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Nucleosomal repeat length in active and inactive genes

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Nucleosomal repeat lengths of total chromatin, H4 histone and β-DR genes have been measured in logarithmically growing HeLa cells. We have detected significant differences in nucleosomal spacing between inactive chromatin and chromatin regions actively engaged in transcription. These differences are also maintained in metaphase chromosomes at times when transcription ceases although a shortening in nucleosomal repeat length is observed in active and inactive chromatin. These observations support a model where DNA-core histone interactions are temporarily altered to allow selective remodelling of chromatin organization.

Nucleosomal spacing; Chromatin structure; Transcription

1. INTRODUCTION

The regular array of nucleosomes seen under the electron microscope would suggest an uniform, periodic and static organization of the genetic material which is also maintained in mitotic chromosomes [1]. Biochemical evidence, however, suggests that within this regular structure, transcriptionally competent regions of chromatin can be identified by their selective solubilization by nucleolytic enzymes and for example DNase I hypersensitive sites have been mapped on the 5′- [2–4] and 3′- [5–7] flanking regions of active or inducible genes. It has yet to be established whether these differences are mostly due to differences at the first level of DNA compaction (the nucleosome) or whether they are the result of a different high ordered structure dictated for example by altered nucleosomal spacing. In some cases shorter repeats [8–10] have been reported for chromatin actively engaged in RNA transcription as compared to inactive chromatin but longer repeats [11] have been also found for active chromatin while in other cases such differences have not been detected [12].

The present results analyze the DNA nucleosomal length of histone coding regions as compared to that of total chromatin at times when histones are synthesized and when transcription ceases in mitotic chromosomes.

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Abbreviation: β-DR, β-chain of the DR I class II histocompatibility antigen

2. MATERIALS AND METHODS

HeLa cells were grown in Dulbecco's modified minimum essential medium and harvested while not confluent (2×10^6 cells/10 cm dish). Nuclei were isolated by swelling the cells in hypotonic buffer followed by homogenization in a tight-fitting Dounce homogenizer and differential centrifugation. Chromosomes were prepared from mitotic cells by the method of Wray and Stubblefield [13]. Nuclei and chromosomal pellet were suspended in 10 mM Tris-HCl, 10 mM NaCl, 3 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.5 (1 mg DNA/ml), and digested with micrococcal nuclease (80 A_{260} units/ml) for the times indicated and the reaction was stopped by the addition of EDTA (10 mM final concentration); nucleosomal particles were extracted from digested chromatin and

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chromosomes following established procedures [14] and DNA isolated by phenol/chloroform extraction. Samples were fractionated electrophoretically in 1.8% agarose gels and bands were visualized by ethidium bromide staining. The PhiX174 RF DNA-*Hae*III digest was employed to calculate the number of base pairs of each stained band. DNA was then transferred to nitrocellulose filters and hybridized with the H4 probe excised from the pHu4A plasmid (kindly provided by Dr N. Heinz from The Rockefeller University, New York). The histone probe contained a TATA box, GACTTC sequence elements in the 5′-flanking region and a dyad symmetry element characteristic of the 3′-ends of most histone genes. Filters were also hybridized with a cDNA probe representing the β-chain of the DR I class II histocompatibility antigen (kindly provided by Dr G. Corte from the same institute). The position of each nucleosomal band was visualized by autoradiography of the filter which also contained as marker the 5′-end labeled PhiX174 RF DNA-*Hae*III digest. The length of the repeating unit was obtained by averaging the differences between the first and the next six nucleosomal bands following the method of Noll and Kornberg [15].

3. RESULTS AND DISCUSSION

In this study we have asked the question whether nucleosome spacing of conformationally competent genes differs from that of total chromatin. Nuclei were isolated from logarithmically growing HeLa cells and digested for the times indicated with micrococcal nuclease. Mononucleosomes and higher oligomers extracted with 2 mM EDTA [14] were separated on agarose gels and after staining with ethidium bromide the number of base pairs was estimated by comparing the migration of the chromatin fragments to that of a PhiX174 RF DNA-*Hae*III digest. The same radioactively labeled markers were employed to evaluate the nucleosomal length of the β-DR and H4 histone genes.

Table 1

Nucleosomal repeat lengths for total chromatin, H4 histone and β DR genes[a]

	Digestion time (min)	Total chromatin	H4 gene	β DR gene
Logarithmically growing cells	3	171.5 (n = 4)	180.0 (n = 4)	172.5 (n = 4)
	5	172.5 (n = 4)	177.3 (n = 3)	157.0 (n = 4)
	7	165.0 (n = 4)	175.7 (n = 3)	170.7 (n = 6)
	10	170.0 (n = 4)	175.0 (n = 4)	173.3 (n = 3)
		$\bar{x}$ = 169.7 ± 7.6	$\bar{x}$ = 177.4 ± 6.9	$\bar{x}$ = 168.4 ± 7.7
		$p < 0.0001$[b]	$p < 0.0001$[b]	$p < 0.0001$[b]
		$p < 0.008$[c]		$p < 0.006$[c]
Metaphase chromosomes	3	186.0 (n = 5)	190.0 (n = 5)	175.0 (n = 4)
	5	182.4 (n = 5)	190.8 (n = 5)	192.0 (n = 4)
	7	189.0 (n = 5)	187.4 (n = 5)	180.0 (n = 6)
	10	179.0 (n = 5)	192.0 (n = 4)	185.0 (n = 3)
		$\bar{x}$ = 184.1 ± 6.7	$\bar{x}$ = 190.0 ± 2.0	$\bar{x}$ = 183.0 ± 7.0
		$p < 0.005$[d]		$p < 0.02$[d]

[a] Nucleosomal repeat lengths have been determined for total chromatin, H4 and β-DR genes in logarithmically growing cells and in mitotic chromosomes and probabilities (p) are given only in the case when significant differences were detected. n indicates sample size

[b] Significant difference compared to the corresponding values in metaphase chromosomes

[c] Significant difference compared to the H4 gene in logarithmically growing cells

[d] Significant difference compared to the H4 gene in metaphase chromosomes

Different incubation times were used to avoid artefacts possibly due to the exonucleolytic activity of the micrococcal nuclease. Values obtained at the earliest and longest times of incubation are identical within the experimental error and the standard deviation. Furthermore digestion conditions and nucleosome extraction were such that sliding of nucleosomes or nucleosome rearrangement are avoided [16].

The nucleosomal length calculated for the total chromatin isolated from logarithmically growing cells appears to be close if not identical to that of the non-expressed gene of β-DR (table 1). Differences in the nucleosomal repeat are however detected between the transcriptionally competent H4 gene and total chromatin. An average of 169 base pair length has been calculated for total chromatin comparable to that of the inactive β-DR gene (168 bp) which is shorter than that found for the H4 gene.

A recent report has presented a careful analysis of the nucleosomal organization of H4 gene during the cell cycle [17] showing that the H4 nucleosomal linker is shorter compared to total chromatin and inactive globin genes. The H4 probe used by Moreno et al. [17] is comparable to that we employed, and the reasons for these discrepancies have still to be established. It must be pointed out that our calculation was carried out on a S_2 extract of digested chromatin [14] while the values given by Moreno et al. [17] were measured following total solubilization of chromatin after micrococcal nuclease digestion.

Nucleosomal length has also been calculated in chromosomes from mitotic cells obtained by selective detachment after exposure to colcemide. In agreement with Moreno et al. [17] H4 gene repeat lengths are longer compared to total chromatin and β-DR gene suggesting that a differential genome organization is still maintained at times when histone transcription ceases.

These results deserve a few final comments. Nucleosomal organization is a dynamic situation and when chromatin condenses into mitotic chromosomes, core particles must slide to provide binding sites for those factors involved in chromosome organization and this may also cause the redistribution of nucleosomes in transcriptionally competent chromatin which is different from that of inactive chromatin. The mobility of nucleosome protein core and/or its dissociation from DNA supports the 'jumping nucleosome' model proposed by Mirzabekov et al. [18] who suggest that chromatin regions are temporarily devoid of core histones to allow transcription.

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