THE REGULATION OF RNA SYNTHESIS DURING MITOSIS

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SUMMARY. The inhibition of RNA synthesis in mitotic cells is accountable, at least in part, by a reduced template capacity for RNA synthesis seen with mitotic chromatin. By reconstituting chromatin from purified DNA and either S-phase or mitotic chromosomal proteins, it is shown that the reduced template capacity is produced by the interaction of the mitotic proteins with DNA. The degree of inhibition of template capacity is related to the dose of mitotic proteins in the reconstituted chromatin.

From the work of several authors (1-3) it is known that RNA synthesis is virtually suppressed during anaphase and metaphase and that protein synthesis is dramatically reduced (2,4). Using Colcemid arrested metaphase cells, it has been shown that the synthesis of nuclear heterogeneous RNA and nucleolar (ribosomal) RNA ceases (5); 4S RNA is synthesized at approximately one-third the rate, and 5S is synthesized at 75% of the interphase rate (6).

This repression of RNA synthesis during mitosis is one of the most striking examples of reversible gene regulation in eukaryotic cells, and it may be analogous to the process of heterochromatization in interphase cells.

There seems to be a strong correlation between highly condensed chromosomal material and genetic inactivity in cells other than those in metaphase (7-11). Further, it has been substantiated that RNA synthesis is more pronounced in euchromatic than in heterochromatic areas of chromosomes (12-14).

The inhibition of RNA synthesis during mitosis, thus, is a convenient model for the study of gene inactivation for two reasons: (1) the magnitude

of the effect is quite large, and (2) it may resemble the process of heterochromatization, a mechanism that is probably central to the control of development and differentiation in eukaryotic cells.

Johnson and Holland (15) have shown that mitotic chromatin is a poor template for RNA synthesis using an exogenous E. coli RNA polymerase. It has been generally assumed (16) that in the process of coiling and condensation the once-exposed DNA becomes inaccessible to RNA polymerase. However, it may be possible that the template inactivity is caused by differences in the chemical constitution of mitotic chromosomes per se and that both the inhibition of RNA synthesis and the chromatin condensation are a reflection of this.

A report from this laboratory has shown that non-histone chromosomal proteins are synthesized at an undiminished rate during mitosis, at variance with other cellular proteins (17). In light of recent studies suggesting that these acidic, non-histone proteins may have a role in the control of gene expression (18-28), we were led to reconsider the role of the chemical constitution of chromatin in the inhibition of RNA synthesis seen during mitosis. In this paper we confirm that mitotic chromatin is a poor template for RNA synthesis. Furthermore, when chromatin is reconstituted from log phase, homologous DNA and either mitotic or S-phase chromosomal proteins (DNA free), the chromatin reconstituted with mitotic proteins has a lower template activity than chromatin reconstituted with S-phase proteins. An activity in between S phase and mitotic levels can be obtained when chromatin is reconstituted from log phase DNA and a mixture of S phase and mitotic chromatin protein fractions. These results indicate that some constituent(s) of the chromosomal protein fraction is responsible for the inhibition of RNA synthesis occurring during mitosis.

Mitotic cells were obtained by synchronizing HeLa cells in suspension culture by a double thymidine block. Three hours after release from the second thymidine block Colcemid (0.5 µg/ml) was added to the cultures.

Mitotic cells were harvested 7 hours later at which time 85% of the cells were in metaphase as determined by light microscopy of stained preparations. The degree of inhibition of RNA synthesis as measured by ^{3}H-uridine incorporation (85%) was proportional to the number of metaphase arrested cells obtained (data not shown).

Using an exogenous E. coli RNA polymerase, chromatin isolated from mitotically arrested cells is a poor template for RNA synthesis as compared to chromatin isolated from S-phase cells (Fig. 1). The assays were run using a fixed amount of RNA polymerase with increasing amounts of chromatin.

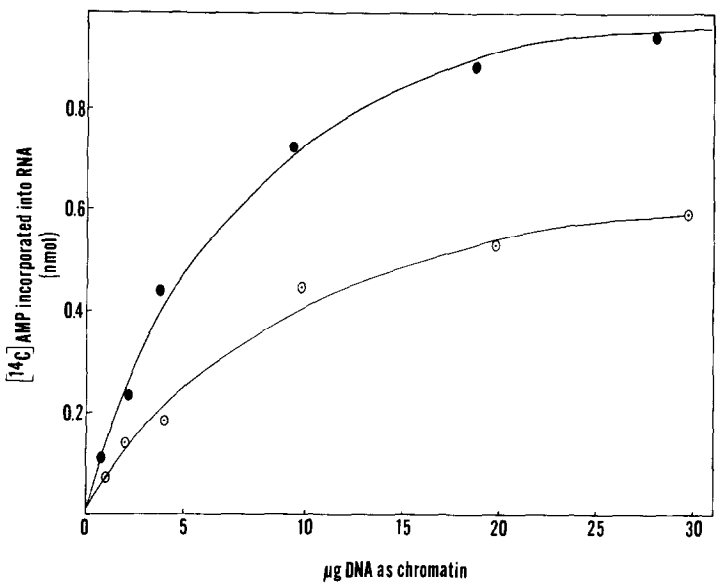

Fig. 1. Template activity of mitotic chromatin. Exponentially growing S_3 HeLa cells were maintained in suspension culture in Eagle's Minimal Essential Medium supplemented with 3.5% calf and fetal calf serum respectively. The cells were synchronized by exposure to 2 mM thymidine for 12 hours, followed by fresh medium for 9 hours and reexposure to 2 mM thymidine for 12 hours. Three hours after release from the second thymidine block, S-phase cells were harvested, and 3 hours later Colcemid (0.5 µg/ml) was added. Mitotic cells were harvested 7 hours after Colcemid addition. 85% of the cells were in metaphase. The cells were washed 4 times with Earle's salt solution and 4 times in 0.080 M NaCl, 0.020 M EDTA, pH 7.2 and 1% Triton X-100; extracted twice with 0.15 M NaCl, 0.01 M Tris-HCl, pH 7.5 and gently homogenized in distilled water. The chromatin was pelleted and washed once in distilled water. RNA polymerase was prepared from early exponential-phase Escherichia coli strain B, purchased from General Biochemicals, Chagrin Falls, Ohio, U.S.A., by the method of Chamberlin and Berg (29), as modified by Bonner et al. (30). The assay of chromatin template activity was as previously described (31). Each point contained 20 µg RNA polymerase. ●——●——●, S-phase chromatin. ○——○——○, mitotic chromatin.

It may be argued that this result is an artefact due to the use of Colcemid in obtaining metaphase arrested cells. To rule out this possibility we repeated the experiment using mitotic cells obtained by selective detachment as described in a previous report (17). An identical result was obtained. Mitotic chromatin isolated from metaphase cells obtained by this procedure had 25% of the template activity for RNA synthesis as S phase chromatin (data not shown). This result legitimated the continued use of Colcemid to obtain large numbers of mitotic cells.

In order to investigate whether the inhibition of template activity seen with mitotic chromatin was due to the chemical composition of the chromatin or to physical changes in the DNA structure per se, we dissociated the chromatin. The DNA was quantitatively removed. Pure DNA isolated from exponentially growing HeLa cells was added back, and chromatin was reconstituted from either mitotic or S-phase chromosomal proteins.

Chromatin was dissociated by suspension in 3 M NaCl, 5 M urea and 0.01 M Tris-HCl, pH 8.3. The DNA was pelleted by centrifugation at 88,000 x g for 48 hours. Table 1 shows the distribution of DNA in the pellet and supernatant. It can be seen that all the ^{3}H-thymidine is found in the pellet. There is no radioactivity in the supernatant. Table 2 shows the distribution of protein in the pellet and supernatant. Ninety-five per cent of the protein has been removed and isolated free of any DNA.

TABLE 1

Dissociation of Chromatin - Recovery of DNA

cpm in pellet	cpm in supernatant	% DNA in pellet
47×10^6	0	100

Exponentially growing HeLa cells were labeled with 0.4 µCi/ml ^{3}H-thymidine (6.7 Ci/mmol) for 24 hours. Chromatin was isolated and dissociated as described in the text. The DNA was pelleted by centrifugation at 88,000 x g for 48 hours.

TABLE 2

Dissociation of Chromatin - Recovery of Protein

cpm in Pellet	cpm in Supernatant	% Protein in Supernatant
42,812	834,724	95%

Exponentially growing HeLa cells were labeled with 0.2 μCi/ml ^{3}H-leucine (58 Ci/mmol) for 24 hours. Chromatin was isolated and dissociated as described in the text. The dissociated chromatin was centrifuged at 88,000 x g for 48 hours.

Using the supernatant fraction containing the chromosomal proteins and DNA isolated from the nuclei of log phase HeLa cells by the method of Quincy and Wilson (32), chromatin was reconstituted by recombination of DNA and proteins in 3 M NaCl, 5 M urea and 0.01 M Tris-HCl, pH 8.3, followed by removal of NaCl by gradient dialysis (33). Dialysis was carried out for a minimum of 3 hours against 200 volumes of 5 M urea; 0.01 M Tris-HCl, pH 8.3 containing 1.5 M, 1.0 M, 0.8 M, 0.6 M, 0.4 M, and 0.2 M NaCl respectively. The chromatin was then dialyzed against 200 volumes of 5 M urea - 0.01 M Tris-HCl, pH 8.3 overnight and pelleted by centrifugation at 30,000 x g for 30 minutes.

Figure 2 compares the adsorption spectrum of native and reconstituted chromatin from S-phase and mitotic cells respectively. It can be seen that in each case the reconstituted chromatin has an identical spectrum to its native counterpart. By this criterion there is a faithful reconstitution.

It can be seen in Fig. 3 that chromatin reconstituted from mitotic proteins shows depressed template activity with respect to chromatin reconstituted from S-phase proteins. The difference in template activity and the absolute amount of RNA synthesized is practically identical to that seen with native S-phase and mitotic chromatin respectively. The depression in template activity seen with mitotic chromatin must, then, be due to the presence of some factor(s) in the protein fraction as isolated in these experiments.

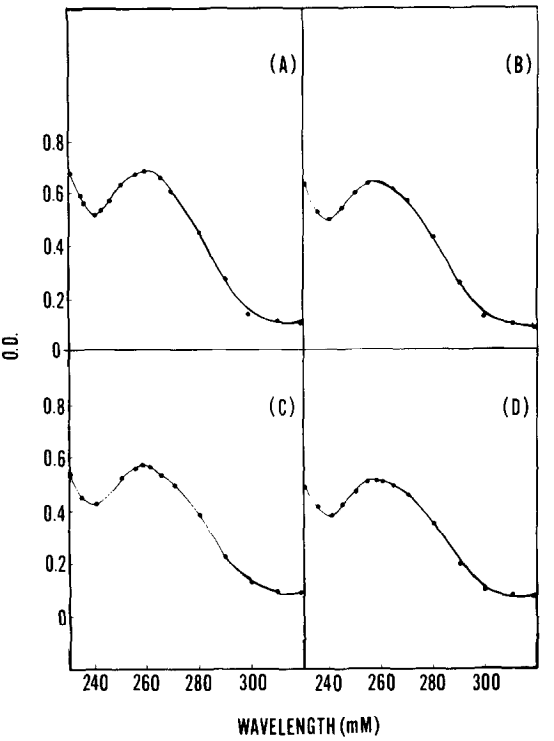

Fig. 2. Adsorption spectrum in 0.91 M Tris-Cl, pH 7.9 of(a) native S-phase chromatin; (b) reconstituted S-phase chromatin; (c) native-mitotic chromatin, and (d) reconstituted mitotic chromatin. Chromatin was isolated, dissociated and reconstituted as described in the text and in legend to Fig. 1.

In one final experiment we show that the magnitude of the depression of template activity depends upon the amount of this factor(s) present in the mitotic protein fraction. Chromatin was reconstituted from log phase DNA and equal amounts of the protein fractions from both S-phase and mitotic chromatin. The reconstituted chromatin showed a template activity intermediate between the fully active S phase and the depressed mitotic chromatin (Fig. 3). The degree of inhibition in template activity appears to depend entirely on the dosage of some factor(s) in the mitotic protein fraction. This confirms that the inhibition of template activity is due to a difference in the chemical composition of the protein fraction in mitotic chromatin with respect to S-phase chromatin. The cell must synthesize this factor late in G_2 because the template activity of mid G_2 chromatin is

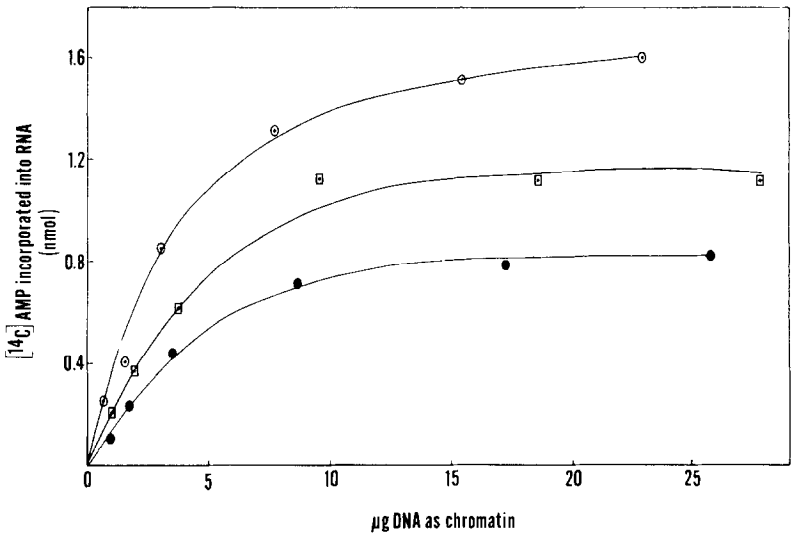

Fig. 3. Chromatin template activity of reconstituted chromatin. Chromatin was reconstituted from homologous log phase DNA and the protein fraction of dissociated chromatin as described in the text. The mixed reconstituted chromatin was prepared at the same initial protein/DNA ratio as used to reconstitute mitotic and S-phase chromatin but half the proteins came from the mitotic and half from S-phase protein fractions. ○——○——○, S phase; ●——●——●, mitotic; □——□——□, mixture of S-phase and mitotic protein fractions.

identical to that of S-phase chromatin (data not shown). The combination of this factor with DNA during mitosis must act to inhibit RNA transcription.

This repressor is most likely a protein, as recent findings indicate that the DNA and chromosomal proteins used for chromatin reconstitution did not contain any detectable amounts of RNA (34). Several reasons implicate an acidic, non-histone protein as the most likely candidate for the repressor. It has been shown previously that chromatin condensation at mitosis occurs without any detectable changes in the quantity or types of histones associated with DNA (35-36). A change in some specific property of existing histone may lead to condensation. However, these same studies showed that metaphase chromatin contains far greater quantities of non-histone protein. In light of the evidence implicating these proteins in the regulation of transcription in other systems (18-28), it seems more likely that a qualitative or simply a quantitative change in these proteins is responsible for

the restriction in template activity observed with metaphase chromatin.

Our system, coupled with further fractionation of the protein supernatant, should allow this question to be settled definitively.

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