Histones Associated with Non-nucleosomal Rat Ribosomal Genes Are Acetylated while Those Bound to Nucleosome-organized Gene Copies Are Not*

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Acetylation of histones bound to rat rRNA genes has been studied relative to their organization in chromatin, either as canonical nucleosomes, containing the inactive copies, or as anucleosomal nonrepeating structures, corresponding to the transcribed genes (Conconi, A., Widmer, R. M., Koller, T., and Sogo, J. M. (1989) Cell 57, 753–761). Nuclei from butyrate-treated rat tumor cells were irradiated with a UV laser to cross-link proteins to DNA, and the purified covalent complexes were immunofractionated by an antibody that specifically recognized the acetylated histones. Upon probing with sequences coding for mature rat 28 S RNA, DNA of the antibody-bound complexes was 5–20-fold enriched relative to the total rat DNA. Since the laser cross-links histones to DNA in both active and inactive genes, one cannot distinguish which one of them, or both, are bound to acetylated histones. Alternatively, purified mononucleosomes were immunofractionated, but DNA from the antibody-bound monosomes was not enriched in coding rDNA. Taken together, these results suggest that nucleosome-organized rRNA genes are bound to nonmodified histones and that the acetylated histones are associated with the active, anucleosomal gene copies.

It is now well established that the regulation of gene expression in eukaryotes occurs at the level of chromatin and that transcription needs changes in chromatin structure (1, 2). There is a consensus in the literature with regard to the nucleosome structure upon transcription of protein-coding genes. In contrast, the picture that emerges from studies of ribosomal RNA genes is rather confusing. Electron microscopy analyses demonstrate the presence of organized histone-containing particles in ribosomal chromatin (3–5), in some cases indistinguishable from coprepared naked DNA (3, 5). Many biochemical studies, however, demonstrate the presence of organized histone-containing particles in ribosomal chromatin (6–10). Such a contradiction is not surprising having in mind that in somatic cells only a portion of the repeated rRNA genes are transcribed (11, 12) and that electron microscopy is restricted to transcribed copies, while biochemical analysis assays the entire set of ribosomal genes. An important contribution in this respect was the demonstration by psoralen photo-cross-linking that cells in vertebrates (13, 14) and in yeast (15) contain two types of ribosomal chromatin, one that consists of nucleosomes and represents the inactive genes and one that lacks a repeating structure and corresponds to the transcribed copies. If, however, nucleosomes disappear as distinct entities, it is not clear whether histones are released from or remain attached to the extended DNA. The existence of nucleosome-free ribosomal chromatin as revealed by psoralen cross-linking does not mean absence of histones (13, 16). Moreover, the same authors have shown that histone-DNA interactions, different from those in intact nucleosomes, do exist and allow extensive access of psoralen to histone-complexed DNA (17). Studies on Drosophila melanogaster active ribosomal RNA genes claimed that they are packaged into unstable nucleosome structure (18) (see, however, Ref. 19). Association of histones with transcribed Xenopus laevis rRNA genes in nucleosome-like structures was demonstrated by the ~200-bp spacing of the cleavage sites of topoisomerase I (20). In a study on the chromatin structure of ribosomal genes of the same organism by UV laser-induced histone-DNA cross-links, we found that coding sequences and spacer enhancers and promoters were associated with histones both in actively transcribed embryonic genes and in their silent counterparts in the erythrocytes (21, 22).

The presence of histones on transcribed ribosomal genes raises the question about their postsynthetic acetylation. Generally, the level of histone acetylation is higher in transcriptionally active than in silent chromatin (23, 24). The numerous correlative evidence communicated during the last 30 years was recently fortified by more direct biochemical studies (25–29) and genetic experiments (30, 31). Very recently, the problem faced a new development connected with the role of acetylation of individual core histone species as well as the modification of different lysine residues on the same histone molecule (32–34). It should be stressed, however, that all of these data come from studies on protein-coding genes. The genes transcribed by RNA polymerase I have not been purposefully studied in this respect merely because it was not clear whether they contain histones at all. Two contradictory results have been reported so far, claiming hyperacetylation of H3 in the active nucleolar chromatin from Physarum polycephalum (9), and a lack of significant difference between histone acetylation in nuclei, nucleoli, and active ribosomal chromatin from the same organism (35). This work presents our results on the acetylation of histones bound to ribosomal genes in rat tumor cells, grown in the presence of butyrate to inhibit deacetylation (36). An antibody capable of recognizing acetylated core histones was used to immunoprecipitate cross-linked protein-DNA complexes generated by irradiation of nuclei with a UV laser. The DNA from the antibody-bound complexes, containing both active and inactive rRNA genes, was enriched in...
Acetylation of Histones Bound to Ribosomal Genes

C. Guerin, C. A. Leblond, J. E. DiDonzio, and H. M. Zillikens

Acetylation of Histones Bound to Ribosomal Genes

EXPERIMENTAL PROCEDURES

Growing Cells in Butyrate and Isolation of Nuclei—Guerin ascites tumor cells were inoculated in albinor rats. On day 7 after transplantation the ascites fluid was collected, and the cells were pelleted by low speed centrifugation and resuspended in Dulbecco’s modification of Eagle medium (Flow Laboratories, UK) containing 5% fetal serum, 20 mM sodium butyrate, heparin (1 unit/ml), and 3 mM sodium butyrate. Heparin. The suspension was incubated 12 h at 37°C under gentle shaking. The viability of the cells was monitored by the trypan blue exclusion test.

To isolate nuclei, the cells were pelleted, washed twice in 0.14 M NaCl, once in 10 mM Tris, pH 7.5, 0.14 M NaCl, 3 mM MgCl2, 1 mM phenylmethylsulfonyl fluoride, 10 mM sodium butyrate and then suspended in the same buffer supplemented with 0.5% Triton X-100. After incubation for 10 min on ice, the suspension was centrifuged, and the pellet was washed twice in the buffer without Triton X-100. The final nuclear pellet was stored in 0.1 M NaCl, 50 mM Tris, pH 7.5, 10 mM sodium butyrate.

Preparation of Nucleosomes—Nuclei were resuspended in digestion buffer containing 10 mM Tris, pH 7.5, 10 mM NaCl, 10 mM sodium butyrate, 1 mM CaCl2, 0.1 mM phenylmethylsulfonyl fluoride at a concentration of 3 mg/ml DNA and digested with 240 units/ml micrococcal nuclease (Sigma) for 15 min at 37°C. The digestion was stopped with 5 mM EDTA, and the suspension was chilled on ice and centrifuged for 10 min at 3000 × g. The supernatant was saved, and the pellet was resuspended in 6 mM sodium butyrate, 0.25 mM EDTA. Following an incubation on ice for 30 min, the suspension was centrifuged as above, and the supernatant was added to the first one. The combined supernatants were concentrated with Centricon microconcentrator (Amicon), loaded on a 5–25% sucrose gradient containing 10 mM Tris, pH 7.5, 0.1 M NaCl, 6 mM sodium butyrate, 0.25 mM EDTA and centrifuged in a Beckman SW28 rotor at 24,000 rpm for 19 h at 5°C. The mononucleosome fraction was collected and dialyzed overnight against the buffer for immunoprecipitation of mononucleosomes.

Cross-linking of Proteins to DNA in Nuclei by UV Laser and Isolation of Protein-DNA Complexes—A nanosecond Nd:YAG laser was used to irradiate nuclei (and, when necessary, the mononucleosomes) suspended in 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 10 mM sodium butyrate at a concentration of about 5 × 106 units/ml. Generally, irradiation at 266 nm was performed in a rectangular fused silica cuvette under constant stirring at a pulse energy of 7 mJ, diameter of the beam 0.5 cm, and repetition rate 0.5 Hz as described elsewhere for the picosecond regime of irradiation (37). In some experiments the cross-linking was performed by single-pulse laser irradiation, using a flow cuvette and computer-commanded laser pulse (a technology to be published elsewhere) formed by single-pulse laser irradiation, using a flow cuvette and computer-commanded laser pulse (a technology to be published elsewhere) with essentially the same result. The cross-linked protein-DNA complex was immunoprecipitated with the antibody as described elsewhere (21). Briefly, 100 μl of IgG Sorb (The Enzyme Center, Malden, MA) were suspended in 0.5 ml of 1% bovine serum albumin (BSA) in phosphate-buffered saline and agitated 30 min at 20°C to block the sites for nonspecific absorption. After centrifugation, the pelleted was suspended in a 0.5-M NaCl mixture of cross-linked material and antibody in 50 mM HEPES, pH 7.5, 2 mM NaCl, 0.1% SDS, 1% Triton X-100, 1% sodium deoxycholate, 5 mM EDTA, 0.1% BSA, and incubated overnight at 4°C under constant shaking. Following centrifugation, the pellet was washed five times in the same solution, three times in 50 mM HEPES, pH 7.5, 0.15 M NaCl, 5 mM EDTA, and the remaining material was eluted with 3.5 M KSCN in 20 mM Tris, pH 8.2.

Purified mononucleosomes were immunoprecipitated following a protocol described elsewhere (25) except that IgG Sorb was used to bind antibodies (see above), and all incubations were carried out overnight at 4°C.

Hybridization—All DNA preparations were purified by treatment with RNase (50 μg/ml, 20 min, 37°C), followed by Proteinase K digestion (100 μg/ml for at least 4 h at 37°C), extraction with phenol-chloroform, and precipitation with ethanol. The DNA samples to be immobilized on membranes were denatured in 0.5 M NaOH, 1.5 M NaCl for 10 min at 37°C and 1 min in boiling water and dotted on Hybond N filters (Amersham Corp.). The filters were immersed in 0.5 M NaOH, 1.5 M NaCl for 5 min and then in 0.5 M Tris, pH 7.2, 2% NaCl for 30 s, blotted dry, and exposed to UV light for 2 min to cross-link DNA to the filter. The filters were prehybridized in 5 × SSC (1 × SSC = 0.15 M NaCl, 0.015 mM sodium citrate), 5 × Denhardt’s solution, 0.5% SDS, 50 mM phosphate buffer, pH 7.0, 200 μM denatured salmon sperm DNA for 2 h at 62°C. Hybridization was carried out under the same conditions for 16 h at 62°C using DNA labeled by random priming to specific activities of 2–9 × 108 cpm/μg at a final concentration of 100 μg/ml. Following hybridization, filters were washed twice in 2 × SSC, 1% SDS for 15 min at 65°C and twice in 1 × SSC, 0.1% SDS for 15 min at 65°C. Filters were blotted dry and autoradiographed at −80°C using intensifying screen. The hybridization signals were quantified by densitometry of the exposed film.

Acetylation—Histones were extracted with 0.25 M HCl, precipitated with 20% trichloroacetic acid, and separated by electrophoresis in 15% polyacrylamide/acytac acid/urea/Triton gels (39).

RESULTS

The experimental approach we followed is outlined in Fig. 1. Cross-linking was used to assay the acetylation of histones, bound to the RNA genes regardless of whether they were wrapped in nucleosomes or existed in an extended nucleosomal conformation. The experiments with the purified mononucleosomes addressed the same question solely for the nucleosome-organized gene copies.

Antibody Characterization—The polyclonal antibody we raised should meet certain criteria in order to serve our purpose. It should be specific for the ε-N-acetyl group of lysine and must not recognize the nonmodified parental molecules. This is illustrated in Fig. 2. Using ELISA, a clear reaction of the antibody with chemically acetylated H4 is observed while the reaction with nonacetylated H4 is similar to that observed with the nonimmune IgG (Fig. 2a). The ability of the antibody to react with the ε-N-acetyl group of proteins other than H4, the histone that was used to raise the antibody, is demonstrated by chemically acetylated bovine serum albumin (Fig. 2b). The antibody was further characterized by inhibition of the immune reaction (Fig. 2c). We show this test not only to confirm the specificity of the antibody but rather to demonstrate its ability to react with the antigen in solution. Such an ability is conditio sine qua non if the antibody is to be used for immunoprecipitation. Besides, as far as the inhibition experiments have been carried out with physiologically acetylated histones, this test justifies the application of the antibody in studying histone acetylation in nuclei. The same result was obtained when the inhibition was carried out with cross-linked protein-DNA complexes. This means that linking of the antigen to DNA by UV laser does not affect the binding ability of the antibody.

1 The abbreviations used are: ELISA, enzyme-linked immunosorbent assay; BSA, bovine serum albumin.
Another characteristic is the reaction of the antibody with histones as a function of the number of acetylated lysines. To test this, acetylated histones from tumor cells grown in the presence of butyrate were separated on a polyacrylamide gel, blotted on filters, and revealed with the antibody. Acetylation of H4, which is best separated in this gel, is presented in Fig. 2d. Again, the antibody showed no reaction with nonacetylated H4. The immunoreaction increases upon increasing the level of acetylation; the most intensive bands on the immunoblot are the tri- and tetraacetylated forms of H4, while the protein pattern of the histone is dominated by its mono- and diacetylated molecules. The antibody, therefore, recognizes any acetylated H4 molecule, but the reaction is much stronger with hyperacetylated forms.

The precipitation ability of the antiacetyl antibody is demonstrated in Fig. 3. The electrophoretic analysis of the antibody-bound fraction shows the presence of acetylated molecules of histones H4, H3, and H2B. This is well illustrated with histone H4, which is best resolved in the electrophoretic system used; the antibody-bound fraction contains mainly tetra- and triacetylated molecules, while the unbound fraction is enriched for unmodified and low acetylated forms of H4.

Antibody-bound DNA from Cross-linked Protein-DNA Complexes Containing Both Nucleosome-organized and Anucleosomal rRNA Genes Is Enriched in Sequences Coding for rRNA—Following irradiation, nuclei were sonicated to reduce the size of DNA to about 300 base pairs and passed through CsCl to separate the covalently linked protein-DNA complexes from free DNA and proteins. Such a fragment size was used to avoid a situation when tandemly arranged rRNA genes could be a part of a long stretch of nonribosomal DNA, carrying covalently linked acetylated histone. After immunoprecipitation, equal quantities of precipitated DNA ("bound" DNA) and nonprecipitated DNA ("unbound" DNA) were screened for the presence of coding rDNA sequences, using as a probe the plasmid p20 containing a 1.6-kilobase pairs BamHI-EcoRI fragment coding for mature 28 S rat rRNA. This fragment has been subcloned from λRrlV into pBR322 (40). Bound, unbound, and total rat DNA (DNA purified from the cross-linked protein-DNA complex).
Acetylation of Histones Bound to Ribosomal Genes

**FIG. 4.** Dot hybridization analysis of DNA immunoprecipitated with antiacetyl antibody from cross-linked protein-DNA complexes. Total rat DNA is DNA purified from the cross-linked complexes, which were fractionated by the antibody into bound and unbound DNA. Three identical filters were prepared for hybridization, each one containing three dots of immobilized total rat DNA (1.0, 0.5, and 0.25 μg) and three dots of p20 DNA, containing BamHI-EcoRI fragment of rat rDNA, coding for 28 S RNA (corresponding to 1.0-, 0.5-, and 0.25-μg insert) and were hybridized to **32P**-labeled total rat DNA (A), bound DNA (B), and unbound DNA (C), respectively. The three hybridization mixtures were prepared in such a way as to contain an equal quantity of DNA (based on *[^3H]*-thymidine incorporation) and an equal amount of **32P**-radioactivity in equal final volumes. The content of ribosomal DNA sequences in the tested DNA preparations is illustrated by their hybridization to p20 DNA (bottom lane), the enrichment of the bound DNA for coding rDNA and, respectively, the depletion of the unbound DNA (see "Discussion").

**DISCUSSION**

Since the presence of histones on transcribed rDNA has been demonstrated, it was reasonable to examine their level of acetylation. The evidence so far reported that links histone acetylation to transcription was based on studies with transcribed and repressed protein-coding genes. Therefore, a study designed to assay the acetylation of histones bound to rDNA is justified only if it addresses the problem active/inactive genes. The interpretation of any biochemical study of ribosomal genes designed to assay the acetylation of histones bound to rDNA is justified only if it addresses the problem active/inactive genes.

**FIG. 5.** Dot hybridization analysis of DNA from immunoprecipitated mononucleosomes. Total rat DNA was isolated from nuclei and sonicated to about 300 base pairs; input DNA is isolated from purified mononucleosomes before their fractionation into unbound and antibody-bound DNA. Filters and hybridization mixtures were prepared as described in the legend to Fig. 4. The content of rDNA in bound and unbound DNA can be seen as C to B and D to B signal ratios, respectively (bottom lane). Upon immunofractionation of monosomes, the input DNA showed somewhat reduced content of rDNA as compared to total DNA (see "Discussion"). The asterisk marks the position of mononucleosomal DNA.

The observed enrichment of bound DNA in ribosomal genes might eventually be due to a specific affinity of rDNA to IgG Sorb, unrelated to the presence of the antibody. This possibility was tested by two experiments in which the antibody was either omitted from the solution with which the cross-linked complexes were incubated prior to addition to IgG Sorb, or replaced by nonimmune IgG. The amount of material absorbed under these conditions did not exceed 5% of that absorbed in the presence of the antiacetyl antibody. What is the nature of this DNA, an average probe DNA or selectively attached rDNA? To determine this, equal amounts of genomic rat DNA and DNA from nonspecifically absorbed complexes were analyzed for the presence of rDNA by hybridization to p20 DNA as described above. The signals obtained with the two DNA preparations did not significantly differ (not shown), i.e. nonspecifically absorbed DNA is bulk DNA. This holds true upon immunoprecipitation of noncross-linked chromatin.

Antibody-bound DNA upon Immunoprecipitation of Monosomes Is Not Enriched in Coding rDNA—Sucrose gradient-purified mononucleosomes were directly immunoprecipitated with the antiacetyl antibody and were further processed as the cross-linked material. A typical autoradiography of a dot hybridization analysis is shown in Fig. 5. In this case the hybridization of antibody-bound and unbound DNA to p20 was compared to the hybridization of "input" DNA (DNA isolated from the purified mononucleosomes before precipitation) to p20. In the above experiments using the cross-linked protein-DNA complexes from irradiated nuclei, the input DNA is in fact a total DNA since the laser cross-linking is a random process. In contrast, nucleases preferentially cleave transcriptionally active chromatin, so that the resulting mononucleosomes might be deficient in certain DNA sequences. In our experiments the input DNA showed somewhat reduced content of rDNA compared to total DNA (Fig. 5, A and B). One explanation of this finding suggests that micrococcal nuclease treatment has preferentially eliminated nuclease-sensitive active (anucleosomal) ribosomal gene copies. The results of Fig. 5 could also be obtained if rDNA has not been cleaved to monosomes upon digestion of nuclei and hence could not have been in this fraction. An efficient protection of rDNA from nuclease attack in comparison to bulk chromatin was reported but, nevertheless, rDNA of monomeric size has been obtained. In our digestion experiments the presence of rDNA in the ladder of DNA fragments, including monomeric ones, was demonstrated by Southern blot analysis using p20 DNA (Fig. 5, inset). Other independent experiments with precipitated mononucleosomal DNA showed a signal-ratio bound DNA/p20 DNA/input DNA of 1 or less (Fig. 5). It follows, therefore, that the histone molecules associated with nucleosome-organized rRNA gene copies are not acetylated. Essentially the same results were obtained upon immunoprecipitation of laser-irradiated monosomes.

**FIG. 6.** Autoradiography of dot hybridization analysis showing the enrichment of ribosomal genes in antibody-bound DNA (A) compared to unbound DNA (D). Three identical filters were prepared for hybridization, each one containing three dots of immobilized total rat DNA (1.0, 0.5, and 0.25 μg) and three dots of p20 DNA, containing BamHI-EcoRI fragment of rat rDNA, coding for 28 S RNA (corresponding to 1.0-, 0.5-, and 0.25-μg insert) and were hybridized to **32P**-labeled total rat DNA (A), bound DNA (B), and unbound DNA (C), respectively. The three hybridization mixtures were prepared in such a way as to contain an equal quantity of DNA (based on *[^3H]*-thymidine incorporation) and an equal amount of **32P**-radioactivity in equal final volumes. The content of ribosomal DNA sequences in the tested DNA preparations is illustrated by their hybridization to p20 DNA (bottom lane), the enrichment of the bound DNA for coding rDNA and, respectively, the depletion of the unbound DNA (see "Discussion").
analyze directly the active genes is a difficult task, one has to isolate a chromatin fraction that is not organized in nucleosomes. Another approach is to compare mammalian somatic cells with markedly different levels of rRNA synthesis. This does not solve the problem, however, because according to the same studies (13, 14), the 1:1 ratio of active versus inactive ribosomal gene copies remained constant, independent of the transcriptional activity of these genes. However, one can easily isolate mononucleosomes, shown to represent the inactive ribosomal chromatin (13). Accordingly, our experimental approach consisted of two parallel procedures. The first one assesses the acetylation of histones bound to both active and inactive ribosomal genes. This was accomplished by cross-linking proteins to DNA in the nuclei by irradiation with UV laser. An important property of the laser is that it cross-links in nanosecond time intervals, thus “freezing” in vivo existing protein-DNA interactions (37, 41). It must be mentioned that the reversible acetylation of histones does not affect their cross-linking to DNA, although the covalent link between histones and DNA was shown to proceed via the N-terminal tails (43), where the acetylatable lysines had been located. After cross-linking, an antibody that specifically recognizes acetylated histones but not their nonmodified parental molecules was used to select DNA fragments linked to acetylated histones. DNA of these fragments was then analyzed for the presence of sequences coding for mature rat 28 S RNA. The second procedure aimed to assay the acetylation of histones bound to the inactive nucleosome-organized rRNA genes. To this end, mononucleosomes from micrococcal nuclease-digested nuclei were immuno-fractionated, and DNA of the antibody-bound nucleosomes was analyzed as above.

To prove that hyperacetylated histones were responsible for the immunoprecipitation, proteins of the antibody-bound fraction were analyzed by polyacrylamide gel electrophoresis (Fig. 3). The preferential precipitation of the hyperacetylated histone molecules is well illustrated with H4. The bands of tri- and tetraacetylated molecules dominated the picture. H2B and H3 were also acetylated. Such a result is to be expected, because histones are the best known acceptors of acetyl groups (23). Beside them, the only nuclear proteins shown to undergo acetylation are HMG proteins (23). The lack of hyperacetylated forms of these proteins as well as their much lower quantity, compared to histones, makes their contribution to precipitation negligible, if any. Nevertheless, the antibody-bound fraction was dotted on filters and reacted with a biotinylated anti-HMG1 antibody, which cross-reacted also with HMG2. No reaction was observed (data not shown). As for HMG14/17, their amount is much lower than that of HMG1/2.

The antibody-bound DNA obtained upon immunofractionation of the cross-linked protein-DNA complexes was 5–20-fold enriched in coding rDNA sequences. This means that rDNA gene copies have been associated with acetylated histones. The question is which of them, the active genes, the inactive ones, or both? By analogy with protein-coding genes one may assume that at least the transcribed copies should be acetylated. However, the possibility that all rDNA genes were associated with acetylated histones could not be ruled out. The question was answered by the alternative approach, immunoprecipitation of monosomes, shown to contain the inactive rDNA (13). The antibody-bound monosomal DNA contained coding rDNA sequences in an amount that did not significantly differ from that of the input DNA. The lack of enrichment is interpreted to mean that nucleosome-organized rRNA genes have been associated with nonacetylated histones. It follows, therefore, that the acetylated histones that enriched the antibody-bound fraction of the cross-linked protein-DNA complexes in coding rDNA have been associated with the rest of the gene copies, the anucleosomal ones, claimed to be transcriptionally active (13).

To check the reliability of both the experimental approach and the antibody in selecting defined DNA sequences, in a control experiment we assayed the distribution of DNA from centromeric heterochromatin, shown to be associated with underacetylated histones (34, 44). To this end, UV laser cross-linked histone-DNA complexes from mouse cells were immunoprecipitated with either the antiacetyl antibody used in this study or an antibody against histone H2A. The two antibody-bound fractions were analyzed for the presence of mouse satellite DNA. The content of satellite sequences in the anti-H2A-precipitated DNA was similar to that in bulk mouse DNA, while in the antiacetyl antibody-bound DNA the amount of satellite sequences was dramatically reduced.2

Another conclusion from the experiments with the ribosomal genes is that immunofractionation on the basis of acetylated histones resulted also in fractionation of DNA, separating a subset of it (antibody-bound DNA) which differs in sequence complexity from total DNA. This can be seen upon comparing the hybridization signals total DNA/total DNA and bound DNA/total DNA (upper lines of Figs. 4, A and B, and 5, A and C). Under the conditions of the experiment, when equal amounts of total rat DNA were dotted on the membranes and hybridized to equal amounts of 32P-labeled total DNA and bound DNA, the signal bound DNA/total DNA has been repeatedly found lower than the signal total DNA/total DNA. The dependence of the filter hybridization on the amount of reiterated DNA on one hand and, on the other, the experimental evidence that the fractionation of chromatin fragments on the basis of acetylated histones results in selection of transcribed protein coding DNA (25, 26, 45), which generally represents single copy genes, suggest an explanation of this finding.

An intriguing observation in the above cited studies of ribosomal chromatin by the psoralen strategy (13–15) was that in vertebrates the changes in the rate of rRNA synthesis did not result in changes in the ratio of active/inactive ribosomal chromatin structures (13, 14), while yeast can rapidly change the portion of active genes in response to altered growth conditions (15). A conclusion was made that the regulation of rRNA synthesis in vertebrates is achieved at the level of transcription initiation of the available anucleosomal genes rather than by activation/inactivation of gene copies (15). It was recently reported that genes which are not transcribed at a moment but are “paused” to transcription have been associated with acetylated histones (26, 45, 46). In the light of this evidence, our data that histones associated with anucleosomal (transcribed) ribosomal genes are acetylated, while those bound to nucleosome-organized (inactive) gene copies are not, support such a view for the modulation of rRNA synthesis.

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