Distribution of nucleosomes on reconstituted chromatin from cloned mouse β-globin DNA

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Relative abundance of nucleosomes on reconstituted chromatin was estimated with cloned mouse β-globin gene DNA. Mononucleosomal DNA was isolated from reconstituted chromatin after digestion with micrococcal nuclease, nick-translated and used as a probe for blot hybridization. DNA fragments of restriction nuclease-digested globin DNA were transferred to DBM-paper and hybridized with mononucleosomal [^{32}P] DNA probe. The results showed non-random distribution of nucleosomes.

Chromatin reconstitution *Nucleosome* *β*-Globin DNA

1. INTRODUCTION

Nucleosomes, the basic units of chromatin [1], are found both in transcriptionally active and inactive genes of chromatin [2–4]. There is, however, good evidence that nucleosomes are not arranged randomly on genomes but are 'phased' at least in certain genes [5–11]. Experiments with minichromatin of SV-40 revealed a nucleosome-free region in replication origin of DNA [11,12].

Even in reconstituted chromatin, it has been reported that nucleosomes are phased [13] and that DNA-sequence specificity of nucleosomal binding has been observed [14,15]. From these findings it is suggested that base sequences or higher structures of DNA affect the formation of nucleosomes.

MβG2, mouse β_{major} globin DNA clone, was isolated in [16]. This cloned globin DNA contains a 7.3 kb-long insert of genomic DNA including β_{major} globin gene and flanking regions. We consider it worthwhile to examine how nucleosomes are formed on this cloned genomic DNA fragment. For this purpose mononucleosomal DNA from reconstituted chromatin was used as a probe to determine the distribution of nucleosomes and it was found that nucleosomes are not assembled randomly on reconstituted chromatin.

Abbreviations: EDTA, ethylenediamine tetracetic acid; SDS, sodium dodecylsulfate; DBM, diazobenzyloxymethyl

2. EXPERIMENTAL

2.1. *Preparation of plasmid DNA and histones*

MβG2 DNA subcloned in pBR322 was obtained from Dr M. Obinata (University of Tokyo). χ1776 cells harbouring the plasmid were grown, and cleared lysate was prepared as in [17]. Closed circular plasmid DNA was isolated by acid–phenol extraction [18]. Restriction nuclease map of MβG2 is shown in fig.1.

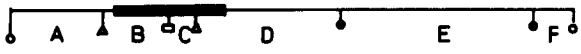

Fig.1. Restriction nuclease map of MβG2 DNA. The thick bar represents coding sequences, and orientation of transcription is from left to right: (○) *Eco*RI; (△) *Hin*dIII; (□) *Bam*HI; (●) *Bgl*II.

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Core histones were prepared from Friend erythroleukemic cells, clone 727. Nuclei were isolated as in [19], and washed thoroughly with 0.6 M NaCl in buffer B [10 mM Tris–HCl (pH 7.9), 2 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride]. Washed chromatin was extracted with 0.4 N H_2SO_4, and the extract was dialyzed against buffer B. The dialyzed sample was adjusted to 2 M NaCl and run through a hydroxyapatite column [20] equilibrated with 2 M NaCl in buffer B. The passed through fractions were pooled and concentrated by ultrafiltration. This histone preparation contained no detectable level of histone H1 and non-histone proteins according to SDS–polyacrylamide gel electrophoresis.

2.2. *Reconstitution of nucleosomes*

Reconstitution of nucleosomes was done as in [21]. Histones and DNA were mixed at 1:0.8 (w/w) (DNA = 50 μg/ml) in 5 M urea, 2 M NaCl in TEP buffer [10 mM Tris (pH 7.9), 1 mM EDTA, 0.1 mM PMSF]. Urea was removed first by dialysis against 2 M NaCl in TEP buffer, and the salt concentration was reduced stepwise by dialysis against 1.5, 1.0, 0.75, 0.5 and 0 M NaCl in TEP buffer. Each step required from 3–4 h.

2.3. *Isolation of mononucleosomal DNA and nick translation*

Reconstituted nucleosomes were digested with micrococcal nuclease (6 units/A_{260}) for 10 min at 37°C, and DNA was isolated after treatment with proteinase K and SDS followed by extraction with phenol/chloroform. DNA was run on a 1.5% low-melting temperature agarose (Sea Plaque) gel, and the mononucleosomal band was cut out. The agarose was melted at 65°C and DNA was recovered by phenol-extraction and ethanol-precipitated. Mononucleosomal DNA was nick translated with [^{32}P]dCTP (Amersham International, Bucks; 3000 Ci/mmol) as in [22].

2.4. *Southern transfer and filter hybridization*

MβG2/pBR322 DNA was digested with an excess amount of *Eco*RI, *Hin*dIII, *Bam*HI and *Bgl*II, and the DNA fragments were separated on a 1.5% agarose gel. DNA was transferred to a DBM-paper [23] as in [24]. This paper was hybridized with nick-translated mononucleosomal DNA, washed and autoradiographed [25].

3. RESULTS

To study the formation of nucleosomes in our reconstitution system, reconstituted chromatin was digested with micrococcal nuclease, and the isolated DNA was run on an agarose gel. Distinct bands of mononucleosomal DNA of about 160 basepairs are visible (fig.2); oligonucleosomal DNA up to tetranucleosomes can also be seen (fig.2).

To determine the relative abundance of nucleosomes on reconstituted chromatin, probes were prepared from mononucleosomal DNA generated from reconstituted chromatin prepared

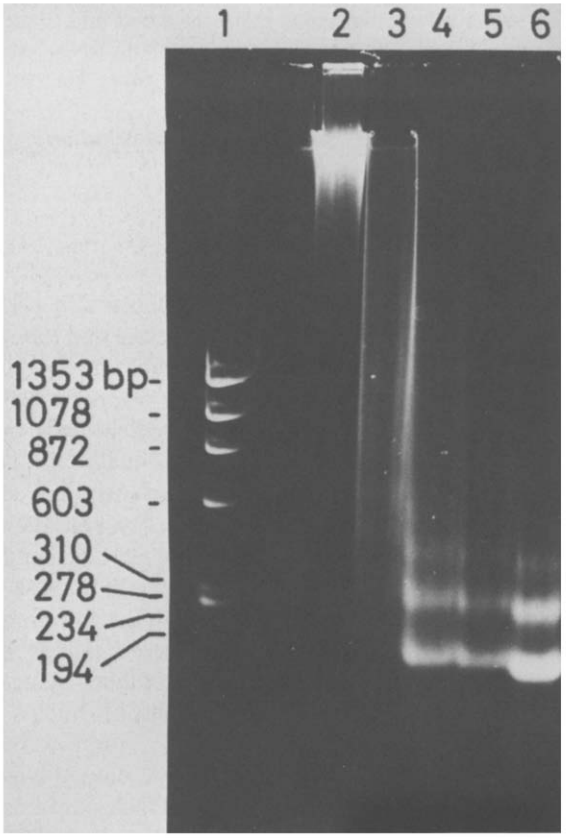

Fig.2. Agarose gel electrophoresis of DNA from micrococcal nuclease-digested chromatin. Reconstituted chromatin prepared by dialysis method was digested with micrococcal nuclease (1.5 units/A_{260}) for 0, 0.5, 1, 2.5 and 6 min at 37°C (lane 2–6, respectively). Lane 1 shows the size marker of ϕX174RF DNA digested with *Hae*III.

either with circular, *Eco*RI or *Bam*HI-digested MβG2 DNA. Reconstituted chromatin was digested extensively with micrococcal nuclease, and DNA was purified. The bands of mononucleosomal DNA on an agarose gel were cut out, and the DNA was isolated. As a probe control, naked MβG2 DNA digested briefly with micrococcal nuclease was used. DNA was nick translated with [^{32}P]dCTP (spec. act. ~1.5 × 10^7 cpm/μg DNA).

On the other hand, MβG2 DNA was digested with restriction nucleases, *Eco*RI, *Bam*HI, *Hin*dIII and *Bgl*II. The fragments were separated on an agarose gel, and then transferred onto DBM-papers. To ensure the qualitative binding of small DNA fragments, a DBM-paper instead of nitrocellulose filter was used as suggested by Alwine [23]. The transferred filters were hybridized with ^{32}P-labeled probes. The results of autoradiography are shown in fig.3. The relative abundance of nucleosomes was calculated by measuring radioactivity of each band and by dividing the counts by the chain length of the restriction fragments.

The results of two typical experiments are shown in fig.4. The radioactivity of hybridized bands with nick-translated total MβG2 DNA was taken as the standard reflecting the efficiency of DNA-transfer and hybridization. By comparing the specific activity of bands from total DNA probe with that from mononucleosomal DNA probes, the relative abundance of nucleosomes formed on MβG2 DNA can be estimated. The relative abundance of nucleosomes on globin gene was not random (fig.4): nucleosome formation seems less in bands B and C on MβG2 DNA, and these bands corres-

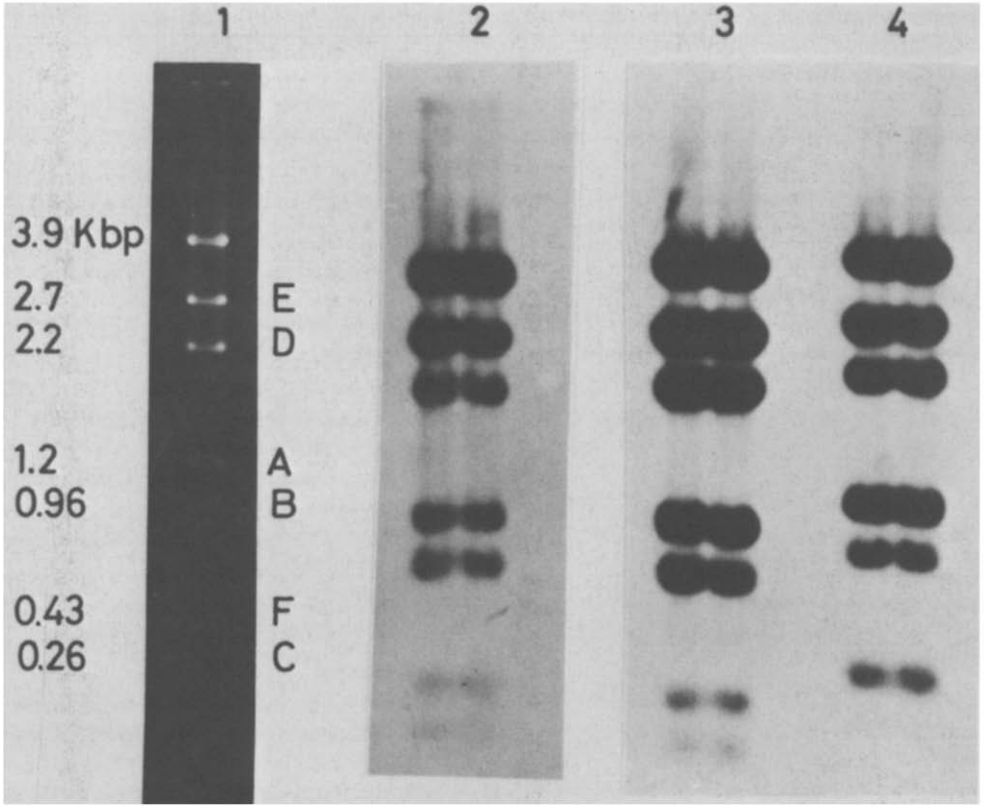

Fig.3. Hybridization of mononucleosomal DNA to restriction fragments on the filters. (1) Ethidium bromide-stained gel; (2–4) DNA fragments were transferred to DBM-paper and hybridized with ^{32}P-labeled mononucleosomal DNA from reconstituted chromatin with: (2) circular DNA; (3) *Eco*RI-digested; or (4) *Bam*HI-digested DNA. The fragment of 3.9 kbp originated from pBR322, and the fragment F was not separated enough from vector fragment.

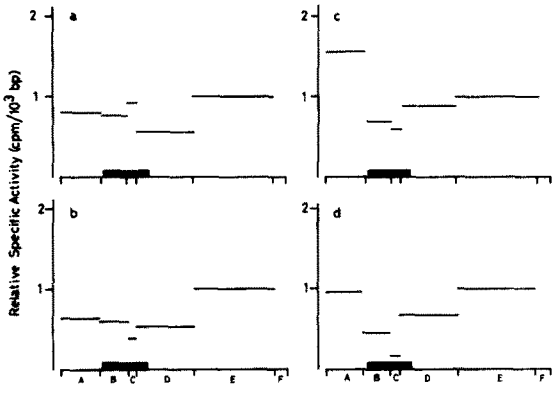

Fig.4. Relative abundance of nucleosomes on reconstituted chromatin. Filters corresponding to the autoradiographic bands shown in fig.3 were excised, and the radioactivity was measured. The values were divided by the chainlength of each fragment and the relative specific activity was expressed on the base of fragment E taken as 1.0. ^{32}P-Labeled probes were: (a) total MβG2 DNA; (b) mononucleosomal DNA from reconstituted chromatin with circular DNA; (c) *Eco*RI-; or (d) *Bam*HI-digested DNA.

pond to the 5′-half of the β_{major} globin gene.

The absolute values of hybridization varied slightly from experiments to experiments, probably due to variations in extent of hydrolysis during processing of gels and/or in the efficiency of DNA-transfer, but the above mentioned difference between naked DNA and reconstituted nucleosomes was reproducibly observed.

4. DISCUSSION

Reconstitution of nucleosomes has been carried out by many authors using various methods. The most common method is the mixing of DNA and histones in a high salt in the presence of urea and removing urea first and then the salt by dialysis. Nucleosomes are formed by this method, and mono- to oligonucleosomal DNA bands are visible (fig.2).

For a comparison of the relative abundance of nucleosomes on reconstituted chromatin, we used mononucleosomal DNA as a probe, and hybridized with restriction fragments of MβG2 DNA transferred to the DBM-paper. The formation of nucleosomes may not occur randomly; some regions of the globin gene were more slowly assembled into nucleosomes, regardless of the physical form of DNA (fig.4).

As a control for estimating relative abundance, naked MβG2 DNA digested briefly with micrococcal nuclease was used. From a comparison of the relative specific activity from blot hybridized filters, the nucleosomes seemed to be less in bands B and C, which correspond to the 5′-half of the 7.3 kb MβG2 DNA. DNA on the filters was in excess of the ^{32}P-labeled probe; consequently the radioactivity hybridized to the filters may be regarded as representing the relative abundance of DNA sequences present in mononucleosomal DNA.

Nucleosomes were preferentially assembled in vitro in AT-rich regions of SV-40 DNA [15]. Since complete nucleotide sequences have not yet been determined using MβG2 DNA, we could not estimate the presence of AT-rich regions on each restriction fragment. Base sequences and/or higher order structure of DNA may possibly influence the assembly of nucleosomes.

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