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Yeast nucleosomes allow thermal untwisting of DNA

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ABSTRACT

Thermal untwisting of DNA is suppressed in vitro in nucleosomes formed with chicken or monkey histones. In contrast, results obtained for the 2 micron plasmid in Saccharomyces cerevisiae are consistent with only 30% of the DNA being constrained from thermal untwisting in vivo. In this paper, we examine thermal untwisting of several plasmids in yeast cells, nuclei, and nuclear extracts. All show the same quantitative degree of thermal untwisting, indicating that this phenomenon is independent of DNA sequence. Highly purified yeast plasmid chromatin also shows a large degree of thermal untwisting, whereas circular chromatin reconstituted using chicken histones is restrained from thermal untwisting in yeast nuclear extracts. Thus, the difference in thermal untwisting between yeast chromatin and that assembled with chicken histones is most likely due to differences in the constituent histone proteins.

INTRODUCTION

In eukaryotic cells, enzymes involved in DNA repair, replication and transcription must contend with the presence of auxiliary chromosomal proteins and particularly with the presence of nucleosomes. In some cases, the canonical nucleosome may be inhibitory to enzymatic processes which use DNA as substrate, as has been found for initiation of transcription (1). This suggests either that a mechanism exists for preventing formation of nucleosomes on certain segments of DNA, or that nucleosomes are altered in structure to allow the enzymatic machinery access to the DNA. These considerations have led us to study the nature of the association between the histone proteins and DNA in the nucleosome and to look for changes in nucleosome structure which could alter the accessibility of the associated DNA.

One sensitive and non-perturbing method of investigating histone-DNA interactions in the nucleosome is to measure the

ability of the DNA to thermally untwist. Naked DNA is known to undergo a temperature-dependent untwisting equal to $-.011^{\circ}/^{\circ}C/bp$ (2,3). Early experiments suggested that the four core histones were sufficient to prevent thermal untwisting of DNA in SV40 minichromosomes (2). Subsequently, Morse and Cantor showed that nucleosomes reconstituted from chicken erythrocyte core histones, with or without histone H5, also prevented thermal untwisting (4,5). Surprisingly, each nucleosome was found to suppress the thermal untwisting of over 200 bp of DNA. More recently, it has been found that from 160 to 180 bp of DNA per nucleosome are suppressed from thermal untwisting in isolated SV40 minichromosomes containing monkey histones (6).

In light of these results, the recent finding by Saavedra and Huberman (7) that the yeast 2 micron plasmid undergoes thermal untwisting *in vivo* was unexpected. These authors were able to show, by isolating 2 micron plasmid from cells incubated at temperatures ranging from 0° to $38^{\circ}C$, that the plasmids showed a shift in linking number which was consistent with about 70% of the DNA being as free to thermally untwist as naked DNA. These results were intriguing to us, inasmuch as they suggested that the yeast genome, which is much more transcriptionally active than the genomes of larger eukaryotes, might also differ substantially in the packaging of its DNA into chromatin. However, since the number of nucleosomes on the 2 micron plasmid in yeast has not been definitively established, one cannot determine from this experiment the amount of DNA suppressed from thermal untwisting in each nucleosome. Furthermore, since the experiments were done in living cells, it could be that other factors present in the yeast nucleus modulate nucleosome structure and thereby free the DNA to thermally untwist. In order to address these issues, it was necessary to ascertain whether the thermal untwisting observed for the 2 micron plasmid could also be observed for other plasmids for which the number of nucleosomes could be accurately determined and whether the observation made *in vivo* would also hold true outside of the cellular milieu.

MATERIALS AND METHODS

Plasmids

The plasmids GAT2, pBRAT2 and TALH, each bearing the selectable TRP1 marker gene, were introduced into SC3 cells (Mat$^{<}$, ura 3-52, his 3-1, trp1, gal2, gal10, cir0) (8) as described by Sherman et al. (9) except that spheroplasts were formed using zymolyase. To construct pBRAT2, the plasmid YRp7, consisting of TRP1ARS1 in the Eco RI site of pBR322 (10) was cut with Bam HI and Ava I and the 4766 bp fragment isolated. The ends were filled with dNTPs, using the large fragment of E. coli DNA polymerase, and the fragment circularized. The resulting plasmid was then cut with Nae I, synthetic Bam HI linkers were added and the fragment recircularized to form the E. coli/yeast shuttle vector we call pBRAT2. To construct GAT2, the plasmid pBM150 (11) was cut with Eco RI. The Eco RI ends were filled with dNTPs using the large fragment of E. coli DNA polymerase and ligated to synthetic Bgl II linkers. The plasmid was then cut with Bgl II and Bam HI, the fragment containing the upstream activating sequence for the GAL1 and GAL10 genes was isolated, and monomers and tandem repeat dimers of this fragment were ligated into the Bam HI site of pBRAT2. After amplifying these plasmids in E. coli, bacterial and yeast sequences were separated by digestion with Eco RI and gel electrophoresis. The 2150 bp and 2835 bp fragments were circularized to form the plasmids named GAT1 and GAT2. TALH is a TRP1ARS1 derivative carrying a fragment of the 5' flanking region of the HIS4 gene and the E. coli lac operator. The Xho I HIS4 promoter fragment from pHYC3(89) (12) was cloned into the Sal I site of pUC19. The Eco RI-Pvu II fragment of pUC19 containing this insertion as well as the E. coli lac operator was excised. The ends of the fragment were rendered flush with the large fragment of E. coli DNA polymerase and Eco RI linkers added. This construct was inserted into the Eco RI site of the TRP1ARS1 plasmid. The plasmid pXBS201, a gift from M. Schlissel and D. D. Brown, consists of a 239 bp fragment containing the somatic 5s rRNA gene and flanking sequences from X. borealis cloned into pBR322 lacking the 356 bp Bam HI-HinD III fragment (13).

Preparation of Yeast Spheroplasts and Nuclei and Isolation of Plasmid DNA

SC3 cultures containing TRP1ARS1, GAT2, pBRAT2 or TALH were grown to A_{600}=1.0, usually in about 100 ml volumes, as in ref. 14. Plasmid DNA was isolated directly from cells using a procedure modified from Saavedra and Huberman (7). All operations up to addition of proteinase K/SDS were carried out at the temperature appropriate for the given sample. Cells were washed with 10 mM N-ethylmaleimide, an inhibitor of topoisomerase (15). The pelleted cells were suspended at a concentration of 1-2 x 10^6 cells/ul in 50 mM Tris-Cl, pH 8.0/50 mM EDTA/10 mM N-ethylmaleimide, added to an approximately equal volume of autoclaved glass beads (0.45-0.5 mm, Thomas Scientific), and vortexed at high speed for 3 min. This operation was usually carried out in Eppendorf tubes. Samples which had been incubated at 0°C or 7°C were vortexed in four 45 sec bursts, each followed by 45 sec on ice. The supernatant was withdrawn from the glass beads, the beads washed once and the wash combined with the original supernatant. Proteinase K (Sigma) and SDS were added to 0.1 mg/ml and 0.1% respectively and the samples incubated 1-2 hrs at 37°C, extracted with phenol and chloroform, and precipitated with ethanol. Nucleic acid was taken up in a volume of about 1 ul 10 mM Tris-Cl/0.1 mM EDTA per original 10^7 cells, treated with 1 ug/10 ul RNase A for 30 min at 37°C, and stored at 4°C.

Crude nuclei were prepared by lysing cells with glass beads in 150 mM KCl/50 mM Tris-Cl, pH 7.2/2 mM $MgCl_2$/17% glycerol and spinning out cellular debris for 2 min at 2000 rpm (325 x g) in an Eppendorf microfuge. After incubation of the supernatant at the desired temperature, DNA was isolated as described above.

Spheroplasts were prepared by treating washed cells with zymolyase 100 000 (Kirin Breweries, Tokyo) at 30°C for 20-30 min and DNA was isolated by treatment with hot guanidinium hydrochloride (16). For those samples incubated at lower temperature, spheroplasts were either transferred directly to the appropriate temperature without removal of zymolyase, or were first spun down and resuspended in deionized H_2O. These preparations are best regarded as partially lysed spheroplasts.

Nuclear Extracts and Purified Plasmid Chromatin

Nuclear extracts containing intact plasmid chromatin were prepared as described (17) by suspending pelleted spheroplasts (2000 x g, 7 min) in chilled 10 mM Tris-Cl pH 8.0/0.1 mM EDTA/150 mM NaCl/10 mM $MgCl_2$/0.1 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride at a concentration of 1-2 x 10^9 cells/ml. After 10 min on ice, the mixture was homogenized by five strokes with a Potter-Elvehejm homogenizer and then incubated for 2 hrs on ice. The suspension was centrifuged for 5 min in an Eppendorf microfuge and the supernatant used as nuclear extract.

Plasmid chromatin was purified from the crude nuclear extract by an affinity method which depends on the presence of an E. coli lac operator insertion into the TRP1ARS1 plasmid. Briefly, a fusion protein containing most of the lac repressor and β-galactosidase is added to the nuclear extract to bind to the lac operator. Addition of rabbit anti-β-galactosidase and goat anti-rabbit IgG coupled to Immunobeads immobilizes the chromatin on the beads. After thorough washing, the plasmid chromatin can be released from the Immunobeads in a highly purified form by treatment with the inducer IPTG. This procedure has recently been described in detail (17).

Nucleosome Reconstitution and In Vitro Relaxation of Minichromosomes

Chick erythrocyte histones were purified and reconstituted onto supercoiled pXBS201 as in ref. 5. Reconstituted plasmid chromatin and affinity-purified TALH chromatin were relaxed with nicking-closing extract prepared from chick erythrocytes (18), and the DNA subsequently purified as in ref. 4 except that carrier tRNA was used in all ethanol precipitations.

Gel Electrophoresis and Topoisomer Analysis

Purified DNA was electrophoresed on chloroquine-containing agarose gels (19) in 50 mM Tris/25 mM acetic acid/1 mM EDTA pH 7.9 at 2 V/cm for 15-24 hrs. Following electrophoresis, DNA (10-20 ng per lane) was depurinated with acid (20), transferred to nylon membranes (GeneScreen) by Southern blotting (21) and cross-linked by UV irradiation (22). Nick-translated probes (23) were hybridized to the membranes overnight according to Church and Gilbert (22), except that Pentex Grade V BSA was used in the

prehybridization buffer, BSA was omitted from the wash buffer and, following four 5 min washes with the second wash buffer, the membranes were washed for 30 min at 65°C in 30 mM NaCl/3 mM sodium citrate, pH 7.0/0.1% SDS. Autoradiograms (Kodak XAR5 film) were exposed for durations such that the optical density of the topoisomer bands was between 0.2 and 2.0, ensuring linear film response. Densitometric scans of autoradiograms, obtained with a Beckman DU-8 or Hoefer Scientific Instruments (San Francisco) GS 300 densitometer, were analyzed as in ref. 4.

RESULTS

In the experiments described below, thermal untwisting is measured by differences in linking number observed on topoisomer-resolving gels. For closed circular DNA,

$$L = Tw + Wr$$

where L is the linking number, Tw is the twist and Wr the writhe of the DNA (24, 25). Since for relaxed molecules, Wr=0, two populations differing in twist will differ equally in linking number. Each unit difference in L corresponds to a 360° difference in Tw.

We first sought to determine whether the thermal untwisting exhibited by the 2 micron plasmid *in vivo* would also be exhibited by other multicopy plasmids in yeast. Yeast cultures carrying the multicopy plasmids TRP1ARS1 or GAT2 were grown to mid-log phase (A_{600}=1.0) at 30°C. A portion of the culture was then incubated on ice or at 7°C for one hour to allow the minichromosomes to be relaxed by intracellular topoisomerase (7). DNA was then purified from one half of the culture at 0°C or 7°C by breaking the cells quickly with glass beads, and the other half was returned to 30°C for 30 min before isolating the DNA. Purified DNAs were electrophoresed on topoisomer-resolving gels, blotted to nylon membranes, and hybridized to probes specific for the plasmid of interest.

Representative data from two such experiments are shown in Fig. 1. The molecules relaxed at 0°C or 7°C are less negatively supercoiled than those originally isolated at 30°C. It can also be seen, in the first and third lanes, that returning the cultures containing TRP1ARS1 from 7°C to 30°C resulted in

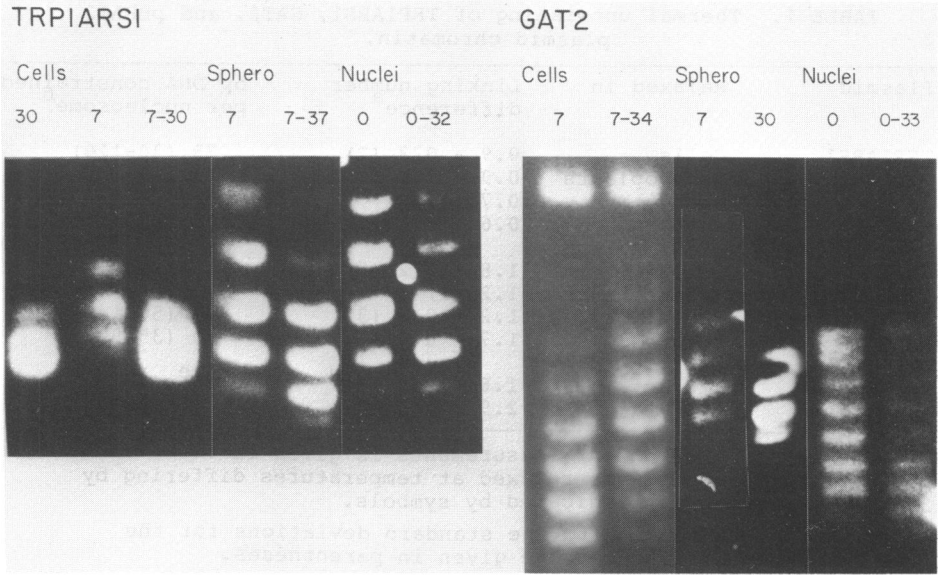

FIGURE 1. Thermal untwisting of TRP1ARS1 and GAT2 in yeast cells, partially lysed spheroplasts, and nuclei. Yeast cultures containing TRP1ARS1 or GAT2 were prepared as described in the text, incubated at the indicated temperatures, and the purified DNA electrophoresed for 15 hrs (lanes 1-3), 17 hrs (4-7), 23 hrs (8-9), or 18 hrs (10-13) in 1.9% (lanes 1-3, 6-7, 12-13), 1.8% (4-5, 10-11) or 1.4% (8-9) agarose containing chloroquine diphosphate at 1 ug/ml (1-3), 2 ug/ml (4-5), 1.6 ug/ml (6-7, 12-13), 40 ug/ml (8-9), or 1.5 ug/ml (10-11). The notation 7-30 indicates that half of a sample incubated at 7°C was returned to 30°C for 30 min before DNA isolation (See text). All the topoisomers in the figure are migrating as negatively supercoiled molecules except for the "GAT2/cells" lanes, which are migrating as positive supercoils. The examples shown for TRP1ARS1 cells and GAT2 partially lysed spheroplasts, although clearly showing thermal untwisting, were not used in our quantitative analyses.

restoration of the original (30°C) topoisomer distribution, indicating that the shift in linking number caused by incubating the culture at 7°C was probably not caused by a loss of nucleosomes. Similar results were obtained when plasmid chromatin was relaxed in partially lysed spheroplasts or nuclei (Fig. 1 and Table 1). Direct comparison of plasmid DNA isolated from cells, partially lysed spheroplasts, nuclei and nuclear extracts (see below) showed the same topoisomer distributions at

TABLE 1. Thermal untwisting of TRP1ARS1, GAT2, and pBRAT2 plasmid chromatin.

Plasmid	Relaxed in	Linking number difference[a]	bp DNA constrained per nucleosome[b]
TRP1ARS1	Cells	0.9 ± 0.3 (2)	75 (30-120)
(1453 bp,	Spheroplasts	0.9 ± 0.1 (3)	75 (60-90)
7 nucleo-	Nuclei	0.7 (1)	100
somes)	Extract	0.6 ± 0.1 (5)	115 (105-135)
GAT2	Cells	1.8 ± 0.2 (3)[c]	35 (15-50)
(2.8 Kbp,	Spheroplasts	1.2 (1)[c]	80
16 nucleo-	Nuclei	1.2 ± 0.7 (3)	95 (50-140)
somes)	Extract	1.7 ± 0.4 (6)	60 (35-90)
pBRAT2	Extract	1.6 ± 0.5 (3)[d]	25[e] (0-70)
(4.8 Kbp)		2.9 ± 0.8 (3)	60[e] (30-90)

[a]The number of independent measurements is given in parentheses; samples were relaxed at temperatures differing by 30-32 C, except where indicated by symbols.

[b]The range corresponding to the standard deviations for the linking number differences are given in parentheses.

[c]Samples were relaxed at temperatures differing by 26-27°C.

[d]Samples were relaxed at 7°C and 20°C.

[e]Based on an estimate of about 29 nucleosomes per plasmid (165 bp of DNA per nucleosome).

a given temperature, demonstrating that nucleosomes are stable to the various isolation procedures (data not shown).

Intact plasmid chromatin can also be prepared in an extract by allowing the plasmids to diffuse out of crude preparations of nuclei, followed by removal of nuclei and residual cellular debris by centrifugation (14). Such extracts contain topoisomerase activity, as can be shown by adding exogenous supercoiled DNA to the extract and seeing that it is relaxed. We routinely added naked DNA to the extract in our experiments as well, to ensure that the endogenous topoisomerase was still active.

Thermal untwisting of GAT2 and TRP1ARS1 in nuclear extracts occurs to the same extent as it does in cells, partially lysed spheroplasts and nuclei, as can be seen in Fig. 2. The average shift in linking number measured for a 30°C temperature increment was 0.8 for TRP1ARS1 and about 1.7 for GAT2 (Table 1). The GAT2 plasmid was determined to have about 16 nucleosomes by linking number analysis, and the TRP1ARS1 plasmid has an average of seven

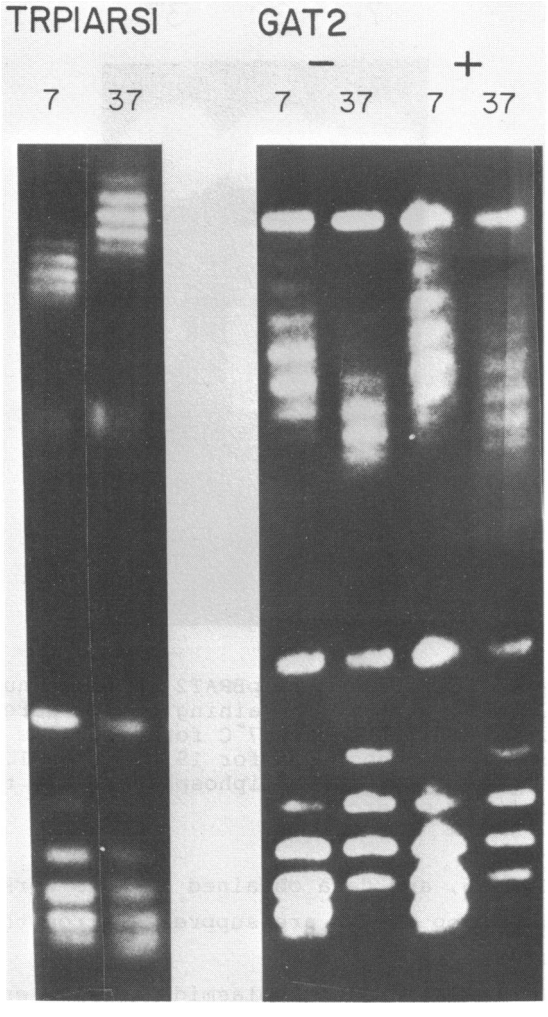

FIGURE 2. Thermal untwisting of TRP1ARS1 and GAT2 in yeast nuclear extracts. Nuclear extracts containing TRP1ARS1 or GAT2 plasmid chromatin were incubated at 7°C overnight or 37°C for 1-2 hrs and the purified DNA electrophoresed for 18 hrs (TRP1ARS1) or 16 hrs (GAT2) on 1.8% agarose gels containing 1.0 (TRP1ARS1) or 1.75 (GAT2) ug/ml chloroquine diphosphate. Naked pBR322 DNA was added to the TRP1ARS1-containing extracts, and naked TRP1ARS1 DNA to the GAT2-containing extracts, prior to relaxation. In these gels, the naked DNA migrated as positively supercoiled molecules, and the minichromosomal DNA as negative supercoils. The faster migrating species is TRP1ARS1. In the GAT2 lanes, the "+" indicates samples to which nicking-closing extract was added before relaxation and the "-" indicates samples incubated in the absence of added nicking-closing extract.

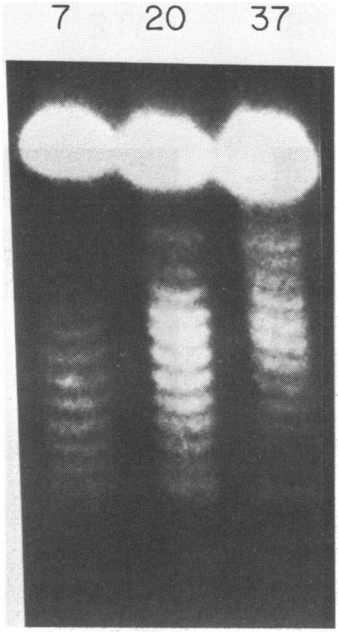

FIGURE 3. Thermal untwisting of pBRAT2 in yeast nuclear extracts. Nuclear extracts containing pBRAT2 were relaxed at 4° C overnight, 20°C for 7 hrs, or 37°C for 4 hrs, as indicated, and the purified DNA electrophoresed for 19 hrs on a 1.6% agarose gel containing 40 ug/ml chloroquine diphosphate. The topoisomers migrated as positive supercoils.

nucleosomes (14, 26, and data obtained in this work); thus, between 50 and 100 bp of DNA are suppressed from thermal untwisting per nucleosome.

Both the TRP1ARS1 and GAT2 plasmids consist entirely of DNA sequences derived from yeast. To further investigate possible effects of DNA sequence on thermal untwisting, we therefore introduced the plasmid pBRAT2 into yeast, prepared nuclear extracts and measured thermal untwisting as before. The plasmid pBRAT2 consists of 69% prokaryotic DNA sequences (from pBR322); nevertheless, it thermally untwists to the same degree as GAT2 and TRP1ARS1 (Fig. 3 and Table 1). The thermal untwisting of yeast plasmid chromatin therefore does not appear to be a sequence dependent phenomenon.

We next wanted to determine whether the increased thermal

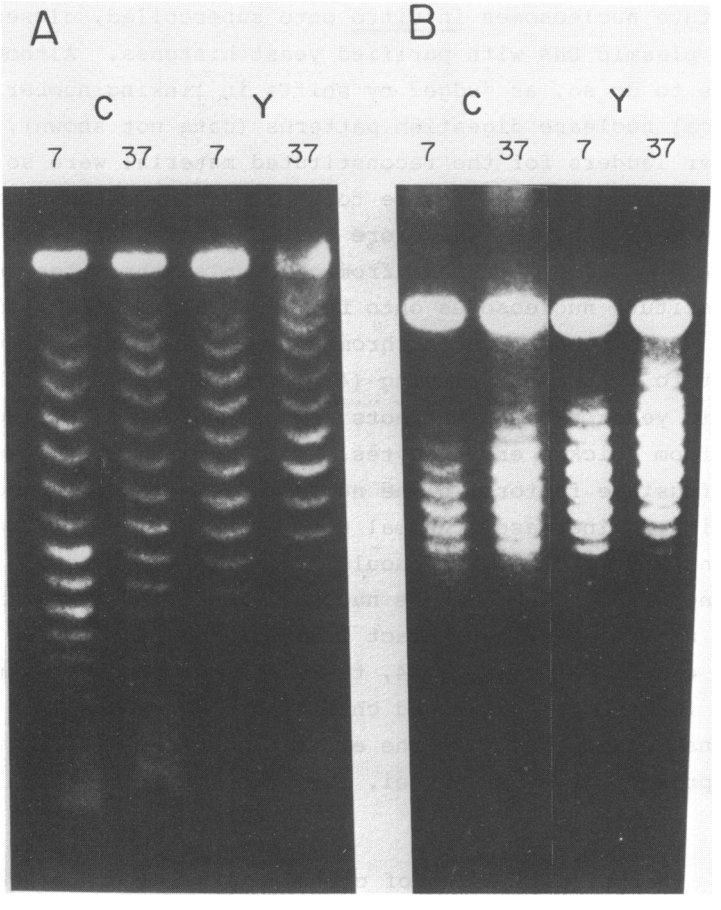

FIGURE 4. Thermal untwisting of reconstituted circular chromatin treated with chicken erythrocyte nicking-closing extract or in yeast nuclear extracts. Panels A and B represent pXBS201 reconstituted with about 15 and 20 nucleosomes, respectively. Samples were relaxed *in vitro* with nicking-closing extract from chicken erythrocytes (lanes C) or in yeast nuclear extracts (lanes Y) at 7°C overnight or 37°C for 1-2 hrs, as indicated. Purified DNA was electrophoresed for 19 hrs on 1.6% agarose gels containing 8 ug/ml (A) or 40 ug/ml (B) chloroquine diphosphate. Under these conditions the topoisomers migrated as positive supercoils.

untwisting of yeast chromatin relative to that of higher eukaryotes was due to differences in chromatin structure *per se* or to other factors present in the extract and *in vivo*, such as yeast topoisomerase. We therefore attempted to

reconstitute nucleosomes in vitro onto supercoiled, closed circular plasmid DNA with purified yeast histones. Although we were able to do so, as judged by shifts in linking number and micrococcal nuclease digestion patterns (data not shown), topoisomer ladders for the reconstituted material were so broadly distributed that we were unable to obtain interpretable data on thermal untwisting. We therefore devised two alternative experiments. First, histones from chicken erythrocytes were used to reconstitute nucleosomes onto the plasmid pXBS201 at high density. Such reconstituted chromatin has been shown to be resistant to thermal untwisting (4). This material was then relaxed in yeast nuclear extracts and with nicking-closing extract from chicken erythrocytes. If the yeast topoisomerase or other diffusible factors in the nuclear extract were responsible for permitting increased thermal untwisting of yeast chromatin, the reconstituted chromatin should exhibit greater thermal untwisting when relaxed in the nuclear extract than when relaxed with the nicking-closing extract from chick erythrocytes. In fact, as can be seen in Fig. 4, the same thermal untwisting was observed for the reconstituted chromatin under these two conditions, indicating that the effect is not due to diffusible factors present in yeast nuclei. The results from several

TABLE 2. Thermal untwisting of circular pXBS201 DNA reconstituted with chicken erythrocyte histones.

Nucleosomes per plasmid (approximately)	Relaxed by	Linking number difference*
15	Chicken erythrocyte nicking-closing extract	1.4 ± 0.8
	Yeast nuclear extract	1.4 ± 0.3
20	Chicken erythrocyte nicking-closing extract	1.1 ± 0.25
	Yeast nuclear extract	1.2 ± 0.25

*Measured between samples relaxed at 7°C and 37°C.

similar experiments are summarized in Table 2. Chromatin relaxed in the yeast nuclear extract at either temperature is shifted to more negative superhelical densities; although we do not know the

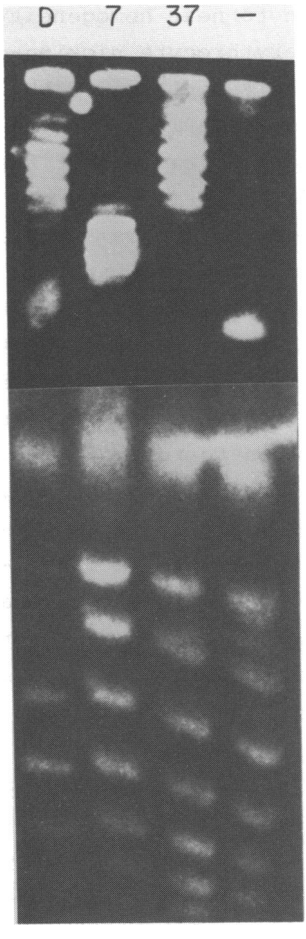

FIGURE 5. Thermal untwisting of yeast TALH plasmid chromatin after affinity-purification. Naked pBR322 was added to purified TALH plasmid chromatin and incubated with nicking-closing extract at 7°C overnight or 37°C for 1 hr, as indicated. The purified DNA was electrophoresed for 18 hrs on a 1.6% agarose gel containing 1.2 ug/ml chloroquine diphosphate. All TALH samples except the relaxed naked TALH DNA, in the lane headed "D", migrated as negative supercoils; the pBR322 DNA migrated as positive supercoils except in the lane "-". The lane headed "-" was incubated at 37°C for 1 hr without nicking-closing extract. All DNA shown was run on a single gel; different photographic exposures were used in making the figure.

cause of this shift, it does not affect the conclusions regarding thermal untwisting in these experiments.

As a second experimental alternative to studying chromatin prepared *in vitro* with purified yeast histones, we purified yeast plasmid chromatin to near homogeneity in order to relax it *in vitro* with chicken erythrocyte nicking-closing extract. For this purpose we used the plasmid TALH, which in addition to the sequences contained in TRP1ARS1 has an insert bearing the *lac* operator sequence and a fragment of the 5' flanking region of the HIS4 gene. The affinity of the operator sequences to the *lac* repressor protein allows the plasmid chromatin to be easily purified from a nuclear extract. When such purified chromatin is incubated with naked pBR322, the pBR322 DNA remains supercoiled, showing that the yeast chromatin is purified away from topoisomerase by this protocol (Fig. 5). When nicking-closing extract from chicken erythrocytes is added to the purified plasmid chromatin to relax it at 7°C or 37°C, results such as those shown in Fig. 5 are obtained. Both samples have average linking numbers differing from the starting material, which is identical to the sample in lane "-" (data not shown), indicating that the isolated plasmid chromatin can be relaxed by chicken erythrocyte nicking-closing extract at both temperatures. The TALH plasmid clearly undergoes a substantial thermal untwisting; quantitation from two separate purifications and several gels yielded an average shift of 1.0 supercoils, or about 60% of the value expected for naked DNA. Since the plasmid has an average of 7 nucleosomes (Fig. 5; compare lanes "D" and "37"), this is equivalent to a protection from thermal untwisting of about 90 bp per nucleosome, consistent with the results already presented. It thus appears that yeast chromatin is considerably less effective than that of higher eukaryotes in suppressing thermal untwisting of DNA.

DISCUSSION

The results presented here strongly suggest that DNA in yeast nucleosomes is more flexible in response to changes in temperature than DNA in nucleosomes from higher eukaryotes. (We emphasize here that this *thermal* flexibility, that is, the

ability of the DNA to respond to changes in temperature by altering its equilibrium helical twist, is distinct from the term flexibility used in relation to persistence length, or in relation to fluctuations in twist or writhe about the equilibrium values for a given temperature and solvent.) Since we have so far been unable to obtain interpretable data on thermal untwisting in chromatin reconstituted in vitro with purified yeast histones, we cannot definitively rule out the possibility that some factor other than histones contributes to the increased thermal flexibility of yeast nucleosomal DNA. However, the observation that chromatin reconstituted using chicken erythrocyte histones does not thermally untwist in yeast nuclear extracts indicates that any such factor would have to be bound tightly to the plasmid chromatin and be unable to diffuse. Furthermore, any such factor would have to bind to the various sequences we have examined in this work, as well as the 2 micron plasmid, with approximately equal affinity. We therefore believe it unlikely that any bound non-histone factor is responsible for the increased thermal flexibility of yeast chromatin.

It is also formally possible that the temperature-dependent changes in linking number which we have measured are not due to changes in twist at all, but rather to a temperature-dependent change in the writhe of the DNA. This could happen, for instance, by a gradual loosening of the ends of the DNA from the nucleosome as the temperature is lowered. We consider this unlikely, particularly since the temperature-dependent changes in linking number remain linear over a wide range of temperatures between 0° and 38°C (this work and ref. 7).

Another possibility which remains untested is that some factor(s) present during in vivo nucleosome assembly in yeast might lead to more thermally flexible chromatin than could be assembled using only purified histones and DNA. This does not seem to be the case for higher eukaryotes (4,6). One way of testing whether such a mechanism could be operating independently of primary histone sequences would be to replace yeast histone genes in vivo with genes encoding histone sequences from higher eukaryotes, and to measure thermal untwisting of the resulting chromatin. Such experiments are now in progress.

However, until evidence to the contrary is obtained, the simplest explanation for the increased thermal flexibility of yeast chromatin relative to that of higher eukaryotes is the difference in amino acid sequence (or secondary modifications; but see below) of the histone proteins.

What is the probable structural basis of the increased thermal flexibility of yeast nucleosomal DNA? The thermal untwisting observed is still less than that of free DNA. This could reflect partial restraint of the entire length of DNA associated with the nucleosome, or it could be due to a region of 50-100 bp being completely restrained from thermal untwisting. Thermal denaturation profiles of yeast nucleosomes exhibit a major intermediate transition at 68°C not seen for chicken erythrocyte or calf thymus nucleosomes, with the higher melting transition being correspondingly reduced in intensity (28). Together with data indicating that the DNA at the ends of canonical core particles is the first to melt (29, 30), this would be consistent with the central 50-100 bp of yeast nucleosomal DNA remaining tightly bound to the histones, with the ends being somewhat freer. This is also consistent with the circular dichroism of the yeast nucleosome being intermediate between the chick erythrocyte nucleosome and free DNA (28), although other interpretations of the circular dichroism are possible.

We have used an average value for the length of DNA constrained from thermal untwisting in yeast nucleosomes. Different nucleosomes on the same plasmid may, however, differ in their physical properties (26). Although we cannot directly address whether the same length of DNA is constrained from thermal untwisting in each yeast nucleosome, the consistency of our data for three different plasmids, along with that for the 2 micron plasmid *in vivo* (7), would argue against this possibility, at least as anything but a minor perturbation.

Not all of the nucleosomal DNA which is able to thermally untwist can be completely free or even truly linker-like, since micrococcal nuclease digestion of yeast chromatin yields a limit digest of about 140 bp (31). Furthermore, the linking number change caused by incorporation of DNA into the yeast nucleosome

is -1.0 (14), the same as for chicken erythrocyte (32) and calf thymus (33) nucleosomes. This latter point, although implying that yeast nucleosomes are not radically different in their topology from those of higher eukaryotes, is puzzling in one respect. One way to account for the change in linking number of -1.0 upon incorporation of DNA into a nucleosome (and relaxation by topoisomerase) is by a change in writhe of about -1.7 offset by a change in twist of about +0.7 (34). The change in twist in this model is from about 10.5 to 10.0 base pairs per turn of the DNA helix, spread over the length of the nucleosomal DNA. In contrast, thermal relaxation of naked DNA by -.011°/°C/bp changes the average twist from 10.54 at 37°C (35) to 10.43 at 4°C. It is not clear whether or why DNA which has already changed its twist (by virtue of incorporation into a nucleosome) to a value considerably different from naked DNA at 4°C or 37°C should still respond in a way similar to naked DNA to changes in temperature. Perhaps, as recent structural data suggests (36, 37), the altered twist of nucleosomal DNA is not uniformly distributed after all.

If the increased thermal flexibility of yeast nucleosomal DNA over that of higher eukaryotes is due to differences in the histones, precisely what differences, at the amino acid level, are likely to be responsible? Chicken and yeast core histones differ at a total of 103 out of just under 500 amino acid residues (38). Yeast apparently has no histone H1 equivalent (39), and the suppression of thermal untwisting by chicken erythrocyte histones is not dependent on the presence of H1/H5 (4), so only the core histones need be considered. However, just under half of these non-conserved residues occur in the amino or carboxyl tails of the histones. Since removal of these tails by trypsin does not affect suppression of thermal untwisting in chicken erythrocyte nucleosomes (5), the altered residues in these regions of the yeast histones are unlikely to be responsible for the differences in thermal untwisting. (By the same token, post-translational modifications to the histones, which are almost completely restricted to the amino tails, are also unlikely to contribute to the greater thermal flexibility of yeast nucleosomal DNA.) Furthermore, hybrid nucleosomes composed of H4 from yeast and H2A, H2B and H3 from calf thymus exhibit a

thermal denaturation profile similar to native calf thymus (or chicken erythrocyte) nucleosomes (28). If the difference between the thermal denaturation profiles of yeast and chicken erythrocyte nucleosomes reflects the structural changes responsible for the different thermal untwisting properties, this would suggest that differences in histone H4 cannot alone account for these changes.

One particularly interesting difference in the amino acid sequence of the yeast histones occurs at position 110 in histone H3. In all other species for which this sequence is known, this residue is a cysteine (38). In yeast, it is an alanine (40). Cys_{110} is found close to the center of dyad symmetry in the nucleosome (36), and has been found to differ in its accessibility to labelling compounds in active versus inactive chromatin from Physarum polycephalum (41). Moreover, carboxymethylation of Cys_{110} has been shown to destabilize nucleosome core particles (42). Possibly this residue is involved in structural changes in chromatin which are connected with functional alterations. In this connection, it is noteworthy that the yeast genome is some ten-fold more transcriptionally active than are the genomes of higher eukaryotes (43-45). If the nucleosomes of higher eukaryotes can provide an impediment to transcription, as alluded to in the introduction, perhaps yeast histones, in order to support a high degree of transcription, have evolved to allow a more flexible chromatin structure. Nucleosomes from higher eukaryotes might also be capable of unfolding, but the process could be energetically less favorable (46). Understanding the precise nature of the physical differences between nucleosomes of yeast and higher eukaryotes might therefore give us new insight into the role that chromatin structure plays in transcriptional regulation.

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