Rapid isolation of yeast plasmids as native chromatin

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ABSTRACT

Many regions of chromatin are subject to dynamic changes. We have developed a rapid method for isolation of small chromatin templates from yeast which will facilitate biochemical analysis of chromatin composition. Using the PH05 promoter we show that templates prepared from cells grown in inducing or repressing conditions show native chromatin structures. This method may be widely applicable as the chromatin structures at a centromere, at ARS1 and at part of the lacZ region on two other plasmids are preserved after chromatin isolation.

There is currently much interest in dynamic chromatin processes such as transcription factor mediated nucleosomal rearrangements (1). In yeast (Saccharomyces cerevisiae), the presence and position of nucleosomes in chromatin is determined by mapping sites sensitive to exogenously added nucleases in nuclei or more recently in permeabilised yeast spheroplasts (2,3). In order to dissect the interactions and modifications in chromatin resulting in events such as transcription it is necessary to isolate specific regions of chromatin away from the bulk. Isolation of a plasmid as chromatin would provide a good starting material for examining the composition of specific regions on the plasmid such as promoters or origins of replication. The methods currently available for chromatin isolation are time consuming (4—6). Here we report on a rapid method for producing chromatin templates with nucleosome organisation resembling that seen in vivo. The technique involves elution of chromatin from spheroplasts lysed with low concentrations of detergents followed by centrifugation to remove the majority of the detergent and proteins from the preparation. We have chosen the PH05 promoter as a suitable template as it shows distinctive changes in nucleosome position which correlate with the presence or absence of phosphate in the growth medium (7—9). Cell cultures of strain 699 (MATa, ade2, trpl, can1-100, leu2-3-112, his3-11-15, ura3-52) containing the PH05—specific probe and a total genomic probe (data not shown). The cleared supernatant contains plasmid DNA (band marked P), chromosomal DNA (band marked G) and the unknown nucleic acid (which also hybridises weakly to a total genomic DNA probe, see Fig. 2) as U.

Figure 1. Composition of nucleic acids in chromatin preparations. Photograph of DNA prepared from a cleared supernatant separated by electrophoresis on a 1.5% agarose gel in 1 TBE and 10 µg/ml ethidium bromide. The identity of the bands were confirmed by Southern blotting (not shown). Plasmid DNA is marker P, chromosomal DNA as G and the unknown nucleic acid (which also hybridises weakly to a total genomic DNA probe, see Fig. 2) as U.

Yields are optimal from fresh transformants maintained on selective medium.

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a probe from the nuclease (up to 180 U/ml) produces a nucleosome ladder. Probing identical to that observed by others (7–9), including discontinuities of the same filter with the genomic DNA probe is compared with migration of the nucleosomal ladder produced after hybridisation (Fig. 2A). The discontinuities become clear when the position of the probe and the nuclease hypersensitivity site (heavy arrows). etc. A map of the promoter region of the PH05 gene is shown indicating the position of the probe and the nuclease hypersensitivity site (Fig. 2B).

Digestion of the final chromatin preparation with micrococcal nuclease (up to 180 U/ml) produces a nucleosome ladder. Probing a Southern blot of DNA isolated from the digested chromatin with a probe from the PH05 promoter reveals a pattern of bands identical to that observed by others (7–9), including discontinuities in the nucleosomal ladder due to hypersensitive sites in the region (Fig. 2A). The discontinuities become clear when the position of migration of the nucleosomal ladder produced after hybridisation of the same filter with the genomic DNA probe is compared with the PH05 specific probe (Fig. 2B). Extensive micrococcal nuclease digestion yields predominantly mono- and dinucleosomes rather than the regular array in the chromosomal chromatin. The pattern of micrococcal nuclease cutting remained identical after incubation of the chromatin in the cleared supernatant. Chromatin templates were isolated from cells grown in high (1.5 g/l; H) or low (0.1 mg/l; L) phosphate and digested with 180 U/ml micrococcal nuclease (lanes 1–5). The DNA was separated on a 1.5% gel in 10 mM TBE and 10 µg/ml ethidium bromide and blotted to Magna membrane. The blot was hybridised to the 370 bp PH05 promoter fragment (A) or to total genomic DNA. An overnight exposure is shown in (A) and a 14 day exposure is shown in (B). The position of migration of the regular nucleosome ladder in chromosomal chromatin is indicated. 1, mononucleosome; 2, dinucleosome; 3, trinucleosome; etc. A map of the promoter region of the PH05 gene is shown indicating the position of the probe and the nuclease hypersensitivity site (heavy arrows).

The PH05 promoter undergoes a characteristic chromatin transition, the loss of four nucleosomes spanning the promoter, when cells are grown in low phosphate (7–9). We used accessibility to ClaI nuclease digestion as a measure of the presence or absence of nucleosome 2 on the PH05 promoter in chromatin templates prepared from cells grown in high or low phosphate (Fig. 2B). Digestion of 100 µl cleared supernatant at 37°C for 30 min with 75 U ClaI in buffer A revealed 95% cutting on a template prepared from cells grown in low phosphate and <5% cutting on a template prepared from cells grown in high phosphate (Fig. 3). This pattern of sensitivity is identical to that described for the PH05 promoter in vivo (8,9).

Two further plasmids were also purified using this method. A 2 µl based GALI–lacZ plasmid showed a nucleosomal repeat ladder at the central region of the lacZ gene identical to that observed in vivo. An indirect end label analysis of a TRP ARS CEN plasmid extracted using this method indicated that the characteristic nuclease resistant structure at the centromere is preserved on the chromatin in vitro, as is the characteristic hypersensitive site at ARS1 and the characteristic nucleosome position throughout the backbone of the plasmid (data submitted but not shown). In <1 h, it is possible to prepare a chromatin template with a micrococcal nuclease and restriction endonuclease sensitivity identical to that observed in the cell, which is substantially free of contaminating proteins and in a buffer with a low detergent concentration. This will facilitate the characterisation of chromatin composition at distinct loci.

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REFERENCES