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Chromatin structure snap-shots: rapid nuclease digestion of chromatin in yeast

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Yeast (*Saccharomyces cerevisiae*) is a focus for research on the structure and function of DNA as chromatin (1,2). There is currently much interest in dynamic chromatin processes such as replication origin function and transcription factor mediated nucleosomal rearrangements (3,4). The presence and position of nucleosomes and other nucleoprotein complexes has traditionally been inferred by mapping DNA cleavage sites produced by digesting chromatin in isolated nuclei with exogenously added nucleases. However, the preparation of yeast nuclei is too time consuming for analysing chromatin structure changes that might take place in the space of a single cell cycle. Methods must therefore be found that allow nucleases to be rapidly introduced into nuclei. Diffley *et al.* have recently used DNase I digestion of chromatin in glass-bead disrupted yeast cells to footprint the ORC complex at the 2μm origin at 10 min time points within a cell cycle (3). However, we show below that glass-bead treatment of yeast cells disrupts nucleosomal organisation. We have previously reported that yeast chromatin can be probed by nuclease digestion of detergent permeabilised sphaeroplasts (5,6). In this paper, we present a new protocol based on the preparation of sphaeroplasts which is as rapid, but less aggressive, than glass-beading. We suggest that this method may be of general utility in observing chromatin structure in a time-resolved manner, and use it to show that the nucleosomal organisation of the constitutively expressed *TRP1* gene is stable throughout the cell cycle.

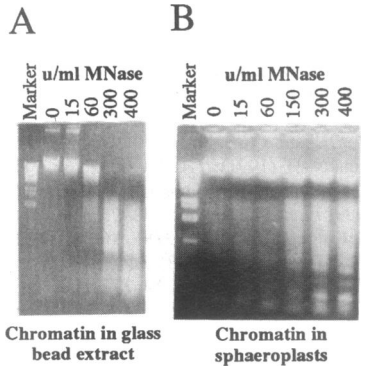

Figure 1. 1% Agarose gels showing *S.cerevisiae* DNA digested with increasing concentrations of micrococcal nuclease in: (**A**) glass bead treated cells prepared using the protocol described in (3); (**B**) detergent permeabilised sphaeroplasts prepared as described in this paper.

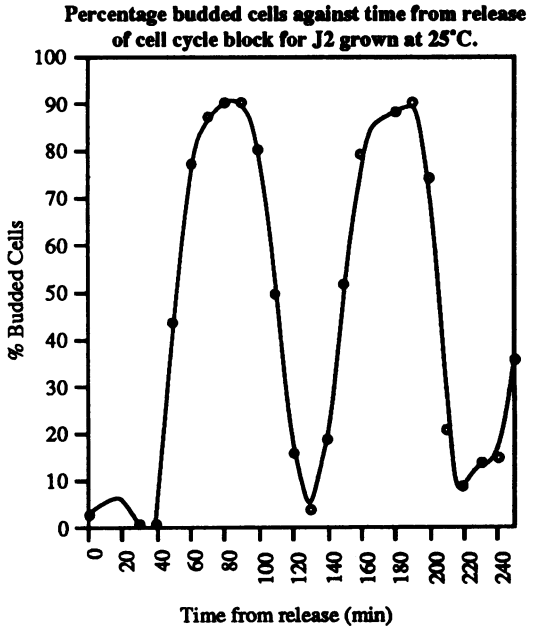

Figure 2. Graph of the percentage of budded cells in the synchronous culture of J2 against time from release of the cell cycle block.

Yeast cells (1×10^8) are harvested from culture in 2 ml screw-cap microcentrifuge tubes (which can be used throughout the procedure) at 12 000 r.p.m. for 10 s. Media is completely removed by aspiration and the cells are resuspended in 1 ml of sphaeroplasting solution containing 1 M sorbitol, 20 mg/ml yeast lytic enzyme (ICN, 20 000 U/g) and 0.5 mM β-mercaptoethanol by pipetting. The cells are incubated for 2–2.5 min at room temperature with constant gentle inversion of the tube. Sphaeroplasts are harvested by microcentrifugation at 12 000 r.p.m. for 10 s and the sphaeroplasting solution pipetted off for re-use. The sphaeroplast pellet is washed twice in 1 ml 1 M sorbitol for a few seconds. It is important at this stage not to disrupt the pellet by pipetting but only to dislodge it from the side of the tube. The sorbitol washes are aspirated after microcentrifugation at 12 000 r.p.m. for 5 s. The sphaeroplast pellet is then quickly resuspended in 200 μl of digestion buffer containing 1 M sorbitol, 50 mM NaCl, 10 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM β-mercaptoethanol, 0.5 mM spermidine, 0.075% NP-40 and the appropriate nuclease. A 1 ml micropipette tip is used at this stage to ensure that the sphaeroplasts are not clumped, although

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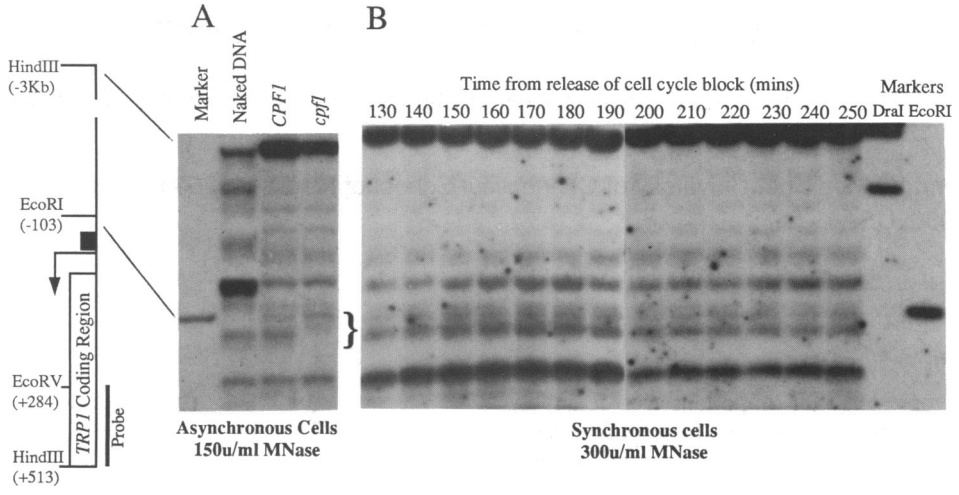

Figure 3. Southern blot indirect-end-label analysis of the *S.cerevisiae TRP1* locus in asynchronous and synchronous cultures. (**A**) Chromatin from asynchronous cultures of DBY745 (*CPF1*) and YAG93 (*cpf1* null) was digested with 150 U/ml micrococcal nuclease as described in (5). DNA was purified, digested to completion with *Hin*dIII and separated on a 1.5% agarose gel for blotting and probing with the fragment indicated on the gene map to the left. A single CPF1 binding site is indicated with a black box. A nucleosome-free region maintained in the presence of CPF1 (6) is marked with a bracket. DBY745 DNA digested to completion with *Eco*RI/*Hin*dIII was included as a marker. (**B**) Chromatin from cells sampled at 10 min time points from a synchronised J2 (*CPF1dbf2-2*) culture was digested with 300 U/ml micrococcal nuclease as described in this paper. The purified DNA was analysed as above. Markers on this blot are J2 DNA digested partially with *Dra*I and then to completion with *Hin*dIII, and J2 DNA digested to completion with *Eco*RI/*Hin*dIII.

care should be taken not to lyse the cells. We use micrococcal nuclease (Pharmacia LKB) or DNase I (ICN) at final concentrations of 150–300 U/ml or 20–60 U/ml, respectively, and incubate the digests at 37°C for 4 min. Nuclease digestion is terminated by adding 20 µl 250 mM EDTA/5% SDS followed by rapid mixing (the solution should clear indicating lysis of the cells).

Given a microcentrifuge with rapid acceleration and braking (Eppendorf 5410 and Sigma 112 work well), the procedure described above can be completed in ~8 min. It is therefore possible to sample repeatedly from a growing yeast culture. The minimum time taken for sphaeroplasting is strain-dependent and must be determined empirically by testing for lysis on addition of the EDTA/SDS stop solution described above. Over-sphaeroplasting should be avoided. We find that after microcentrifugation, the sphaeroplasting solution contains minimal cell contamination and can be re-used for up to 10 digestions.

The resulting samples are treated with Proteinase K and RNase A and extracted three times with phenol/chloroform (1:1). DNA is precipitated with 40 µl 5 M NH_4Ac and 200 µl isopropanol and resuspended in 20 µl TE. The DNA can either be digested to completion with an appropriate restriction enzyme for indirect end label analysis or subjected to primer extension for higher resolution footprinting.

Figure 1 shows the result of micrococcal nuclease digestion of chromatin prepared by the method of Diffley *et al.* (3), and the method described above. Digestion in sphaeroplasts, as described above, yields a clear nucleosomal ladder when the purified DNA is separated on an agarose gel. Digestion in glass bead treated cells, on the other hand, produces a smear indicating that this method disrupts nucleosomal chromatin structure. Figures 2 and 3 show an analysis of the nucleosomal organisation of the constitutively expressed *TRP1* gene throughout the cell cycle using the method described in this paper. A temperature sensitive *dbf2-2* strain J2 (7) was arrested at late M-phase by incubation in YPD at 37°C for 1 h 45 min. Arrested cells (2.5×10^9) were harvested by centrifugation in a rotor warmed to 37°C and resuspended in 100 ml of fresh YPD at 25°C. This synchronised culture (Fig. 2) was incubated at 25°C with shaking, and samples containing 1×10^8 cells taken at 10 min intervals during the second synchronous cycle for treatment as described above. Digestion patterns were analysed by indirect-end-labelling with a probe specific for *TRP1*. The chromatin organisation of the *TRP1* promoter, including a nucleosome-free region maintained by the DNA binding factor CPF1 (6), appears to remain stable throughout the cell cycle (Fig. 3).

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REFERENCES

1 Winston, F. and Carlson, M. (1992) *Trends Genet.* **8**, 387–391.
2 Svaren, J. and Hörz, W. (1993) *Curr. Opin. Genet. Dev.* **3**, 219–225.
3 Diffley, J.F.X., Cocker, J.H, Dowell, S.J and Rowley, A. (1994) *Cell* **78**, 303–316.
4 Schmid, A., Fascher, K-D. and Hörz, W. (1992) *Cell* **71**, 853–864.
5 Kent, N.A., Bird, L.E. and Mellor, J. (1993) *Nucleic Acids Res.* **21**, 4653–4654.
6 Kent, N.A., Tsang, J.S.H., Crowther, D.J. and Mellor, J. (1994) *Mol. Cell Biol.* **14**, 5229–5241.
7 Johnston, L.H., Eberly, S.L., Chapman, J.W., Araki, H. and Sugino, A. (1990) *Mol. Cell Biol.* **10**, 1358–1366.