Sliding-end-labelling

A method to avoid artifacts in nucleosome positioning

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A method, termed 'sliding-end-labelling', has been devised to avoid a frequent artifact in nucleosome positioning by indirect end labelling, namely the appearing of DNA fragments originated by two nuclease cuts, one of them lying within the region covered by the probe. The method is applied to the nucleosome positioning in the yeast *SUC2* gene for invertase.

Chromatin *Nucleosome positioning* *Indirect end labelling* SUC2 *gene* (Saccharomyces cerevisiae)

1. INTRODUCTION

The term 'positioning' was suggested by Van Holde [1] to refer to sequence-specific locations for nucleosomes on non-repetitive DNA. Its occurrence, possible causes and implications have been recently reviewed [2].

Positioning of nucleosomes was first studied by Nedospasov and Georgiev [3], by using a method similar to the indirect end-labelling technique of Wu [4] combined with digestion with MNase, which preferentially digests the linker DNA [5]. Several artifacts may appear when using this method, and some of them have been reviewed by Reeves [6]. The first one arises from the sequence specificity of the enzyme [7,8] and it can be circumvented by using control digestions of naked DNA as discussed by Simpson [2]. The second, and yet unresolved, artifact may arise when MNase cutting occurs within the DNA region covered by the probe. In this instance, one single cut would generate two fragments that hybridize with the probe, although only one of them would abut on the restriction site used as reference end. We describe in this paper a procedure that we have termed 'sliding-end-labelling' to avoid this artifact, as it allows one to check whether a given DNA fragment detected after hybridization shares with the probe the same restriction end.

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Abbreviations: MNase, micrococcal nuclease; bp, base pairs

2. MATERIALS AND METHODS

2.1. *Materials*

Saccharomyces cerevisiae strain X2180 1A (*MATa, SUC2, CUP1, malO*) was used in these studies. All the enzymes were from Boehringer and they were used as described by Maniatis et al. [9].

2.2. *Growth of yeast and isolation of nuclei*

Yeast was grown in YEPG medium (1% yeast extract, 2% bactopeptone, 2% glucose). To obtain cells repressed and derepressed for *SUC2* gene, the method of Carlson et al. [10] was used. Nuclei were isolated as described elsewhere [11].

2.3. *Digestions with MNase*

Nuclei were suspended in 10 mM NaCl, 3 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM Tris-HCl, pH 7.4 to a concentration of about 100 μg DNA/ml.

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Variable amounts of the enzyme were then added and the samples were incubated at 37°C for 5 min. For control digestions, DNA was diluted in the same buffer to a concentration similar to that used for nuclei. Digestions were stopped by adding a cold EDTA solution to a final concentration of 10 mM. SDS was then added to a final concentration of 1% and DNA was isolated [11].

2.4. *Electrophoretic analysis*

DNA was restricted (see below) and analysed in 1.8% agarose slab gels containing 89 mM Tris, 89 mM borate, 0.2 mM EDTA, pH 8.3. Transfer of DNA from agarose gels to nylon filters (Hybon-N, Amersham) was performed as described by the manufacturer. Probes were isolated from low melting-point agarose (BRL, ultrapure) [12]. ^{32}P-labelled probes were prepared by nick-translation [13]. Filters were hybridized in 6 × SSC (1 × SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7), 1 × Denhardt's solution, 50% (v/v) formamide, 10% (w/v) dextran sulphate, 100 mg/ml of sonicated and denatured salmon sperm DNA, and 10^7 cpm/ml of heat-denatured ^{32}P-labelled probe. Hybridization was carried out at 42°C overnight. After hybridization, filters were washed twice with 2 × SSC (200 ml each) for 15 min, once with 2 × SSC, 0.1% SDS for 30 min, and once with 0.1 × SSC for 10 min. Washing was always done at 65°C. Filters were then air-dried and autoradiographed.

3. RESULTS AND DISCUSSION

The rationale of the sliding-end-labelling method is outlined in fig.1. Let us suppose that the vertical arrows mark the preferential cutting sites of MNase. Should the digestion be carried out under mild conditions, so that only a single cut per DNA molecule occurred, once restricted with *E*1 the cutting sites would be unambiguously located, because only the bands *a, b, c* and *d* would have been detected. If, on the other hand, digestion with MNase had been more extensive fragments like that marked *x*, generated by two cleavages on the same DNA molecule, could have been originated. Fragment *x* would hybridize, because its left end lies within the probe, and this would lead to the erroneous conclusion that there was a cutting site *x* bp downstream from the site *E*1. Now, supposing

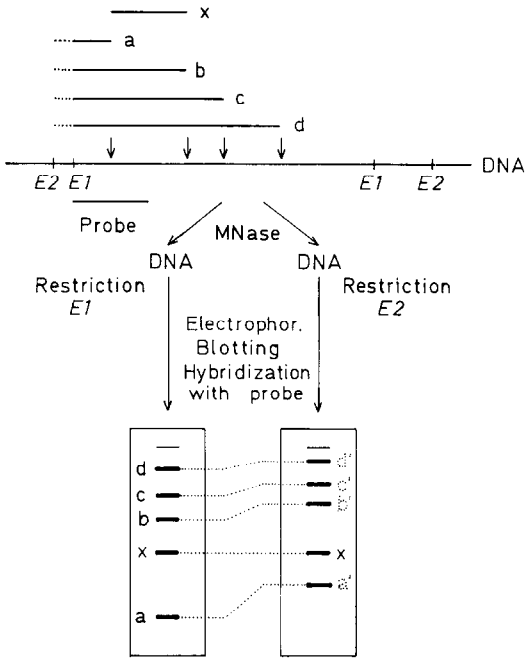

Fig.1. Outline of the sliding-end-labelling method. See text for details.

that a restriction site of a second endonuclease, *E*2, exists close to the left of *E*1, DNA can be restricted with *E*2 and probed as before. In this instance, fragments *a'*, *b'*, *c'* and *d'*, rather than *a, b, c* and *d*, will be formed and the appearance of the autoradiogram will be as if the bands had slid upwards. On the contrary, the size of fragment *x* will remain unchanged and this will allow one to conclude that fragment *x* does not abut on the *E*1 site.

The validity of the sliding-end-labelling method has been checked in the course of studies on nucleosome positioning in yeast *SUC2* gene for invertase. MNase cutting sites were located relative to the *Xba*I site (+829) with the aid of a *Xba*I-*Kpn*I probe 330 bp long. Fig.2 (lanes 1 and 2) shows the results of this experiment. Several bands appear whose sizes would allow one to map the potential cutting sites relative to the *Xba*I site. A *Bam*HI site is located 42 bp upstream from the *Xba*I site [14]; when *Bam*HI was used as the second enzyme, the sliding of most of the bands (fig.2, lane 3) is apparent. Only the bands marked with an asterisk remain with their mobility unchanged and, therefore, they correspond to fragments released by two nuclease cuts. In fact,

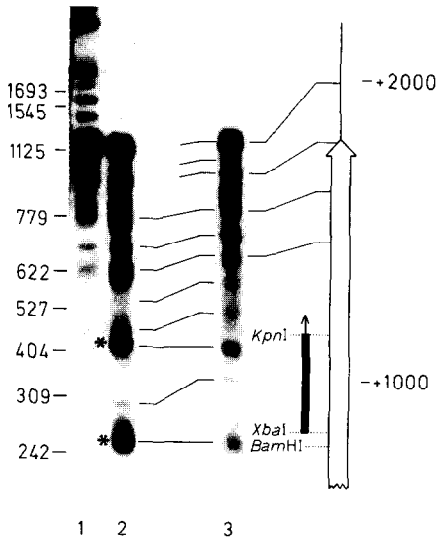

Fig.2. Application of the sliding-end-labelling method to the positioning of nucleosomes in the yeast *SUC2* gene. Yeast nuclei were digested with 50 (lane 1) or 250 units MNase per mg DNA (lanes 2 and 3), and the DNA samples restricted with *Xba*I (lanes 1 and 2) or *Bam*HI (lane 3). The bands showing no difference in their mobility between lanes 2 and 3 are marked by an asterisk. The bands not appearing in naked DNA (not shown) are assumed to represent MNase cutting sites in the linkers and they have been mapped in the schematic representation of the gene. The migration of size standards is shown to the left.

their sizes correspond to those of mono- and dinucleosomes. The true cutting sites, tentatively ascribed to linkers, are mapped in fig.2. Nucleosome positioning on the *SUC2* gene will be confirmed by additional mappings (manuscript in preparation).

It is obvious that if only mild digestions had been done, monomers would not have appeared (lane 1), but information on the cutting sites close to the *Xba*I site would have been lost.

The only limitation of this method is that a second restriction site is required. It is essential for this second site to lie outside of the region covered by the probe and close to the first restriction site, but, at least, 20–30 bp apart from it; otherwise the sliding will be hardly noticeable. It is also desirable that the second enzyme does not possess an additional cutting site within the region under study. The present availability of a vast number of restriction endonucleases makes it possible to meet these requirements for most of the genes studied.

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