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Effect of Sodium Butyrate Treatment of *HeLa* Cells on the Transcriptional Activity of their Isolated Nuclei

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Sodium n-butyrate has been used as a tool to study the presumptive effects of the *in vivo* hyperacetylation of histones from HeLa cells on the transcriptional activity of their isolated nuclei. No gross differences were found in either initiation or RNA synthetic capacity among the nuclei from control or butyrate treated cells. The electrophoretic pattern of the RNA synthesized in nuclear suspensions was markedly affected by prior exposure of the cells to the fatty acid.

The mechanisms by which eukaryotic cells modulate specific gene transcription are basically unknown. A possible mechanism for gene activation involving histone acetylation was first proposed by ALLFREY *et al.* (1). YAMAMOTO and ALBERTS (20) extended this model and postulated that the reversible acetylation of histones could provide a flexible mechanism to modulate gene transcription *via* a local perturbation in the structure of chromatin. As a crucial test for this hypothesis several authors have been able to show that the hyperacetylation of histones brings about structural changes in chromatin, with patterns of susceptibility to DNase I which resemble those observed in active genes (8, 15, 16, 18). It has also been reported that the multiacetylated forms of H4 his-

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tone are preferentially associated with template active chromatin (5). In an attempt to bridge these structural findings with the transcriptional functionality, it has been shown that the chemical hyperacetylation of chromatin increases the rate of RNA chain elongation (7) and the number of initiation sites (9) assayed with DNA-dependent RNA polymerase from *E. coli*. Criticism arising from both, the use of chemical reagents for the acetylation and heterologous polymerase for the transcriptional studies, led us to reexamine this question, using the butyrate induced hyperacetylation of histones *in vivo* (14) and the endogenous capacities for RNA synthesis in isolated nuclei.

Materials and Methods

HeLa cells were grown as monolayers in 700 ml Roux flasks, in 50 ml of Dulbecco's modified Eagle's medium supplemented with 10 % fetal calf serum. When indicated, sodium butyrate at the final concentration 7 mM was added 24 hours before harvesting the cells. The fatty acid was also present in all the subsequent handling of the cells and nuclei. Cells were harvested near confluence and washed three times in sodium phosphate pH 7.

Nuclei were isolated by differential centrifugation of cell lysate as described by BUSIELLO and DI GIROLANO (3). The nuclear concentration was estimated by direct haemocytometer counting and by the measurement of DNA with diamino benzoic acid (4). Histones were extracted from nuclei with 0.4 M sulphuric acid, collected by ethanol precipitation and analyzed electrophoretically by the method of PANYIM and CHALKLEY (10), using long gels in order to resolve the multiacetylated forms of H4.

RNA synthesis in nuclear suspensions was assayed (3) in the presence of 0.1 mM ATP, GTP, CTP and 0.006 mM UTP. The reaction was started with 0.005 mCi of ^{3}H UTP (10 Ci/mmol, from Amersham). After incubation at 26° C, the reaction was stopped with 10 % trichloroacetic acid. The precipitates were collected on Whatman CF/G filters, washed with 5 % trichloroacetic acid containing 2 % sodium pyrophosphate and counted for radioactivity by liquid scintillation. In studies of RNA chain initiation (17), ATP and GTP were substituted by their corresponding sulfur containing nucleosides (γ-S) triphosphates (from Boehringer).

Electrophoresis of RNA samples was carried out in 0.6 % agarose-2.4 % acrylamide gels (14). After densitometric scanning at 260 nm, the gels were cut in 1.5 mm slices. The radioactivity in the gel slices was measured by liquid scintillation after an overnight incubation at 40° C with 5 ml of a mixture composed of 50 parts of toluene-Permablend (from Packard), 5 parts of NCS tissue solubilizer (from Nuclear Chicago) and 1 part of ammonia.

The RNA synthesized in the presence of the thiol analogues of the nucleotides was isolated and desalted by gel filtration on G 10 Sephadex as described (17). The newly initiated RNA chains, with the thiol group at the 5′ end, were isolated by affinity chromatography on mercury-agarose (11), prepared as described (13). All the glassware was sterilized and the solutions pretreated with macaloid or diethylpyrocarbonate (Sigma) to prevent RNase action during the incubation of the nuclei and the isolation of RNA.

Results and Discussion

RIGGS *et al.* (12) first reported the occurrence of an extensive acetylation of chromatin bound histones H3 and H4 in mammalian cells grown in the presence of n-butyrate. Later it was shown that this effect was the result of an inhibition of histone deacetylase activity by the fatty acid (14). The electrophoretic analysis of

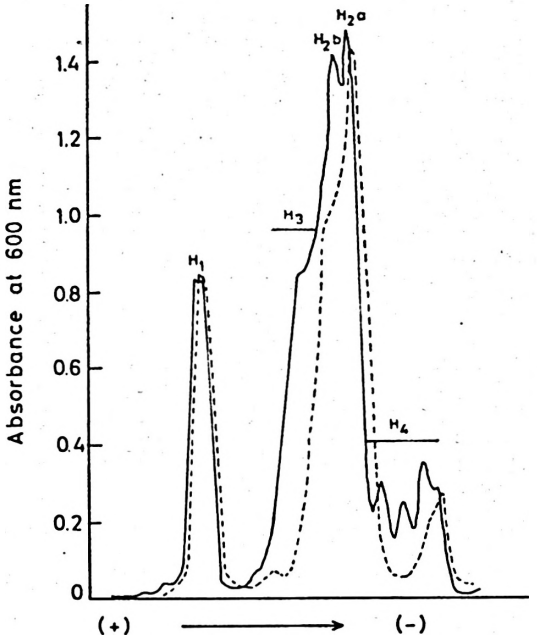

Fig. 1. *Electrophoretic analysis of histones extracted from butyrate treated cells.* Histones were extracted from nuclei (3×10^7 cells). Seventy micrograms of histone protein were electrophoresed in 20 cm gels as described in methods. After staining with amidoblack, the portion of the gel containing the histones was scanned at 600 nm in a Gilford 2400 spectrophotometer. Butyrate (——), control (----).

histones extracted from butyrate treated cells (fig. 1) shown that there is a extensive acetylation of H4 as ascertained by the lower mobility of these histone subfractions.

To explore the influence of the hyperacetylation of histones *in vivo* on the synthesis of RNA *in vitro*, the transcriptional activity of nuclei from control and butyrate treated cells was studied. Both the test and control nuclei exhibit a similar RNA synthetic capacity (fig. 2) and elongation rates when assayed at three different nuclear inputs. A similar observation was reported (6) while this manuscript was in preparation. It was also observed (data not shown) that RNA synthesis in these nuclei was equally affected by α-ammanitin (40 inhibition at a concentration of 0.0035 mg/ml). This observation suggests a similar transcriptional activity for the DNA-dependent RNA polymerase II in control and hyperacetylated nuclei.

The pattern of RNA initiation in isolated nuclei was studied with the methodology developed by REEVE (11), in which RNA synthesis was allowed to proceed in the presence of the analogues of ATP and GTP with sulfur at the γ-phosphate and ^{3}H UTP as the radioactive precursor. The newly initiated RNA chains, with the thiol group at the 5′ end and the radiolabel throughout the entire chain length, were isolated by affinity chromatography as described in methods. The pattern of elution from mercury-agarose of the RNAs synthesized *in vitro* is shown in figure 3. No major differences were observed, although the relative amount of RNA initiated by the control was slightly higher than in test nuclei (29 per cent of

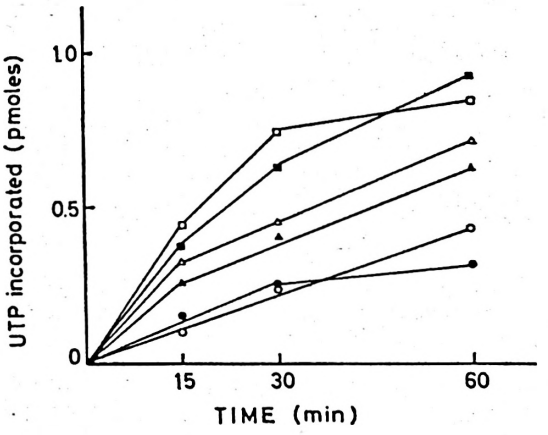

Fig. 2. *RNA synthesis in isolated HeLa cell nuclei.* RNA synthesis was assayed as described in methods with 0.25 (circles), 0.5 (triangles) and 1 (squares) $\times 10^6$ nuclei, in a final volume of 0.115 ml. Open and closed symbols stand for nuclei prepared from control and butyrate treated cells.

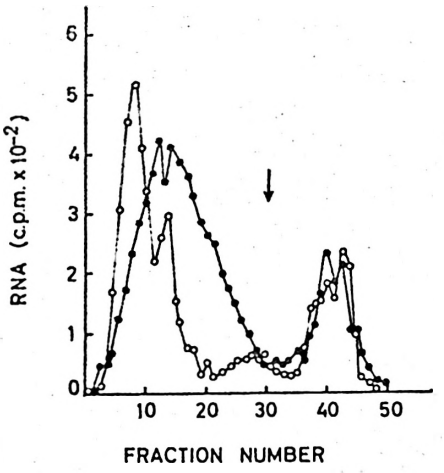

Fig. 3. *Mercury-agarose chromatography of RNA initiated* in vitro.
The assay conditions were as indicated in methods, with 8×10^6 nuclei, in a final volume of 0.8 ml. After 40 min incubation, the RNA was extracted and desalted before chromatography in 8 ml column of mercury-agarose. The fractions (1 ml) were assayed for radioactivity by liquid scintillation counting. Open and closed symbols stand for the control and butyrate treated cells respectively. The arrow indicates the start of the specific elution of newly initiated RNA chains with 10 mM dithioerytritol.

the total RNA output of the column in controls, against a 21 per cent for the RNA initiated in butyrate treated nuclei).

These results indicate that the general parameters of RNA transcription are not affected by the hyperacetylation of chromatin bound histones in nuclei from cells exposed to n-butyrate. This conclusion does not exclude the occurrance of more subtle changes in the nature of the genomic response to the hyperacetylation of histones. As a preliminary approach to this question, the size distribution of RNA chains synthesized by isolated nuclei was studied. Control experiments, in which nuclei from cells grown with or without butyrate were incubated with cytoplasmic RNA in the standard conditions for RNA synthesis, did not show any significant amount of nuclease activity and RNA degradation, as ascertained by the absorbance at 260 nm after acid precipitation of RNA or by the electrophoretic criterium (data not shown).

The electrophoretic pattern of the RNAs synthesized *in vitro* (fig. 4) indicates that the average size of the RNA transcripts was affected by the prior exposure of the cells to butyrate, with a consistent redistribution towards RNA molecules of lower size, ranging from 4 S to 15 S. Whether this pattern of RNA transcripts represents a new family of informational RNA or the increased synthesis of the «small nuclear» RNA (2) in response to the hyperacetylation of histones, remains to be ascertained.

This result is compatible with the hy-

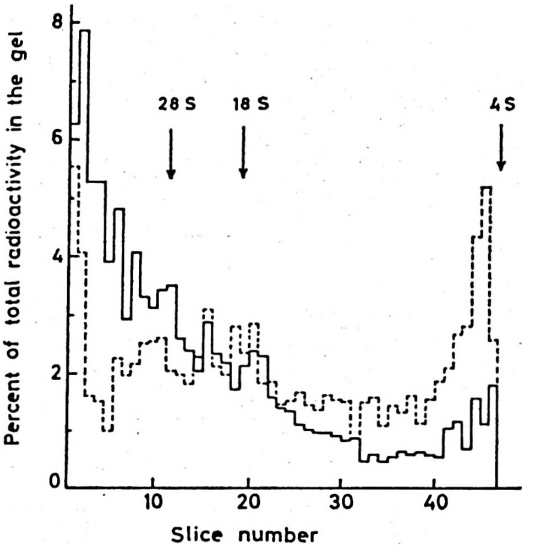

Fig. 4. *Electrophoretic analysis of RNA transcripts.*
RNA synthesized by isolated nuclei (5×10^6) was extracted after 40 min incubation, as described in methods. Aliquots of this RNA (15000 cpm) were electrophoresed as described in methods. Solid and broken lines correspond respectively to RNA synthesized in control and butyrate treated nuclei.

pothesis that the acetylation of histones may play a role, necessary but not sufficient, in the regulation of gene expression in eukariots.

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Resumen

Se estudian los efectos de la hiperacetilación de histonas *in vivo* con n-butirato, sobre la actividad de transcripción medida en núcleos aislados de células HeLa. Los resultados indican que no hay diferencias apreciables en cuanto a capacidad total o iniciación de síntesis de RNA, entre células crecidas en presencia de butirato y sus controles. Sin embargo, el ácido graso parece afectar marcadamente al patrón electroforético de los RNAs sintetizados *in vitro*.

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