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DIFFERENTIAL STABILITY OF THE HIGHER ORDER STRUCTURE OF CHROMATIN ASSOCIATED WITH GENES HAVING DIFFERENT TRANSCRIPTIONAL ACTIVITY

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We have investigated the stability of the higher order structure of chromatin associated to genes which display a different transcriptional activity in adult rat liver. Nuclei were digested with micrococcal nuclease and chromatin was fractionated by sedimentation in sucrose gradients. Specific DNA sequences were revealed by dot-blotting. In conditions of physiological ionic strength the distribution of the inactive γ-casein gene sequences is similar than the bulk of chromatin. In the same conditions the relative content of the albumin gene, highly expressed in adult rat liver, revealed an enhanced instability of the chromatin superstructure. The distribution of the potentially active but silent α-fetoprotein sequences in adult liver showed an intermediate unfolding of its chromatin superstructure. These distinct behaviour was not observed in non-physiological ionic strength conditions. Our results suggest that distinct folding of the local higher order structure of chromatin actually occurs in the region of active, potentially active and inactive genes.

The use of nucleases such as DNase I or micrococcal nuclease as probes for chromatin structure has allowed to show that genes active in transcription in a given system adopt a distinct conformation as compared with the bulk of chromatin. The effects observed are either located in precise zones in the vicinity of coding sequences, such as DNase I hypersensitive sites (1), or occuring in a large area surrounding the transcribed sequences. The maintenance of the nucleosome structure in the active sequences has been proposed except for the hypersensitive sites or very actively transcribed genes. The obvious corolary to this proposition is that the structural changes observed may correspond to effects on the H1-dependent higher-order structure of chromatin (2).

It has been shown that chromatin preparations extracted at low temperature and at physiological ionic strength, after mild digestion of nuclei with micrococcal nuclease, show patterns of sedimentation in sucrose gradients that are very sensitive to conditions that may alter the higher-order structure of chromatin (3,4). In the present study this approach has been used to check whether genes having a different state of transcriptional

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activity show a different behaviour that may be interpreted as the consequence of being present in chromatin domains with a differential stability in their higher-order structure. In particular the correlation of the distribution of active sequences with the presence of HMG-proteins studied in a previous study (5) has been investigated.

MATERIALS AND METHODS

Chromatin preparation. Adult rat liver nuclei and chromatin have been prepared by homogenization and mild micrococcal nuclease digestion at 4ºC with 40 or 140 mM KCl as described (5). The chromatin was extracted by dialysis against 40 or 140 mM KCl, 0.2 mM EDTA, 0.5 mM PMSF, 10 mM Tris-HCl, pH 7.4 and sedimented through 5-20 % sucrose gradients in the same buffer. 0.5 ml samples were loaded onto the gradients and centrifuged in a Beckman SW40 Ti rotor for 90 min at 39000 rpm. The gradients were monitored at 254 nm trough an ISCO monitor and 1 ml fractions were collected.
Hybridization conditions. DNA from the different chromatin fractions was prepared by treatment with 0.2 % SDS, 0.2 mg/ml of Proteinase K (Merck) at 37ºC overnight, extracted with phenol/chloroform and ethanol precipitated. Serial dilutions of DNA samples were dotted onto nitrocellulose filters (BA 85, Schleicher and Schüll) according to Kafatos et al. (6). Prehybridization and hybridization was done as described elsewhere (7). DNA probes were prepared by nick-translation and they routinely had a specific activity of 1.5×10^8 dpm/ µg (8). Cloned cDNA probes were used, for albumin a 1:1 mixture of plasmids pRSA57 and pRSA13 (9), for α-fetoprotein a 1:1 mixture of plasmids pRAFP87 and pRAFP65 (10) and finally for γ-casein plasmid pC031 (11) used as a control. After hybridization sheets were washed, dried and exposed to X-ray films for 1 to 5 days at -80ºC. The autoradiographies were scanned with a Chromoscan 3 (Joyce Loebl) microdensitometer. Results were expressed as the average densitometric value obtained from 8 and 4 µg of DNA from each fraction or as the relative enrichment of active versus inactive genes in each chromatin fraction.

RESULTS

The stability of the higher order structure of chromatin associated to genes with a different state of activity in adult rat liver has been studied by analyzing the distribution of their sequences in chromatin fractions prepared by micrococcal nuclease digestion of nuclei and fractionation by centrifugation in sucrose gradients. This approach has been used before to measure the distribution of HMG-proteins in similar fractions of chromatin (5). Under these conditions, depending on the extent of digestion up to 80 % of the total DNA may be solubilized. The presence of specific gene sequences in each fraction was measured by the dot-blot hybridization technique. Three probes were used to measure the presence of specific sequences in the different chromatin fractions. One corresponds to the albumin gene, expressed in adult rat liver (12), the second to α-fetoprotein (AFP), a gene expressed in fetal liver (12) and the third to γ-casein, a gene not expressed in the liver (13). Controls on the fixation to nitrocellulose of DNA fragmented to mono-, tri- and 15-mer nucleosome sizes showed that after a treatment of the

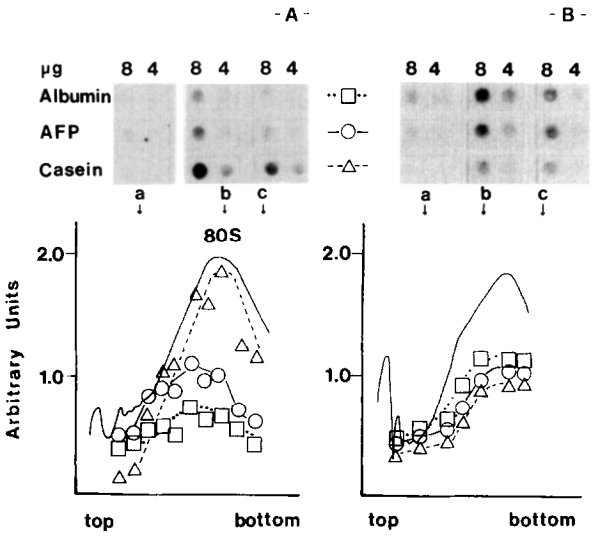

Figure 1 . Distribution of the sequences of the albumin, α -fetoprotein (AFP) and γ-casein genes in different chromatin fractions from a sucrose gradient sedimentation analysis. Chromatin was prepared at 140 mM KCl (A) and 40 mM KCl (B) from rat liver nuclei, and extracted at the same ionic strength, by solubilization after digestion with 160 (A) or 100 (B) units of micrococcal nuclease during 45 min at 4ºC. Detection of specific genes was done by hybridization of the dot-blots with the respective probes albumin (□), AFP (○) and γ-casein (△). Results are expressed as the average densitometric value obtained from 8 and 4 µg of DNA from each fraction. At the top of each graphic are shown autoradiographies of the dot-blots for three characteristic fractions (a,b,c) after hybridization with the three probes. The DNA distribution along the gradient was determined by the absorbance at 260 nm. The vertical arrow points to the position of the hexanucleosome.

filters identical to that used for the hybridization measurement, the relative signal obtained differs in less than 25 % between the different DNA sizes.

Figure 1A shows a typical example of sequence distribution of the three genes after micrococcal nuclease digestion at physiological ionic strength. The time of digestion was chosen to obtain a bimodal pattern with one of the maxima at 80S (3,5). The distribution of γ -casein gene sequences is similar to that of the bulk of chromatin. Albumin and α -fetoprotein gene sequences appear shifted to polynucleosomes sedimenting slightly slowly than the bulk of chromatin. The higher proportion of the non expressed gene (γ -casein) observed may be the consequence of the digestion of a large proportion of the active gene sequences into subnucleosomal particles.

Figure 1B shows that no difference is observed in the distribution of the same genes, through chromatin fractionated after digestion of nuclei at 40 mM KCl ionic strength. Under these conditions, independently of the time of digestion, fractionation of solubilized chromatin never gives rise to a stabilization of the maximum S value. This one progressively decreases as a result of the instability of the high order structure of chromatin at this lower ionic strength (3,5).

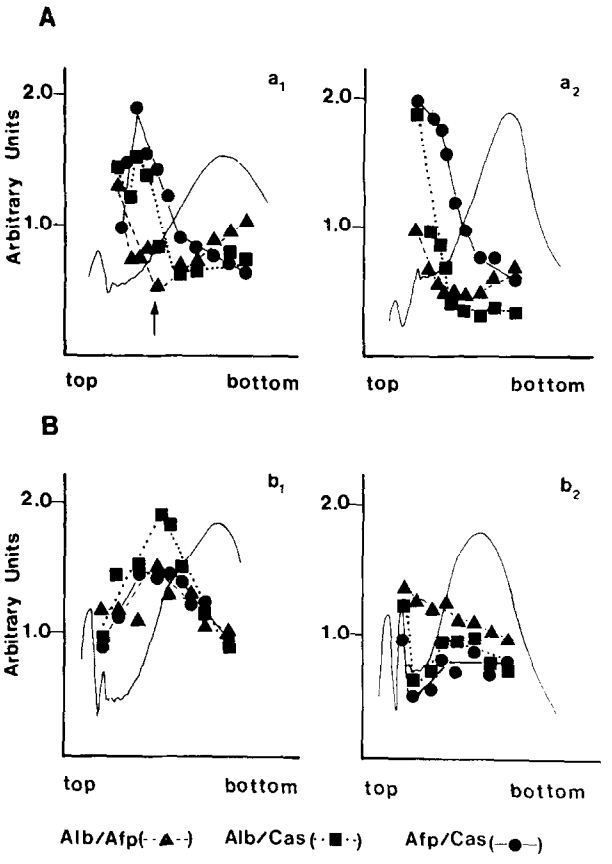

Figure 2 . Relative distribution of the albumin, α-fetoprotein and γ-casein gene sequences in different chromatin fractions from sucrose gradient sedimentation analysis. The relative distribution of the gene sequences was calculated as the ratios albumin/casein (■), AFP/casein (●) and albumin/AFP (▲) using the average densitometric values obtained with 8 and 4 μg of DNA from each fraction. Genes were detected in chromatin fractions from sucrose gradients at 140 mM KCl (A) or 40 mM KCl (B). Chromatin had been obtained by solubilization from nuclei after their digestion with 160 (A) and 100 (B) units of micrococcal nuclease during 30 (a_1), 90 (a_2), 45 (b_1) and 120 (b_2) min.

The relative distribution of the albumin, AFP and γ-casein genes was compared by plotting the ratio between them in the different fractions. Figure 2 shows gradients obtained after two times of digestion by micrococcal nuclease at physiological (A) and intermediate (B) ionic strength. At physiological ionic strength and at short times of digestion the highly expressed albumin gene shows an enrichment in the fractions containing small oligonucleosomes in relation to the γ-casein gene sequences (Fig. 2,a1). A similar observation can be made when α-fetoprotein and γ-casein genes are compared. The relation of the albumin versus α-fetoprotein gene shows a distribution with a minimum in chromatin fragments sedimenting around six nucleosomes (shown with an arrow in the figure). At longer times of digestion (Figure 2,a2) the relative distribution of albumin versus α-fetoprotein gene

sequences remains quite similar, however albumin gene sequences are much more digested than the γ-casein sequences.

With chromatin prepared and analyzed at 40 mM KCl (Figure 2B) the relative distribution of the albumin, α-fetoprotein and γ-casein gene sequences appear very similar at short times of digestion. The albumin/AFP ratio becomes uniform for all gradient fractions at longer digestion times.

DISCUSSION

In our previous works (3-5) we have defined a sensitive method to study the stability of the higher order structure of chromatin by analyzing the sedimentation patterns in sucrose gradient of chromatin preparations extracted at physiological ionic strength and low temperature after nuclei digestion with micrococcal nuclease. In the present study we have used this method to analyze the stability of chromatin associated to active and inactive genes in adult rat liver.

Our results show that at physiological ionic strength, active, potentially active and inactive gene sequences in adult rat liver can be distinguished. Activity correlates with an increased instability in the higher order structure of chromatin, since chromatin associated to active genes (such as albumin) or potentially active genes (such as α-fetoprotein) sediment more slowly than chromatin associated to inactive genes (as γ-casein) which behaves as bulk chromatin. The instability of active genes is also confirmed by a relatively more abundant presence of albumin and α-fetoprotein genes in the smaller oligonucleosomes compared to the γ-casein gene sequences. When the relation of albumin versus α-fetoprotein gene sequences is considered, a distribution reaching a miminum at around six nucleosomes is observed. These results may be the consequence of the behaviour of α-fetoprotein gene intermediate between the albumin and γ-casein gene (see Fig. 1,A) and it could reflect structural differences between a gene who is being actively transcribed and another one that had been transcribed by liver during rat development but it is silent at the adult stage.

Structural chromatin changes associated to the activity of albumin and α-fetoprotein genes as global gene sensitivity to DNase I (14) and micrococcal nuclease (15) or DNase I hypersensitivity (16-18) have been reported. Nevertheless the knowledge on the local higher order structure of chromatin associated to active and inactive genes has been until know poorly studied. By using a similar approach Kimura et al. (19) have demonstrated a permanent unfolding of the chromatin structure of the α-globin gene, after it has been expressed, in mature chicken erythrocyte cells in relation to

inactive genes. These results suggest that potentially active genes are present in a region more labile to structural changes than the inactive genes, but less than the highly expressed genes. Our results show that the genes examined in rat liver behave in agreement with this proposal.

Variations in DNA stress (20), binding of specific histone subpopulations (21) and/or HMG proteins may induce distinct levels of conformational states in chromatin of the active albumin gene, the potentially active AFP gene and the inactive γ-casein gene, inducing distinct conformations of the genes having a different state of activity. In particular, the distribution along the gradient of specific gene sequences described in the present work departs from that of HMG proteins in similar chromatin fractions as reported elsewhere (5). However the relative pattern of active versus inactive genes (Figure 2) is similar to the distribution of HMG proteins (5). The presence of these protein in at least a part of the active chromatin zones or indirectly acting on the DNA structure may be a hypothesis to explain this behaviour.

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