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Nucleosomes represent a physical barrier for cleavage activity of DNA topoisomerase I *in vivo*

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DNA topoisomerase I together with the other cellular DNA topoisomerases releases the torsional stress from DNA caused by processes such as replication, transcription and recombination. Despite the well-defined knowledge of its mechanism of action, DNA topoisomerase I *in vivo* activity has been only partially characterized. In fact the basic question concerning the capability of the enzyme to cleave and rejoin DNA wrapped around a histone octamer remains still unanswered. By studying both *in vivo* and *in vitro* the cleavage activity of DNA topoisomerase I in the presence of camptothecin on a repeated trinucleotide sequence, $(TTA)_{35}$, lying in chromosome XIII of *Saccharomyces cerevisiae*, we can conclude that nucleosomes represent a physical barrier for the enzyme activity.

Key words: chromatin, DNA structure, DNA topoisomerase I, nucleosome, *Saccharomyces cerevisiae*, trinucleotide repeats.

INTRODUCTION

DNA topology in eukaryotic nuclei is controlled by a specific class of proteins, DNA topoisomerases [1]. These enzymes are classified into two groups depending on their catalytic activity: type I enzymes change DNA topology by breaking and rejoining one DNA strand at a time, whereas type II enzymes act simultaneously on both strands. In eukaryotes, DNA topoisomerase IB reduces the torsional stress arising from basic DNA processes such as transcription, replication and recombination [2]. Although DNA topoisomerase I is involved in such important functions, the enzyme is not essential in *Saccharomyces cerevisiae* [3].

Despite the well-known mechanisms of DNA topoisomerase I activity and the susceptibility to different inhibitors [4], its accessibility to the DNA *in vivo* has been poorly investigated. To date, evidence regarding the *in vivo* DNA topoisomerase I cleavage activity only refers to nucleosome-free regions [5–7] and it is not known whether nucleosomes could represent a barrier for DNA topoisomerase I to access DNA. In fact it has been demonstrated that nucleosomal complexes interfere with the accessibility of transcription and replication factors on DNA [8,9]. Recently, Roca and co-workers [10] have demonstrated that DNA topoisomerase II relaxes nucleosomal DNA much faster than DNA topoisomerase I. By analysing the relaxing activities on different nucleosomal substrates they suggested that, *in vivo*, DNA topoisomerase I more than DNA topoisomerase II, could be impeded by nucleosomal structures in cleaving and rejoining DNA [10]. Actually the DNA topoisomerase I cleavage reaction on *in vivo* sequences has not yet been studied on substrates containing or not nucleosomes. In order to answer this question, in the present study we have evaluated the DNA topoisomerase I cleavage activity on the natural $(TTA)_{35}$ repeated sequence, both *in vitro* and *in vivo*. This sequence contains structural features presumably favouring both the DNA topoisomerase I site-specific cleavage activity and nucleosome assembly. In fact, the TTA sequence has been shown to be intrinsically flexible [11] and the TA step is locally bent [12]. These two features (i.e. flexibility and local bending) and the previous observation that DNA topoisomerase I *in vitro* efficiently cleaves the bent TA step [13] rendered the $(TTA)_{35}$ repeat a good substrate by which to evaluate the capability of DNA topoisomerase I to cleave a specific sequence, possibly in the presence of nucleosomes. In addition, using a strain where the H4 synthesis is under the control of the GAL1 promoter [14], we were able to study the DNA topoisomerase I activity on a sequence where the nucleosome organization is lost or maintained in glucose or galactose respectively. This approach allowed us to study, *in vivo*, the DNA topoisomerase I reaction on different chromatin arrangements.

EXPERIMENTAL

Strains, plasmids and culture medium

The strains used were W303-1a (*Mata, ade 2-1, ura 3-1, his 3-11,15, trp1-1, leu 2-3,112, can1-100*), AMR51 (*Mata, ade 2-1, ura 3-1, his 3-11,15, trp1-1, leu 2-3,112, can1-100, TOP1::leu2*) kindly provided by R. Sternglanz (Department of Biochemistry and Cellular Biology, Stony Brook University, Stony Brook, NY, U.S.A.) and UKY403 [*Mata, ade 2-101, his -Δ200, leu 2-3,112, lys2-801, ura3-52, trp1-Δ419,Δhhf1::his3* and *Δhhf2::leu2/pMH3110* (*TRP1, CEN3, ARS1, UAS gal-HHF2*)] kindly provided by M. Grunstein (Department of Biological Chemistry, Geffen School of Medicine and the Molecular Biology Institute, University of California, Los Angeles, CA, U.S.A.).

The sequence −422 to +612 [from the *PRE8* (proteinase yscE) ATG] encompassing the $(TTA)_{35}$ region, was PCR amplified and cloned into the EcoRI site of the yeast vector pADH426 [15] yielding the plasmid pFDFG1a.

Cells were grown in minimal YNB (yeast nitrogen base) [16], supplemented with 2% glucose or 2% galactose when appropriate.

Enzymes and chemicals

Restriction enzymes, T4 polynucleotide kinase and MNase (micrococcal nuclease) were from Roche. Vent(exo$^-$) polymerase was from New England Biolabs. Zymolyase 100T was

Abbreviations used: CPT, camptothecin; MNase, micrococcal nuclease; RIS, RNA initiation site.

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from Seikagaku. CPT (camptothecin) was from Sigma and radiochemicals were obtained from Amersham.

Chromatin analysis

MNase treatment was performed as described previously [17]. MNase (2 and 4 units) was added to different aliquots of spheroplast preparation and the samples were incubated at 25 °C for 20 min. The DNA was then deproteinized and ethanol precipitated. RNase treatment was also performed.

Low-resolution analysis

Indirect end-labelling analysis [18] was performed as follows: after treatment with the appropriate restriction enzymes, the samples were electrophoresized in 1.2 % agarose gels (1.75 V/cm), transferred on to nitrocellulose membrane, hybridized to the TTA probe and detected by autoradiography.

In vivo induction of CPT-dependent DNA topoisomerase I cleavage sites

Cells were treated for cleavage-site induction as previously described [7]. Three different aliquots were incubated with 50, 100 or 200 μM CPT for 2 min at room temperature (25 °C). The reaction was stopped with 1 % SDS containing 5 mM EDTA. The DNA was then deproteinized and ethanol precipitated. RNase treatment was also performed.

Oligonucleotide primers and probes

The oligonucleotide sequences used in the extension reactions were VEC, 5′-GGTATCGATAAGCTTGATATCG-3′ and r3, 5′-CGCGTTTCCGTATTTTCCGC-3′. The oligonucleotide VEC lies at position −449 from the *PRE8* ATG of the cloned sequence containing the $(TTA)_{35}$ in pFDFG1a. The r3 oligonucleotide used lies at position −267/−248 bp from the 35S RNA RIS (RNA initiation site) complex. 5′-End labelling using [γ-^{32}P]ATP and T4 polynucleotide kinase was performed according to [19].

The probe TTA, annealing at positions +436/+232 bp from the ATG of the *PRE8* gene, was labelled by random priming.

Multiple-round primer extension and high-resolution analysis of *in vivo* MNase and DNA topoisomerase I cleavage sites

Genomic DNA (1–2 μg) was reacted with Vent polymerase and end-labelled oligonucleotide as described previously [20]. The samples were cycled 25 times through the following steps: 95 °C for 5 min, 58 °C for 10 min and 76 °C for 3 min (conditions for oligonucleotide VEC). The extension products were phenol extracted, ethanol precipitated, dissolved in formamide buffer and analysed by 6 % denaturing PAGE.

In vitro DNA topoisomerase I cleavage sites

The reactions were performed as described previously [21] by reacting 1, 5 and 10 units of purified DNA topoisomerase I (from calf thymus) with 1–2 ng of end-labelled DNA. The reactions were carried out in 10 mM Tris/HCl (pH 8.0), 10 mM NaCl, 1 mM $MgCl_2$ and 100 μM CPT for 15 min at 37 °C. The DNA substrate for the cleavage reaction was EcoRI excised from pFDFG1a. After restriction digestion with XhoI/XbaI the fragment was 3′-labelled, purified and further digested with SmaI or HindIII in order to uniquely orient the labelling.

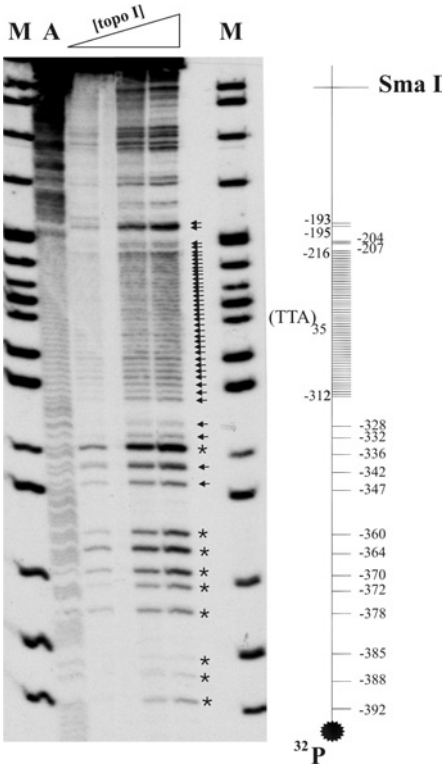

Figure 1 ***In vitro*** **DNA topoisomerase I cleavage site on TTA sequence**

Increasing amounts of calf thymus DNA topoisomerase I (1, 5 and 10 units; indicated by the triangle) were reacted with the 3′-labelled TTA-containing DNA sequence in the presence of CPT. Mapping of the fragments, separated by a sequencing gel, was obtained by comparing the migration of samples with the size marker (M) and sequencing lane (A). Black arrows and asterisks indicate *in vitro* DNA topoisomerase I cleavages on TTA or different sequences respectively. A schematic map containing the genes flanking the TTA repeated sequence is reported at the right side. Numbering refers to the *PRE8* ATG.

RESULTS

We selected the trinucleotide repeat $(TTA)_{35}$ lying in the intergenic region of chromosome XIII of *S. cerevisiae*, between the *RPM2* (RNase P mitochondrial 2) and *PRE8* genes. This sequence, as reported above, contains the structural elements presumably suitable to be susceptible to DNA topoisomerase I site-specific activity and to assemble nucleosomes.

In vitro high-resolution analysis of DNA topoisomerase I cleavage sites on the $(TTA)_{35}$ repeat

The DNA tract in chromosome XIII containing the sequence $(TTA)_{35}$ was amplified by PCR with two oligonucleotides annealing at positions −474 and +755 relative to the *PRE8* ATG. The resulting fragment was cloned into the yeast vector pADH426, yielding the pFDFG1a plasmid. The XhoI/XbaI fragment from pFDFG1a was 3′-labelled, purified and further digested with SmaI in order to uniquely orient the labelling and to study the DNA topoisomerase I cleavages with a nucleotide level of resolution. The fragment was reacted *in vitro* with increasing amounts of calf thymus DNA topoisomerase I (indicated by the triangle in Figure 1) in the presence of CPT; this drug inhibits the rejoining reaction of DNA topoisomerase I thus creating, in the presence of a denaturing agent, single-strand breaks and behaving like a single-strand nuclease. As shown in Figure 1, DNA topoisomerase I strongly recognized the TTA repeats (indicated

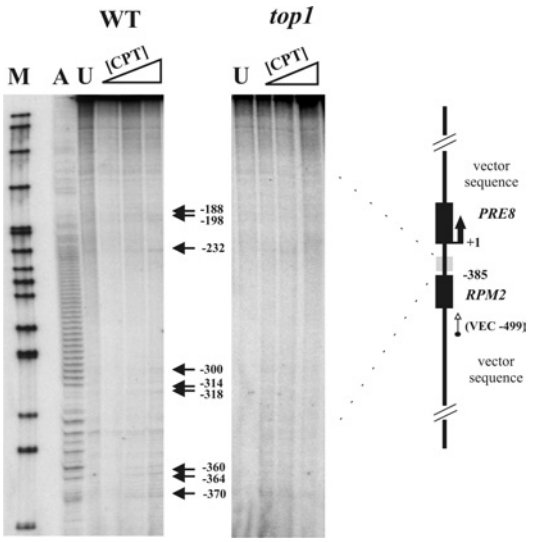

Figure 2 ***In vivo*** **DNA topoisomerase I cleavage site on TTA sequence**

Spheroplasts from wild-type (WT) or Δ*top1* cells were treated with 0 (indicated on the Figure as U), 50, 100 and 200 μM CPT (indicated by the triangles). The fragments resulting from DNA topoisomerase I activity were primer-extended from oligonucleotide VEC (white arrow) annealing at position −499 from the *PRE8* ATG. Black arrows indicate DNA topoisomerase I cleavage sites. M, pBR322/MspI size marker; A, sequencing lane. The right-hand side of the Figure shows a representation of the analysed region.

by arrows in Figure 1). The regularly repeated sequence was highly cleaved (from −312 to −216) and TTA repetitions in the flanking regions were also efficiently cleaved. The asterisks on Figure 1 indicate cleavage sites not occurring in a TTA sequence.

In vivo analysis of DNA topoisomerase I cleavage sites on the $(TTA)_{35}$ repeat

Given the results shown in Figure 1 we evaluated the DNA topoisomerase I cleavage activity on the same DNA sequence *in vivo*. Wild-type and AMR51 (Δ*top1*) cells, transformed with pFDFG1a, were analysed for cleavage-site induction as previously reported [7,20]. Briefly, spheroplasts from wild-type and Δ*top1* cells were permeabilized with nystatin [17] and treated with different amounts of CPT (indicated by the triangle in Figure 2). DNA was then purified and primer-extended from the labelled oligonucleotide VEC (annealing on the vector sequence at −449 bp from the *PRE8* ATG contained in the fragment cloned in pADH426). Only a weak cleavage profile was observed in the wild-type strain (Figure 2). The only detectable sites were localized at the flanking regions of the triplet (indicated by arrows in Figure 2). In the Δ*top1* strain no cleavage sites were observed. Taken together, these results suggest that DNA topoisomerase I poorly reacts *in vivo* with the $(TTA)_{35}$ repeat despite its *in vitro* high reactivity towards the same sequence (Figure 1).

The $(TTA)_{35}$ repeat is engaged in a positioned nucleosome *in vivo*

Results shown in Figure 2 indicate that DNA topoisomerase I does not efficiently react with the $(TTA)_{35}$ tract *in vivo*. Could this low cleavage activity on the repeated sequence be due to its chromatin organization? In order to clarify this point we studied the nucleosome positioning on this DNA sequence by *in vivo* analysis with MNase.

Spheroplasts from wild-type cells, transformed with pFDFG1a, were treated with nystatin, digested with different amounts of MNase (indicated by the triangles in Figure 3) and DNA was purified. Results are shown in Figure 3(A). The *in vivo* treated samples (chromatin) were compared with those digested *in vitro* (naked DNA) to indicate the susceptibility of deproteinized

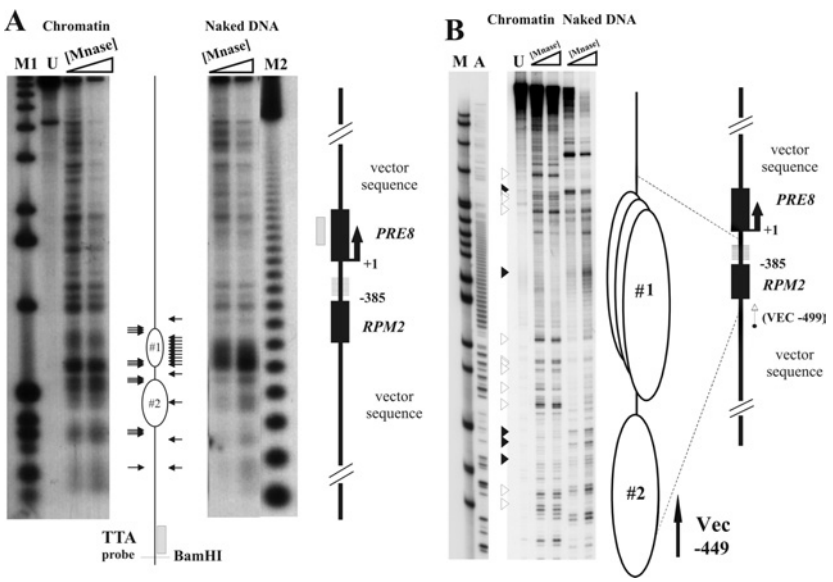

Figure 3 Nucleosome positioning on the TTA sequence

(**A**) Nystatin-permeabilized spheroplasts were treated with 0, 2 and 4 units of MNase. Purified DNA was restricted with BamHI and run on a 1.2% agarose gel, transferred on to a nitrocellulose membrane and hybridized with the TTA probe. Chromatin, *in vivo* digested samples; Naked, *in vitro* digested samples of deproteinized DNA; U, untreated DNA; M1, size marker (1 kb ladder); M2, size marker (123 bp ladder). Black arrows indicate MNase cleavage sites. (**B**) DNA was digested *in vivo* (Chromatin) and *in vitro* (Naked DNA), after deproteinization, with 0, 1, 2, 4 or 8 units of MNase and was primer extended with the labelled oligonucleotide VEC (position −449). M, size marker (pBR322/MspI); A, sequencing lane; U, untreated DNA. Filled and empty arrowheads indicate protection and enhancement respectively. The map on the right-hand side shows the nucleosome with multiple positions (indicated as ellipses). All numbering refers to the *PRE8* ATG.

DNA to MNase. This comparison showed protected regions representing nucleosomal particles based on their dimension (140–160 bp) (indicated as ellipses in Figure 3). The nucleosome reported as #1 occupied positions from − 160 bp to − 330 bp in the chromatin samples, whereas in the naked DNA the same region appeared strongly digested. This region encompasses the $(TTA)_{35}$ region (− 214 to − 318 bp).

To increase the mapping resolution, we primer-extended the DNA of different aliquots from the same samples with the labelled oligonucleotide VEC. The high-resolution results reported in Figure 3(B) shows that the $(TTA)_{35}$ repeat was almost completely protected by the nucleosome #1 (from − 349/−324 bp to − 189 bp on multiple positions; indicated by filled arrowheads in Figure 3B), as shown by comparing the chromatin samples with the naked DNA. In addition MNase-hypersensitive sites (indicated by the empty arrowheads) were localized at the flanking regions of the mapped nucleosome.

Taken together the results reported in Figure 3 demonstrate that the $(TTA)_{35}$ repeat is actually organized in a family of positioned nucleosomes covering multiple positions, all sharing the TTA repeat in the middle of the particle.

In vivo chromatin organization of the $(TTA)_{35}$ sequence after nucleosome removal

Could the positioned nucleosome on $(TTA)_{35}$ be responsible for DNA topoisomerase I failure in generating efficient cleavage sites *in vivo*? In order to verify this hypothesis we have analysed the *in vivo* DNA topoisomerase I cleavage activity in a yeast strain lacking both H4 genes and carrying one copy of H4 histone under the control of the GAL1 promoter [14]. This implied that in the presence of glucose as a carbon source, the chromatin organization would be lost and DNA should become accessible *in vivo* to both MNase and DNA topoisomerase I.

Yeast UKY403 cells, transformed with pFDFG1a, grown in galactose, were divided into two aliquots, washed and resuspended in a growth medium containing galactose or glucose. After 4 h of growth, spheroplasts were treated with different amounts of MNase. The digestion profiles shown in Figure 4(A) (bulk DNA), indicate that in the galactose-grown cultures the nucleosomal ladder is obtained, whereas in the glucose-grown cultures, a smear appears in the corresponding region of the gel. This suggests that MNase freely accesses DNA when the nucleosomal barriers are absent.

When the samples from galactose-grown or glucose-grown cultures were analysed using an end-labelling technique [18] as in Figure 3(A), a specific difference was observed (Figure 4B). Galactose-grown samples showed protection of the TTA region towards MNase (nucleosome #1), as reported in Figure 3; conversely, glucose-grown samples showed a diffuse accessibility to MNase on the $(TTA)_{35}$ sequence similar to that of the naked DNA.

DNA topoisomerase I *in vivo* cleaves the $(TTA)_{35}$ sequence after nucleosome removal

The results reported in Figure 4(B), demonstrate that the chromatin organization of the $(TTA)_{35}$ repeat was completely lost when cells were grown in glucose. Since in these conditions MNase accesses the $(TTA)_{35}$ repeat with the same efficiency *in vivo* and *in vitro*, we wanted to evaluate whether DNA topoisomerase I could also reach this sequence when chromatin was no longer organized. We then analysed the same cells for DNA topoisomerase I cleavage-site induction at high resolution (Figure 5A). The samples were primer-extended starting from

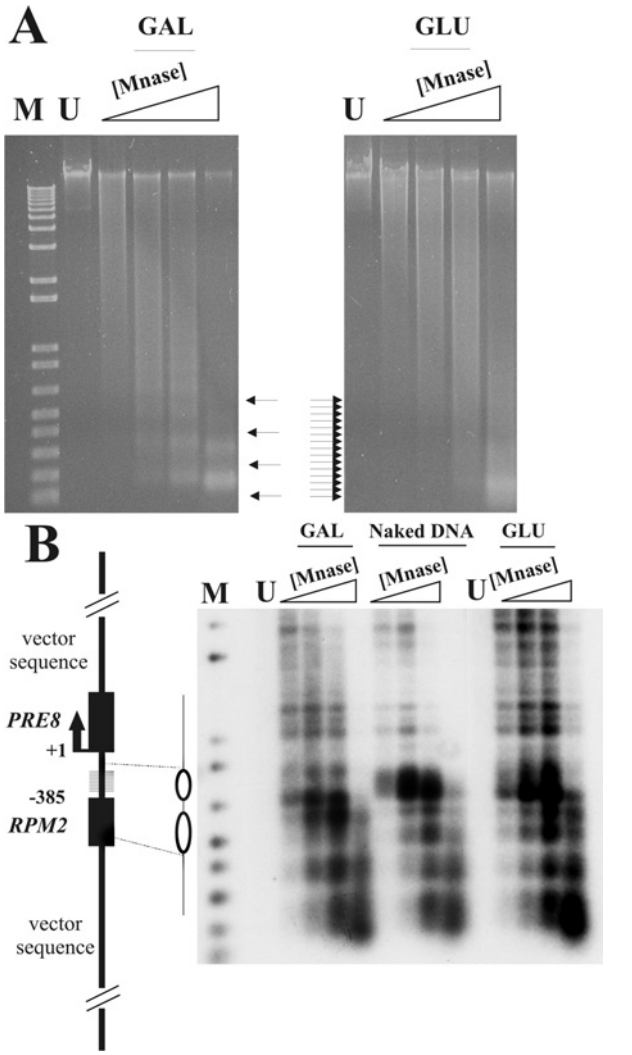

Figure 4 Nucleosome organization on TTA sequence upon nucleosome removal

(**A**) Spheroplasts from cells grown in galactose (GAL; transcribing the H4 gene) or in glucose (GLU; non-transcribing the H4 gene) were subjected to MNase digestion (as in Figure 3). DNA was purified and analysed by agarose gel electrophoresis. M, size marker (1 kb ladder); U, untreated DNA. Arrows show cleavages of MNase on DNA. (**B**) Spheroplasts from cells grown in galactose (GAL; transcribing the H4 gene) or in glucose (GLU; non-transcribing the H4 gene) were subjected to MNase digestion as reported in Figure 3 and compared with MNase digestions on deproteinized DNA (Naked). M, size marker (1 kb ladder); U, untreated DNA; ellipses indicate nucleosomes.

the labelled oligonucleotide VEC. The reactivity of DNA topoisomerase I in the glucose-grown culture was highly enhanced compared with that observed in the galactose-grown culture. In addition, the cleavage profile of glucose-grown samples (indicated by arrows in Figure 5) almost completely overlapped that obtained *in vitro* (see Figure 1). We then analysed the same samples in a different chromosomal location (the NTS2 region of the ribosomal DNA) as a control. In fact, we have previously shown [7,20] that in this nucleosome-free region [22] a strong DNA topoisomerase I cleavage site occurs at − 171 bp from the RIS [7]. Results are shown in Figure 5(B). When the oligonucleotide r3 [−268 bp from the 35S RIS] was primer-extended, both conditions (glucose-grown and galactose-grown, i.e. nucleosome present/absent respectively) showed the − 171 cleavage site, demonstrating the same reactivity of DNA topoisomerase I in

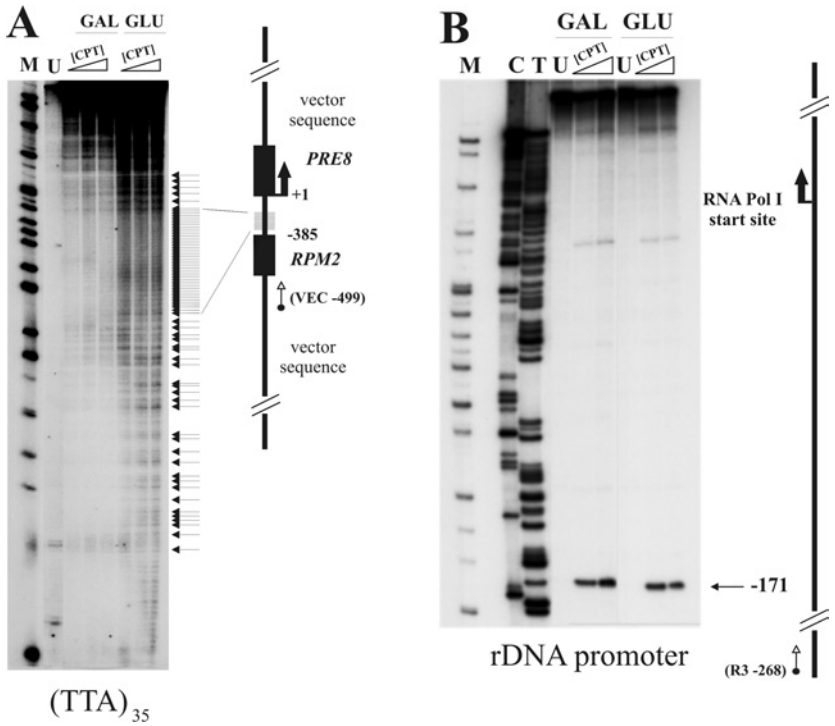

Figure 5 DNA topoisomerase I cleavage activity on the TTA sequence upon nucleosome removal

(**A**) Spheroplasts from cells grown in galactose (GAL; transcribing the H4 gene) or in glucose (GLU; non-transcribing the H4 gene) were subjected to DNA topoisomerase I cleavage sites in the presence of CPT (induction as in Figure 2). M, size marker (pBR322/MspI); U, DNA from untreated cells. Arrows indicate DNA topoisomerase I cleavage sites. Numbering refers to the *PRE8* ATG. At the right-hand side of the Figure a schematic representation of the analysed region is shown. (**B**) Samples as in (**A**) were primer-extended from oligonucleotide r3 in order to show DNA topoisomerase I cleavage sites on the NTS2 region of ribosomal genes. C and T, sequencing lanes; U, untreated sample; M, size marker.

a nucleosome-free region when H4 transcription is on or off (Figure 5B). Taken together the results reported in Figures 5(A) and 5(B) demonstrate that nucleosomes represent a barrier for localized DNA topoisomerase I activity.

In order to exclude that the observed differences in accessibility to the TTA region were due to different amounts of DNA topoisomerase I in the two growth conditions, we measured both TOP1 mRNA production and DNA-relaxing activity (results not shown). All evaluations showed a slight increase in mRNA and DNA topoisomerase I activity in cells from glucose-grown cultures; however, such a small difference did not justify the strong enhancements observed in the *in vivo* cleavage profile (Figure 5A).

DISCUSSION

It is generally accepted that all sequences in DNA can be substrates for DNA topoisomerase I relaxing activity, even though DNA topoisomerase I seems to have a higher reactivity towards some particular sequences showing structural features such as DNA bending [13,23]. The high-resolution mapping of *in vivo* DNA topoisomerase I site-specific activity concerns nucleosome-free regions [5–7]. In order to verify whether nucleosomes affect DNA topoisomerase I site-specific cleavages, we studied a natural DNA sequence repeating the TTA trinucleotide 35 times. Such a sequence provides the locally bent TA step [12], presumably a good substrate for local DNA topoisomerase I cleavage induction and an intrinsic flexibility [11] potentially useful to efficiently assemble the nucleosome.

The TTA trinucleotide repeat has proved to be efficiently cleaved *in vitro* by DNA topoisomerase I (Figure 1) in the repeated tract and also in its surrounding regions, confirming the preference of the enzyme for this sequence. Conversely, an *in vivo* approach showed a reduced reactivity of the same sequence towards DNA topoisomerase I. Thus it is conceivable to hypothesize that chromatin structure affects DNA topoisomerase I cleavage. Recently Roca and co-workers [10] observed that DNA topoisomerase II is more efficient than DNA topoisomerase I in releasing topological stress from nucleosomal substrates; this supports the hypothesis that nucleosomes may represent a barrier for DNA topoisomerase I activity. Because of this a distinction between the global relaxing activity and the local site-specific cleavage reaction should be taken into consideration. In fact a different chromatin organization in a given substrate may not be a determinant for the whole relaxing activity of DNA topoisomerase I; indeed the enzyme can release torsional stress acting on different sites in the substrate. Conversely, when a given sequence is analysed in terms of cleavage activity exerted by DNA topoisomerase I, the absence/presence of a nucleosome could be very relevant; this latter point was investigated by the experimental system in the present study.

In order to verify this hypothesis, we studied the *in vivo* chromatin organization of the $(TTA)_{35}$ tract. As shown in Figure 3, a positioned nucleosome occupies the $(TTA)_{35}$ region possibly hindering the DNA topoisomerase I cleavage activity. We then employed a yeast strain carrying a plasmid in which the H4 gene is under the control of the GAL1 promoter. This allowed us to switch on/off the synthesis of this histone, depending on the carbon source. In the galactose-grown condition the presence of nucleosomes impairs the DNA topoisomerase I approach to the $(TTA)_{35}$ sequence. Conversely, in the glucose-grown condition, when H4 production is repressed, no organized chromatin is

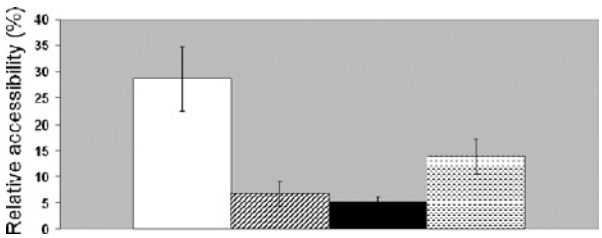

Figure 6 DNA topoisomerase I accessibility in different contexts

Different DNA topoisomerase I digestion profiles from *in vitro* (white bar), *in vivo* (dashed bar), *in vivo* glucose-grown (non-transcribing the H4 gene; black bar) and *in vivo* galactose-grown (transcribing the H4 gene; dotted bar) were evaluated by densitometric scanning. Results represent the percentage of cleavage activity on the TTA repeat.

observed on the TTA repeat and DNA topoisomerase I increases its sequence-specific activity, particularly on the $(TTA)_{35}$ tract (Figure 4).

In order to evaluate the cleavage difference of DNA topoisomerase I *in vivo*, *in vitro* and when chromatin is destructured (i.e. H4 is no longer synthesized in glucose medium), we quantified the DNA topoisomerase I digestion profiles and the results are compared in Figure 6. As shown, when chromatin is regularly organized (*in vivo* and *in vivo* galactose-grown; see also the chromatin profiles in Figures 3 and 4) approx. 5–6 % of the digested material is represented by the TTA repeat. Conversely the relative digestion of the TTA repeat when samples are reacted *in vitro* with DNA topoisomerase I reaches values of approx. 30 %. Also the dissolution of regularly organized chromatin allows DNA topoisomerase I to digest the TTA repeat *in vivo*, but with an efficiency of 15 % (approx. 3-fold higher than on the regular chromatin).

Thus we can conclude that: (i) DNA topoisomerase I efficiently reacts with the TTA repeat; (ii) the $(TTA)_{35}$ sequence, the longest and most stable among the simple repeated sequences in *S. cerevisiae* [24], is organized in a positioned nucleosome and this can possibly account for its high stability, in fact each nucleosome stores one negative supercoil [25,26], thus preventing DNA denaturation and induction of conformational alterations responsible for genetic instability [27]; and (iii) the positioned nucleosome on the $(TTA)_{35}$ sequence represents a hindrance to the DNA topoisomerase I activity. This last conclusion, based on the glucose/galactose experiments, represents the first formal evidence that DNA topoisomerase I cannot react with nucleosomal DNA.

Despite the well-known chemistry concerning the DNA topoisomerase I mechanism of action and the interference with small inhibitors largely employed in chemotherapy, so far no details are known about the possible interference that nucleosomes could represent *in vivo* towards the topoisomerization reaction. In the present study we provide direct evidence clarifying this task.

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