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CHROMATIN STRUCTURAL CHANGES PRECEDE REPLICATION IN INITIATED REPLICONS DURING INHIBITION OF DNA ELONGATION[1].

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ABSTRACT Partial inhibition of replicative DNA synthesis by hydroxyurea or other agents produces changes in the composition and structure of bulk chromatin. We have begun to investigate the structural changes in specific regions of the genome using synchronized cells and cloned genomic probes. Current results indicate changes in chromatin structure occur preferentially in initiated replicons and can precede the replication fork during inhibition of DNA elongation.

INTRODUCTION

Synchrony of CHO cells in early S phase by allowing G1 cells to enter S phase in the presence of 1.0 mM hydroxyurea or other agents for 10 h produces (a) a 30% depletion of histone H1 compared with exponentially growing cells, (b) abnormally short repeat lengths in the 3-5% of the total DNA that is replicated, and (c) a shortening in the measured repeat lengths in unreplicated bulk chromatin (1,2). These are especially interesting results, because synchrony of cells in this way appears to produce an accumulation of initiated, partially elongated replicons that arise from normally early replicating DNA. In terms of this model, changes in H1 content and repeat lengths can be "explained" if it is assumed that loss of histone H1 and changes in chromatin structure normally occur throughout a replicon at

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initiation, or they can move ahead of the fork during inhibition of DNA elongation (1).

We have begun to test these models by investigating whether changes in nucleosome repeat lengths occur preferentially in initiated replicons and whether they can precede the replication fork during inhibition of DNA elongation.

METHODS

Chinese hamster (line CHO) suspension cultures were synchronized in G1 by the isoleucine deficiency method. They were further synchronized in early S phase by releasing G1 cells into complete medium containing 1.0 mM hydroxyurea for 10 h, or they were subjected to prolonged block in early S phase by releasing G1 cells into the presence of 1.0 mM hydroxyurea for 24 h. Cell synchrony and average DNA content per cell were determined by flow cytometry (1,2).

Isolated nuclei were digested with micrococcal nuclease at 37°C as functions of time, resulting in different percentages of acid soluble bulk DNA (1). Isolated DNA was further treated with RNase before being analyzed in 1.5% agarose flat-bed gels (Tris-borate-EDTA buffer), photographed, and transferred to Zetabind membranes with 0.4 N NaOH.

The blots were hybridized with a ^{32}P-labeled nick-translated 1076 bp metallothionein II (MTII) CHO genomic probe which begins at position -247 and extends 62 bp beyond the "transcribed" sequences. After autoradiography the blots were stripped of the MTII probe and rehybridized with a ^{32}P-labeled nick-translated plasmid probe containing multiple copies of the repeated sequence pHuR-093. Nucleosome repeat lengths were calculated using a linear regression method (1).

RESULTS

Metallothionein (MTII) is a single-copy unexpressed, noninducible gene that is replicated during early S phase in wild type CHO cells (3). CHO pHuR-093 is a repeated sequence or a set of repeated sequences that is detected by hybridization with a cloned hexameric repeated sequence called pHuR-093, derived from human fibroblasts. The pHuR-093 repeated sequence does not appear to be replicated until >1.5 h after synchronized early S phase cells are released to resume cell cycle traverse (unpublished results). Based

on the proposed models, (a) synchrony of cells in early S phase should produce a collection of initiated replicons arising from early replicating DNA (including MTII), (b) changes in chromatin structure should occur specifically in initated replicons and not in later replicating DNA (including pHuR-093), and (c) the changes in the early replicating MTII should exceed those observed for bulk chromatin, because only early replicating DNA should contribute to changes in bulk chromatin as cells progress from G1 to early S phase.

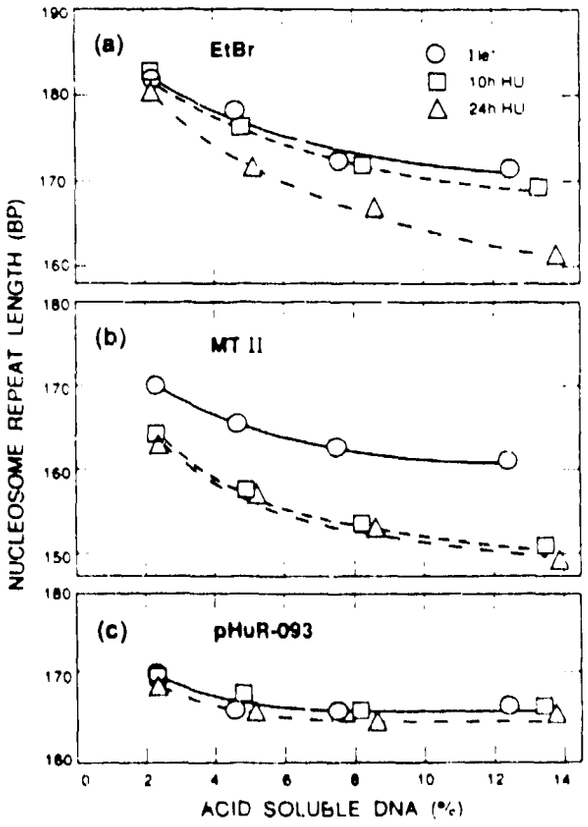

FIGURE 1. Measured nucleosome repeat lengths of bulk chromatin (a), the MTII gene region (b), and pHuR-093 (c) as functions of the percentage acid soluble bulk DNA from G1 cells subjected to prolonged block in S early phase (24h HU).

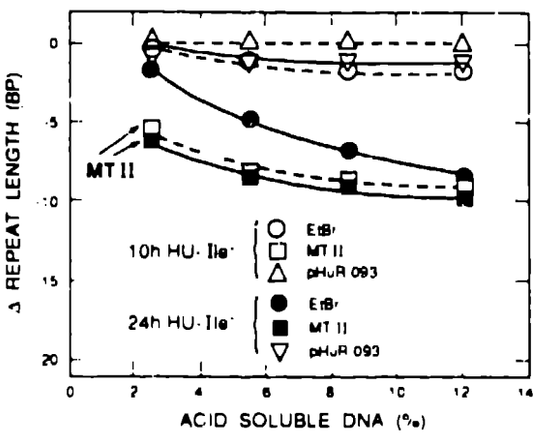

FIGURE 2. Changes in repeat lengths between early S phase cells and G1 cells (10h HU - Ile^-) and between cells subjected to prolonged block and G1 cells (24h HU - Ile^-) for bulk chromatin (EtBr), MTII, or pHuR-093 plotted as functions of acid soluble bulk DNA. The curves were computed from data in Figure 1.

Measurements of changes in the repeat lengths between G1 and early S phase cells (FIGURES 1 and 2) indicate that the results are consistent with the predictions. The early replicating MTII gene exhibits 5-9 bp reductions in repeat lengths between early S phase and G1 cells. These reductions are the same as those seen in the MTII region after prolonged 24 h S phase block when there are substantial changes in both bulk chromatin and the early replicating MTII gene region. Also, the changes in MTII between early S phase and G1 cells exceed those measured for bulk chromatin. In contrast, the repeat lengths of the later replicating pHuR-093 are nearly constant.

Whereas these results indicate that changes are occurring preferentially in the MTII region, compared with bulk chromatin and CHO pHuR-093, they do not tell us whether MTII was replicated as G1 cells entered and became synchronized in early S phase.

Slot blot analysis of MTII contents in G1 and early S phase cells and slot-blot analysis of BrdU-labeled replicated DNA and unlabeled old DNA from early S phase

cells indicate that 10±10% of the total MTII is newly replicated in the early S phase cells synchronized with hydroxyurea. Thus, the results indicate that structural changes precede MTII replication and, apparently, the replication fork by a, yet, undetermined distance.

DISCUSSION

The results are consistent with the notions that changes in chromatin structure (a) occur preferentially in initiated replicons and (b) can precede the fork during inhibition of DNA elongation. Whereas the changes in front of the fork indicate that structural changes or "replicon unfolding" can be uncoupled from DNA elongation in initiated replicons, we do not know whether (a) the changes are normal events that occur at initiation during unperturbed replication or (b) they are reversible or irreversible perturbations of normal processes. In either case, they could be important components in the production/induction of genomic damage by chemical or physical agents and genomic rearrangements produced by transient inhibition of DNA Synthesis (4).

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