CELL-CYCLE-SPECIFIC CHANGES IN CHROMATIN ORGANIZATION

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SUMMARY: Cell-cycle-specific changes in the organization of chromatin have been observed using the natural polyanion heparin as a probe. The heparin-mediated release of DNA from intact nuclei has been measured in mitotically synchronized populations of Chinese hamster (line CHO) cells during early interphase. Changes in the patterns of release of DNA from nuclei at different times post-mitosis suggest that significant changes in the organization of chromatin occur during G_1. These changes may be related to G_1-specific histone modification.

INTRODUCTION

Changes in chromatin organization during the cell cycle have been reported by several investigators (1-4). Results of ultrastructural studies show an increasing dispersion of chromatin fibers as cells progress through G_1 into S phase (1). Accessibility of DNA to [^{3}H]actinomycin D and DNase I has been found to increase in S-phase chromatin compared with G_1 chromatin in synchronized HeLa cells (2,3). Also, an increased binding of *Escherichia coli* RNA polymerase in proliferating WI38 cells, compared with cells in non-cycling confluent monolayers, has been observed (4). These findings suggest a loosening of chromatin structure as cells progress through the pre-DNA synthetic phase of the proliferation cycle. With the exception of the ultrastructural studies, the probes of chromatin structure mentioned above interact with the DNA component of chromatin. Polyanions constitute a different class of probes which interact primarily with the histone component of chromatin (5-9). The usefulness of polyanions for observing changes in chromatin organization is the subject of this report.

Both the natural polyanion (heparin) and synthetic polyanions (polystyrene sulfonate, polyethylene sulfonate, dextran sulfate) have been reported to produce specific effects on nuclei and chromatin (5-15). These include stimulation of template efficiency for both endogenous and exogenous RNA polymerases (5,10-12); increased availability for exogenous DNA polymerase (13); induction of nuclear swelling (8,14); release of DNA from nuclei (8,14); and enhancement of [^{3}H]actinomycin D binding by nuclei (9). All of these effects can be attributed to specific interaction of polyanions with histones (5-9). This finding has been confirmed in our Laboratory and will be reported elsewhere (16).

Previous reports have shown that treatment of rat liver nuclei with the natural polyanion heparin causes DNA to be released in a highly dispersed state (14). In the present studies, the heparin-mediated release of DNA from isolated nuclei has been quantitated. The efficiency with which heparin disperses DNA from nuclei has been observed to change during early interphase in mitotically synchronized populations of Chinese hamster cells. The relationship between this observation and G_1-specific changes in chromatin composition via histone modification or changes in non-histone chromosomal proteins is discussed.

MATERIALS AND METHODS

Chinese hamster cells (line CHO, obtained from Dr. T. T. Puck) were maintained free of *Mycoplasma* contamination in F-10 medium (Gibco) supplemented with 10% calf and 5% fetal calf sera (both supplied by Flow Laboratories). The cells were synchronized by mitotic selection without drugs or trypsin, as reported earlier (17). Uniform labeling of DNA was accomplished by growth in [^{14}C]-thymidine at 0.006 Ci/ml (New England Nuclear Corporation) for two generations prior to mitotic selection. Following mitotic selection, the cells were resuspended in radioisotope-free medium at 37°C. Entry of cells into S phase was prevented by treating the cells prepared by mitotic selection with 10^{-3} M hydroxyurea commencing at 1 h after release from mitosis in suspension culture (18).

Nuclei were prepared as described previously (19) by treatment with 1% NP-40 in 0.01 M Tris-Cl (pH 7.4 at 24°C), 0.01 M NaCl, 0.0015 M $MgCl_2$. Nuclei were washed by resuspension and centrifugation (800 x g for 5 min) with 0.02 M Tris-Cl (pH 7.5 at 24°C), 0.25 M sucrose buffer and resuspended in the same buffer at 3 x 10^7 nuclei/ml.

Samples containing nuclei at 1.5 x 10^7/ml and heparin at the indicated concentration (final concentrations of Tris and sucrose in the reaction were 0.01 M and 0.125 M, respectively) were incubated at 2°C for 5 min. The treatment causes both nuclear swelling (14) and release of DNA from nuclei (14), both of which correlate with the removal of histones from DNA (16). Fifty volumes of 0.01 M Tris (pH 7.5 at 24°C) were added to the samples to terminate heparin-mediated release of DNA. Each sample was mixed vigorously with a vortex mixer for 15 sec. The samples were centrifuged at 60 000 x g (average) for 25 min at 4°C. Aliquots were taken from each sample before and after centrifugation for liquid scintillation counting. Aliquots (1.0 ml) were counted in 15 ml of Aquasol (New England Nuclear Corporation). The percent DNA remaining in the supernatant after centrifugation was designated the released, dispersed, or decondensed DNA.

RESULTS

The efficiency of heparin-mediated release (or decondensation) of DNA from nuclei was measured at various times as mitotically synchronized cells traversed early interphase. The results of these experiments are shown in Fig. 1A. The percent of bulk DNA which is released in 5 min at 4°C by a given concentration of heparin decreases considerably as the cells progress from early G_1 (1 h post-mitosis) into early S phase (7 h post-mitosis). Two additional features of these data are that the fraction of bulk DNA which is resistant to release by

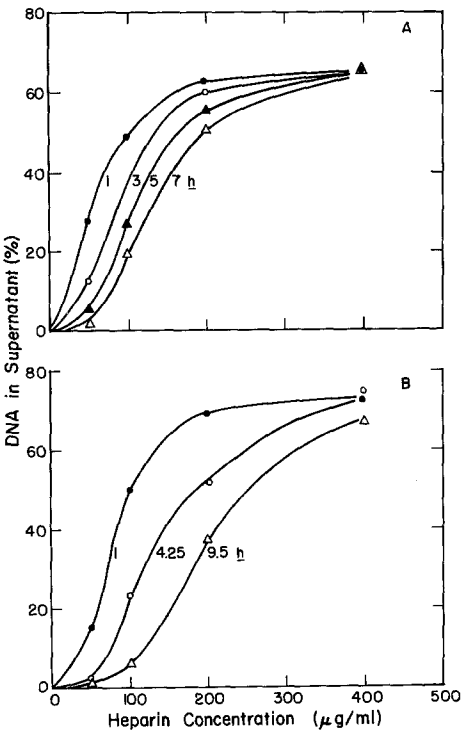

Fig. 1. Changes during early interphase in the heparin-mediated release of DNA from nuclei. The percent of DNA released from isolated nuclei was measured, as described in Materials and Methods, for mitotically synchronized cells traversing interphase in the absence (A) or the presence (B) of hydroxyurea. Samples were removed from the synchronized populations at times after release from mitosis as indicated.

heparin is approximately the same at large heparin concentrations, regardless of the position of nuclei in interphase, and that the release of DNA from nuclei appears to occur in a cooperative fashion. Further studies are in progress to clarify the implications of these two points. Because the primary target of heparin action is the histone component of chromatin (5-9), the results in Fig. 1A suggest that the accessibility of histones changes significantly during G_1.

The natural synchrony decay in a mitotically selected population of cells (17,19) results in entry of some cells into S phase as early as 4 h post-mitosis. When cells enter S phase, histone synthesis begins concomitantly with the initiation of DNA replication (20). The increase in histone (chromatin) mass per nucleus in cells during S phase alters the histone:heparin stoichiometry and could account for part of the decrease in heparin-mediated DNA dispersion illustrated in Fig. 1A. To determine how much of this effect could be attributed to increased histone per nucleus, mitotically synchronized cells were

treated with hydroxyurea at 1 h post-mitosis, and samples were taken for assay at various times. In the presence of hydroxyurea, the cells progress through the G_1 phase of the cell cycle and accumulate in late G_1 at the G_1/S boundary (18). Both detectable DNA replication and histone synthesis are inhibited during hydroxyurea treatment (20). Hence, for a given heparin concentration, the histone:heparin ratio remains constant in samples assayed during G_1 traverse. The results of these experiments are recorded in Fig. 1B. It is clear that the decreased heparin-mediated dispersion of DNA from nuclei during G_1 traverse is not inhibited in the presence of hydroxyurea and that, therefore, it does not result from an increase in the histone or DNA component of chromatin mass.

The timing of the G_1-specific changes in chromatin organization described above has been compared with the timing of entry of cells into S phase. This was done by determining the heparin concentration at which 50% of the maximum amount of DNA is released from the nuclei (i.e., the heparin concentration at 50% of the maximum level of supernatant DNA observed at large heparin concentrations). The values were measured from data such as those shown in Fig. 1. These results (Fig. 2) show that the timing of the chromatin organizational change is similar in cells traversing G_1 in the presence and absence of hydroxyurea. Entry of cells into S phase is given by the labeled fraction determined from autoradiographic measurements on non-prelabeled replica cultures handled otherwise exactly as the prelabeled cultures. If the curve for heparin-mediated chromatin dispersion in Fig. 2 is extrapolated to the early G_1 level (60 μg heparin/ml), the change in heparin-mediated chromatin dispersion can be estimated to begin 1 to 2 h post-mitosis. Hence, this change precedes by 2 to 3 h entry into S phase, estimated to begin between 3 and 4 h post-mitosis (from extrapolation of the labeled fraction data to 0).

DISCUSSION

Significant changes in the organization of chromatin beginning in early interphase are detectable by treatment of isolated nuclei with an agent which interacts preferentially with histones. Current evidence suggests that release of DNA from nuclei occurs as a result of removal of histones from chromatin (5-9,14). The decreased susceptibility of chromatin to the heparin-mediated release of DNA as cells progress through early interphase suggests several possible explanations: (a) the affinity of histone for DNA could be increased by modification of histones [specifically, phosphorylation of histone f1 (19,21)]; (b) the accessibility of histones to heparin could be altered by histone modification in the absence of changes in the affinity of histones for DNA; and (c) the accessibility of histones to heparin could be reduced by association

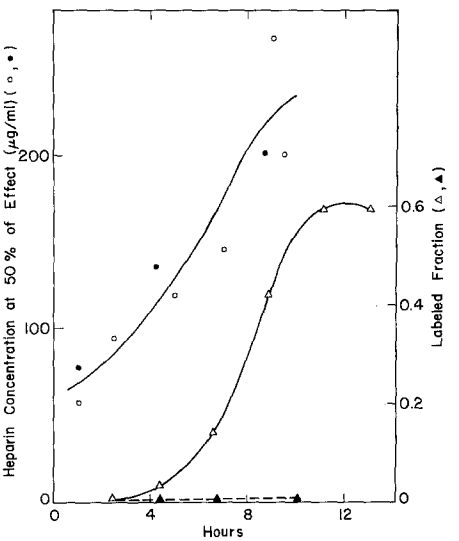

Fig. 2. Cell-cycle dependence of heparin-mediated release of DNA from nuclei. The heparin concentrations required to release 50% of the maximum level of DNA released at large heparin concentrations (greater than 400 μg/ml) were determined from curves such as shown in Fig. 1 for cells synchronized by mitotic selection and treated (-●-) or not treated (-○-) with hydroxyurea. Labeled fraction determinations (fraction of cells in the population labeled with a 15-min pulse of [^{3}H]thymidine) were performed on non-prelabeled synchronized replica cultures simultaneously with the above experiments [hydroxyurea present (-▲-) and hydroxyurea absent (-△-)].

of non-histone chromosomal proteins with chromatin. Studies by other investigators in this Laboratory have demonstrated a G_1-specific phosphorylation of histone f1 which precedes S phase by 2 h (20,21). Hence, the close correspondence in the timing of the G_1-specific phosphorylation event and the observed change in chromatin organization reported here suggest that these events are related. However, the possibility that the change (or part of the change) in chromatin arrangement measured by heparin-mediated chromatin release is a result of G_1-specific synthesis of non-histone proteins and their subsequent association with chromatin during G_1 must be considered (22,23). Ongoing experiments are designed to evaluate these possibilities.

While studies of chromatin organization during the cell cycle using DNA-specific probes suggest an increasing accessibility of DNA as cells traverse G_1 (2-4), the present studies using an agent which interacts primarily with histones indicate that a progressive change occurs in the arrangement of chromatin during G_1. Although these results cannot at present be accounted for by a specific model of chromatin, further studies using probes specific for both of the major components of chromatin (i.e., DNA and histones) should provide insight into the determinants of changes in the organization of chromatin during the cell cycle.

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REFERENCES

1. Dewey, W. C., Noel, J. S., and Dettor, C. M. (1972) Radiation Res. 52, 373-394.
2. Pederson, T., and Robbins, E. (1972) J. Cell Biol. 55, 322-327.
3. Pederson, T. (1972) Proc. Natl. Acad. Sci. (U.S.) 69, 2224-2228.
4. Hill, B. T., and Baserga, R. (1974) Biochem. J. 141, 27-34.
5. Miller, G., Berlowitz, L., and Regelson, W. (1971) Chromosoma 32, 251-261.
6. Miller, G. J., Berlowitz, L., and Regelson, W. (1972) Exp. Cell Res. 71,, 409-421.
7. Berlowitz, L., Kitchin, R., and Pallotta, D. (1972) Biochim. Biophys. Acta 262, 160-168.
8. Arnold, E. A., Yawn, D. H., Brown, D. G., Whyllie, R. C., and Coffey, D. S. (1972) J. Cell Biol. 53, 737-757.
9. Tubbert, M. A., and Berlowitz, L. (1974) Exp. Cell Res. 85, 205-211.
10. Chambon, P., Ramuz, M., Mandel, P., and Doly, J. (1968) Biochim. Biophys. Acta 157, 504-519.
11. Chambon, P., Karon, H., Ramuz, M., and Mandel, P. (1968) Biochim. Biophys. Acta 157, 520-531.
12. Spelsberg, T. C., and Hnilica, L. S. (1969) Biochim. Biophys. Acta 195, 55-62.
13. Kraemer, R. J., and Coffey, D. S. (1970) Biochim. Biophys. Acta 224, 553-567.
14. Kraemer, R. J., and Coffey, D. S. (1970) Biochim. Biophys. Acta 224, 568-578.
15. dePomerai, D. I., Chesterton, C. J., and Butterworth, P. H. W. (1974) FEBS Letters 42, 149-153.
16. Hildebrand, C. E., Gurley, L. R., Tobey, R. A., and Walters, R. A. (1974), manuscript in preparation.
17. Tobey, R. A., Anderson, E. C., and Petersen, D. F. (1967) J. Cell. Physiol. 70, 63-68.
18. Tobey, R. A., and Crissman, H. A. (1972) Exp. Cell Res. 75, 460-464.
19. Hildebrand, C. E., and Tobey, R. A. (1973) Biochim. Biophys. Acta 331, 165-180.
20. Gurley, L. R., Walters, R. A., and Tobey, R. A. (1974) Arch. Biochem. Biophys. 164, 469-477.
21. Gurley, L. R., Walters, R. A., and Tobey, R. A. (1974) J. Cell Biol. 60, 356-364.
22. Gerner, E. W., and Humphrey, R. M. (1973) Biochim. Biophys. Acta 331, 117-127.
23. Choe, B.-K., and Rose, N. R. (1973) Exp. Cell Res. 83, 271-280.