UNCOUPLING OF NONHISTONE CHROMOSOMAL PROTEIN SYNTHESIS AND DNA REPLICATION IN HUMAN DIPLOID WI-38 FIBROBLASTS

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Received 1 May 1973
Revised version received 15 May 1973

1. Introduction

The genome of differentiated eukaryotic cells is a nucleoprotein complex referred to as chromatin consisting of DNA, RNA, histone, and nonhistone chromosomal proteins. While histones have been shown to be responsible for the repression of DNA-dependent RNA synthesis [1–3], recent evidence suggests that quantitative [4–7] and qualitative [8–10] variations in the transcriptional capacity of the genome may be mediated by nonhistone chromosomal proteins. The role of nonhistone chromosomal proteins in the control of gene expression has been the subject of several recent reviews [11–14]. It has been demonstrated previously that in continuously dividing cells [15–17], as well as in quiescent cells which are stimulated to proliferate [18], histone polypeptide synthesis is restricted to the S phase of the cell cycle and ceases if DNA replication is inhibited by cytosine arabinoside or hydroxyurea. In continuously dividing cells it has been shown that the synthesis of nonhistone chromosomal proteins occurs in the cytoplasm [19] during all phases of the cell cycle on pre-existing and newly synthesized mRNA's [20] and is not affected by the inhibition of DNA replication [17]. However, the dependency of nonhistone chromosomal protein synthesis on DNA replication in models of stimulated DNA synthesis has not been examined previously.

The aim of the present investigation was to define further the relationship between chromosomal protein synthesis and DNA replication by studying the biosynthesis of these macromolecules in density-inhibited human diploid WI-38 fibroblasts stimulated to proliferate by a change of medium.

2. Materials and methods

Human diploid WI-38 fibroblasts were grown to confluence in Eagle's Basal Medium (BME) supplemented with 10% foetal calf serum in one liter Blake culture flasks in a moist CO_2 incubator. Seven days after plating, the confluent monolayers were stimulated to proliferate by replacing the old medium with fresh BME containing 10% foetal calf serum [6, 21, 22]. Nuclei and chromatin were isolated at 4°C as described previously by Stein et al. [6].

Chromatin was solubilized in 1% SDS, 1% β-mercaptoethanol, 0.01 M sodium phosphate, pH 6.8; dialyzed against 0.1% SDS, 0.1% β-mercaptoethanol, 0.01 M sodium phosphate, pH 6.8 and the chromosomal proteins were then separated according to their molecular weight by SDS polyacrylamide-gel electrophoresis [23]. In one experiment, the histones were separated according to charge, utilizing the high resolution acetic acid urea method of Panyim and Chalkley [24].

3. Results and discussion

Table 1 indicates that 16 hr after density-inhibited human diploid WI-38 fibroblasts are stimulated to proliferate by a change of medium, the cells actively incorporate [^{3}H] thymidine. This is in agreement with reports from several laboratories that DNA synthesis occurs at this time [6, 21, 22]. It is also evident from the data in table 1 that cytosine arabinoside at a concentration of 40 μg/ml is effective in suppressing com-

Table 1
Effect of cytosine arabinoside on DNA synthesis in WI-38 human diploid fibroblasts.

Sample	Cpm/100 μg DNA
Unstimulated control	5 130
S-phase control	42 768
S-phase cytosine arabinoside	3 231

The rates of [^{3}H]thymidine incorporation into DNA were determined in density-inhibited WI-38 human diploid fibroblasts, in S-phase cells (16.5 hr after the stimulation of density-inhibited fibroblasts to proliferate by a change in medium), as well as in S-phase cells following 30 min of pretreatment with 40 μg/ml of cytosine arabinoside. One-liter Blake bottles, each containing 10^7 cells, were labeled for 30 min with [^{3}H]thymidine (0.1 μCi/ml, 6.0 Ci/mM). Cells were harvested, washed twice with cold (40°C) 0.3 N perchloric acid, and nucleic acids were extracted with hot (90°C) 1 N perchloric acid.

pletely this increased rate of DNA synthesis. Cytosine arabinoside is a potent inhibitor of DNA replication, and at a concentration of 40 μg/ml it has no significant effect on the bulk rates of RNA or protein synthesis. To resolve directly the relationship between the synthesis of nonhistone chromosomal proteins and DNA replication in a model of stimulated DNA synthesis, 2 hr (G_1) and 16 hr (S) after density-inhibited monolayers of human diploid WI-38 fibroblasts were stimulated to proliferate by a change of medium the cells were pretreated with cytosine arabinoside (40 μg/ml) for 30 min and then pulse labeled with [^{3}H]L-tryptophan for 30 min. Cytosine arabinoside was included in the labeling medium. Chromatin was prepared and the total chromosomal proteins were resolved according to molecular weight on SDS polyacrylamide gels [23]. The rationale for these experiments was that, since histones do not contain tryptophan the distribution of radioactivity throughout the gels reflects solely the synthesis of nonhistone chromosomal proteins. It is evident from figs. 1A and 1B that cytosine arabinoside at a concentration of 40 μg/ml does not influence the incorporation of [^{3}H]L-tryptophan into the various chromosomal proteins during the prereplicative phase of the cell cycle (G_1), eliminating the possibility that the antimetabolite has a direct effect on the synthesis of these polypeptides. Furthermore, when DNA synthesis is blocked by cytosine arabinoside during S phase, the synthesis of nonhistone chromosomal proteins in control and cytosine arabinoside-treated cells is virtually identical (figs. 1C and 1D). It should be noted that there is no significant incorporation of [^{3}H]L-tryptophan in the regions of the gel where the histones are located. Electrophoresis of purified histones on these gels under similar conditions demonstrates that such polypeptides are limited to fractions 62–96 (data not shown). A comparison of the distribution of the [^{3}H]L-tryptophan or [^{3}H]L-leucine (not shown) radioactivity among the G_1 and S-phase nonhistone chromosomal proteins also suggests that there are variations in the nonhistone chromosomal proteins synthesized and associated with DNA during these two distinct periods of the cell cycle.

An identical experiment using [^{3}H]L-leucine clearly demonstrates that the inhibition of DNA synthesis during S phase with cytosine arabinoside is effective in reducing the incorporation of [^{3}H]L-leucine into proteins which migrate in the histone region of the gel to the G_1 level, and the incorporation of [^{3}H]L-leucine into the nonhistone chromosomal proteins (fractions 1–61) is not altered (not shown). To establish conclusively that the synthesis of histones is dependent on concomitant DNA synthesis in this system, S-phase WI-38 fibroblasts were incubated in the presence of cytosine arabinoside (40 μg/ml) for 30 min and then pulse labeled for an additional 30 min with [^{3}H]L-leucine and cytosine arabinoside. Histones were extracted with 0.4 NH_2SO_4 from the chromatin of control and cytosine arabinoside-treated cells and resolved by the high-resolution acetic acid—urea method of Panyim and Chalkley [24]. Fig. 2 shows that cytosine arabinoside completely blocks the incorporation of [^{3}H]L-leucine into the major classes of histone polypeptides.

The present experiments demonstrate that in density-inhibited human diploid WI-38 fibroblasts which are stimulated to proliferate by a change in medium, the synthesis of nonhistone chromosomal proteins which occurs during the S phase of the cell cycle is not affected when DNA synthesis is blocked by cytosine arabinoside. This is in contrast to the synthesis of histone polypeptides, which is restricted to the S phase of the cell cycle and is inhibited completely when DNA synthesis is interrupted by the antimetabolite. These results are in agreement with previous findings by this investigator which showed that the synthesis of nonhistone chromosomal proteins is not reduced when DNA synthesis is inhibited by cytosine arabi-

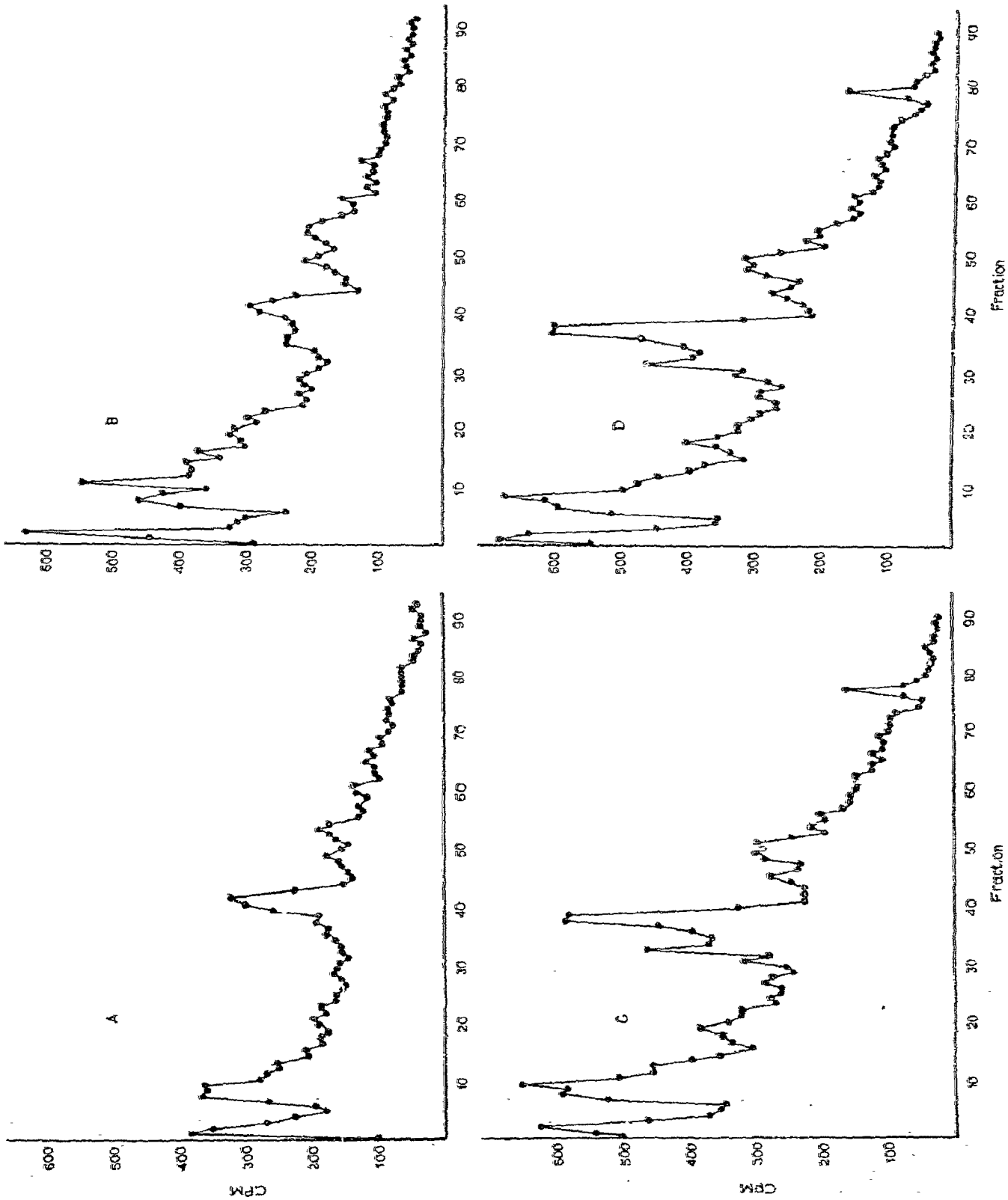

Fig. 1. A) SDS polyacrylamide-gel electrophoretic profile of [^{3}H]L-tryptophan-labeled G_1 total chromosomal proteins. B) Effect of cytosine arabinoside on the SDS polyacrylamide-gel electrophoretic profile of [^{3}H]L-tryptophan G_1 total chromosomal proteins. C) SDS polyacrylamide-gel electrophoretic profile of [^{3}H]L-tryptophan-labeled S-phase total chromosomal proteins. D) Effect of cytosine arabinoside on the SDS polyacrylamide-gel electrophoretic profile of [^{3}H]L-tryptophan-labeled S-phase total chromosomal proteins.

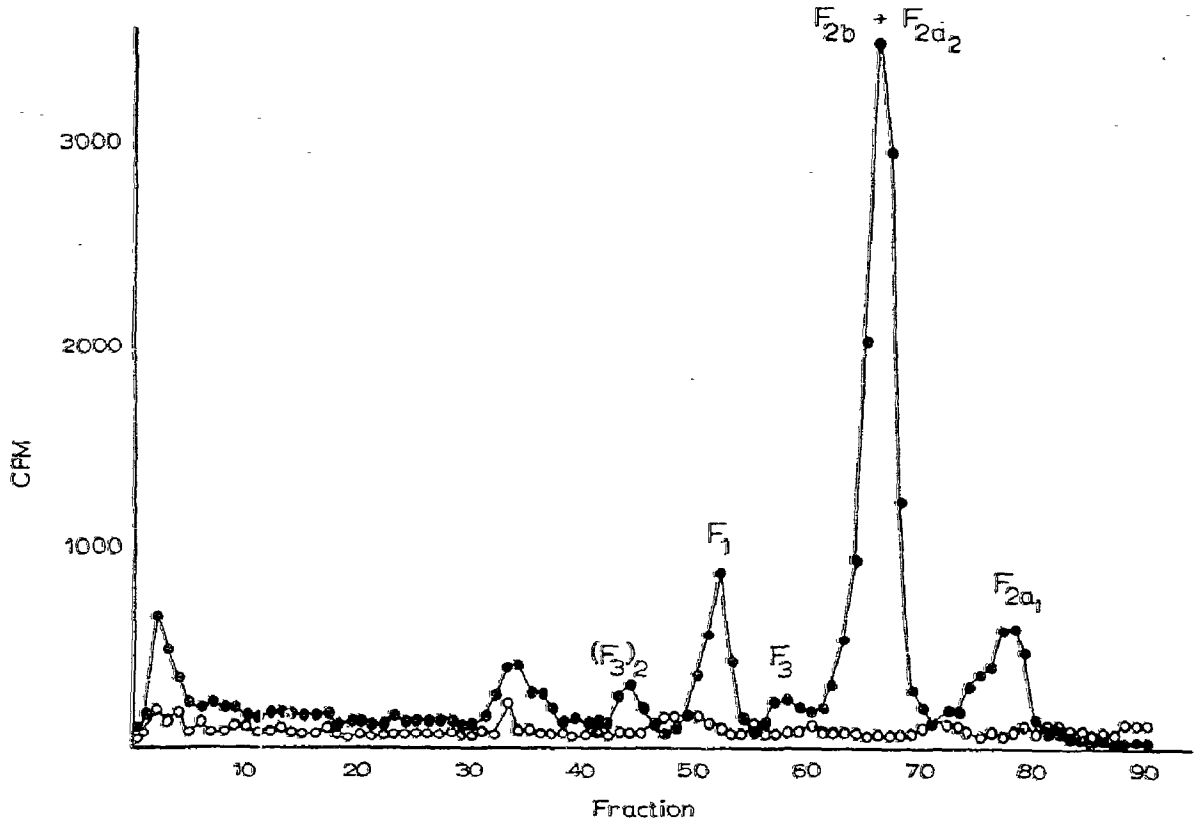

Fig. 2. Effect of cytosine arabinoside on the polyacrylamide-gel electrophoretic profile of [^{3}H]L-leucine-labeled S-phase 0.4 N H_2SO_4 soluble chromosomal proteins. Electrophoresis was carried out according to the method of Panyim and Chalkley [24]. Cytosine arabinoside-treated S-phase histones (o—o—o); control S-phase histones (•—•—•).

noside or hydroxyurea in continuously dividing HeLa S_3 cells [17]. Such findings, taken together, indicate that in quiescent cells which are stimulated to proliferate, as well as in continuously dividing cells, the synthesis of nonhistone chromosomal proteins — unlike that of the histones, which is tightly coupled with DNA replication — occurs independently, suggesting the possibility of a regulatory function for these acidic chromosomal proteins.

Reasoning of this nature is consistent with recent evidence from several laboratories which suggests that the nonhistone chromosomal proteins may play a significant role in the regulation of gene expression in general, and specifically, in the control of transcription during the cell cycle [11–14]. The regulatory function of these proteins is supported by their tissue and species specificity [25–30]; their presence in greater quantities in active rather than in inactive tissues [31], as well as in active (euchromatin) rather than in inactive (heterochromatin) chromatin [32]; qualitative and quantitative differences in their rates of synthesis, turnover and phosphorylation throughout the cell cycle [17, 33–37]; and their capacity to regulate transcription in a manner characteristic of the tissue of origin [8–10] or the particular state of the cell cycle [6, 7]. The specific manner in which nonhistone chromosomal proteins interact with the genome remains to be elucidated.

Acknowledgements

The authors wish to express their appreciation to Mrs. Gale Hunter and Mrs. Lena Lavie for their expert technical assistance. This research was supported by grants from the Damon Runyon Memorial Fund for Cancer Research, DRG-1138, from the National Science Foundation, GB-38349, and the American Cancer Society, F73UF-6.

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