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DNA Topoisomerase I Controls the Kinetics of Promoter Activation and DNA Topology in *Saccharomyces cerevisiae*

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Inactivation of the nonessential *TOP1* gene, which codes for *Saccharomyces cerevisiae* DNA topoisomerase I, affects the rate of transcription starting at the *ADH2* promoter. For both the chromosomal gene and the plasmid-borne promoter, mRNA accumulation is kinetically favored in the mutant relative to a wild-type isogenic strain. The addition of ethanol causes in wild-type yeast strains a substantial increase in linking number both on the *ADH2*-containing plasmid and on the resident 2μm DNA. Evidence has been obtained that such an in vivo increase in linking number depends on (i) the activity of DNA topoisomerase I and of no other enzyme and (ii) ethanol addition, not on the release from glucose repression. A direct cause-effect relationship between the change in supercoiling and alteration of transcription cannot be defined. However, the hypothesis that a metabolism-induced modification of DNA topology in a eukaryotic cell plays a role in regulating gene expression is discussed.

Regulation of eukaryotic gene expression at the level of transcription involves several different mechanisms, all based on the complex interplay among different sets of proteins: RNA polymerases, chromatin components, ubiquitous and specific transcription factors, and DNA topoisomerases. It has been shown, especially in the yeast *Saccharomyces cerevisiae*, that this last group of enzymes controls the topology of DNA when perturbed by processes such as transcription, replication, and recombination (reference 43 and references therein). Restoration of the steady-state DNA conformation and topology is expected to occur via dynamic pathways involving temporary modifications of defined protein-DNA interactions.

The imbalance among the enzymatic activities controlling DNA topology probably has transient and reversible effects. In the yeast *S. cerevisiae*, phenotypic consequences of the absence of only one or more DNA topoisomerases have been shown to include recombination (11, 26, 42), chromosomal DNA replication (45), and transcription. In this process, it is not yet clear whether the activity of topoisomerases is relevant only in the elongation step or also in the assembly of the transcription machinery on the DNA template and/or in the enzymology of RNA initiation.

It has been shown that changes in the topology of episomal domains occur when DNA topoisomerase I alone or both DNA topoisomerases I and II are mutated (4, 21). These analyses demonstrate the previously proposed model (28), which predicted the generation of positive and negative supercoiling as a consequence of RNA polymerases tracking along the double strand. It is believed that DNA topological modifications in *S. cerevisiae* are associated mainly with transcriptional elongation, this process being the major cause of the generation of supercoiling in eukaryotic systems.

From these models and observations, the existence of sets of genes whose activity is influenced by topological modifications can be predicted. In this case, topological changes would be a cause (and not a consequence) of differential promoter utilization. A recent study has indeed pointed out that transcription of rRNA minigenes is stimulated in both *top1-top2* and *top1* cells, possibly because of the accumulation of negative supercoiling, indicated to favor initiation by RNA polymerase I (35).

We have investigated the relationship between RNA polymerase II-dependent transcription and DNA topology by comparing these two parameters in two pairs of isogenic strains, the only difference between the two components of each pair being a mutation in DNA topoisomerase I. The gene analyzed is *ADH2*, which codes for alcohol dehydrogenase II. This gene is regulated at the level of initiation of transcription, being repressed in glucose-containing medium and activated almost 100-fold when glucose is substituted by ethanol (46). We have previously observed a correlation between the properties of the wild type and of up-promoter mutants of *ADH2* and DNA structural alterations (12); in addition, in a partially purified yeast RNA polymerase II-based transcription assay, DNA topology modifies the transcription efficiency of the *ADH2* gene (8). It was therefore of interest to investigate the *ADH2* system by altering in vivo the DNA conformation through a genetic approach, i.e., by the use of DNA topoisomerase I mutants.

We observe that in the absence of the major DNA-relaxing activity, (i) the kinetics of both the chromosomal and episomal *ADH2* mRNA synthesis are markedly favored and (ii) the topology of ethanol-grown episomal DNA is different in the wild type relative to mutant strains. The potential link between these two observations is discussed.

MATERIALS AND METHODS

Yeast strains and growth conditions. Two different groups of isogenic strains were used. Strains CH335 (**a** *his4-539 lys2-801 ura3-52 TOP1-TOP2*) and its derivative TG107 (*top1::URA*) (30) were kindly provided by J. C. Wang. Strains W303-1a (**a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112*

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can1-100) and its derivatives *top1-8*::*LEU2* (4) and *top1-9*::*URA3*, also named RS188, AMR51, and YAP1, respectively, were kindly provided by R. Sternglanz.

Cells were grown overnight at 30°C in YPD (1% yeast extract, 2% Bacto Peptone, 3% glucose) or YNB (0.68% yeast nitrogen base) medium supplemented with the required amino acids and with 3% glucose (or 2% raffinose where indicated). For induction of the *ADH2* gene, the cells were diluted to a concentration of 1.5×10^6 to 6×10^6 cells per ml, resuspended in fresh medium containing glucose (or raffinose), and kept for 1 h at 30°C. The cells were then washed with the same volume of fresh medium without the carbon source. After removal of two 10-ml control aliquots (one for DNA extraction and the other for RNA extraction), ethanol was added at a final concentration of 3%. For each time point, two 10-ml aliquots of the culture were pelleted and quickly chilled in a dry ice-ethanol bath.

For yeast transformations, cells were made competent by treatment with lithium acetate (24).

Plasmids. Plasmid YRp31 (17), a kind gift of L. Panzeri and G. Lucchini, contains the *LYS2* and *TRP1* genetic markers. PADH-322 (shown in Fig. 2) was obtained as follows. The 190-bp *Eco*RV-*Bam*HI DNA fragment was removed from pBR322 and substituted with the 1,270-bp *Bam*HI-*Eco*RV DNA fragment from ADR2-BS-pBR322 (44), kindly provided by E. T. Young. The latter fragment contains the entire regulatory region of the *ADH2* gene and the first 62 bp of the coding sequence. DNA A and DNA B (shown in Fig. 2) were obtained as follows. The ca. 6.5-kb *Cla*I fragment from YRp31 was inserted in the *Cla*I site of PADH-322 in both orientations. To introduce the *TOP1* gene in strain TG107 on a centromeric plasmid, the same *Cla*I fragment from YRp31, filled in with Klenow enzyme, was inserted in the *Sma*I site of pCC10, a generous gift of R. Sternglanz; this plasmid was derived from YCp50 (32) by inserting in its *Hin*dIII site a 3,787-bp *Hin*dIII fragment containing the entire *TOP1* gene and its own promoter and termination signals (39). Plasmid YEptopA-PGPD (21), containing the *Escherichia coli topA* gene downstream of the promoter of the yeast *GPD* gene (encoding glyceraldehyde-3-phosphate dehydrogenase), was kindly provided by P. Benedetti.

Circular DNA extraction and analysis. The preparation of circular DNA from yeast cells was routinely performed by alkaline lysis of spheroplasts. The method involving the mechanical disruption of entire cells by means of glass beads was tested for comparison; identical results were obtained, thus indicating that the observed topological effects were not artifactual consequences of DNA isolation, as already described by Giaever and Wang (21). In the first method, the pellet was resuspended in 0.9 M sorbitol–0.1 M EDTA. 2-Mercaptoethanol (14 mM) and Zymolyase (2.5 mg/ml) were immediately added, and the suspension was incubated for 30 min at 37°C. Two volumes of 0.2 N NaOH–1% sodium dodecyl sulfate were added to the spheroplasts, and the mixture was kept on ice for 10 min; 1.5 volumes of 3 M potassium acetate (pH 4.8) was then added, and the mixture was kept on ice for 45 min. After 10 min of centrifugation to pellet the cellular debris and the chromosomal DNA, 2 volumes of ethanol were added to the supernatant in order to precipitate the circular DNA forms. In the other method, we followed the procedure described by Brill and Sternglanz (4).

The DNA was analyzed through horizontal gel electrophoresis in 1% agarose and TPE buffer (50 mM Tris-phosphate [pH 7.2], 1 mM EDTA) (36). Chloroquine was added to the gel matrix and to the recirculating buffer at a final concentration of 2 μg/ml. Southern blotting was performed by the standard procedure, using Schleicher & Schuell BA-S 85 nitrocellulose paper.

RNA extraction and analysis. Total RNA from yeast cells was prepared as described by Schmitt et al. (34). After ethanol precipitation, the RNA samples were resuspended in 20 μl of sterile water, and 1 μl was used to determine the A_{260}.

For Northern (RNA) analysis, one aliquot corresponding to 10 μg for each sample was dried, resuspended in 50% formamide–12.3 M formaldehyde–1× morpholinepropanesulfonic acid (MOPS) buffer (20 mM MOPS, 5 mM sodium acetate [pH 5.0], 1 mM EDTA), and denatured at 60°C for 15 min. After addition of 1/10 (vol/vol) loading buffer (50% glycerol, 0.05% xylene cyanol, 0.05% bromphenol blue), the samples were subjected to electrophoresis in 1% agarose–1× MOPS buffer–12.3 M formaldehyde–0.25 μg of ethidium bromide per ml. The gel was run at 3.5 V/cm for 15 min, then at 4.5 V/cm for 25 min, and finally at 7.5 V/cm for 1 h. After photographing, Northern analysis was performed by the standard procedure, using Schleicher & Schuell BA-S 85 nitrocellulose paper.

For primer extension analysis, 10 μg of total RNA was annealed with a labeled primer (10^5 cpm, 0.1 pM) and treated with reverse transcriptase as described by Townes et al. (40). The extended products were analyzed on 8% sequencing gels.

RESULTS

***ADH2* mRNA synthesis is kinetically favored in a yeast topoisomerase I mutant strain. (i) The chromosomal gene.** We have analyzed the induction of transcription of the chromosomal *ADH2* gene; this gene is specifically activated by the addition of ethanol to the culture medium (46).

Figure 1 shows the results of the primer extension analysis performed on total RNA extracted from *S. cerevisiae TOP1-TOP2* strain CH335 or from its *top1-TOP2* derivative (TG107) deficient in DNA topoisomerase I activity. Cells were grown in YPD medium to a density of 1.5×10^7 cells per ml, washed with YP and diluted about 10 times in YPE (Fig. 1A) or YPD (Fig. 1B) as a control, at the same cell density for both strains. Total RNA was extracted at different intervals after ethanol or glucose addition and annealed with a 22-mer (see the legend to Fig. 1) which can hybridize both with *ADH1* and *ADH2* mRNAs. For each time point, a comparison is shown between wild-type strain CH335 (odd-numbered lanes) and *top1* strain TG107 (even-numbered lanes). In the DNA topoisomerase I-deficient strain, a stronger *ADH2* gene activation clearly occurs relative to the wild type 120 and 180 min after the glucose-to-ethanol shift (Fig. 1A). The *top1* mRNA/wild-type mRNA ratio is about 15 at 120 min and about 4 at 180 min. Transcription in the wild type and in the *top1* strain initiates at exactly the same multiple sites and with similar relative strengths.

The increase in *ADH2* promoter activity in the *top1* strain coincides with the decrease of *ADH1* mRNA (Fig. 1A); this inverse proportionality is expected (15) and provides an internal control for RNA loading. Figure 1B shows a parallel experiment in which glucose instead of ethanol was added to the YP cell suspension; primer extension was performed with the same oligonucleotide. In this case, only the *ADH1* mRNA is visible and no major difference between the two strains is observed.

When the same type of analysis shown in Fig. 1 was repeated with another pair of isogenic strains (*TOP1-TOP2*

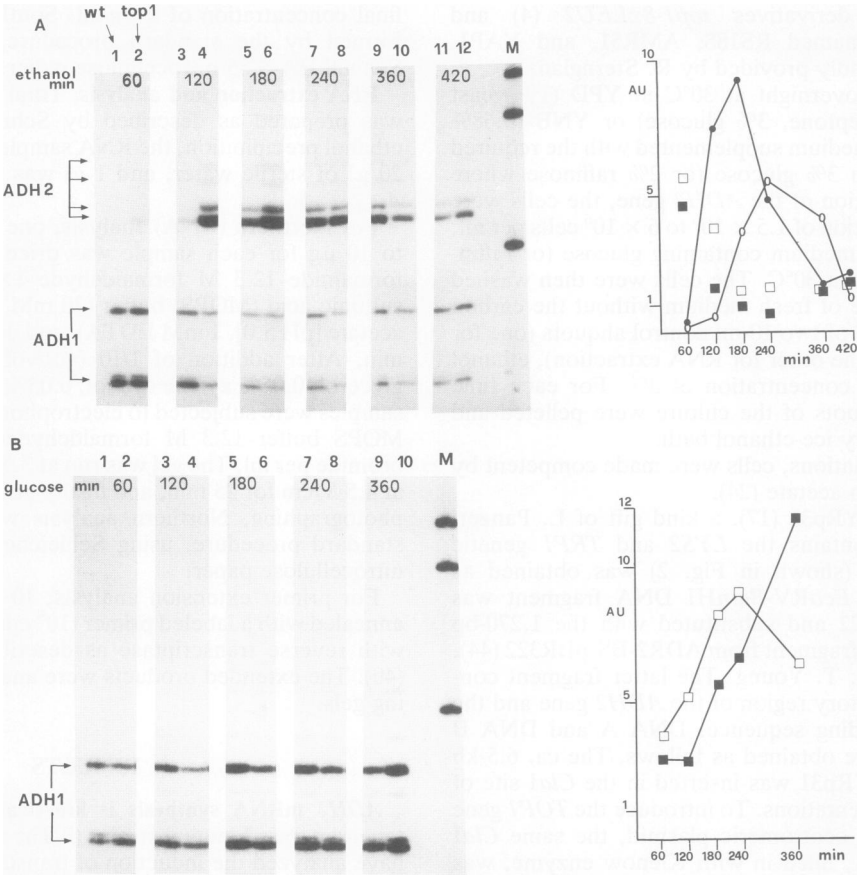

FIG. 1. Primer extension analysis of chromosomal *ADH1* and *ADH2* transcripts. (A) Cells from strains CH335 (wild-type [wt]; odd-numbered lanes) and TG107 (*top1*; even-numbered lanes) were grown overnight in YPD, washed, and resuspended in YP. At the indicated times after the addition of ethanol (3%), aliquots were removed from the culture and total RNA was prepared. The four arrows for the *ADH2* gene and the two arrows for the *ADH1* gene indicate the products of reverse transcription run on an 8% polyacrylamide sequencing gel; they start with a primer spanning the ATG of both genes (5′GAGTTTCTGGAATAGACATTGT3′) and terminate at the 5′ ends of the mRNAs. *ADH2* transcription starts at two major (indicated by the two lower arrows) and two minor (upper arrows, visible only on longer exposures) sites. (For locations of the RNA initiation sites, see references 12 and 46). Lane M, molecular weight markers (110, 97, and 67 nucleotides) from pUR250 DNA digested with *Hpa*II. A densitometric analysis of the data is shown in the graph. ○, wild-type *ADH2*; ●, *top1 ADH2*; □, wild-type *ADH1*; ■, *top1 ADH1*; AU, arbitrary units. (B) Same as panel A except that the cells were resuspended in YPD after washing.

W303-1a and *top1-TOP2* W303-1a; see Materials and Methods), a more efficient kinetics of induction of the *ADH2* mRNA was also observed for the mutant, although the difference between the two strains was less pronounced (data not shown). The two pairs of isogenic strains differ genetically; nevertheless, in both cases, a deletion in the DNA topoisomerase I gene is sufficient to confer the same specific phenotype, i.e., a facilitated activation of the *ADH2* gene.

(ii) The episomal promoter. The effect described above involves the chromosomal copy of the *ADH2* gene. To analyze the involvement of DNA topoisomerase I (which can be shown to be the sole cause of the differential phenotype observed; see below), we studied the topology and transcription of an episomal copy of the *ADH2* promoter.

Essentially, it was of interest to test whether a change in DNA topology could explain the transcriptional difference between the two strains. To perform this test, it was necessary to reproduce the different kinetics of *ADH2* mRNA synthesis on a DNA template susceptible to topological analysis. Figure 2 shows the strategy used to insert the *ADH2* promoter on a plasmid suitable for transformation into the two pairs of isogenic strains (see below). The first construct made was PADH-322, consisting of the promoter region and the first 62 bp of the *ADH2* gene inserted between the pBR322 *Bam*HI and *Eco*RV sites. The *Cla*I site of this construct was used to insert a ca. 6.5-kb *Cla*I fragment excised from YRp31 (17) containing both the *LYS2* and *TRP1* genes. The plasmid obtained was called DNA B (to distinguish it from DNA A, in which the same *Cla*I fragment has the opposite orientation). The two genetic markers allow transformation of both CH335-TG107 (*lys2*) and W303-1a (*trp1*) pairs of isogenic strains.

ADH2 transcription in the DNA B construct includes a short tract of pBR322 sequence and ends at the 3′ extremity of the *LYS2* gene, where the termination signals of an unidentified open reading frame are present (18). The mRNA synthesized on this transcription unit is about 1,300 nucleotides long (Fig. 3A).

ADH2 gene activity was analyzed in strains CH335 (wild type) and TG107 (*top1*) transformed with DNA B. Figure 3A

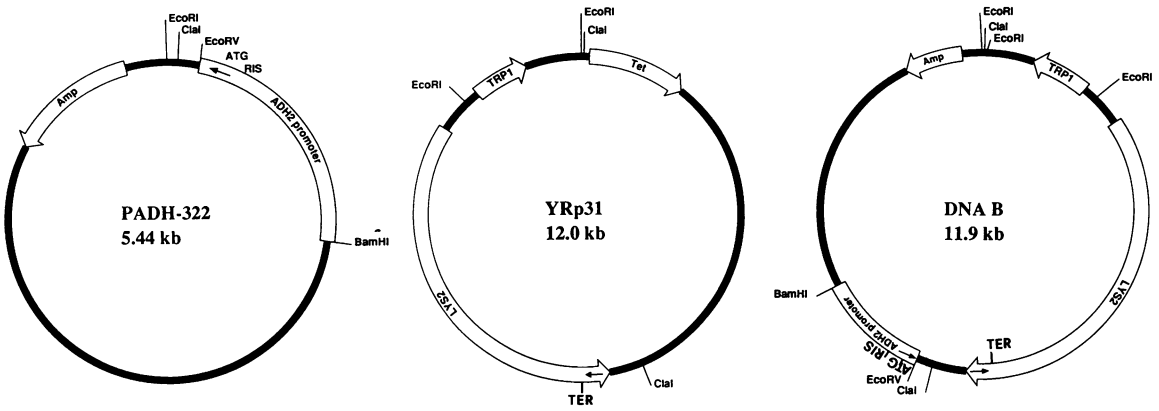

FIG. 2. Maps of the plasmids used for construction of a yeast episome containing the *ADH2* promoter. PADH-322 is a pBR322 derivative containing a 1,270-bp fragment from ADR2-BS-pBR322 (see Materials and Methods) between its *Eco*RV and *Bam*HI sites. DNA B was obtained from PADH-322 via the insertion of the *Cla*I fragment of YRp31 (containing the *LYS2* and *TRP1* genes) in its *Cla*I site. RIS, RNA initiation site for the *ADH2* promoter; TER, termination signal of an unidentified open reading frame inversely oriented at the 3′ end of the *LYS2* gene.

shows the results of the Northern analysis of total RNA isolated from the transformants. The cells were grown in YNB medium containing glucose to a density of 6×10^7 cells per ml, diluted 10 times in fresh YNB medium plus glucose, and kept at 30°C for 1 h. Aliquots were withdrawn after washing and resuspending the cells in YNB medium without a carbon source (lanes 1 and 7) or at different times after addition of ethanol (lanes 2 to 6 for the wild type and 8 to 12 for the *top1* strain). The probe used (the 162-bp *Cla*I-*Eco*RV segment of pBR322 present in DNA B; see the map in Fig. 2) can hybridize only with the 1,300-nucleotide mRNA starting from the episomal *ADH2* promoter. Activation in the DNA topoisomerase I mutant strain is stronger, as observed for the chromosomal copy. The kinetics of *ADH2* induction in the chromosome and in the episome differ (compare Fig. 1A and Fig. 3A; also data not shown). We attribute this diversity to the fact that the *ADH2* promoter requires ADR1 protein activity only in the chromosomal location, as shown by a genetic approach (2). Nevertheless, in both cases, transcription is more efficient in the topoisomerase I mutant background.

In addition to the Northern analysis, we performed primer extension analyses to ascertain that the 5′ ends of the transcripts initiated at the *ADH2* episomal promoter are the same as in the chromosome (data not shown).

Cells transformed with DNA A (the other construct containing the *ADH2* promoter, obtained from YRp31 and PADH-322) were also analyzed. In this DNA, the *Cla*I fragment with the *LYS2* and *TRP1* markers has the opposite orientation with respect to DNA B (see the map in Fig. 2). Also, on DNA A the initiation of RNA synthesis is more efficient in the DNA topoisomerase I mutant strain, although the overall transcription of DNA A is lower than that of DNA B in both strains (data not shown). This finding indicates that the relative positions of the genes in the domain modify their functional behaviors.

The topology of ethanol-stimulated episomal DNA differs between wild-type and DNA topoisomerase I-deficient strains.

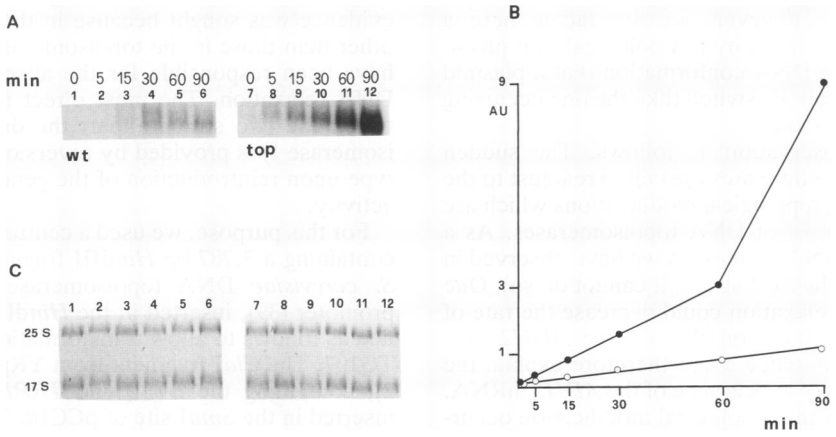

FIG. 3. *ADH2* transcription from DNA B in CH335 (wild type [wt]) and TG107 (*top1*) cells. (A) Northern analysis showing the steady-state level of RNA extracted from the two strains at the indicated intervals after the addition of ethanol and hybridized with the *Cla*I-*Eco*RV segment of pBR322 to detect *ADH2* mRNA. (B) Densitometric analysis of the data shown in panel A. ○, wild-type *ADH2*; ●, *top1 ADH2*. The values (expressed in arbitrary units [AU]) were corrected for the amount of rRNA calculated from a scanning of panel C. (C) Ethidium bromide staining of the RNA samples analyzed in panel A. 25S and 17S indicate the two major yeast rRNA species.

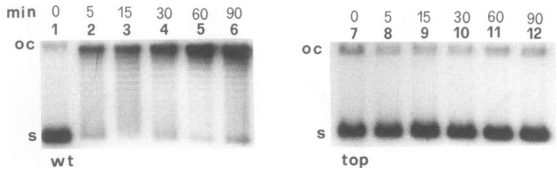

FIG. 4. Analysis of the topology of DNA B prepared from CH335 (wild type [wt]) and TG107 (*top1*) cells grown in ethanol for the indicated times. Circular DNA was extracted from the samples analyzed in Fig. 3, run on a 1% agarose–TPE gel containing 2 μg of chloroquine per ml. After Southern blotting, the filter was hybridized with the *Cla*I-*Eco*RV segment of pBR322. Two DNA forms are indicated: open circular (OC) and supercoiled (S).

Topology and transcription are linked. Because of the isogenic nature of the two strains analyzed, the difference in their transcriptional responses is presumably related to the absence of DNA topoisomerase I. Analysis of the topology of the transcribed plasmids was therefore of interest.

We report here the analysis of the topology of DNA B extracted from strains CH335 (wild type) and TG107 (*top1*) before and after ethanol addition.

Circular DNAs prepared from the samples analyzed in Fig. 3 are shown in Fig. 4. The chloroquine-agarose gel analysis reveals a striking result: the topoisomer distribution of the DNA B from the wild-type strain (which is negatively supercoiled before the addition of ethanol to the cell suspension; lane 1) undergoes a strong decrease of supercoiling immediately after that addition (lane 2).

In contrast, the same DNA B in the *top1* mutant shows no relaxation but, rather, an increase of negative superhelicity after the addition of ethanol, as expected from its transcription (4). This was seen when the samples shown in Fig. 4 were analyzed in the presence of a higher chloroquine concentration: the intercalating agent not only resolves the topoisomers in the negative range but also shows the production of positive supercoiling in the wild-type strain after ethanol addition (data not shown). The strong relaxation observed in the wild-type strain is unexpected and unprecedented.

A small increase in negative (and not in positive) supercoiling due to transcription of the *CYC1* promoter on a yeast plasmid has been observed (29). A shift in the topoisomer distribution toward the positive range is actually caused by a temperature shift (33). However, we are facing here a different phenomenon (induced by physiological, not physicochemical, causes): the DNA conformation that a plasmid assumes following a metabolic switch (like the one occurring after the addition of ethanol).

We interpret this observation as follows. The sudden change of carbon source stimulates the cell to readjust to the new condition, inducing topological modifications which are normally under the control of DNA topoisomerases. As a consequence, plasmid DNA relaxes (as we have observed in the wild-type strain; in the *top1* strain, it cannot do so). One can speculate that this relaxation could decrease the rate of formation of a transcription complex on the *ADH2* promoter. A topological difference could therefore explain the difference in the kinetics of appearance of the *ADH2* mRNA.

To determine whether the topological modification occurring in wild-type strain CH335 is a peculiarity of DNA B and of the genes that form that domain, we analyzed the topoisomer distribution of the endogenous 2μm DNA present in the same circular DNA preparations. When the filter shown in Fig. 4 was rehybridized with a 2μm probe, the topological

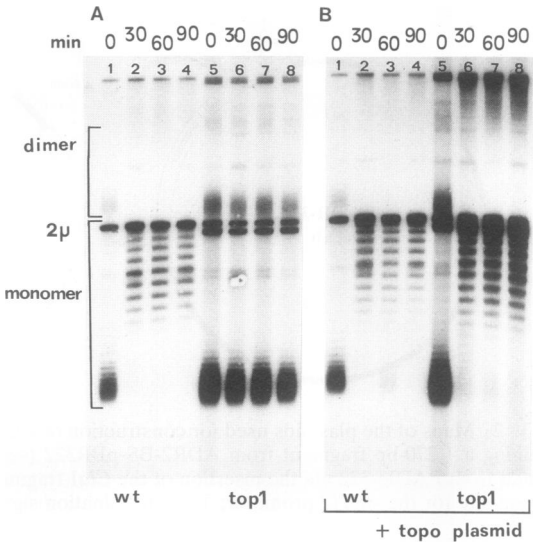

FIG. 5. Ethanol-dependent 2μm DNA relaxation in CH335 (wild type [wt]) and TG107 (*top1*) cells before (A) and after (B) transformation with a plasmid containing the entire *TOP1* gene with its own promoter (topo plasmid). Cells were grown in YNB medium containing glucose, washed, and analyzed immediately (lanes 1 and 5) or at increasing times (as indicated) after the addition of ethanol. Circular DNAs were subjected to chloroquine-agarose gel electrophoresis as described in the text.

behaviors of the *ADH2*-containing plasmid (Fig. 4) and of the 2μm DNA (not shown) are identical. In the wild-type strain, both DNAs are immediately relaxed after ethanol addition; in the *top1* strain, both DNAs remain in the negatively supercoiled initial condition.

This result implies that the change in metabolism due to the glucose-ethanol shift induces an increase of linking number mediated by DNA topoisomerase I, no matter which genes are present on the two circular DNA populations. In strain TG107, in which DNA topoisomerase I is missing, no such change is observed.

The ethanol-dependent topological relaxation of 2μm DNA circles is caused by DNA topoisomerase I. The topological difference caused in the plasmids by the shift to ethanol is indeed related to the DNA topoisomerase I gene. Direct evidence was sought because in the *top1* strain, mutations other than those in the topoisomerase I gene could possibly have been responsible for the absence of ethanol-induced DNA relaxation. The most direct proof of isogenicity between the two strains (apart the difference in DNA topoisomerase I) is provided by reversion to the original phenotype upon reintroduction of the gene coding for the missing activity.

For this purpose, we used a centromeric plasmid (pCC10), containing a 3,787-bp *Hin*dIII fragment spanning the entire *S. cerevisiae* DNA topoisomerase I gene with its own promoter (39), inserted in the *Hin*dIII site of YCp50, modified as follows to allow transformation of strains CH335 and TG107. The *Cla*I fragment from YRp31 (see the map in Fig. 2), containing the *LYS2* and *TRP1* genetic markers, was inserted in the *Sma*I site of pCC10. The four strains (CH335 and TG107 and their derivatives transformed with this construct) were analyzed for ethanol-induced DNA relaxation (Fig. 5). Cells were grown in YNB medium containing glucose to a density of 4.5×10^7 cells per ml, washed with YNB, and diluted 7.5-fold in YNB (lanes 1 and 5) or YNB

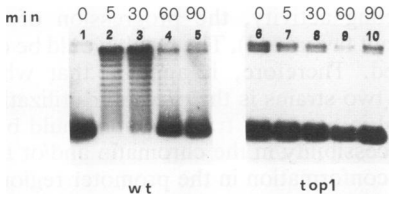

FIG. 6. Ethanol-dependent 2μm DNA relaxation in RS118 (wild type [wt]) and YAP1 (*top1*) cells transformed with a plasmid containing the *E. coli topA* gene (see Materials and Methods). Cells were grown in YNB medium containing glucose, washed, and analyzed immediately (lanes 1 and 6) or at increasing times (as indicated) after the addition of ethanol.

containing ethanol. Aliquots were withdrawn at 30, 60, and 90 min after ethanol addition, and the topoisomer distributions of the 2μm DNA circle of all four strains were analyzed by chloroquine-agarose gel electrophoresis.

The results clearly show that reintroduction of the DNA topoisomerase I gene in strain TG107 is sufficient to restore the expected phenotype: after the shift to the ethanol-containing medium, the 2μm DNA circle is again subjected to an increase of linking number due to the resumed relaxing activity (Fig. 5B, lanes 5 to 8).

***E. coli* DNA topoisomerase I cannot substitute for the *S. cerevisiae* enzyme in the ethanol-dependent DNA relaxation.** If the DNA relaxation observed after addition of ethanol is due to the transient accumulation of unconstrained torsional stress, the same effect should be produced also by a heterologous DNA topoisomerase I. In this case, the need for a relaxing activity would be satisfied by a nicking-closing enzyme of whatever origin. On the other hand, no relaxing activity of foreign origin will substitute for *S. cerevisiae* DNA topoisomerase I if the observed topological modification involves (i) a change in the DNA structure or in the episomal DNA-protein complex that can be recognized only by the resident DNA topoisomerase I or (ii) an interaction between the enzyme with a yeast-specific factor(s) that occurs after ethanol addition.

To test this hypothesis, we introduced a plasmid carrying the *E. coli topA* gene under the control of a constitutive yeast promoter in strains RS188 (wild type) and YAP1 (*top1* mutant). In the wild-type strain (Fig. 6, lanes 1 to 5), the expected DNA relaxation is observed following addition of ethanol. In contrast, no relaxation occurs in the *top1* strain (lanes 6 to 10), even though an active *E. coli* DNA topoisomerase I is present. In this experiment, we used a pair of isogenic strains different from the one used for Fig. 5. The different genetic background is presumably responsible for the rapid recovery of the initial DNA conformation. We measured the activities of both yeast and bacterial enzymes by preparing cell extracts from the samples shown in Fig. 6 (data not shown).

The data indicate that in the yeast *S. cerevisiae*, DNA topoisomerase I is specifically required to mediate the topological modification induced on minichromosomes in the presence of ethanol. Moreover, we have observed that in a temperature-sensitive DNA topoisomerase II mutant strain, the ethanol-dependent DNA relaxation occurs at both the permissive and restrictive temperatures with the same efficiency (data not shown).

Taken together, these results imply a specific role for *S. cerevisiae* DNA topoisomerase I in the shift of topoisomer distribution accompanying the glucose-to-ethanol metabolic switch.

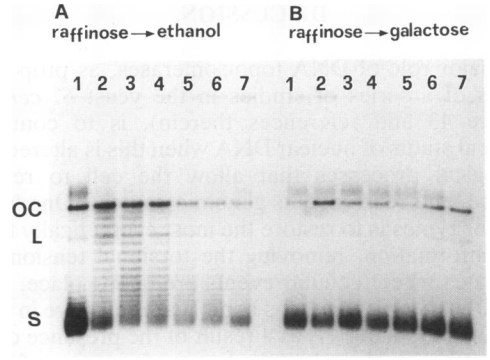

FIG. 7. Analysis of the 2μm DNA topology in wild-type CH335 cells grown in 2% raffinose (lanes 1) and then shifted to 3% ethanol (A) or 2% galactose (B). Lanes 2 to 7 (both panels) show the topology of 2μm DNA at different times (5, 15, 30, 60, 120, and 180 min) after the change of carbon source. Gel electrophoresis and hybridization were performed as described in the text. Positions of open circular (OC), linear (L), and supercoiled (S) DNA are shown.

The relaxation of 2μm DNA occurs upon ethanol addition, not upon release from glucose repression. The relaxation that occurs immediately after the change of carbon source from glucose to ethanol could be caused not by the presence of ethanol but by the removal of glucose and the repression exerted by this nutrient. Alternatively, the relaxation could be a direct consequence of the presence of ethanol. To distinguish between these two possibilities, we tested raffinose as an initial carbon source, thus avoiding the carbon catabolite repression induced by glucose.

Wild-type cells (strain CH335) were grown overnight at 30°C in YNB medium containing 2% raffinose, diluted to a concentration of 4×10^6 cell per ml in fresh medium containing raffinose, and grown for an additional hour. The culture was then washed with the same volume of sterile water and split in half; ethanol (3%) or galactose (2%) was added to each half, and the cells were incubated at 30°C for different intervals. The result shows that the 2μm DNA is relaxed immediately after the addition of ethanol (Fig. 7A, lane 2) but remains negatively supercoiled upon addition of galactose (Fig. 7B, lanes 2 to 7).

This finding shows that (i) the removal of glucose repression is not sufficient to induce the increase of linking number observed for the 2μm DNA and (ii) the change of carbon source from raffinose to galactose is not by itself sufficient to cause the topological relaxation. The same is true with a change from glucose to either galactose or raffinose (data not shown). Therefore, ethanol is the cause of the observed phenomena.

We have noticed that other nonfermentable carbon sources, such as glycerol, lactate, and acetate, are able to induce a similar but much less pronounced effect compared with ethanol (data not shown). In other words, the molecular event occurring in the nucleus and mediated by DNA topoisomerase I appears to be linked to the process of respiration.

An additional indication supporting the link between the shift in the DNA topoisomer distribution and cell respiration is provided by the fact that after several hours of growth in glucose, the 2μm DNA undergoes a relaxation (data not shown). Presumably, when the cells exhaust most of the glucose in the medium and start to accumulate ethanol, a change in DNA topology in the nucleus provides a molecular signal for mitochondrial functions.

DISCUSSION

The major role of DNA topoisomerases, as proposed on the basis of a series of studies in the yeast *S. cerevisiae* (reference 43 and references therein), is to control the topological status of nuclear DNA when this is altered by the physiological processes that allow the cell to replicate, transcribe, and rearrange its genomic content. One function of these enzymes is to restore the most energetically favored DNA conformation, removing the torsional tension which accumulates where cellular events are taking place.

Both DNA topoisomerases I and II are sensitive in vitro to supercoiling (5, 6, 30, 47) as a result of the presence of DNA sites characterized by a certain degree of curvature for DNA topoisomerase I (7) and the preferential interaction with DNA crossovers in the case of DNA topoisomerase II (47). It therefore appears that DNA topoisomerases are powerful sensors of DNA structural modifications: their activity is continuously in equilibrium with the molecular events undergoing in the cell nucleus.

In prokaryotes, there is a tightly controlled balance between relaxing and supercoiling functions which is mediated by enzymes whose genes are transcriptionally regulated by the DNA conformation itself (reference 31 and references therein). Moreover, it has been shown that environmentally induced changes in supercoiling can regulate gene expression (1, 16, 22, 23).

In eukaryotes, little is known about controls that operate on the genes coding for DNA topoisomerases or directly on the enzymes. It can be reasonably expected that changes in the activity of DNA topoisomerases cause various phenotypes.

Trying to find additional phenotypes for eukaryotic DNA topoisomerase I, we were particularly interested in understanding the influence on gene expression of mutation of this gene. DNA topoisomerase I is not essential for viability, and in most cases DNA topoisomerase II can substitute for it. Nevertheless, a series of phenotypes has been attributed to DNA topoisomerase I gene deletions: (i) a 200-fold increase in ribosomal DNA recombination (11); (ii) a marked increase in the negative supercoiling of episomal DNA undergoing intense transcription (4); (iii) a slower decrease in mRNA synthesis for many genes that are entering the stationary phase of the cell cycle (10); and (iv) a stimulation of transcription of rRNA minigenes (35).

We sought to find one or more genes whose expression would be different between a wild-type and a DNA topoisomerase I-deficient isogenic strain in order to define an in vivo relationship between regulation of transcription and DNA conformational modifications. The choice of the *ADH2* gene is linked to previous analyses that have indicated a remarkable in vitro sensitivity of its promoter to topological alterations (12).

***ADH2* gene expression.** In this report, we show that the expression of *ADH2* is favored in the DNA topoisomerase I-deficient strain, both in the normal chromosomal location and when the promoter is present on an episome.

Several possibilities should be taken into account to explain this result, given that gene expression is controlled at various steps. A difference at the posttranscriptional level is difficult to explain since the effect is observed also when only the *ADH2* promoter plus the first 62 bp of the coding region are present on an episome (that is, the majority of the mRNA synthesized on the DNA B construct, including terminations signals, is heterologous). A difference in the elongation rate is unlikely, since it is expected that in the absence of the major swiveling activity, the progression of RNA polymerase II is not favored (3). The result would be opposite the one observed. Therefore, it appears that what changes between the two strains is the promoter utilization.

Differential initiation of transcription could be due to an increased accessibility in the chromatin and/or to an appropriate DNA conformation in the promoter region caused by the absence of DNA topoisomerase I. Alternatively, a different topology at the promoter level could influence directly the functional interaction between DNA and RNA polymerase II, or some factor(s) required for the induction of a cellular response to ethanol could indirectly be influenced by the mutation. In this regard, the following arguments could be considered, keeping in mind that it is currently impossible to distinguish among the three alternatives.

In prokaryotes, many examples of genes which are sensitive to DNA supercoiling are known (31). In eukaryotes, a recent review has analyzed in detail the relationship between DNA supercoiling in chromatin structure and gene expression (19). It is believed (19) that the torsional tension present at the level of a promoter could help the assembly of the transcription initiation complex or could assist the RNA polymerase in the strand separation process.

It has been shown that a mutation in DNA topoisomerase I leads to increasing negative superhelicity in plasmids undergoing transcription (4). Therefore, the possibility exists for such available torsional tension to be utilized as a controlling or enhancing element for the initiation of transcription on defined sets of promoters belonging to the same topological domain. If the torsional tension in a wild-type strain is continuously removed by the action of DNA topoisomerases, the initiation of mRNA synthesis from this supercoiling-regulated set of promoters would be kinetically unfavored. It is expected that for each domain, only certain promoters would be sensitive to alteration of DNA conformation, as found in prokaryotes. We have indeed noticed that in the case of DNA B, only *ADH2* transcription differs between wild-type and *top1* mutant cells, whereas *LYS2* transcription drastically drops in both strains after ethanol addition; in the case of the 2μm DNA, constitutive transcription of the genes belonging to this domain is not significantly affected in either strain (data not shown).

Several genetic loci have been implicated in the regulation of *ADH2* (9, 13, 14, 25, 38). Among the various loci, *ADR1* codes for the only known protein which binds directly to the *ADH2* promoter (37). This activating factor is a good candidate for explaining the DNA topoisomerase I mutant phenotype that we have observed. However, it is not required for the expression of the *ADH2* gene when present on plasmids (2).

Thus, any of the other known factors could be directly or indirectly influenced by the absence of DNA topoisomerase I and therefore play a role in the differential utilization of the *ADH2* promoter. Interestingly, some of the functions of the cascade controlling *ADH2* expression (CRE2 = SPT6 and ADR6 = SWI1) have been implicated in the control of chromatin organization (41).

Ethanol-dependent topological shift. We observe that the DNA topology of the analyzed circular DNAs is profoundly modified upon variation of the carbon source from glucose to ethanol. A stringent proof that this is the cause of the different transcriptional behaviors of the *ADH2* promoter in the two isogenic strains is lacking; nevertheless, the delay in the kinetics in mRNA appearance for the wild type correlates well with the increase of linking number that occurs after the addition of ethanol. A recent report provides a good

correlation between the decrease of mRNA synthesis at certain promoters and the accumulation of positive DNA supercoiling (20).

An identical shift in topoisomer distribution is observed also for the 2μm circle, a totally different genetic system present in the same DNA preparations. This finding argues against a dependence of the observed structural modification upon a particular combination of genes: there is no known common genetic feature between 2μm DNA and DNA B.

The most likely explanation of the observed common behavior is that both circular DNAs sense the addition of ethanol through the action of DNA topoisomerase I. In the case of the *ADH2* promoter, this dynamic DNA response could cause a delay in the start of mRNA synthesis. The same explanation could possibly apply also to the *ADH2* chromosomal situation; we are currently looking for changes in high-resolution DNA footprinting at the proper genomic location.

An unexpected feature of the topological shift observed after ethanol addition is that neither bacterial topoisomerase I or endogenous topoisomerase II seems to be able to substitute for the endogenous topoisomerase I activity. As far as the bacterial enzyme is concerned, at least two possible explanations can be provided: (i) if the ethanol-induced modification of the minichromosome structure implies the generation of unconstrained positive supercoiling, this supercoiling could not be removed by *E. coli* DNA topoisomerase I; or (ii) if the action of ethanol is not a direct one (we are currently testing this hypothesis in an in vitro system consisting of reconstituted minichromosomes and topoisomerase I in the presence or absence of ethanol), a mediator (a protein kinase, a component of the cytoskeleton or of the nuclear envelope, etc.) capable of interacting only with the resident DNA topoisomerase I would be required. The latter explanation could apply also to the inability of DNA topoisomerase II to perform the relaxation induced by ethanol. Alternatively, the change in ATP production and utilization due to the metabolic switch could inhibit DNA topoisomerase II, whose activity is known to be strongly ATP dependent.

More work is needed to answer other important questions concerning, for instance, the potential rearrangements that plasmid chromatin undergoes during the topological shift. One could expect (i) a dramatic loss of nucleosomal structure that would render the minichromosome totally unconstrained and (ii) a more subtle modification at the level of each individual linker due to a nonhistonic component of chromatin.

In conclusion, the major topological shift observed is (i) caused by a metabolic stimulus, not by transcription itself, and (ii) due to DNA topoisomerase I. Therefore, the direct involvement of this enzyme in regulatory processes can be hypothesized.

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