CHANGES IN THE PHOSPHORYLATION OF NON-HISTONE CHROMATIN PROTEINS DURING THE CELL CYCLE OF HeLa S_3 CELLS

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Received February 20,1973

SUMMARY

The phosphorylation of non-histone chromatin proteins in synchronized HeLa S_3 cells was studied in 5 phases of the cell cycle: mitosis, G_1, early and late S, and G_2. The rate of non-histone chromatin protein phosphorylation was found to be maximal during G_1 and G_2, somewhat decreased during S phase, and almost 90% depressed during mitosis. Analysis of the phosphorylated non-histone chromatin proteins by SDS-acrylamide gel electrophoresis showed a heterogeneous pattern of phosphorylation as measured by labeling with ^{32}P. Significant variations in the labeling pattern were seen during different stages of the cell cycle, and particular unique species appeared to be phosphorylated selectively during certain stages of the cycle.

Recent studies have suggested that non-histone chromatin proteins play a key role in the regulation of both gene expression and cell proliferation in eukaryotic organisms (1-3). Specifically, it appears that these proteins are responsible for variations in the transcriptional capacity of the genome during the cell cycle in continuously dividing populations of cells (4), as well as in quiescent cells which are stimulated to proliferate (5). This non-histone fraction is now known to contain many proteins which are extensively phosphorylated, and these phosphoproteins exhibit many properties expected of molecules involved in the regulation of gene transcription. For example, changes in their phosphorylation have been found to correlate with changes in gene activity in a number of different systems (6-9), they are heterogeneous and tissue specific (10-11), they bind specifically to DNA (11,12), and they alter the rate of RNA synthesis in cell-free systems (11,13-16).

In order to obtain information on the possible involvement of the phosphorylation of non-histone proteins in the regulation of cell division, we have begun an investigation on the phosphorylation of these chromatin proteins during various stages of the cell cycle of synchronously dividing HeLa S_3 cells. The present results demonstrate that dramatic changes in both the over-all rate and the qualitative pattern of non-histone protein phosphorylation occur during different stages of the cell cycle.

METHODS

Exponentially growing HeLa S_3 cells maintained in suspension culture were synchronized either by selective detachment of mitotic cells or by two cycles of 2 mM thymidine block as described elsewhere (1). The degree of synchrony obtained utilizing these procedures has also been described previously (1). Synchrony was assayed by determining the mitotic index and ^{3}H-thymidine incorporation at 60-minute intervals. Cells were pulse labeled for 30 minutes with $^{32}P_i$ (200 µCi/ml) in phosphate-free Minimal Essential Medium supplemented with 2% foetal calf serum, harvested by centrifugation at 1000 g for 5 minutes, and washed 4 times with Earle's balanced salt solution. Nuclei were isolated by 4 washings with 80 mM NaCl, 20 mM EDTA, 1% Triton X-100, pH 7.2, and washed twice with 0.15 M NaCl, 0.01 M Tris, pH 8.0. This procedure yields nuclei which are free of cytoplasmic contamination when examined by phase-contrast and electron microscopy.

The phosphorylated non-histone chromatin protein fraction was isolated from these nuclei according to the procedure of Gershey and Kleinsmith (17) involving salt extraction and purification on calcium phosphate gel. The purified phosphoprotein fraction was electrophoresed in a 10% acrylamide gel containing sodium dodecyl sulfate (SDS) as described by Weber and Osborn (18).

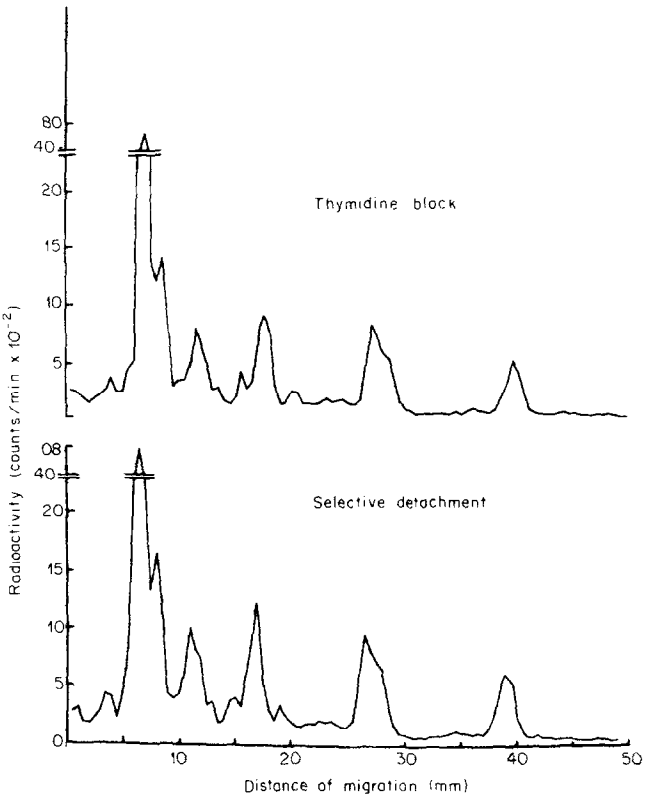
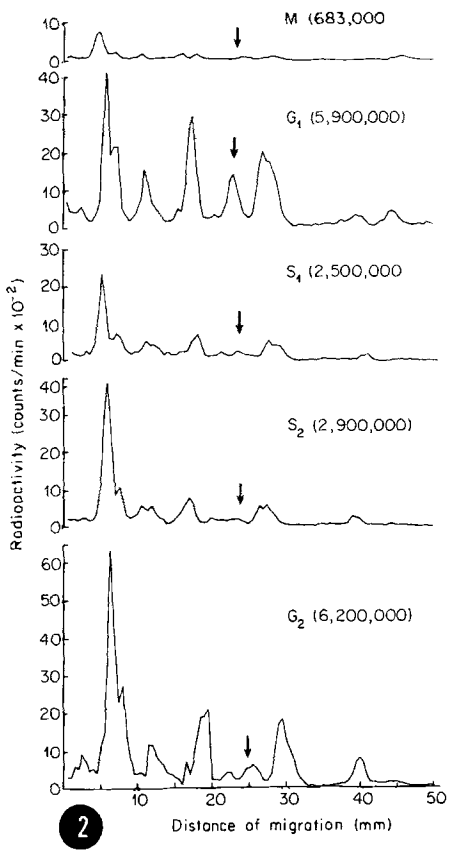


Figure 1. Comparison of ^{32}P labeling patterns of non-histone chromatin phosphoproteins from HeLa S_3 cells synchronized by either selective detachment of mitotic cells or by double thymidine block. In the thymidine block experiment, cells were harvested 3 hours after release from the second thymidine block, at which time more than 98% of the cells were in S phase. In the selective-detachment experiment, cells were harvested 11 hours after detachment, where again 98% of the cells were in S phase. Harvested cells were pulsed with ^{32}P for 30 minutes, after which nuclei were isolated and the non-histone chromatin phosphoprotein fraction prepared. The protein fractions were electrophoresed in 10% SDS-acrylamide gels, which were sliced at 0.5-mm intervals and the slices counted in a liquid-scintillation counter. Radioactivity in each slice is plotted as a function of distance of migration. Note that the labeling patterns for the two different types of synchronization procedures are indistinguishable.

RESULTS AND DISCUSSION

Cells in mitosis and G_1 were obtained by mitotic selective detachment and cells in S phase and G_2 were obtained following release from 2 cycles of 2 mM thymidine block. Such an approach is necessary, since a decay of synchrony occurs as selectively detached mitotic cells



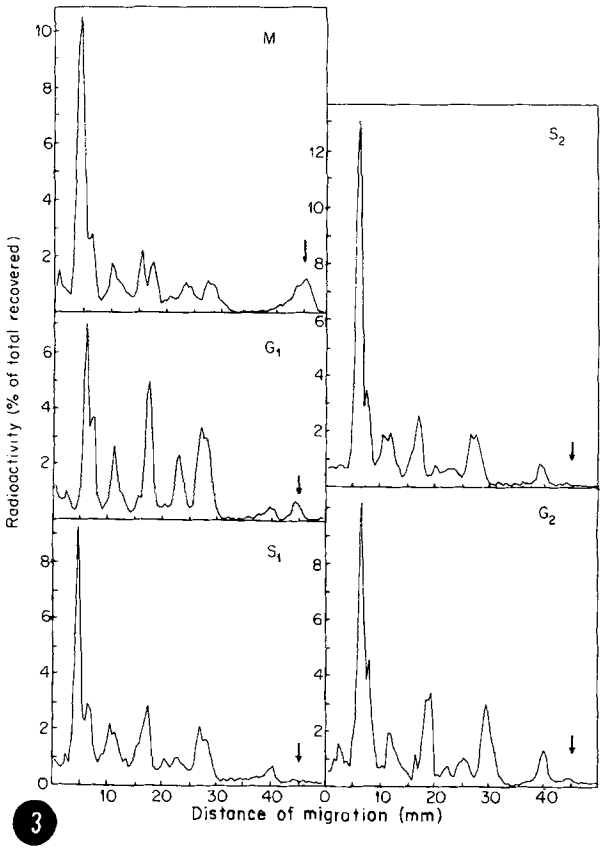


Figure 2. Comparison of ^{32}P labeling patterns of non-histone chromatin phosphoproteins from HeLa S_3 cells at different stages of the cell cycle. Mitotic cells were obtained by selective detachment from semiconfluent monolayers, an aliquot was immediately pulse labeled for 30 minutes with ^{32}P (mitosis), and the remaining cells were permitted to progress into G_1 and labeled after an additional 2.5 hours. Early S-phase, late S-phase, and G_2 cells were labeled 2.5, 5, and 7.5 hours, respectively, after release from 2 cycles of thymidine block. Phosphoprotein samples of 10 µg from each stage were electrophoresed as described in Fig. 1. Radioactivity in each slice is plotted as a function of distance of migration. Note the major quantitative differences in the phosphorylation of different protein species at different stages. The numbers in parentheses are the specific activities in counts/min/mg of the total phosphoprotein preparation at each stage. The arrows indicate the position of a protein peak which is selectively phosphorylated during G_1 (and, to a lesser extent, during G_2).

Figure 3. The data from Fig. 2 are replotted, expressing the radioactivity in each slice as a percentage of total radioactivity in the gel. Note that there are significant quantitative changes in the relative distribution of radioactivity in various regions of the gel. The arrows indicate the position of a protein peak which is phosphorylated selectively during mitosis (M), and, to a lesser extent, during G_1.

progress through S phase and into G_2. Figure 1 compares the ^{32}P labeling patterns of S phase non-histone chromatin proteins from HeLa S_3 cells synchronized by mitotic selective detachment and double thymidine block. Inasmuch as the electrophoretic radioactivity profiles are indistinguishable in both instances, we feel justified in utilizing both procedures in these studies.

When the rates of phosphorylation of the non-histone chromatin proteins are compared at different stages of the cell cycle, dramatic differences are seen (Fig. 2). The over-all rate of phosphorylation is maximal during G_1 and G_2, moderately decreased during S phase, and almost 90% depressed during mitosis. When one examines the pattern of phosphorylation of these proteins by labeling with ^{32}P followed by electrophoresis in SDS-acrylamide gels, striking differences in the patterns are evident (Fig. 2). In addition to the obvious large quantitative changes in the rate of phosphorylation of specific protein peaks, one particular protein component appears to be phosphorylated selectively during G_1 (and, to a lesser extent, during G_2).

If the data from each gel are replotted expressing the radioactivity in each slice as a percentage of the total radioactivity in the gel, then it is possible to make comparisons between the different stages independent of their over-all rates of labeling. As can be seen in Fig. 3, such an analysis also shows significant changes in the relative percentages of activity in various regions of the gel during different stages. Especially noteworthy is the appearance of a specific peak of phosphorylation which is associated primarily with mitosis, and, to a lesser extent, with G_1.

The present results thus show that both the rate and the pattern of phosphorylation of non-histone chromatin proteins change dramatically during the cell cycle. The low rate of phosphorylation during mitosis

correlates with the known suppression of *in vitro* and *in vivo* RNA synthesis during this period (4,19-22). In addition, the selective phosphorylation of unique protein species during specific stages suggests that the phosphorylation of unique non-histone proteins may be involved in changes in nuclear functions at different times of the cell cycle, and indicates that further studies of the cell cycle may be useful in assigning particular functions to the phosphorylation of specific non-histone proteins.

ACKNOWLEDGEMENTS

This research was supported by Grant GB-23921 from the National Science Foundation and Grant DRG-1138 from the Damon Runyon Memorial Fund for Cancer Research. R. D. Platz was supported by USPHS Training Grant 5-T01-GM-71-13. The illustrations were prepared by Sandra Beadle. We are indebted to Ms. Lena Lavie and Ms. Gale Hunter for their expert technical assistance.

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