RANDOM ARRANGEMENT OF NUCLEOSOMES ON DNA IN CHROMATIN

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1. Introduction

The location of nucleosomes on the bulk of the DNA [1] as well as on 5 S [2] and ovalbumin genes [3] and on polypyrimidine/polypurine stretches [4] was found to be random. This has led to the suggestion that the packaging of DNA in nucleosomes is unlikely to bear directly on the control of gene activity but may serve primarily a structural purpose [1]. It has been observed [5], however, that this randomness could be, at least in part, a consequence of a rearrangement of histones induced by micrococcal nuclease digestion. This difficulty is obviated only in the particular cases of SV40 [6–8] and polyoma virus minichromosomes [8] where the same conclusion was obtained using a different method based on restriction endonuclease digestions. The possibility for such a rearrangement is indicated by the observation that extensive digestion of chromatin with micrococcal nuclease generates compact dimers [9,10]. Some insight into the way compact dimers are formed comes from an experiment in which dimers from an early micrococcal digest of chromatin are trimmed with exonuclease III from *E. coli* [11]: no sliding is detected as long as dimers retain their full complement of HI (A. P., R. D. Kornberg, submitted); later in the digestion, HI is released and nucleosomes slide towards each other, generating compact dimers in high yield (A. P., unpublished data). The lower yield of compact dimers obtained with micrococcal nuclease may not reflect a lower ability of this enzyme to cause sliding but is due to the susceptibility of dimers to cleavage into monomers.

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In summary, the extent of histone rearrangement in micrococcal nuclease digestion seems to depend on the balance between the exonucleolytic (this activity is responsible for the release of HI) and endonucleolytic activities of the enzyme (see [12] for a discrimination between the two mechanisms). Since exonucleolytic trimming becomes important late during digestion [12], the question of histone rearrangement is especially relevant in the case of core particles mainly used in the work quoted above.

The location of nucleosomes was investigated here by using DNA fragments obtained from chromatin digested with DNase I instead of micrococcal nuclease, and was again found to be random. It is important to notice that those two enzymes act on chromatin in markedly different ways: whereas micrococcal nuclease inflicts double-strand cuts primarily in the linker DNA, followed by exonucleolytic trimming of the ends, DNase I nicks the two strands of both linker and core DNAs (reviewed [13,14]). This suggests that, at least in an early phase of digestion, DNase I, as a pure endonuclease, does not lead to the release of HI and is unable, in contrast to micrococcal nuclease, to induce a rearrangement of histones.

2. Materials and methods

Nuclei from rat livers were prepared and incubated with DNase I (Sigma Chemical Co., 240 U and 5×10^8 nuclei/ml) for 6 min at 37°C as in [15]. The DNA was extracted by addition of 1% SDS and 1 M NaCl, shaking with chloroform–isoamyl alcohol (24:1), and precipitation with ethanol.

Single strands 80 nucleotide-long were purified by preparative electrophoresis of the DNA in 7 M urea–

12% polyacrylamide gels [16]. Gels were stained with ethidium bromide, the appropriate band cut out and the DNA recovered by electroelution as in [1]. Recovery of the fragments, relative to the amount of DNA loaded on the gels, was 3%. Purified 80 nucleotide-long single strands were dissolved (10 mg/ml) in 0.24 M sodium phosphate (pH 6.8), sealed in a glass capillary, heated for 5 min at 100°C and allowed to reassociate at 55°C for 100 h. The double stranded fraction (90% of the total) was purified by chromatography on a hydroxyapatite column as in [17] (the column temperature was 52°C) and digested with S1 nuclease (see the figure legend).

3. Results and discussion

Single strands 80 nucleotide-long, which constitute the most abundant class of discrete size fragments, were fractionated from a DNase I digest of chromatin in nuclei as in section 2. These fragments originate mainly* from cleavages at nucleotides no. 10 and 90, 20 and 100, 40 and 120, and 50 and 130, with the 5′-termini of the nucleosome core particle as the origin [19,20]. If nucleosomes have, relative to DNA sequences, the same location in all cells, the 80 nucleotide-long fragments which originate from different copies of the genome will overlap in sequences by multiples of 10 nucleotides, and will reassociate into molecules having double stranded regions of length

*It is assumed here, and supported by reconstruction calculations [18], that discrete fragments resolved by gel electrophoresis of the DNA extracted from a DNase I digest of chromatin in nuclei originate from the core of nucleosomes, in which case the relative susceptibilities of the sites have been derived [19,20]

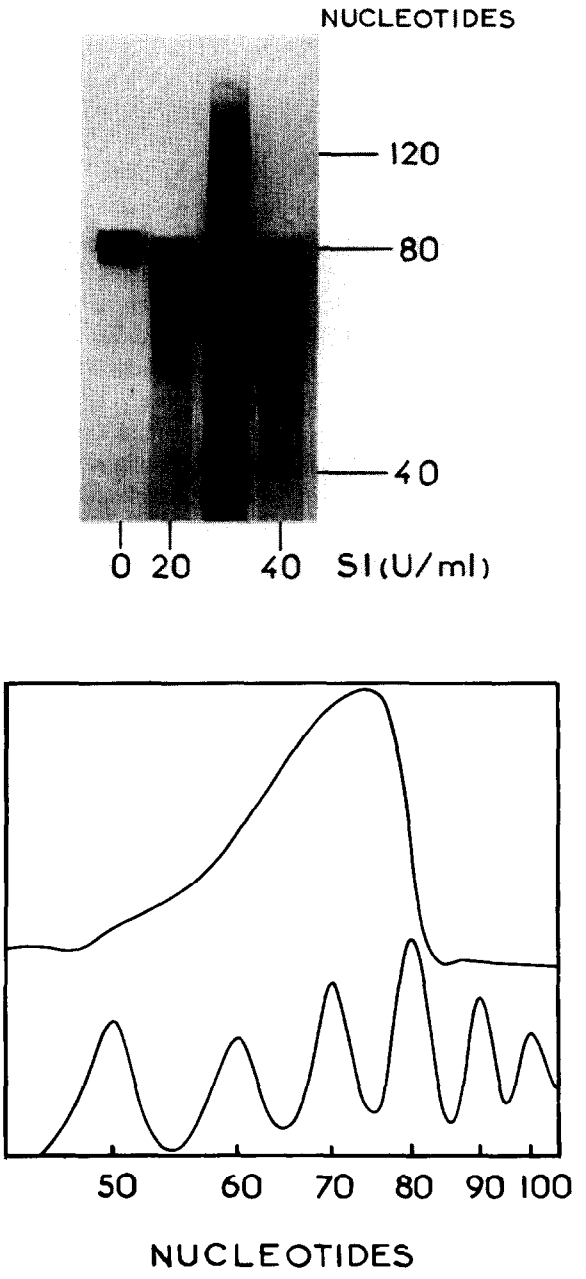

Fig.1. Random distribution of sequence overlaps in 80 nucleotide-long single strands. After purification, the double-strand fraction (20 μg DNA/ml) of the reassociation mixture was digested with S1 nuclease (Sigma Chemical Co.) for 1 h at 37°C as in [1]. This results in an accurate removal of single stranded tails as shown by the following experiment (not shown): Nucleosome monomers were treated with exonuclease III, DNA extracted and digested with S1 nuclease under similar conditions. Gel electrophoresis of the digest resulted in a pattern of sharp bands spaced by ~10 basepairs down to 30 basepairs ([1] and A. P., R. D. Kornberg, submitted). S1 digests were submitted to electrophoresis in a 7 M urea–12% polyacrylamide gel [16] along with the total DNase I digest from which the 80 nucleotide-long fragments originate. A photograph of the ethidium bromide stained gel and traces (they are identical for both 20 and 40 units of S1/ml digests) are shown.

multiple of 10 nucleotides minus 2*. S1 nuclease digestion and gel electrophoresis of the digest will result in a pattern of bands corresponding approximately to the sizes 80, 70, 60, 50 and 40 nucleotides. In contrast, if nucleosomes are randomly located on the DNA, all sequence overlaps between the fragments will have the same probability. S1 nuclease treatment will lead to a uniform distribution of the number of molecules of size between 0 and 80 basepairs. The weight distribution, given in the stained gel, will be wedge-shaped, reaching a maximum at a size of 80 basepairs and decreasing at lower sizes.

Figure 1 shows that the result obtained conforms well to the one predicted from a random location of nucleosomes on the DNA**.

The experiment performed here is conceptually identical to that in [1] where the sequence overlaps between DNA fragments extracted from nucleosome trimers were investigated. Whereas the same general conclusion is obtained, this experiment further excludes a particular type of relation of nucleosomes to sequences, similar to the one suggested for SV40 and polyoma minichromosomes [8], in which nucleosomes would occupy a limited number of specific positions spaced by ~10 basepairs. This possibility would not have been distinguished from a fully random arrangement in the experiment with nucleosome trimers quoted above.

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* Those two nucleotides correspond to the staggering observed between DNase I cleavage sites on opposite strands [19–22]

** As pointed out [1], this conclusion applies only to single copy sequences, which are ~2/3 rds of the rat genome

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