EFFECT OF HOMOLOGOUS RNA POLYMERASE ON THE INCREASE IN CHROMATIN TEMPLATE ACTIVITY OF STIMULATED FIBROBLASTS*

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SUMMARY: Confluent, non-dividing monolayers of WI-38 human diploid fibroblasts can be stimulated to synthesize DNA and divide by a change of medium and serum. Within one hour of stimulation there is an increase in the template activity for RNA synthesis of chromatin. This change in chromatin template activity is seen using either a heterologous E. coli or a homologous HeLa cell RNA polymerase. This strengthens the conclusion that genome activation is one of the earliest changes in the stimulated cells.

Confluent, non-dividing monolayers of human diploid fibroblasts (WI-38) can be stimulated to divide by a change of medium containing 10% serum (1-4). This system provides a convenient experimental model in which to study the molecular events that lead to the initiation of DNA synthesis in non-dividing (G_0) cells stimulated to proliferate. Our studies have concentrated on the biochemical phenomena that occur during the 12-15 hour pre-replicative phase preceding the initiation of DNA synthesis.

We have previously reported that chromatin template activity for RNA synthesis increases within one hour after stimulation of confluent WI-38 fibroblasts (4). Cycloheximide inhibited the increase in chromatin template activity, indicating a requirement for protein synthesis, whereas the stimulation of template activity was not inhibited by actinomycin D, suggesting that the change was independent of RNA synthesis (5).

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We obtained these results using an exogenous E. coli RNA polymerase. Associated with and preceding the rise in chromatin template activity was an increase in the synthesis of non-histone chromosomal proteins (3). These findings were taken to indicate that an increased amount of genetic material is available for transcription very early in the pre-replicative phase of WI-38 cells stimulated to proliferate.

Genome activation has been implicated as an early event in several systems in which non-dividing cells are stimulated to proliferate (4,6-9). Recent studies in our laboratory have shown that the increased chromatin template activity is strictly correlated with the subsequent initiation of DNA synthesis (10).

There has been some criticism in the literature of the use of an heterologous polymerase from E. coli to transcribe mammalian chromatin (11,12) and, as the increased chromatin template activity is felt to be central to all subsequent events leading to DNA synthesis, it is important to show that the quantitative change in the template properties of chromatin in stimulated cells is not simply an artifact of the use of the E. coli polymerase. In this report we show that a similar increase is obtained with the use of a human RNA polymerase.

WI-38 human diploid fibroblasts, purchased from Flow Laboratories (Rockville, Md.), were grown as described previously (3,4) in 1-liter Blake bottles. Confluent monolayers of cells were stimulated to proliferate by replacing the old medium with fresh medium containing 10% fetal calf serum. The chromatin was isolated, with minor modifica-

tions, by the method of Marushige and Bonner (13) as described previously (4). Bacterial RNA polymerase was prepared from early log phase E. coli, strain B (General Biochemicals, Chagrin Falls, Ohio) (11). The assay for chromatin template activity has been previously described (4).

Figure 1 shows the increased template activity of chromatin

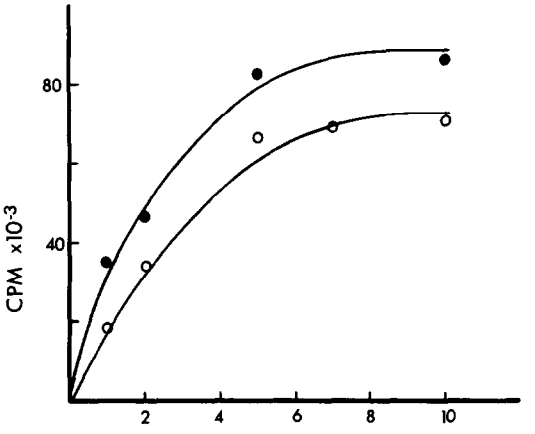

Figure 1. Template activity (using E. coli RNA polymerase) of chromatin from stimulated (●—●—●) and unstimulated (○—○—○) WI-38 fibroblasts. Confluent monolayers were stimulated for one hour by a change of medium and serum. Chromatin was isolated and the template activity determined as described in the text. Each point contained 20 µg of DEAE-cellulose purified (F_4) RNA polymerase.

isolated from WI-38 cells stimulated for one hour by a change of medium with 10% serum. These data were obtained with E. coli RNA polymerase. To compare this result with the template activity of the same chromatins using a homologous polymerase, RNA polymerase was solubilized by the method of Rutter and Roeder (15) from the nuclei of HeLa S_3 cells grown in suspension culture. The enzyme was purified through the step immediately prior to the resolution of the separate

polymerase activities and contained both α-amanitin sensitive and resistant polymerase activities and had a 100-fold dependency on exogenous DNA.

Figure 2 shows that the chromatin from the stimulated

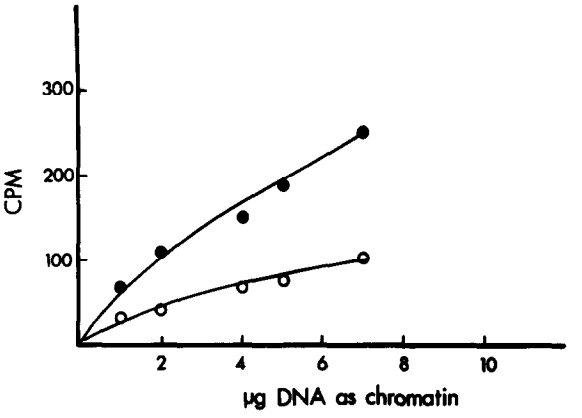

Figure 2. Template activity (using HeLa cell RNA polymerase) of chromatin from stimulated (●—●—●) and unstimulated (○—○—○) WI-38 fibroblasts. Preparation of the human polymerase was as described in the text. Each assay contained 800 µg RNA polymerase. One µg of this enzyme incorporated 0.2 pmole of UMP into RNA in 10 minutes with calf thymus DNA as a template.

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cells still possesses an increased template activity when assayed with HeLa cell RNA polymerase. The extent of RNA synthesis was much less with the homologous polymerase than with the bacterial polymerase, presumably reflecting a lowered affinity of the crude enzyme for chromatin.

The results of these experiments demonstrate that the increased template activity of chromatin from WI-38 fibroblasts stimulated to divide is not an artifact of the use of the heterologous E. coli RNA polymerase but is reproducible even when an homologous RNA polymerase is used for the assay.

This supports our conclusion that genome activation is one of the earliest changes in cells that have been stimulated to initiate DNA synthesis and subsequently divide.

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