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Poly(dA)·poly(dT) rich sequences are not sufficient to exclude nucleosome formation in a constitutive yeast promoter

Riccardo Losa+, Stefano Omari and Fritz Thoma*
Institut für Zellbiologie, ETH-Hönggerberg, CH-8093 Zürich, Switzerland

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ABSTRACT

It was suggested that poly(dA)·poly(dT) rich sequences in yeast *Saccharomyces cerevisiae* act as elements of constitutive promoters by exclusion of nucleosomes (Struhl, K. (1985). Proc. Natl. Acad. Sci. USA 82, 8419 – 8423). We have mapped the chromatin structure of the *pet56-his3-ded1* region in minichromosomes and show that the poly(dA)·poly(dT) sequences are located in nuclease sensitive regions. DNA fragments from the nuclease sensitive promoter region of DED1 were used for nucleosome reconstitution *in vitro*. We show that all sequences can form nucleosome cores and that the poly(dA)·poly(dT) sequence can be incorporated in nucleosome cores. The results suggest that the nuclease sensitivity found *in vivo* is not established by poly(dA)·poly(dT) mediated exclusion of nucleosomes.

INTRODUCTION

In a first level of chromosome organisation, the DNA is packaged by histone proteins into a linear array of nucleosomes. Since the accessibility of the DNA in nucleosomes is limited, the arrangement of nucleosomes with respect to the DNA sequence might play a decisive role in the regulation of DNA dependent processes. Indeed, nuclease digestion experiments revealed that promoter regions of genes (5′ ends) and origins of replication were frequently hypersensitive to nucleases and by this criterium not folded into stable nucleosomes. The exclusion of nucleosomes from regulatory regions suggests an easier access or landing for RNA polymerases (for review 2,3). We need to know what the mechanisms are by which nucleosomes arrange on the DNA sequence and by which nuclease sensitive regions are formed.

Three determinants of nucleosome positions have been established, namely histone-DNA interactions, influences of flanking structures and chromatin folding (reviewed by 4, 5, 6). Since nuclease sensitive regions are devoid of (stable) nucleosomes, one might ask what the mechanisms are which prevent nucleosome formation. The facts that nuclease sensitive regions correlate to regulatory regions and that protein factors which bind there were identified, suggest that nuclease sensitivity may be established by factors (regulatory proteins) which compete with histones for DNA binding. Alternatively, the histone-DNA interactions might be locally weakend by histone modifications (for review 2,3). Furthermore, DNA sequences which have lower affinities for histone octamers (7) and therefore form unstable nucleosomes have been identified. In an extreme case, physical properties (e.g. bendability) of a the DNA sequence might prevent its wrapping around a histone octamer. Best known examples of these are long polymers of poly(dA)·poly(dT) which are not folded into nucleosomes during *in vitro* reconstitution (8, 9, 10). Short poly(dA)·poly(dT) tracts are excluded from the center of nucleosomes, but found at the ends (10, 11).

Poly(dA)·poly(dT) tracts are sequence elements which occur frequently in the yeast *Saccharomyces cerevisiae*. It was demonstrated that poly(dA)·poly(dT) tracts which occur in the promoter regions of the HIS3 gene and the DED1 gene in yeast are elements of constitutive promoters (1). The ded1-poly(dA)·poly(dT) element was shown to enhance transcription *in vivo* (12) and *in vitro* (13). It was suggested that these elements might affect chromatin structure by excluding nucleosomes and facilitating entry of the transcription machinery (1, 12). The naturally occurring ded1-poly(dA)·poly(dT) tract, however, is not a poly(dA)·poly(dT) homopolymer, but interrupted by several G and C: 5′TTTCCTTTTTTCTTTTTG-CTTTTTCTTTTTTT3′ (14). We call this sequence a poly(dA)·poly(dT) tract throughout this paper. Those interrruptions might facilitate wrapping around a histone octamer. In this work, we show that the poly(dA)·poly(dT) tracts are indeed located within nuclease sensitive regions *in vivo*. However, *in vitro* reconstitution experiments using the naturally occurring poly(dA)·poly(dT) tract of the DED1 promoter show that this poly(dA)·poly(dT) tract can be folded in nucleosomes and therefore does not exclude nucleosome formation.

MATERIALS AND METHODS

Constructions: YRpCS1: A 1765 bp long BamH1 fragment, which contains the HIS3 gene flanked by the 5′ends of the PET56 and DED1 genes (14), was inserted into the BamH1 site of

* To whom correspondence should be addressed

+ Present address: F.Marti AG, CH-9001 St Gallen, Switzerland

pBRAT2. Plasmid pBRAT2 (15) is a pBR322 derivative containing the TRP1ARS1 sequence in the EcoRI site and a 10 bp BamHI linker inserted in the NaeI site (nucleotide 1069 of the TRP1ARS1 sequence, 16). The yeast sequences were purified as an EcoR1 fragment, circularized and introduced into yeast *Saccharomyces cerevisiae* (Strain Sc-3: mat alpha, trpl, ura3, his3) by transformation (17). **YRpFT60 to YRpFT68** are derivatives of YRpCS1 which contain additional inserts of various sizes in the HindIII site of the TRP1 gene (Thoma, to be published elsewhere).

Mapping of nucleosome positions: The procedures have been published elsewhere (15, 18, 19). Briefly, plasmid containing yeast strains were grown on selective media, converted to spheroplasts by zymolyase. Spheroplasts were lysed in ice cold buffer A (20 mM TrisHCl (pH 8),150 mM NaCl, 5 mM KCl, 1 mM EDTA, 1 mM PMSF) containing 0.2% Triton X-100. The genomic chromatin was pelleted by centrifugation (15000 rpm, 30 minutes, 0°C) and the minichromosomes were separated from small molecular contaminants by fractionation on a Sephacryl-S300 column (equilibrated and eluted with buffer A). Chromatin and deproteinized DNA were digested with different amounts of micrococcal nuclease in Buffer A supplemented with 5 mM $CaCl_2$ at 37°C for 5 minutes. The DNA was purified, cut by a restriction endonuclease to completion, fractionated according to size on 1% agarose gels, blotted to nitrocellulose and hybridized to a short radioactively labelled probe abutting on the restriction site. A DNA ladder consisting of multiples of 256 bp (18) was used for calibration. Radioactive bands were detected using Fuji X-ray films and enhancer screens.

Construction and Preparation of DNA for reconstitution: DNA fragments from the *pet56-his3-ded1* region were subcloned in the polylinkers of pUC8 or pUC9. The constructs were cut at restriction site (HindIII or EcoR1). To radioactively label the 3'ends, the recessed ends were filled using alpha ^{32}P-dATP, dGTP, dCTP, dTTP and the Klenow fragment of DNA polymerase I. 5' ends were labelled by T4- polynucleotide kinase using gamma-^{32}P-dATP (20). The labelled fragments were released from the plasmid by restriction at a second site, and further purified by preparative polyacrylamide gelelectrophoresis. **HISAT-long:** The 173 bp PstI-XhoI fragment (nucleotide 1206 to1379) of the HIS3-BamHI fragment was inserted between SalI and PstI of pUC9 to give plasmid p9HISAT. The EcoR1-HindIII fragment used for reconstitution is 197 bp long (without single strand overhangs) or 205 bp with filled ends. **HISAT-short:** HISAT-short is the AccI-XhoI fragment (nucleotide 1263 to 1379). It was derived from HISAT-long by AccI cleavage. The AccI-EcoRI fragment is 130 bp (without single strand overhangs). **ATDED-long:** The 185 bp SfanI-DdeI fragment (nucleotide 1309 to 1494) was filled in with Klenow and ligated into the HincII site of pUC8 to give plasmid p8ATDED. The Hind III- EcoR1 fragment is 214 bp (without single stranded overhangs). **ATDED-short:** ATDED-short is the SfanI-HphI fragment (nucleotide 1309 to 1442). It was derived from ATDED-long by Hph I cleavage. The Hind III- HphI fragment is 144 bp (without single strand overhangs). **HIS-185:** The 185 bp AluI-AluI fragment (nucleotide 1016 to 1201) was ligated into the pUC9 vector at the HincII site to give plasmid p9HIS187. The EcoR1 – HindIII fragment is 211 bp (without single stranded overhangs). **XHODED:** XHODED is the XhoI-DdeI fragment (nucleotide 1379 – 1494). It was cut out of p8ATDED using XhoI and EcoR1. The fragment is 128 bp (without single strand overhangs).

Preparation of nucleosome core particles: Soluble chromatin depleted of histones H1 and H5 was prepared from chicken erythrocytes nuclei as described by Bates et al. (21). Native nucleosome core particle were isolated from the soluble chromatin by the method of Lutter (25). The concentration of the core particle was 1.7 mg DNA/ml in 100mM NaCl, 10 mM TrisHCl (pH 8), 1 mM EDTA.

In vitro reconstitution: The protocol was similar to that described by Drew and Travers (22) and Losa and Brown (23). The end-labeled DNA was incubated with about a 100-fold excess of nucleosome core particles in a buffer containing 10mM Tris (pH 7.5), 1 mM EDTA, 1.3 mM β-mercaptoethanol and 800 mM NaCl. Histone octamer exchange from the donor core particle to the end-labeled DNA fragments was carried out for 30 min at 37°C followed by a stepwise reduction to 600, 500, 400, 300, 200, and 100 mM NaCl at room temperature with at least 10 minutes incubation times at each step. The end volumes were usually between 150 and 500 μl depending on the amount of DNA and the concentration at the beginning of the reaction. The efficiency of reconstitution was monitored by electrophoresis through a 0.7% agarose gel run at a low voltage in Tris-Borate-EDTA (20) as described by Drew and Travers (22) and by centrifugation through a sucrose gradient (see below). The relative amount of the core histones was the same at the beginning and at the end of the reconstitution as checked by SDS-PAGE (24). In reconstitution experiments with long fragments (HISAT-long, ATDED-long) and high amount of core particles (more then 100 fold excess), we observed that the reconstituted material had a slower migration in agarose gels and a faster sedimentation in sucrose gradients, suggesting additional binding of histones. Since DNaseI footprinting (see below) did not reveal any difference in nucleosome positions, we believe that additional histones or histone octamers were loosely associated.

Competition Experiments: For the experiment described in Fig. 5, 30 ng ATDED-short (labelled by fill in at HindIII) and 30 ng XHODED (labelled by fill in at XhoI) were mixed in a total volume of 16 μl 10mM Tris, 1 mM EDTA, 1 mM β-mercaptoethanol (pH 8). Aliquots of 2μl of DNA-fragments (7.5 ng) were mixed with different amounts of donor core particles (from 0 to 45μg) in a total volume 40 μl to give core DNA/fragment ratios of 0 to 6000. Reconstitution was done as described above to an end volume of 340 μl. 300 μl of the reconstituted material was run through a 5.5 – 28.8% linear sucrose gradient containing 100mM NaCl, 10mM Tris (pH 7.5) and 1 mM EDTA at 4°C (33000rpm, 13hrs in a SW-60 Beckmann rotor). Fractions a 0.4 ml were collected and the absorbance at 260 nm and the radioactivity distibution were monitored. To determine the relative amounts of XHODED and ATDED-short in each fraction, the DNA was purified by phenol extraction and ethanol precipitation, separated on 8%-polyacrylamid-7M urea gels (1500 V 3.5 hrs) (20). The bands were detected by autoradiography.

DNase I footprinting

Digestions of the reconstituted material with DNaseI were performed in parallel with the digestions of protein free DNA controls. The nucleosome cores (and the free DNA) were adjusted to 5 mM $MgCl_2$ and then incubated with DNaseI (5 μg enzyme) at room temperature for different times, usually between 30 seconds and 5 minutes. The reaction was stopped by addition of 10 mM EDTA. After phenol extraction and ethanol precipitation, the digestion products were resuspended in 99%

formamide containing 1 mM EDTA, 0.03% xylene cyanol and 0.03% bromophenol blue. After boiling for 1 min, the samples were elecrophoresed through an 10% polyacrylamid-7 M urea gel at 1500 V for 4–5 hr. The gels were dried and the bands detected using Fuji X-ray films and enhancer screens at −70°C.

RESULTS

Chromatin structure of the *pet56-his3-ded1* region

The chromatin structure of the HIS3 gene and its flanking regions was studied in a series of minichromosomes that occur at high copy number (Fig 1A). They are all derivatives of the small TRP1ARS1-circle and contain the HIS3 region on a BamH1 fragment inserted in a region of unknown function (UNF, 15). The minichromosomes are between 3227 bp (YRpCS1) and 3766 bp (YRpFT61, YRpFT63) long and differ in small inserts (276 to 539 bp) in the HindIII site of the TRP1 gene (Thoma, unpublished).

For structural analysis, minichromosomes were partially purified from lysed spheroplasts and the accessibility of the DNA was tested by digestion with micrococcal nuclease. The cutting sites obtained in digests of chromatin and deproteinized DNA were displayed by the indirect endlabeling technique (Fig. 2). Protection of cutting within 140 to 200 bp in chromatin is interpreted as the footprint of a histone octamer of a positioned nucleosome (boxes, Fig. 2). The averaged cutting sites and a structural interpretation are given in figure 1. The chromatin structure of the HIS3 region was similar in all the constructs. The small inserts in the HindIII site had no influence on the HIS3 region. Five precisely positioned nucleosomes were found on the coding region of the HIS3 gene, and two each on the PET56 and DED1 fragments. All these nucleosomes were stable at low and high levels of nuclease digestion (not shown).

The nucleosomal regions were separated by nuclease sensitive regions (NSR). One NSR spanned the common promoter region of HIS3 and PET56 and was characterized by two strong cutting sites for micrococcal nuclease (Fig. 2B). The second nuclease sensitive region spanned the 3′ end of HIS3 and the promoter region of DED1 (cutting sites from 1270 to 1434, Fig. 2C). Both poly(dA)·poly(dT) tracts were located in DNA sequences which are poor substrate for m. nuclease (no cutting on deproteinized DNA, see D lanes) and therefore did not allow a direct footprinting. However, since histone octamers are expected to protect about 140 bp of DNA, and since the cutting sites mapped on both sides were only 110 bp and 80 bp apart, we conclude that the poly(dA)·poly(dT) tracts were not folded in nucleosomes *in vivo*.

Since the poly(dA)·poly(dT) tract was shown to be part of the constitutive promoter of the DED1 gene and since long tracts of poly(dA)·poly(dT) can exclude nucleosome formation *in vitro*, it was asked whether the poly(dA)·poly(dT)-tract acts as constitutive promoter by exclusion of nucleosome formation (1). To address this question, we used DNA fragments isolated from this region (Fig. 1B) and tested their ability to form nucleosomes *in vitro*.

Small DNA fragments containing poly(dA)·poly(dT) sequences form nucleosomes *in vitro*

The formation of nucleosomes was tested on two small DNA fragments, HISAT-short and ATDED-short (Fig. 1 and 3), which were just long enough to form a nucleosome. HISAT-short was 136 bp long, which includes the filled and labelled EcoR1 site

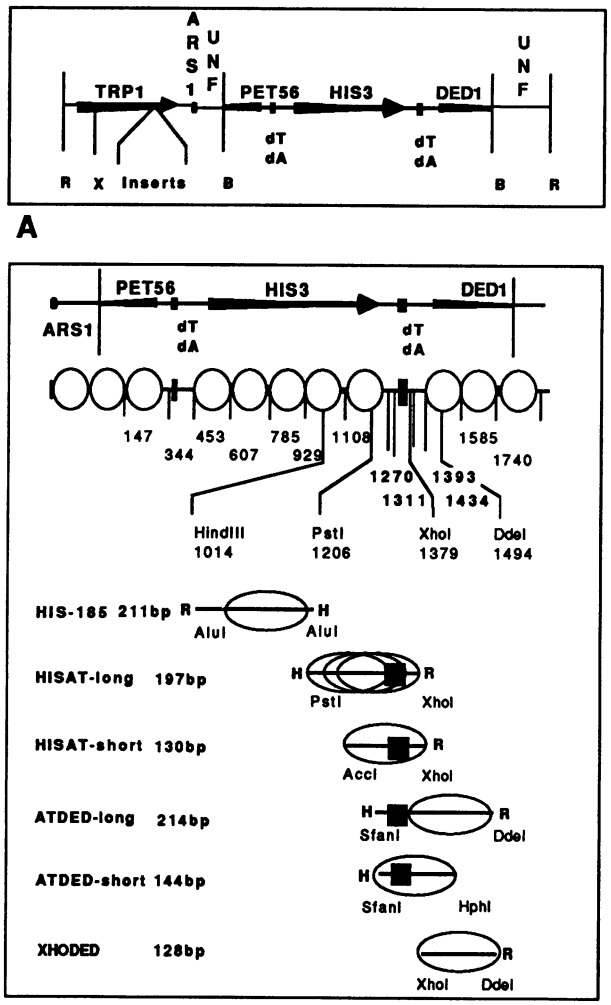

Figure 1. Constructions and Chromatin structures of the *pet56-his3-ded1* region. A linearized drawing of the constructs is shown (A): A BamH1 fragment of the *pet56-his3-ded1* region (14) was inserted into the TRP1ARS1 circle using an artifical BamH1 site (15). Inserts of different lengths were cloned into the HindIII site of the TRP1 gene. R, EcoR1; X, Xba1; B, BamH1; dT/dA, poly(dT)·poly(dA). Summary of the chromatin structure (B). The HIS3 gene contains 5 precisely positioned nucleosomes (circles) flanked by nuclease sensitive regions at the 5′ end and 3′ end. The numbers below the nucleosomes refer to the cutting sites for micrococcal nuclease in the linker DNA (Fig. 2) with respect to the sequence published in the EMBO genebank (SCHIS3G, K. Struhl. Nucleotide 500 on SCHIS3G = nucleotide +1 in (14)). The common promoter region of HIS3 and PET56 as well as the region around the 3′end of HIS3 and the promoter of DED1 are sensitive to micrococcal nuclease and by this criterium free of nucleosomes. Heavy arrows in the top line indicate orientation and length of the open reading frames. Fragments of the HIS3-DED1 region were subcloned in pUC vectors and used for *in vitro* reconstitution. Name and length (without single stranded overhangs) are indicated. Relevant restriction sites are: HindIII, 1014; AluI,1016, 1201; PstI, 1206; AccI, 1263; SfaNI, 1309; XhoI, 1379; HphI, 1442; DdeI, 1494. H and R are HindIII and EcoRI sites of the polylinker used for endlabeling the DNA. Black boxes indicate the 34 bp long poly(dA)·poly(dT) tract (Nucleotides 1337–1370 of SCHIS3G). The putative locations of nucleosome(s) after *in vitro* reconstitution are shown as ellipses.

on the right side and a two bases overhang of the AccI cut on the left side. It contained the poly(dA)·poly(dT) tract on the right side (72 to 105 bp from the left end). ATDED-short was 144 bp long and contained in addition 4 bases overhang at the left (HindIII) side and a one base overhang on the right side (HphI).

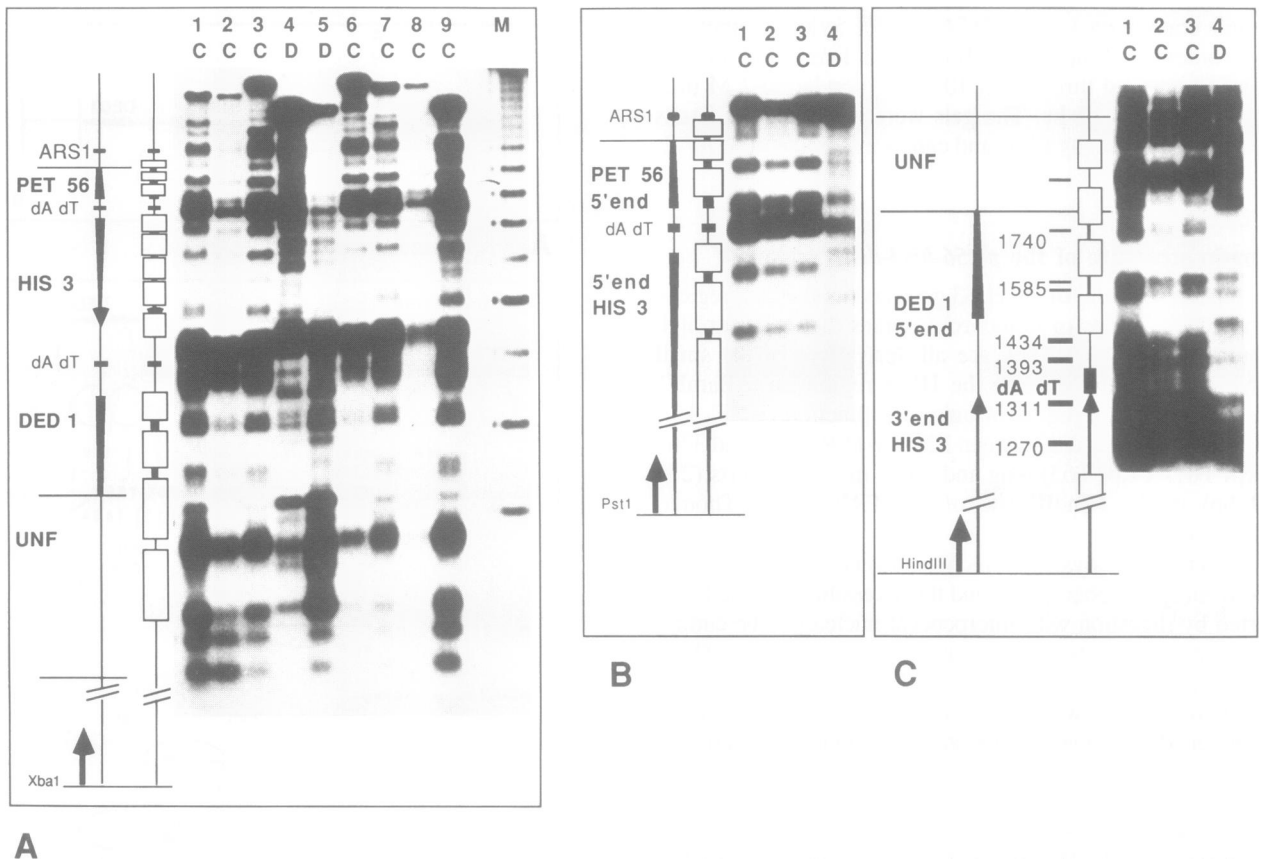

Figure 2. Chromatin structures determined by mapping the accessibility of the DNA to micrococcal nuclease. Chromatin (C) and deproteinized DNA (D) was digested with m. nuclease and the cutting sites are displayed by indirect endlabeling from the Xba 1 site **(A)**, from an internal Pst I site **(B)** and an internal Hind III site **(C).** Boxes show positioned nucleosomes. The intergenic regions are sensitive to nucleases, heavily cut and by this criterium free of nucleosomes. The open reading frames are shown as heavy arrows, and decapitated arrows. The 34 bp long poly(dA)·poly (dT) tract (1337−1370) of the DED1 promoter and of the PET56-HIS3 promoter are indicated as black boxes. Thin arrows in (C) show the 3'end of the transcripts (14). Numbers in (C) indicate the averaged cutting site for m. nuclease. A marker lane showing multiples of 256 bp (15) is included in (A). Lane 1, YRpFT60; lane 2, YRpFT62; lane 3, YRpFT63; lane 4, 5, 9, YRpCS1; lane 6, YRpFT61, lane 7 YRpFT66; lane 8, YRpFT65.

The poly(dA)·poly(dT) tract was located on the left side (39 to 72 bp from the left end, Fig. 1).

The double stranded DNA fragments were labeled with ^{32}P at one end of one strand and nucleosomes were reconstituted by histone octamer transfer from chicken erythrocyte core particles (23). The reconstitution products were identified as nucleosome-like structures by non-denaturing gel electrophoresis, by sedimentation in sucrose gradients (see Fig.5 for sucrose gradients) and by DNase I footprinting. We show DNaseI footprints of the histone octamer, since they allow to monitor nucleosome formation and to deduce their actual position on the DNA sequence. Since the DNA is wrapped on the outside of the histone octamer, DNaseI cleavage occurs on one strand approximately every ten bases giving rise to a characteristic 'ten base ladder' on a polyacrylamide gel (25). Deviations from a 'ten base ladder' occur in unique sequence nucleosomes due to the sequence preference of DNase I (26).

The reconstitution products of both fragments, HISAT-short and ATDED-short, produced such a 'ten base ladder' upon DNase I digestion (Fig. 3) demonstrating that nucleosomes were formed. In HISAT-short, eight maxima can readily be observed (dashes and numbers from 1368 to 1284). The DNA between the maxima was protected from cutting by binding of histone proteins (compare D and C lanes). The frequency of cutting varies with its position on the surface of the nucleosome (25) and is further modulated by the sequence preference of DNase I (see D lanes). In HISAT-short, two positions in the 'ten base frame' appeared not to be cut extensively, presumably because those sequences were located on the nucleosome surface where cutting was low (arrows at about 1325 and 1295). The fact that cutting was low at those positions argues for a unique nucleosome position on HISAT-short.

The reconstituted ATDED-short fragment shows a series of bands in about 10 bases intervals (from about 1325 to 1394 and higher), which also demonstrates the existence of a positioned nucleosome. The poly(dA)·poly(dT) tracts were clearly located within the 'ten base ladder' and hence included in the nucleosome, both in HISAT-short and in ATDED-short. The poly(dA)·poly(dT) tract of the constitutive DED1 promoter was therefore not sufficient to exclude nucleosome formation.

Although HISAT-short and ATDED-short share about half of their DNA sequence (1309−1379), their nucleosomes were not positioned identically with respect to that sequence: the digestion maxima in HISAT-short/ATDED-short were around 1346/1343, 1357/1354, 1368/1363. This result suggests that the common sequence in the two constructs does not contain a dominant positioning signal.

Flanking sequences modulate nucleosome positions

Since the histone octamer might have been forced on the poly(dA)·poly(dT) tracts on the small fragments, reconstitution

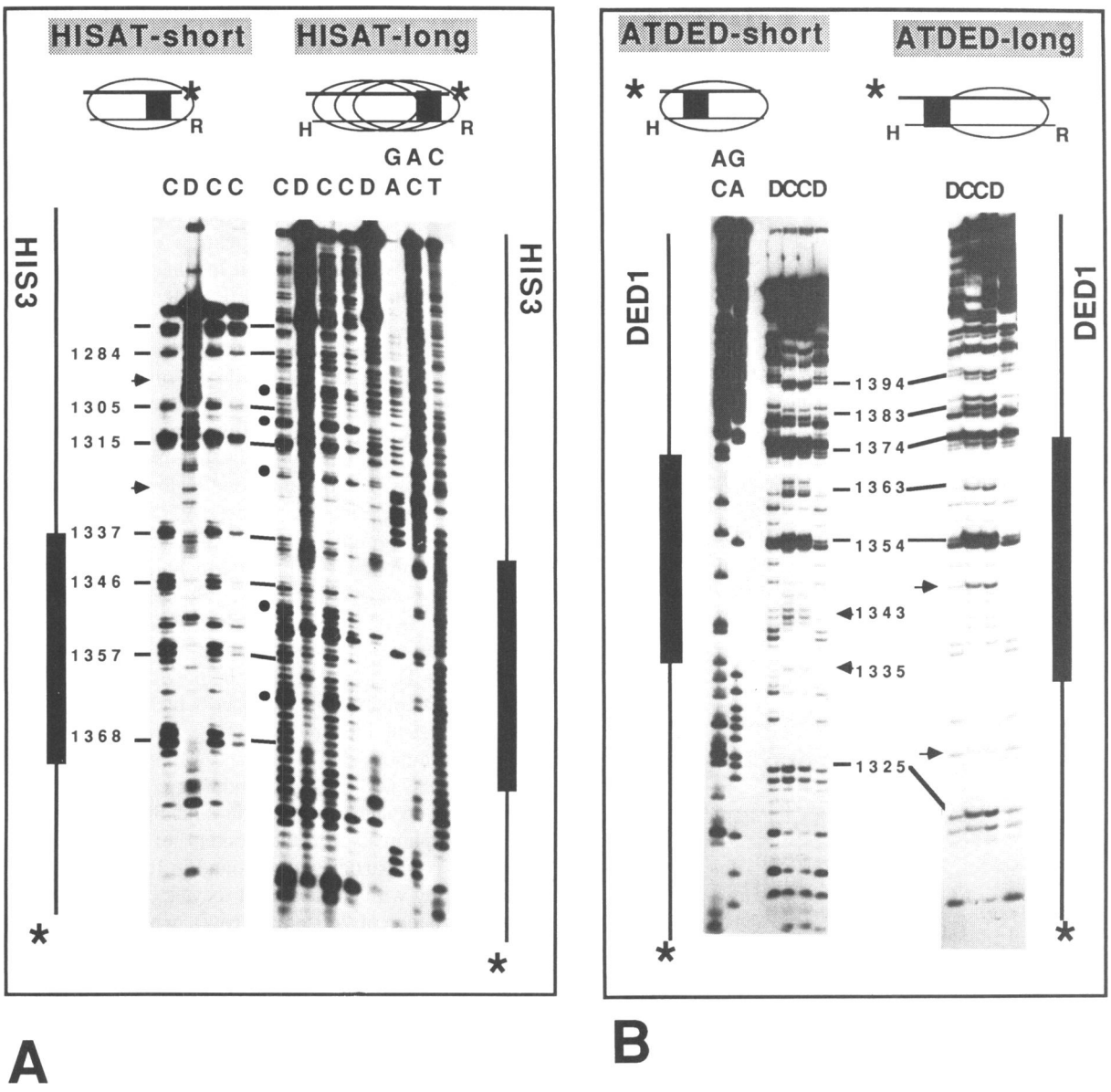

Figure 3. DNAase I footprints of reconstituted nucleosome cores. The 3′ ends of the topstrands of HISAT-short and HISAT-long were labelled with 32-P (*) by fill in the EcoR1 site. The 5′ends of topstrands of ATDED-short and ATDED-long were labelled with 32-P (*) using polynucleotide kinase. The labelled fragments were mixed with chicken erythrocyte core particle in high salt and reconstitution was achieved by stepwise dilution of salt. Reconstituted core particles (C lanes) and protein free DNA (D lanes) were digested to a limited extent by DNase I. The digestion products were fractionated on denaturing polyacrylamide gels and visualized by autoradiography. Numbers indicate cutting sites with respect to the SCHIS3G sequence. The black box represents the poly(dA)·poly (dT) tract. AG,GA, sequencing lanes showing AG and GA, respecively.

was repeated with longer fragments to allow exclusion of the poly(dA)·poly(dT) tracts.

HISAT-long was 205 b(p) long, which includes the 197 bp long double stranded region, 4 bp of the filled EcoR1 site and a 4 base overhang on the HindIII site. Since the poly(dA)·poly(dT) tract was located 131 nucleotides from the left end, HISAT-long should allow nucleosome formation with almost complete exclusion of the poly(dA)·poly(dT) tract. The footprints of a reconstitution experiment produced a complex pattern (figures 3A and 4). The HISAT-long digestion contained the DNase I maxima of HISAT-short (connective lines in Fig. 3A), but in addition further maxima (dark circles) were evident. This DNAse I pattern suggests that the histone octamers bound at multiple positions on HISAT-long, but with a preference for one or two positions. Those positions had a different rotational setting of the DNA sequence: a sequence which faces the histone octamer and is protected in one nucleosome position, is exposed in an other position. This complex pattern and, hence, the nucleosome positions extend almost completely towards the left end (Fig. 4). However, the poly(dA)·poly(dT) tract was not excluded in all nucleosomes of that population.

ATDED-long was a 218 b(p) long, which includes the 214 bp long double stranded region and 4 bases overhangs each at the EcoRI and HindIII ends (Fig.3B). For the experiment shown in figure 4, the bottom strand was labelled by filling in the EcoR1 site. The poly(dA)·poly(dT) tract was located 142 bp to 175 bp from the right end. After reconstitution, a clear DNase I footprint was recorded which extended from about 1354 (Fig. 3B) almost completely towards the right end (1485 in Fig. 4). Below 1354, the digestion pattern of the poly(dA)·poly(dT) region did not

show a characteristic '10 base repeat'. Cutting around 1343 was missing, while a new cut appeared (arrow).These results indicated that the histone octamer bound at a single position on ATDED-long only partially overlapping the poly(dA)·poly(dT) region. These results demonstrate that upon elongation of the DNA-fragment, the positions of reconstituted nucleosomes were changed, but the poly(dA)·poly(dT) tract could be excluded towards the end of the nucleosome in ATDED-long, only, and not in HISAT-long.This modulation of nucleosome positioning towards DED1, but not towards HIS3 suggests that the poly(dA)·poly(dT) tract is not sufficient to exclude nucleosomes from that region.

We like to emphasize that multiple positions were observed on HISAT-long which belongs to the 3'end of the HIS3 gene, while a single position was inferred on ATDED-long which belongs to the DED1 promoter. ATDED-long might therefore contain a positioning signal on the DNA sequence (outside of the poly(dA)·poly(dT) tract). It remains to be investigated whether this putative positioning signal relates to the *in vivo* position of the first DED1 nucleosome, since the sequence from approximately 1434 towards the right end of ATDEDlong corresponds to half of the sequence of the first nucleosome of the DED1 gene mapped *in vivo* (Fig. 2).

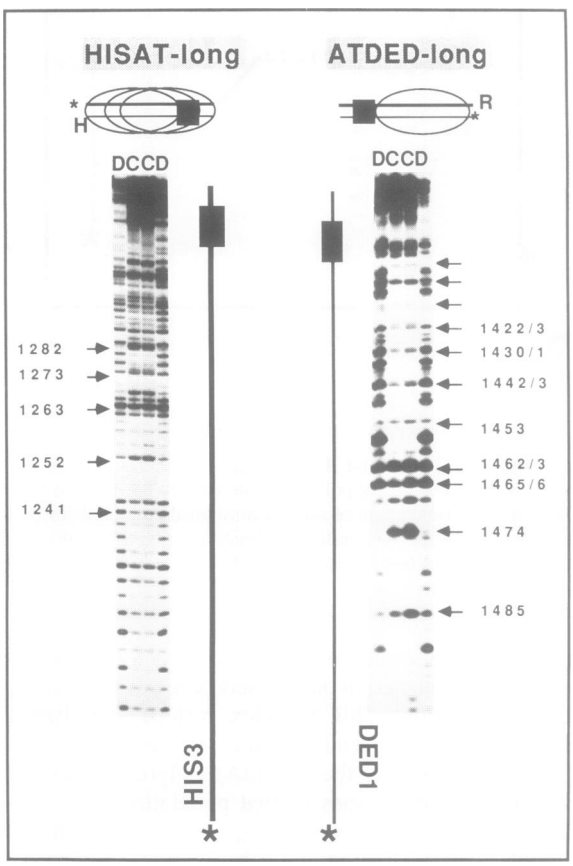

Figure 4. DNAaseI footprints of nucleosome cores reconstituted on HISAT-long and ATDED-long. The 5'ends of the topstrand (HISAT-long) and of the bottomstrand (ATDED-long) were labelled using polynucleotide kinase. Reconstitution and DNaseI footprinting was as described in Fig. 3. While a clear 'ten base repeat' indicates a unique position on ATDED-long, multiple positions appear to be formed on HISAT-long.

Histone octamer bind to DNA fragments with the similar efficiency irrespective of the presence of a natural poly(dA)·poly(dT) tract

Once nucleosomes are formed on a DNA fragment, the optimal position might be found by sliding along the DNA sequence. A fundamental question, however, is whether DNA sequences containing or missing the poly(dA)·poly(dT) tract do form nucleosomes with the same efficiency. We have therefore performed a series of competition experiments. Two DNA fragments of slightly different length were reconstituted in the same reaction mixture with limiting amounts of donor core particles and the reconstitution products were separated on sucrose gradients. A DNA fragment with a low efficiency of nucleosome formation was expected to sediment predominantly in the DNA peak, while fragments with high efficiency were expected to be found in the core particle fraction. Only short (about nucleosome sized) DNA fragments were useful, since longer fragments tend to associate with more than one histone octamer (not shown).

A typical experiment compares ATDED-short and XHODED (Figl 5). Both fragments were close to nucleosome core length. ATDED-short was 149 bp long including the filled HindIII site and a one base overhang at the HphI-end. XHODED was 136 bp long including a filled EcoRI-end and a 4 base overhang at the XhoI end. Both fragments shared 63 bp DNA-sequence, but only ATDED-short contained the poly(dA)·poly(dT) tract, while XHODED contained a sequence which *in vivo* forms half of a nucleosome of DED1 (Fig. 1).

Reconstitutions shown in Fig. 5 were performed in the presence of no cores, 600 ng cores and 3μg cores. The products were separated on sucrose gradients and the fractions analysed by gelelectrophoresis for the content of the labeled fragments. Labeled core particles were found in fraction 5 of the gradient, comigrating with chicken erythrocytes core particles (determined by absorbance at 260 nm, arrow), while free DNA peaked in fraction 3. In the reconstitution with 600 ng cores, similar amounts of material migrated as free DNA and as core particles. If the histone octamer were to bind preferentially to one DNA fragment, this fragment is expected to be underrepresented in the free DNA fractions and overrepresented in the core fraction. The autoradiogram of the DNA gel shows that the relative intensities of the two bands (Fig. 5b) was similar in all fractions. The same was true at 3 μg cores where almost all the labelled material migrated in the core particle fractions. These results suggested that both DNAs bind histone octamers with similar efficiency and that presence or absence of the poly(dA)·poly(dT) tract had no detectable influence on the efficiency of nucleosome formation.

DISCUSSION

We have determined the chromatin structure of the *pet56-his3-ded1* gene region in detail by mapping the accessibility of the DNA to micrococcal nuclease. We have found precisely positioned nucleosomes, five on HIS3, and two each on the PET56 and DED1 regions. In contrast to the TRP1 gene (18), but similar to the URA3 gene (19), the nucleosomes on HIS3 were stable during extensive digestion with m. nuclease and did not rearrange or disintegrate. It is noteworthy that two and three nucleosomes were found tightly packed in HIS3 and in URA3 (19), respectively. The significance of this observation is not known. In a series of differentially sized minichromosomes, it was demonstrated that nucleosome positions might depend on

chromatin folding (27). The structure of the *pet56-his3-ded1* gene region was examined in minichromosomes which were between 3.2 kb and 3.8 kb long, but no differences were detected. We therefore conclude, that the observed chromatin structure is an inherent property of the region.

A nuclease hypersensitive site at the 'TATA' sequence of the genomic HIS3 gene was previously reported (28,29). Our detailed mapping showed that the nuclease sensitive regions as estimated from the distance between the m. nuclease cuts were at least 110 bp (NSR2) and 160bp (NSR1)long and included the poly(dA)·poly(dT) tracts. It is unlikely that the poly(dA)·poly(dT) sequence *per se* is responsible for the establishment of the whole nuclease sensitivity, since the NSR are much longer than the poly(dA)·poly(dT) tracts. On the other hand, we cannot exclude that the nuclease sensitive regions are folded in nucleosomes, which are unstable during nuclease digestion, because of modified histones, for example.

The central question was, whether the naturally occurring poly(dA)·poly(dT) sequences *per se* avoid nucleosome formation. This hypothesis was based on the observations that upon *in vitro* reconstitution, synthetic poly(dA)·poly(dT) polymers (9) or an 80 bp long poly(dA)·poly(dT) polymer on a recombinant plasmid (8) excluded nucleosome formation while a short stretch of 20 bp poly(dA)·poly(dT) was included in a nucleosome (8; 10). Using small DNA fragments from the NSR1 and a standard *in vitro* reconstitution protocol, we clearly demonstrate that the naturally occurring 34 bp poly(dA)·poly(dT) tract can be folded in nucleosomes.

Since no uncovered, overlapping DNA ends were detected in the short fragments (HISAT-short, ATDED-short), we assign the centers of those nucleosomes close to the middle of the DNA-fragments, approximately to nucleotides 1385 (ATDED-short) and 1325 (HISAT-short). The poly(dA)·poly(dT) tract must therefore be close to but not overlapping the center of the nucleosomes. Since the long poly(dA)·poly(dT) polymers did not form nucleosomes, we think that the G and C nucleotides which interrupt the poly(dA)·poly(dT) tract (at positions 1340/41, 1348, 1354/55; (14)) might break the unusual properties of poly(dA)·poly(dT) (30) and facilitate the wrapping around the histone octamer. Different nucleotides were exposed to DNase I at the surface in HISAT-short and ATDED-short (e.g. 1357 in HISAT-short, 1354 in ATDED-short), which indicated that the rotational setting of the DNA on the nucleosome surface was different. The poly(dA)·poly(dT) tract itself was not uniquely positioned nor did it exhibit a dominant influence on the rotational setting.

In vivo, the histone octamers might be able to slide along the DNA and to find an optimal, high affinity position, while reconstitution with small fragments might have forced the histone octamers on their positions. This is consistent with the observation that nucleosome positions were different in ATDED-long and ATDED-short as well as in HISAT-short and HISAT-long. To test whether the right side of ATDED-long had a higher affinity for histone octamers than the poly(dA)·poly(dT) tract, we compared nucleosome formation in a direct competition experiment between ATDED-short and XHODED in the same test tube and found no remarkable difference. When the experiment was repeated with a 10 time molar excess of one fragment, no significant difference in nucleosome formation was found (not shown). The differences in binding energies between the left and right side of ATDED-long must be too small to be detected in this competition experiment. A quantitation of the competition expriments, e.g. by cutting out and counting the radioactive bands of the DNA-peak and core peak, was deliberately omitted, since minor differences in nucleosome formation might rather be due to 'end effects' or due to the slightly different lengths of the DNA-fragments.

One might ask whether DNA from a nucleosomal region is more efficient in nucleosome formation than DNA from a nuclease sensitive region. To test this hypothesis competition was performed between ATDED-long (214 bp) which originates from a nuclease sensitive region, and the 211 bp long HIS187 fragment, which originates from the nucleosomal region of HIS3. Again, no dramatic difference was observed in the binding efficiencies

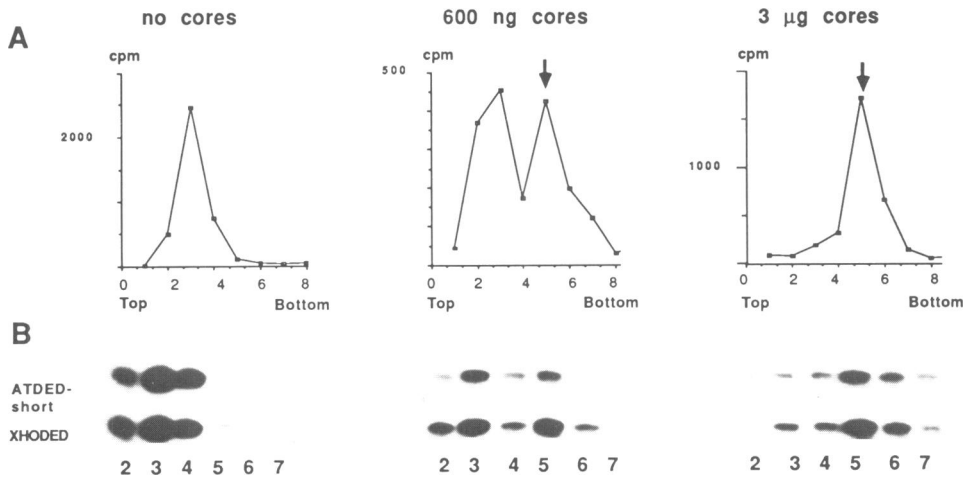

Figure 5. Competition for nucleosome formation between ATDED-short and XHODED. Endlabeled ATDED-short and XHODED fragments were mixed at a molar ratio of 1 and reconstitution was performed in presence of no cores, 600 ng cores , and 3µg cores. The reconstitution products were fractionated on sucrose gradients (A). Core particles were found in fraction 5 comigrating with chicken erythrocyte core particles (determined by absorbance at 260 nm). Free DNA was found in fraction 3. The radioactivity of the fractions was counted and is displayed (A). An aliquot of each fracton was run on a denaturing polyacrylamide gel. The autoradiography of the gel is shown in (B). The relative intensities of the ATDED-short and XHODED bands within a fraction remained unchanged, which suggests that both DNAs form nucleosomes with a similar efficiency.

for the histone octamers (not shown). This results suggested that DNA fragments from a non-nucleosomal region can bind histones with similar efficiency as DNA sequences from a nucleosomal region.

Reconstitution on HISAT-long yielded multiple positions of nucleosomes all of which have presumably similar binding energies. So it is likely that binding of factors might influence nucleosome positions *in vivo*. It was shown that natural sequences had a rather modest binding strength compared with artificial sequences (7). The small differencies in binding energies might be important for a chromatin region where nucleosomes have to be moved or removed to make DNA sequences accessible.

What than determines the nuclease sensitivity of NSR1? NSR1 probably serves a double function, one in termination of HIS3 transcription and another one in promotion of transcription of the DED1 gene. In analogy to the 3′ end of URA3, which consists of a stable nucleosome and a nuclease sensitive region with two strong cutting sites (19), we correlate the last HIS3 nucleosome and two cutting sites to the 3′end of HIS3. Since the sequences *per se* is able to form nucleosomes, we invoke protein factors which might successfully compete with histones and establish nuclease sensitivity. 'Datin', an oligo(dA)·oligo(dT) binding factor (31), might bind the poly(dA)·poly(dT) tract. We find it unlikely that 'datin' alone opens up the nuclease sensitive region in its full length. ABF1 is a second candidate, since it binds immediately to the left of the poly(dA)·poly(dT) tract (32). Since ABFI also binds to the nuclease sensitive ARS1 region which overlaps with the 3′end of the TRP1 gene (18, 32), ABF1 might eventually be involved in the termination of transcription. The nuclease sensitivity between the poly(dA)·poly(dT) tract and the DED1 nucleosomes is presumably related to promotion of transcription. Since the first nucleosome begins approximately 40 bp downstream of the TATA sequence (1393–1398, Struhl, K., EMBL:SCHIS3G), a putative TATA factor might be involved.

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