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The premelting of nucleoprotein: role of non-histone proteins

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ABSTRACT

In native nucleoprotein, the premelting structural changes of DNA are not observed by circular dichroism measurements. In order to determine which protein fraction of chromatin is responsible for the absence of premelting we have examined a series of nucleoproteins depleted of different protein fractions by treatment with sodium chloride or sodium deoxycholate.
The premelting reappears as soon as non-histone proteins are removed or in residual complexes from which the two slightly lysine-rich histone fractions (F2a2+F2b) have been removed. On the other hand, it is shown that histone F1 alone is not able to suppress the premelting phenomenon. It is thus concluded that the absence of premelting is a property of native nucleoprotein where interactions between the different proteins complexed with DNA can occur and especially between the non-histone proteins and the two slightly lysine-rich histone fractions.

INTRODUCTION

A number of circular dichroism studies (C.D.) of chromatine have shown that the secondary structure of DNA inside the nucleoprotein complex was different from that of isolated DNA in solution (1 - 10).
Circular dichroism has also been used to follow the conformational changes of DNA and proteins during the melting of nucleoprotein (7 - 10). Circular dichroic melting curves were obtained by recording the variations of ellipticity at 280 nm or 227 nm where the structural changes of DNA and protein can be seen separately. In a recent report (10) we have studied the C.D. melting

curves of native nucleoprotein and of a series of nucleoproteins partially dissociated by NaCl.
For all the histone depleted complexes we have shown that the C.D. melting curve recorded at 280 nm exhibits two maxima. The first maximum has been attributed to the premelting of DNA and the second one was interpreted as a change from the C to the B form of histone bound DNA. In contrast with the histone depleted complexes, no premelting was observed by C.D. measurements for the native nucleoprotein.
Since the first histones to be extracted with sodium chloride are released together with non-histone proteins, we could not determine which protein fraction was responsible for the absence of premelting in the native nucleoprotein.
In the present study we have therefore compared the properties of native and non-histone depleted nucleoprotein.
We have also studied the properties of chromatin dissociated with deoxycholate, a reagent which has been shown to remove histones in a sequence different from that obtained with sodium chloride (11). In this manner the properties of complexes containing mainly the lysine-rich histone F_1 could be studied.

It will be shown that histone F_1 alone is not able to suppress the premelting phenomenon and that the absence of premelting is a property of native chromatin where interactions between the different proteins complexed with DNA can occur.

MATERIAL AND METHODS

Preparation and assay of material

Native calf thymus chromatin was prepared according to

the method of Zubay and Doty (12) except that diisopropylfluorophosphate 10^{-4} M was added to the standart solvant (0.024 M EDTA - 0.075 M NaCl pH 8) to avoid proteolytic degradation.
The non-histone proteins were removed from the chromatin by repeated washing, in 0.35 M NaCl as described by Goodwin and Johns (13). At the end of the preparation the native chromatin and the non-histone depleted chromatin were dialysed against sodium phosphate buffer 10^{-3} M - EDTA 2.10^{-4} M pH 6,8 and thermal denaturation studies were performed in this medium.
The preparation of chromatin dissociated by sodium deoxycholate was made according to Smart and Bonner (11) with the following modifications : the chromatin pellet which was obtained at the end of the Zubay - Doty preparation was directly resuspended in solutions of the desired concentration of deoxycholate.
These chromatin solutions were vigourously stirred and then centrifuged to remove the insoluble material. The subsequent operations were then as described by Smart and Bonner and finally the different partially depleted complexes were dialysed against sodium phosphate buffer 10^{-3} M - EDTA 2.10^{-4} pH 6,8.
The DNA content of chromatin solutions was determined spectrophotometrically by their absorbance at 260 nm using a molar extinction coefficient per nucleotide residue of 6700. Protein content of chromatin was determined by the Lowry method (14).
Protein to DNA weight ratios of native chromatin, non-histone depleted chromatin and deoxycholate treated chromatin are given in table I.

Table 1

Sample	Protein/DNA (w/w)		Histone/DNA (w/w)	Non-histone/DNA (w/w)	Premelting
Native nucleoprotein (1)	1,55		1,15 no histone removed	0,35 no non histone removed	-
0,35M NaCl-nucleoprotein (2)	1,35		1,15 no histone removed	0,15 half of the non-histone removed	+
Deoxycholate treated nucleoproteins	Exp. 1	Exp. 2			
DNP DOC 0,02 (2)	1,21	1,32	F_{2a2}-F_{2b} partly removed		+
DNP DOC 0,03 (2)	0,71	0,65	F_{2a2}-F_{2b} removed F_3-F_{2a1} partly removed		+
DNP DOC 0,04	0,54				+
DNP DOC 0,05	0,33				+
DNP DOC 0,06	0,24				+
DNP DOC 0,07 (2)	0,23	0,28	Traces of F_3-F_{2a1} F_1 still complexed		+

(1) mean value of four experiments
(2) The bound histones were removed by HCl 0,25 N and analysed by disc gel electrophoresis.

For native chromatin the Protein/DNA ratio falls in the range 1,50 to 1,60 and for non-histone depleted chromatin, in the range 1,30 to 1,40. Since calf thymus chromatin has a histone/DNA ratio of 1,15, it appears that about half of the non-histone proteins cannot be removed after repeated washings in 0,35 M NaCl.
A similar result was obtained when the amount of histone and non-histone proteins was measured by using hydroxyapatite chromatography as described by McGillivray et al. (15).
For 0.35 NaCl washed chromatin the amount of non-histone proteins eluted by sodium phosphate buffer 0.05, Urea 5 M, NaCl 2 M was about half of the amount obtained for native chromatin.

Melting curves

Absorbance and C.D. values at a given wavelength were directly recorded in function of temperature as previously described (10). Plots of the absorbance derivative dA/dt versus temperature were computed according to a program made by J. Pouyet.

RESULTS

I. Thermal denaturation of native and 0.35 M NaCl treated nucleoprotein

1. Absorbance melting curves

Figure 1 shows the absorbance melting curves and the corresponding derivative plots of native and non-histone depleted nucleoproteins in sodium phosphate buffer 10^{-3} M EDTA 2.10^{-4} M (pH 6,8). The derivative plot of native nucleoprotein is composed of two melting bands located at 65°C and 82°C with a shoulder near 97°C. For the non-histone depleted nucleoprotein a new melting band appears at 72°C and the main melting band at 82°C is sharpened ; the melting band at 65°C and the shoulder near 97°C are still present. In the same figure we show the melting pattern of native DNA which present a band at 52°C and a shoulder near 61°C.

No melting band is centered close to the melting temperature of free DNA for the two chromatin samples examined. Thus they are no long streches of DNA free of proteins in the native nucleoprotein and furthermore the removal of non-histone proteins does not uncover very long regions of DNA.

On the other hand, it is clear that the removal of non-histone proteins affects the melting profile of well-defined DNA regions without modifying the stability of the bulk of DNA.

2. C.D. melting curve

The C.D. spectra of native and non-histone depleted nucleoprotein are shown in figure 2. The positive bands of the

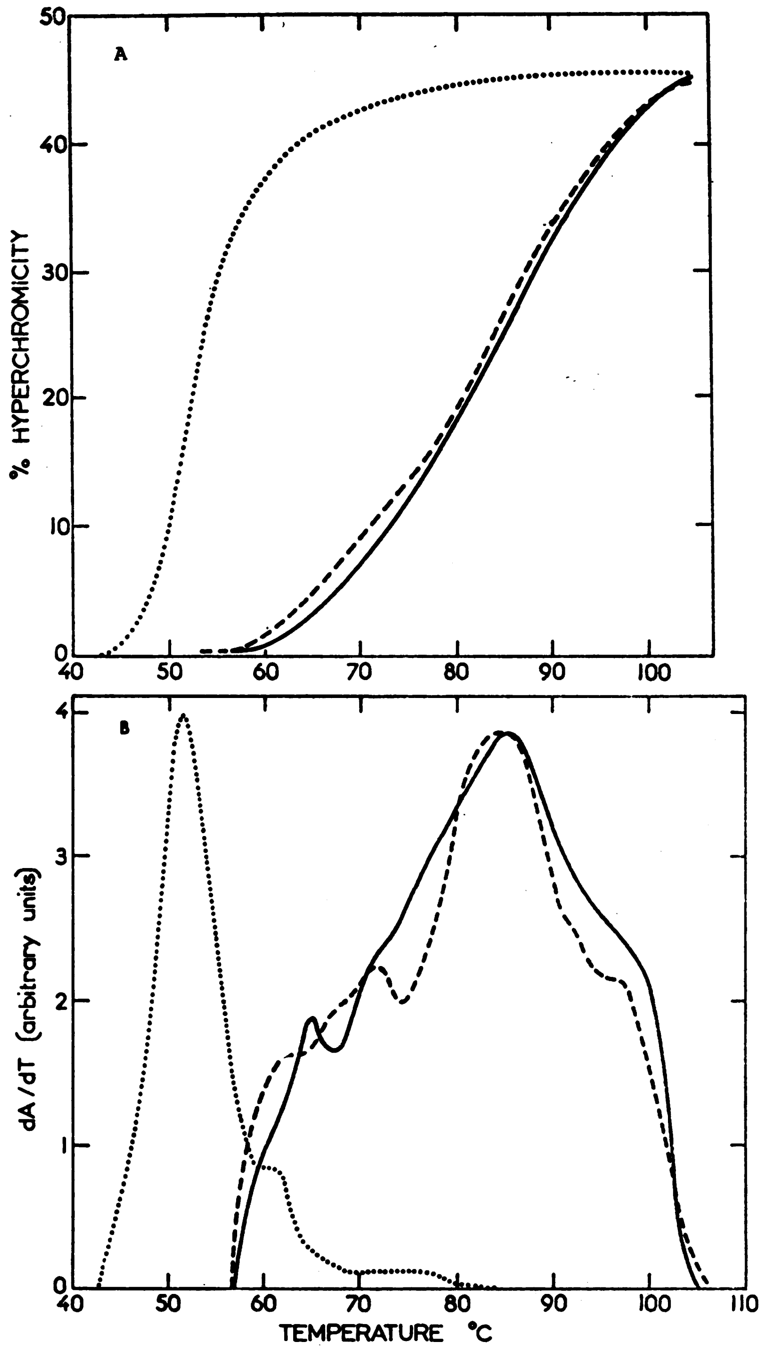

Figure 1 : Absorbance melting curves (A) and derivative melting plots (B) of DNA (....), native nucleoprotein (——) and non-histone depleted nucleoprotein (-----).

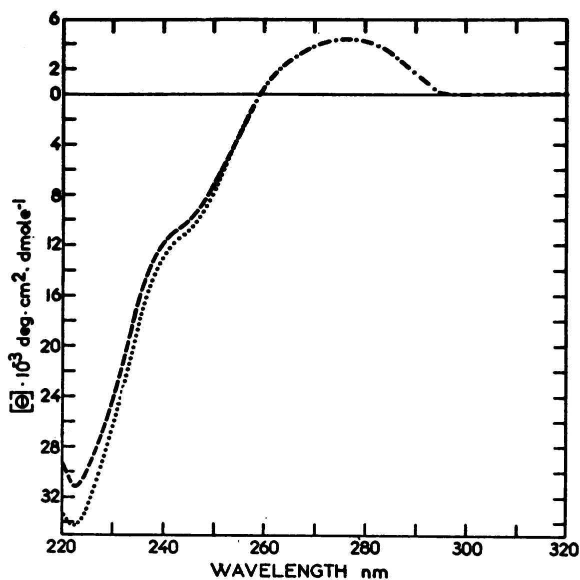

Figure 2 : Circular dichroism spectra of native (.....) and non-histone depleted nucleoprotein (-----).

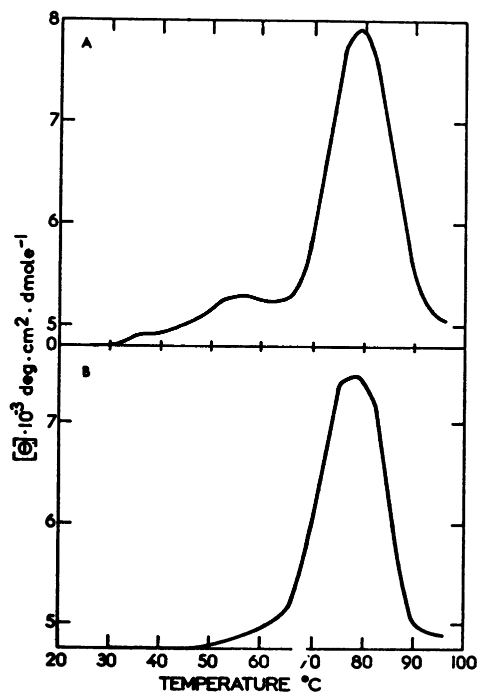

Figure 3 : Dichroic melting curves recorded at 280 nm of (A) non-histone depleted nucleoprotein and (B) native nucleoprotein.

two spectra are very similar and within the experimental errors the value of the ellipticity at the maximum for λ = 280 nm is the same for the two samples. The difference of the intensity of the negative bands can be accounted for by the difference in protein content.

In figure 3 are shown the dichroic melting curves recorded at 280 nm of native and non-histone depleted nucleoproteins. As previously reported the premelting is totally absent in the native nucleoprotein. In the non-histone depleted nucleoprotein an increase of ellipticity is observed in the premelting temperature range, nevetheless the intensity of the ellipticity change is only 1/3 to 1/2 of the increase obtained for pure DNA or for histone depleted nucleoproteins. This result shows that the non-histone proteins removed by washing in NaCl 0.35 M do play a role in the premelting behavior of nucleoprotein. The fact that some non-histone proteins are still bound to the 0.35 NaCl treated nucleoprotein could explain that the premelting is not entirely present in these samples. On the other hand, the question can be raised of a peculiar role of F_1 which would also contribute to the lowering of the intensity of the premelting change. In order to clarify the issue, we have studied the properties of a series of nucleoproteins dissociated by increasing concentration of sodium deoxycholate, a process in which the lysine-rich histone fraction is the last to be removed (11).

II. Circular dichroism and thermal denaturation of nucleoproteins dissociated by sodium deoxycholate

1. Circular dichroism

Figure 4 shows the values of the ellipticity at 280 nm of a series of nucleoprotein samples which have been partially dehistonized by deoxycholate. These values are compared to the values obtained for chromatin samples depleted by NaCl. The data of figure 4 indicate that the removal of a given weight fraction of total protein by deoxycholate results in an increase of the C.D. signal greater than that observed when an equivalent weight fraction of protein is removed by NaCl.
According to Smart and Bonner, 80% of the non-histone proteins are still present in the deoxycholate treated samples and since these proteins were shown not to affect the positive band of the C.D. spectrum of the nucleoprotein, it is necessary to correct the Protein/DNA values of the different samples to estimate the actual contribution of the histones in the C.D. changes of DNA.
When plotted in this manner (that is, ellipticity at 280 nm versus the calculated histone/DNA ratio) the data of figure 4 indicate that the increase of C.D. is only slightly more important in the case of sodium deoxycholate as in the case of NaCl. This result shows that all the histone fractions, including F_1, have a nearly equivalent role on the circular dichroism change of DNA. This conclusion was already postulated on the basis of C.D. melting curves studied previously (10)

2. Melting curves

Absorbance melting curves of nucleoproteins dissociated by sodium deoxycholate have been reported by Smart and Bonner

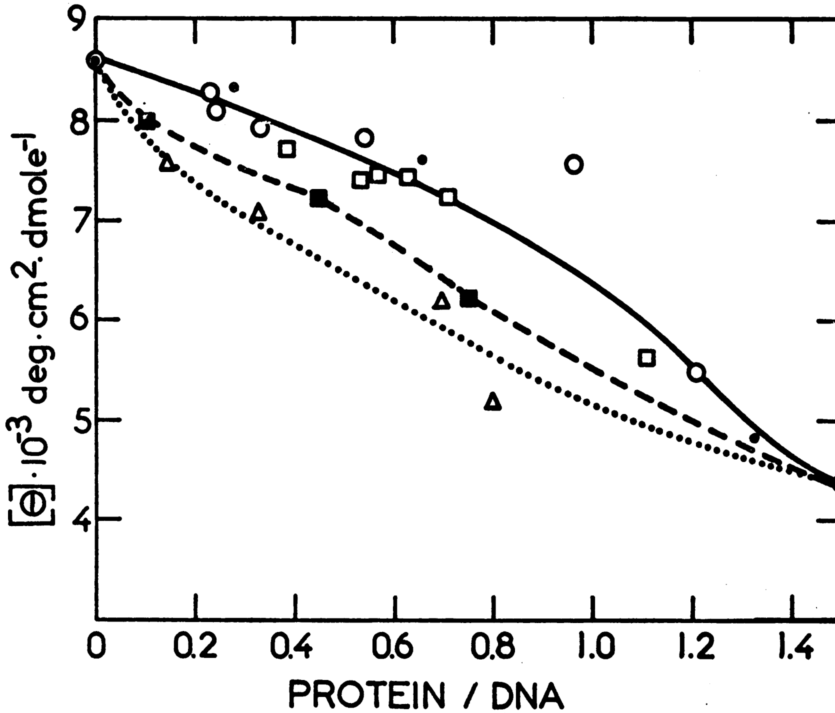

Figure 4 : The variation of ellipticity at 280 nm in function of the Protein/DNA ratio of dissociated nucleoproteins :

(....) nucleoprotein dissociated by sodium chloride
(——) nucleoprotein dissociated by sodium deoxycholate
(----) calculated curve obtained for deoxycholate treated nucleoprotein.

(11) and we have obtained essentially similar results with the samples prepared in our hands (figure 5).

Figure 6 shows the C.D. melting curves recorded at 280 nm, obtained with three different samples of deoxycholate treated nucleoprotein. As in the case of chromatin depleted of histones by NaCl the C.D. melting curves present two distinct melting bands.

A first increase of ellipticity at 280 nm is observed in the premelting range and the maximum occurs at a temperature below that corresponding to the beginning of the hyperchromic effect. The intensity of the C.D. premelting change is the same for all the complexes studied. Of peculiar interest is the fact that the complex dissociated in 0.02 M sodium deoxycholate gives rise to the same premelting C.D. change as the other complexes. In this complex only the slightly lysine-rich histones F_{2b} and F_{2a2} and a small amount of non-histone proteins have been removed. This means that these two histone fractions are of great importance in the premelting behaviour of DNA. On the other hand, the lysine-rich histone fraction F_1 is integrally present in this complex and is the main histone fraction complexed with the nucleoprotein dissociated by 0.03 M and 0.07 M sodium deoxycholate. Our result thus shows that F_1 alone is not able to change the premelting behaviour of DNA.

The second band observed in the C.D. melting curves takes place in the range 65°C - 69°C where a trough occurs in the derivative plots of the absorbance melting curves and before the melting of the most stabilized DNA regions. As in the case of nucleoproteins dissociated by NaCl (10) this band can thus be interpreted as a change from the C to the B form of the

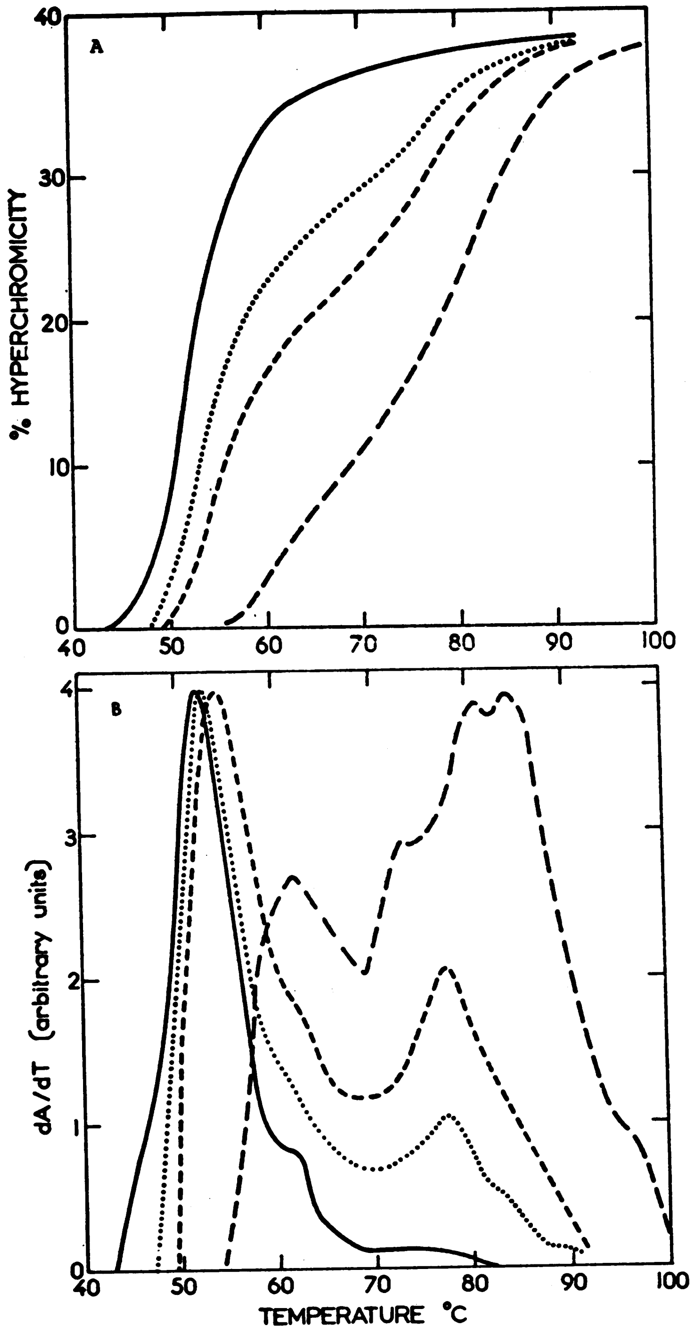

Figure 5 : Absorbance melting curves (A) and derivative melting plots (B) of DNA (——) and three samples of nucleoproteins dissociated by increasing concentration of deoxycholate : (— — —) 0.02 M, (- - - -) 0.03 M, (·········) 0.07 M.

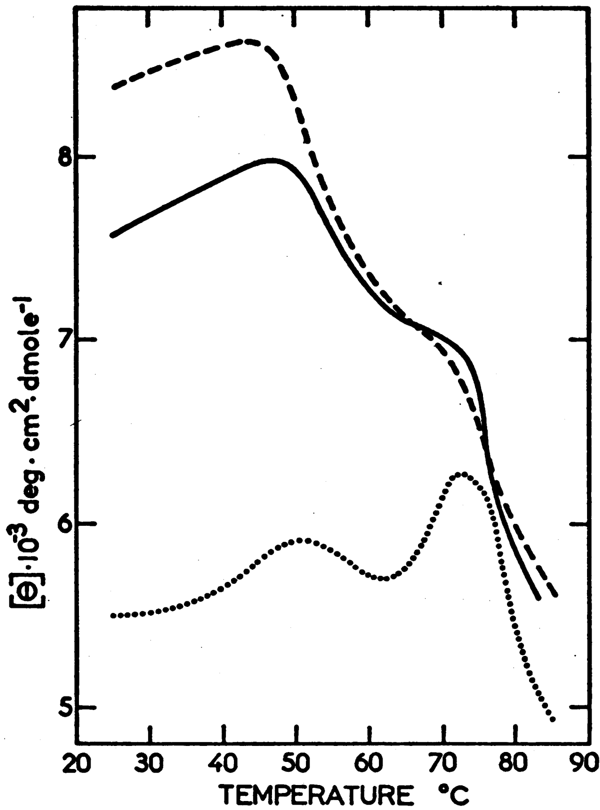

Figure 6 : Dichroic melting curves recorded at 280 nm of nucleoprotein samples dissociated by increasing concentrations of deoxycholate : (....) 0.02 M, (——) 0.03 M, (----) 0.07 M.

histone-bound DNA regions and is not related to the premelting phenomenon.

CONCLUSIONS

The aim of the present study was to determine which protein fraction of chromatin was responsible for the absence of C.D. premelting changes in native chromatin.
We have examined a series of nculeoproteins depleted of different protein fractions and the premelting appears as soon as non-histone proteins have been removed or in complexes where the slightly lysine-rich histones have been removed. Of course when non-histone proteins are removed together with other histone fractions (like F_1 in the case of nucleoproteins dissociated by NaCl) the premelting is also present.
On the other hand, it is clear that the lysine-rich histone fraction F_1 is not able to suppress the premelting. We can therefore assume that the absence of premelting in native nucleoprotein is the result of the interaction between non-histone proteins and the slightly lysine-rich histones F_{2a2} and F_{2b}.

In order to go further a thorough study of the premelting is necessary. For the moment no definite explanation can be given to the phenomenon (16). However recent experiments (de Murcia et al., to be published) (17) give clear evidence of the disappearance of any premelting of the DNA alone in presence of tetramethylammonium chloride. This quaternary ammonium ion is known to be preferentially bound to AT pair , and to stabilize them preferentially to G-C pair (18, 19). In line with these results we can associate the C.D. premelting to the dynamic state of AT regions (20) and assume accordingly that any ligand

able to affect the AT "breathing" would change also the premelting effect. It is thus tempting to say that the role of non-histone proteins would be to change the dynamical opening of the DNA and therefore to regulate the transcriptional events in chromatin.

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