

***In vitro* DNase I footprinting (Fig. S1)**

The protocol was similar to that described by Losa *et al.* (1). Briefly, the 210 bp BglIII-PstI fragment containing the hybrid *tk* promoter was isolated from the pLHC4/TLN-6. The 5' end of top strand of the fragment (BglIII site) was labeled using [γ -³²P]ATP (6000 Ci/mmol; Amersham Pharmacia Biotech) and polynucleotide kinase. The labeled fragment was incubated with about 2-fold excess of HeLa core histones, which had been purified by the conventional method, in 10 mM Tris-HCl (pH 7.5), 1mM EDTA, 1.3 mM β -mercaptoethanol and 1.05 M NaCl. Reconstitution was achieved by a 14 step dilution of NaCl from 1.05 to 0.11 M at room temperature, with 10 minutes incubation times at each step. Digestion of the reconstituted material with DNase I was performed in parallel with the digestion of protein-free DNA control. The nucleosome cores and the free DNA were adjusted to 5 mM MgCl₂ and then incubated with 4 or 0.4 units DNase I (TaKaRa) at 37°C for different times (between 1 and 6 min). The reaction was stopped by addition of EDTA (final concentration; 10 mM). The digestion products were extracted and analyzed. The Maxam-Gilbert method (2) was used to obtain sequencing lanes.

REFERENCES

- 1 Losa, R., Omari, S. and Thoma, F. (1990) Poly(dA)•poly(dT) rich sequences are not sufficient to exclude nucleosome formation in a constitutive yeast promoter. *Nucleic Acids Res.*, **18**, 3495-3502.
- 2 Maxam, A. M. and Gilbert, W. (1977) A new method for sequencing DNA. *Proc. Natl. Acad. Sci. USA*, **74**, 560-564.

Fig. S1

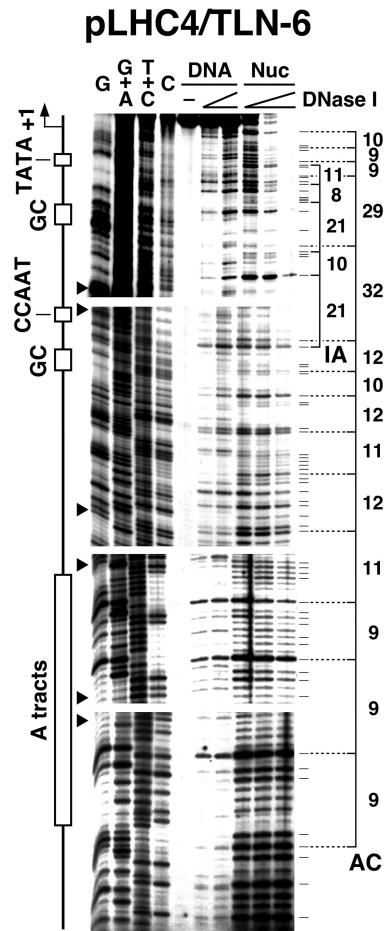
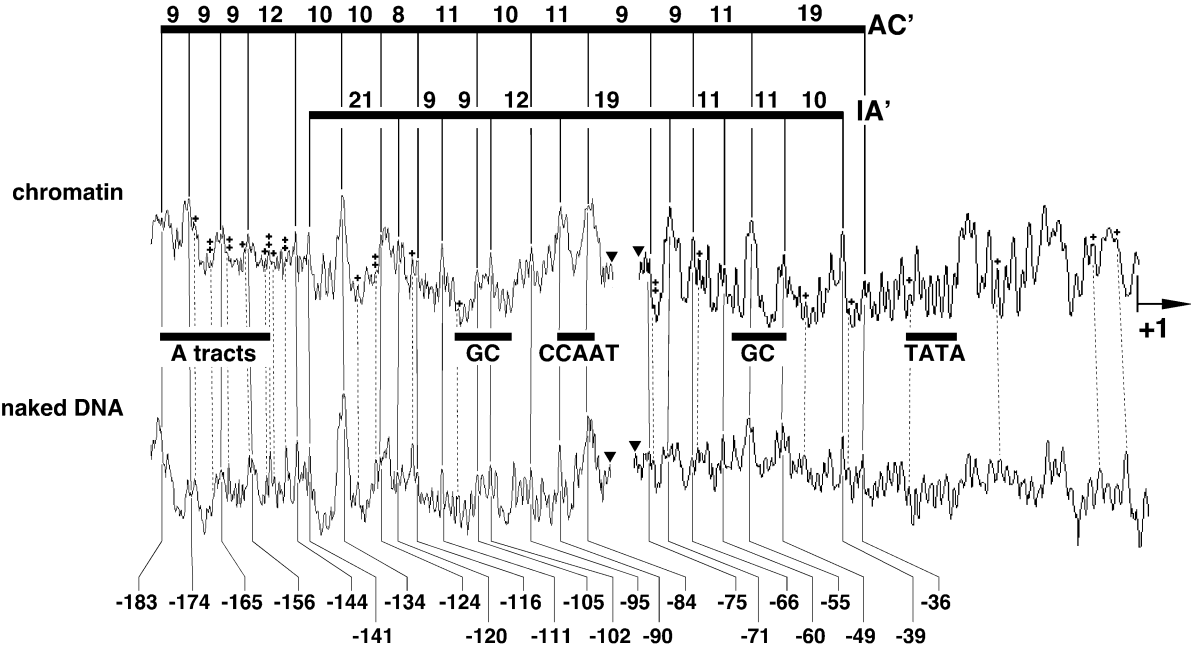


Figure S1. DNase I footprints of the nucleosomes reconstituted on the fragment of pLHC4/TLN-6. The reconstituted nucleosomes and protein-free DNA were digested with DNase I for various times (lanes labeled 'Nuc' and 'DNA' respectively). The undigested DNA fragment was electrophoresed in '-' lane. The distinctive cleavage products obtained from the nucleosomes are indicated on the right-hand side, and distances (bp) between these cleavage signals are indicated in cases where they were between 8 and 12, or near multiples of 10. 'AC' and 'IA' indicate active and inactive forms of nucleosomes respectively. The small filled triangles on the left-hand side of gels indicate overlap points of the signals.

Fig. S2

pLHC4/TLN-6



pST0/TLN-7

