THE PROTEIN COMPOSITION OF RAT SATELLITE CHROMATIN

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1. Introduction

Although most attempts to fractionate chromatin have been designed to prepare transcriptionally active chromatin, the advent of restriction endonuclease has provided the technology to isolate chromatin fractions enriched in highly repeated satellite DNA [1–5]. Two approaches have been used: the first of which involves digestion of nuclei or chromatin with a restriction enzyme which cuts within the satellite sequence. Since this sequence is repeated very frequently within the genome, it follows that this region of the chromatin will be cut into much smaller fragments than the bulk of the chromatin. By centrifugation of the digested chromatin through sucrose gradients, purification of the satellite chromatin to 70–100% has been reported for calf [1], rat [2] and African green monkey [3] tissues. Alternatively, a restriction endonuclease can be chosen such that main band DNA rather than satellite DNA is digested and satellite chromatin is found enriched in the insoluble pellet [4,5].

The function of satellite DNA is unknown (reviewed in [6]) but in situ hybridisation of radioactive satellite DNA probes shows that these sequences are largely associated with permanently condensed chromatin, which suggests that they are not involved in gene expression ([6] but see [7,8]).

A detailed knowledge of the protein associated with satellite DNA will undoubtedly be a useful complement to the studies on the composition of transcriptionally active chromatin, and may afford further insight into the function of highly repeated DNA. The α-satellite DNA-containing chromatin of African green monkey cells lacks histone H1 [3] and is enriched in a set of low molecular weight tightly bound non-histone proteins. We have prepared satellite chromatin from rat liver nuclei to determine whether the protein composition of this fraction differed significantly from that of bulk chromatin. The protein composition of satellite I chromatin has been examined to some extent [19] but here a more detailed analysis was undertaken to include the high mobility group proteins.

2. Materials and methods

2.1. *Preparation of satellite chromatin*

The preparation of rat liver nuclei and satellite chromatin was as in [2], with the following modifications:

(i) Nuclei were digested at 2.5 mg DNA/ml with 8000 units *Eco*RI/ml for 30 min at 37°C;
(ii) After digestion, soluble chromatin (enriched in highly repeated sequences) was extracted by suspending nuclei in extraction buffer (10 mM Tris–HCl (pH 8.0), 0.2 mM EDTA, 0.5 mM PMSF) at 0.5 mg DNA/ml, and leaving the suspension for 90 min at 4°C with occasional shaking by hand before centrifuging;
(iii) Soluble chromatin from the extraction was concentrated 3- or 4-fold with an Amicon ultrafiltration apparatus, and 0.5 mg (in terms of DNA) sedimented through a 19 ml 5–20% sucrose gradient for 16 h at 62 000 × *g*.

The fractions from 3 such gradients were pooled for DNA and protein isolation.

2.2. *Recovery of protein and DNA from sucrose gradients*

Gradient fractions were dialysed for 18 h against 2 changes of 0.1 mM EDTA/0.5 mM phenylmethylsulphonyl fluoride at 4°C and lyophilised. Protein

and DNA was recovered by phenol extraction as in [9].

2.3. *Electrophoresis of DNA*

DNA prepared from the gradient fractions was electrophoresed through 1.5% agarose gels and stained with ethidium bromide [2].

2.4. *Polyacrylamide gel electrophoresis of protein*

Phenol-extracted protein samples were analysed on 15% or 20% polyacrylamide acetic acid–urea slab gels [9] which included an upper loading gel of 7.5% acrylamide, 0.05% bisacrylamide, and stained with Coomassie brilliant blue R. For quantitative work, a 0.2% Procion Navy stain was used [10].

3. Results

Fig.1 shows the absorbance profile of the sucrose-gradient fractionation of the *Eco*RI-solubilised chromatin at 260 nm. The gradients were cut into 4 fractions (A–D) and the DNA from these fractions was analysed by agarose gel electrophoresis. The low molecular weight region of the gradient (A) did not

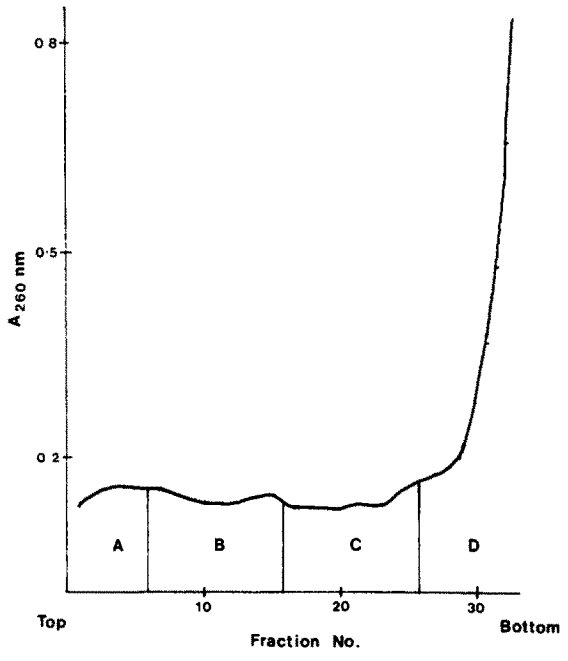

Fig.1. Sucrose gradient profile of *Eco*RI-solubilised rat liver chromatin: (——) A_{260}; 0.7 ml fractions collected.

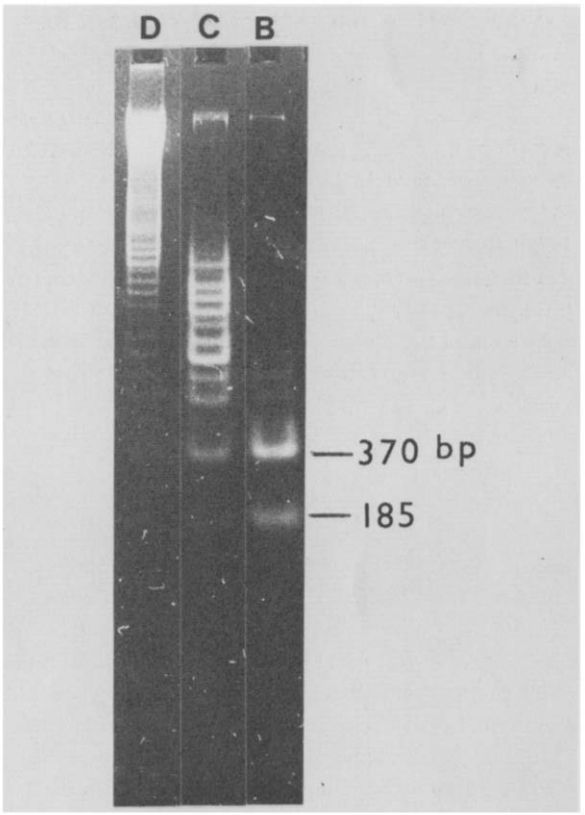

Fig.2. Agarose gel electrophoresis of DNA extracted from sucrose gradient fractions B–D.

contain detectable amounts of DNA. Fractions B–D (fig.2) displayed a series of discrete bands corresponding to satellite DNA. Fraction B (fig.2B) contained two prominent bands of 185 and 370 base pairs and several other high order bands, while in fractions C (fig.2C) the higher order bands predominate. The DNA of fraction D (fig.2D) also has a register of discrete bands, but is substantially contaminated by a continuous smear of very high molecular weight fragments corresponding to non-satellite DNA. Isolation and sequencing of the rat satellite DNA has shown [11] that it is composed of tandemly repeated 370 base pair blocks, which consist of alternating 92 and 93 base pair units with homologous but not identical sequences. In satellite DNA-containing chromatin, cleavage by *Eco*RI is suppressed at distances of 92/93 and 277 base pairs, which should lie within nucleosomes [2,11]. Although fractions B and C from the gradient contain traces of high molecular weight DNA at the top of the gel (fig.2B,2C) which may represent

non-satellite DNA, it is clear that most of the DNA in these fractions is composed of highly-repeated sequences.

The protein composition of the satellite chromatin was then analysed by polyacrylamide gel electrophoresis. We were particularly interested to see whether, as has been reported for African green monkey cells [3], histone H1 was absent or greatly reduced in this fraction. Gels were stained with Procion Navy (which has proved reliable for histone quantification [10]), scanned and photocopies of the scan peaks cut out and weighed. Fig.3 shows scans of phenol-extracted

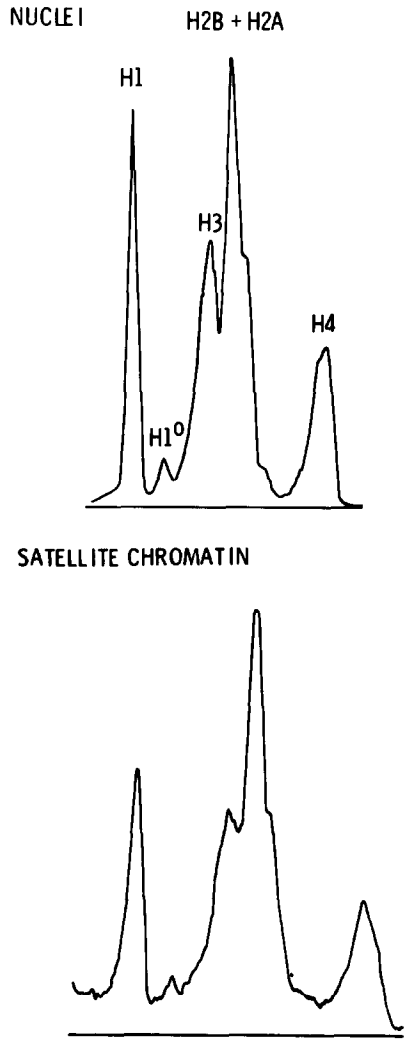

Fig.3. Scans of Procion Navy-stained 15% polyacrylamide acetic acid–urea gels of phenol-extracted protein from nuclei and satellite chromatin fraction C.

proteins from rat liver nuclei and satellite chromatin (C). All 4 core histones are present, as is histone H1. An additional feature of the scans is the minor protein component which migrates midway between histones H1 and H3 on the gels. Both the mobility and quantity of this protein are very like that reported for histone H1° in rat liver [12] using the same gel system. Table 1 gives the composition of histone H1 in nuclei and satellite chromatin expressed as a percentage of total histone. The values are very similar to each other, and to that reported for rabbit thymus tissue [10].

The proteins of 4 gradient fractions were analysed on 20% polyacrylamide acetic acid–urea gels and stained with the more sensitive Coomassie blue stain (fig.4). Both standard and high loadings of protein were used, the latter in order to detect possible minor components, such as the high mobility group non-histone proteins. Phenol-extracted proteins from rat liver nuclei have been included for comparison (fig.4a,e). Gradient fraction B, which contains the lower molecular weight satellite chromatin, shows no evidence of enrichment in non-histone proteins (fig.4b,4g) such as reported for African green monkey chromatin [3]. The highly loaded track from this fraction (fig.4g) shows that histone H1 is considerably depleted. This is not surprising since most of this chromatin is only 1–2 nucleosomes in length (see fig.2B), and so any cooperative binding effect of H1 molecules would be lost [13]. At the low protein loading of this fraction (fig.4b) the histone H4 band is seen to be split into a doublet, which suggests that at least the mono-acetylated form of H4 may be present in satellite chromatin, but there is no suggestion that H4 acetylation is any different to that observed in bulk chromatin.

The protein content of satellite C (fig.4c,4h) also shows no evidence of an enrichment in any non-histone proteins. The highly loaded track (fig.4h)

Table 1
Histone H1 composition of nuclei and satellite chromatin

	Histone H1 / Total histone
Rat liver nuclei	17.6%
Satellite fraction C	17.3%
Rabbit thymus nuclei	17.2%[a]

[a] Taken from [10]

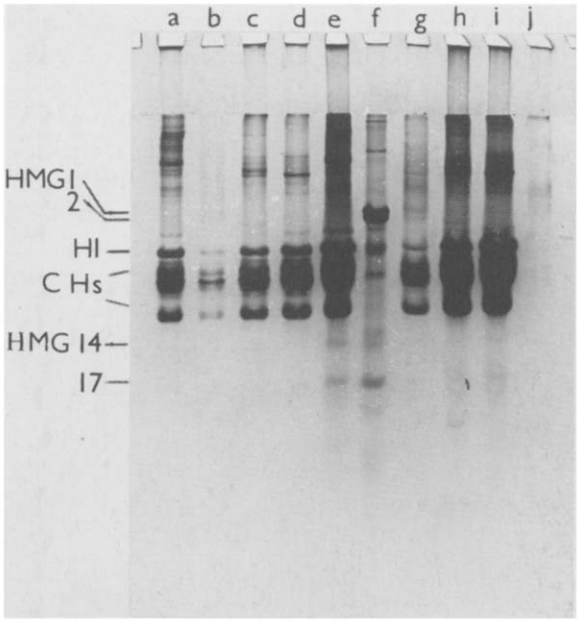

Fig.4. 20% Polyacrylamide acetic acid–urea gel of rat liver nuclear protein, and protein from sucrose gradient fractions: (a) total rat liver nuclear protein; (b) gradient fraction B; (c) fraction C; (d) fraction D; (e) 10 × loading of (a); (f) rat thymus HMG proteins; (g) 6 × loading of (b); (h) 6 × loading of (c); (i) 6 × loading of (d); (j) fraction A. CHs, core histones.

shows traces of the high mobility group proteins HMG 14 and 17, but if this pattern is compared to that of unfractionated rat liver nuclear protein at a similar loading (fig.4e), it is apparent that the satellite chromatin contains reduced amounts of these two proteins. This is also true for gradient fraction D, which is enriched for satellite DNA. Neither the phenol-extract of rat liver nuclei nor the satellite chomatin fractions contain significant amounts of the other major HMG proteins, HMG 1 and 2. This may be because the nuclei of this tissue contain very small amounts of these proteins, or because they are loosely bound to the chromatin, and therefore easily washed out of the nuclei during preparation [9].

4. Discussion

The results of this study indicate that the histone composition of satellite chromatin is similar to that of unfractionated chromatin. All 5 histones are present, and there is no significant reduction in the histone H1 content of large satellite chromatin fragments. Furthermore, there is no evidence for the specific association of any non-histone proteins with satellite DNA, although this region of the chromatin apparently does contain reduced amounts of the high mobility group proteins HMG 14 and 17. These findings conflict with those in [15] using an immunofluorescent technique to demonstrate the specific association of a nuclear protein [15] with a satellite sequence in *Drosophila*, and with those in [3], reporting the enrichment of several tightly bound non-histone proteins in the α-satellite chromatin fraction of African green monkey cells. The reason for this difference in findings is not clear, but may be related to differences in the tissues being studied. In [5], all 5 histones were reported in satellite chromatin from mouse brain, but the purity of the satellite fraction from which the proteins were isolated was not specified.

The similarity between the gross protein composition of satellite and unfractionated chromatin is not surprising, since satellite DNA is folded into a nucleosome structure with the same repeat length as the bulk of chromatin [16,19,20].

The findings of small quantities of HMG 14 and 17 in the satellite chromatin fractions warrants some comment. Although it is difficult to estimate the relative quantities in the satellite and unfractionated chromatin a reasonable estimate is that there is ~50% as much HMG 14 and 17 (relative to the histones) in satellite chromatin. Their presence in this fraction is not due to main band contamination and therefore suggests two possibilities: either HMG 14 and 17 are not exclusively associated with transcribed sequences as suggested by DNase I digestion studies [14]; or some protein rearrangement has occurred during chromatin fractionation. Both of these interpretations are compatible with our findings that isolated nucleosomes highly enriched in HMG 14 and 17 (10–15-fold) from chicken erythrocytes are not correspondingly enriched in globin sequences (2-fold at most) [17] and with the finding that all (or most) of the nucleosomes in the cell nucleus have HMG 14 and 17 binding sites [18].

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