THYMUS HISTONES *

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SUMMARY. DNA synthesis in isolated HeLa nuclei was measured by ^{3}H-TTP incorporation in the presence of cytosol from S-phase cells. The addition of total calf thymus histone at low concentrations stimulated incorporation. Higher levels of added histone markedly inhibited DNA synthesis. The effects of added histone were dependent on the physiological state of the cells from which nuclei were isolated.

Maximal DNA synthesis in isolated HeLa nuclei is dependent upon the presence of all four deoxynucleoside triphosphates, ATP, Mg^{++}, and a 100,000 x g supernatant prepared from S-phase cytoplasm (1,2,3,4). Attempts to isolate the soluble factors in the cytosol which potentiate in vitro DNA synthesis have yielded both low molecular weight, heat resistant (5) and high molecular weight, heat sensitive (2) factors.

In vivo studies have shown that continued DNA synthesis requires protein synthesis (6,7,8). Of the proteins produced in large quantities during the S-phase, histones have been best characterized. Many experiments indicate interdependence of histone synthesis and DNA synthesis (9,10,11,12), yet little information exists which elucidates the mechanisms controlling these processes.

Studies using isolated chromatin have consistantly demonstrated a dose-dependent inhibition of DNA synthesis by added histone (13,14). Inhibition of DNA synthesis in isolated nuclei by exogenously added histone has also been described (13,15). However, since histone synthesis seems to be required for in vivo DNA synthesis, it is conceivable that a positive effect of histone on DNA synthesis should be demonstrable in vitro. Because intact nuclei theoretically possess controlling elements of intact cells without the transport

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problems presented by the plasma membranes, studies were initiated with this system. This communication reports that low levels of added histone stimulate DNA synthesis in isolated nuclei.

MATERIALS AND METHODS

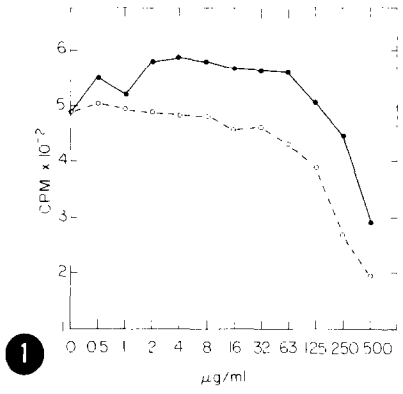
Deoxyribonucleoside triphosphates, ATP, and polylysine hydrobromide (mol. wt. ~30,000) were purchased from Sigma. Calf thymus histone was purchased from Worthington. Methyl-^{3}H-TTP (Schwarz/Mann) had a sp. act. of 17.3 Ci/mM.

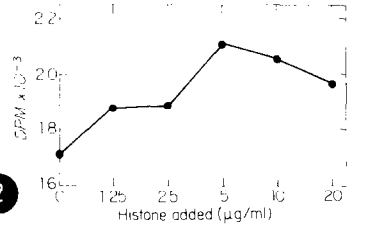
HeLa S-3 cells were propagated in S-MEM containing 3.5% each of calf and fetal calf serum (Gibco) and pen./strep. Spinner cultures were maintained at a density of 2-3.5 x 10^5 cells/ml. Synchrony was achieved by the technique of Mueller, et al. (7): cells were blocked with 10^{-6} M methotrexate with 5 x 10^{-5} M adenosine and were reversed with 1 mg/liter thymidine. Isolation of nuclei and the assay for DNA synthesis were performed according to the methods of Hershey, et al. (2,3). The assay for DNA synthesis contained washed nuclei, 0.4 ml of cytosol and 0.2 ml of assay mixture (20 mM $MgCl_2$, 300 mM NaCl, 100 mM HEPES buffer, 15 mM ATP, 0.3 mM each of dATP, dCTP, dGTP and TTP with 2.5-5 Ci/ml ^{3}H-TTP). After incubation at 37° for 30 min. nuclei were extracted with cold 0.8 M perchloric acid-0.01 M sodium pyrophosphate. Pelleted material was washed 4 times with this solution at half the above concentrations. Radioactivity was released by boiling samples for 30 min. Samples were counted in Aquasol (New England Nuclear) with a Beckman LS-250 spectrometer. All assays were in duplicate except for samples without histone in dose-response experiments which were in triplicate. The average percent error (as the mean of $\frac{SE}{\bar{x}}$ x 100 for each replicate set of assays) is given in the legend to each figure.

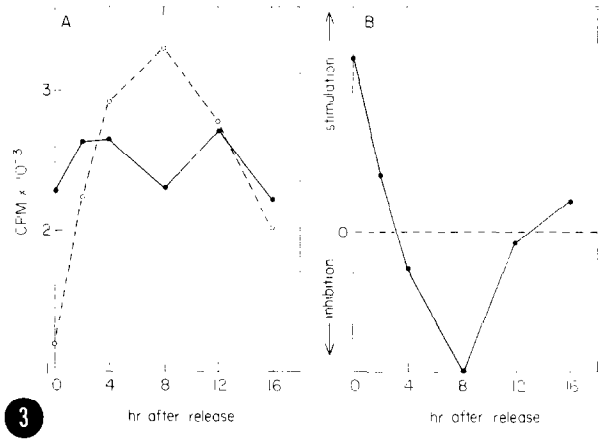
RESULTS

The effects of added total calf thymus histone and polylysine on DNA synthesis are shown in Figure 1. A 20% stimulation of DNA synthesis by histone occurred over a range of concentrations up to 63 µg/ml. In accord with previous findings (16) polylysine was able to mimic the inhibitory effect of histone which occurred in this experiment at concentrations greater than 63 µg/ml. However, polylysine did not mimic the stimulation of DNA synthesis shown by histone: addition of lower concentrations of added polylysine resulted in the same amount of incorporation as the controls without additions.

Much criticism has been directed at studies which employ chemical methods to achieve cell synchrony. To determine whether the observed stimulation of DNA synthesis by added histone might be an artifact of chemical synchrony, randomly growing cells were used as a source of nuclei. Results of this experiment (Figure 2) showed that low concentrations of added histone did stimulate DNA synthesis 17.5% in nuclei from randomly growing cells, although incorp-







Legend to Figure 1. Dose-response of calf thymus histone and polylysine. Two-fold dilutions of histone or polylysine were made in assay mixture with cytosol. 0.6 ml of these containing 0.5 µCi ^{3}H-TTP was incubated in duplicate with 7 x 10^{6} nuclei. The average error was ±2.8%. Closed circles, histone; open circles, polylysine.

Legend to Figure 2. Histone stimulation of nuclei from randomly growing cells. Cells were collected from a culture growing at 1.6 x 10^{5} cells/ml. Duplicate assays contained 1 x 10^{7} nuclei, 1.23 x µCi ^{3}H-TTP and various concentrations of histone in assay mixture. Samples were counted to 3% error and recounted with ^{3}H-hexadecane as an internal standard for determination of DPM. Average error was ±7.3%.

Legend to Figure 3. A. Role of cytosol in the stimulation and inhibition of DNA synthesis by histone. At different times after release of a 5 L. culture from methotrexate block, cytosol was prepared from 2 x 10^{8} cells and frozen. Nuclei were prepared from a culture two hr after release and assayed with the various cytosol preparations with or without 10 µg/ml histone. ^{3}H-TTP was present at 0.6 µCi/assay. The error was ±3.1%. Open circles, cytosol; closed circles, cytosol + 10 µg/ml histone.

B. Cooperative effects of histone and cytosol. Incorporation in the absence of added histone was subtracted from the incorporation with 10 µg/ml histone for the different preparations of cytosol.

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oration per nucleus was lower than that obtained with nuclei from synchronized cells.

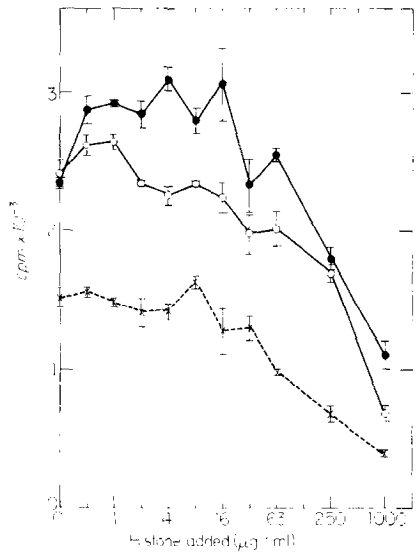
Contribution to the total soluble histone in the assay system comes from nuclear pools, S-phase cytosol, and what is added. To determine the contri-

bution by the cytosol, use was made of the fact that there is a lag after release of blocked cells before histone is synthesized (17). Cytosol was prepared at various times after release of blocked cells and tested in the presence or absence of histone at 10 μg/ml for its ability to stimulate DNA synthesis in nuclei prepared 2 hr after release. The results in Figure 3a show that the ability of cytosol alone to support DNA synthesis changed with time after release of the cells. The changing ability of cytosol alone to support DNA synthesis followed the reported time course of histone synthesis after release (17,12). The level of DNA synthesis in the presence of cytosol with 10 μg/ml added histone did not change with time after release as much as DNA synthesis with cytosol alone. It is significant that the greatest stimulation by added histone occurred with cytosol preparations which least supported DNA synthesis. Added histone inhibited synthesis of DNA at that time after release when cytosol alone supported the greatest incorporation of TTP. These findings are more clearly demonstrated in Figure 3b where the difference between the curves in Figure 3a is plotted.

The effect of the nutritional state of the cells on histone stimulation of DNA synthesis in nuclei was examined by growing cells at three different cell concentrations before synchrony was produced. Nuclei were prepared and assayed at equivalent viable cell concentrations. Figure 4 shows the response of nuclei from the three cultures to increasing concentrations of added histone. Error bars were included because of apparent variation among replicate values. Very little stimulation of DNA synthesis due to added histone was seen in nuclei from cells growing logarithmically. In this case stimulation was only seen at the lowest concentrations of added histone. Nuclei from cells grown at the intermediate density were stimulated over 25% by added histone at concentrations up to 63 μg/ml. High density cells yielded nuclei which incorporated poorly and which were unresponsive to exogenous histone.

DISCUSSION

Isolated nuclei provide a useful system for directly testing factors which



Legend to Figure 4. Effect of culture density on histone stimulation. Cells at 5 x 10^5/ml were maintained both undiluted and diluted 1:2 and 1:4 in fresh medium for 30 hr. Cultures were blocked for 16 hr and released. At 2 hr after release, nuclei were prepared from suspensions adjusted to the same viable cell concentration. Nuclei were diluted to the same concentration and distributed among assay tubes. Each assay contained 1.5 x 10^7 nuclei. Histone dilutions were made in assay mixture. Each assay tube received 0.6 ml of the various histone dilutions containing 0.5 µCi ^{3}H-TTP. All assays were duplicated except those without histone which were in triplicate. Bars show the standard error of the mean for each replicate set. The average error for the entire experiment was ±4.4%. Open circles, 3.1 x 10^5 cells/ml at the time of release; closed circles, 4.8 x 10^5 cells/ml; crosses, 6.3 x 10^5 cells/ml.

may control eukaryotic DNA synthesis. The results above have shown that addition of low concentrations of histone to this system can stimulate DNA synthesis.

Very small amounts of histone are synthesized in cells which are blocked by methotrexate. After release from the block, there is a 3-fold increase in the synthesis of histone, coinciding with the course of DNA synthesis after release (17). The ability of added histone to supplement cytosol taken soon after release (Figure 3) suggests that the lag in reaching rapid rates of DNA synthesis in vivo may be in part due to this lag in histone production. When excessive amounts of histone are present in the system, DNA synthesis was inhibited. This inhibitory effect of histone may act through a mechanism

different from that of stimulation since polylysine can mimic the inhibitory but not the stimulatory effect.

It would appear from Figure 4 that an early consequence of cells reaching saturation densities is a curtailment of available histone. In this experiment, nuclei from cells approaching saturation responded best to added histone, whereas logarithmically growing cells did not respond well. Those nuclei which could be stimulated by histone displayed higher levels of incorporation than nuclei from logarithmically growing cells in spite of similar incorporation in the absence of added histone. If reduced histone levels were a limiting factor during starvation, then accumulation might occur in other biochemical pathways convergent on DNA synthesis, or the number of initiation points might accumulate. Replacement of a histone deficiency could in this situation result in greater incorporation than in controls.

Although these results are preliminary, they provide direct evidence for suggesting that histone may exert a measure of positive control over eukaryotic DNA synthesis.

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