Circular Minichromosomes Become Highly Recombinogenic in Topoisomerase-deficient Yeast Cells*

Received for publication, September 29, 2000
Published, JBC Papers in Press, October 27, 2000, DOI 10.1074/jbc.M008930200

Sonia Trigueros‡ and Joaquim Roca§
From the Institut de Biologia Molecular de Barcelona, Consejo Superior de Investigaciones Científicas, Jordi Girona 18-26, 08034 Barcelona, Spain

In topoisomerase-deficient yeast cells, we have found that circular minichromosomes are present as broad distributions of multimeric forms, which consist of tandemly repeated copies of their monomeric sequences. This phenomenon selectively occurs in ∆top1 cells, and is highly magnified in double mutant ∆top1 ∆top2–4 cells. No multimers are observed in single mutant ∆top2–4 or ∆top3 cells, or in ∆top1 cells that express a plasmid-borne TOP1 gene. Interconversion among multimeric forms takes place rapidly in double mutant ∆top1 ∆top2–4 cells, and the multimeric distributions are readily reverted to the monomeric form when a plasmid-borne TOP1 gene is expressed from an inducible promoter. These observations are a new example of the interplay between DNA topology and genome stability, and suggest that the cell capacity to modulate DNA supercoiling is limited when DNA is organized in small topological domains. Yeast minichromosome multimerization provides an appropriate system in which to study mechanistic aspects of DNA recombination.

Three distinct families of DNA topoisomerases disentangle DNA strands and modulate DNA supercoiling inside the cell. Type IA topoisomerases transiently cleave single-stranded DNA to allow the passage of other DNA segments. Type IB topoisomerases cleave one DNA strand in a double-helix and allow the rotation of the duplex by the uncleaved strand before rescaling the DNA. Type II topoisomerases transiently cleave both strands in a double-stranded DNA segment and transport another double-stranded DNA segment across the gated duplex (for a review, see Ref. 1).

The main biological roles of eukaryotic DNA topoisomerases has been inferred from studies in yeast cells. Saccharomyces cerevisiae has three topoisomerases: topoisomerase I, a type IB enzyme encoded by the TOP1 gene (2, 3); topoisomerase II, a type II enzyme encoded by the TOP2 gene (4); and topoisomerase III, a type IA enzyme encoded by the TOP3 gene (5). One function of these enzymes is to remove DNA supercoiling. During replication, DNA ahead of the replication fork becomes positively supercoiled as the parental DNA strands come apart behind it. During DNA transcription, the hindrance to the rotation of the transcription ensemble drives positive supercoiling of DNA ahead of the polymerase and negative supercoiling behind it (6). Yeast topoisomerase I serves as the major DNA swivel during replication and transcription, but this role can be fulfilled by topoisomerase II (7, 8); thus, both enzymes are capable of relaxing positively and negatively supercoiled DNA in vivo (9, 10). Because untwining of parental DNA strands is often incomplete at the end of DNA replication, the other main role of topoisomerases is to unlink the new pairs of replicated DNA molecules. Decatenation activity of topoisomerase II is essential for proper chromosome segregation during mitosis (11, 12) and meiosis (13). The less abundant topoisomerase III has weak DNA relaxation activity and does not contribute to supercoil removal in vivo (14); it is, however, required to complete the final stages of meiotic recombination (15).

Whereas TOP2 is essential for cell viability, cell growth is not blocked by null mutations of the yeast TOP1 or TOP3 genes. However, deficiency of any of the three yeast topoisomerases is found to affect genome stability (for a review, see Refs. 16 and 17). A null top1 mutant and a top2 thermo-sensitive (ts)1 mutant, grown at a semipermissive temperature, were shown to have a high frequency of mitotic recombination in the rDNA cluster (18). In double mutant yeast cells ∆top1 ∆top2-ts, grown at permissive temperature, destabilization of the rDNA gene cluster was revealed by the presence of over half of the rDNA genes as extrachromosomal rings containing one or more copies of the 9-kb rDNA unit (19). These excised rings were found to integrate back into the rDNA locus, presumably through homologous recombination, when plasmid-borne TOP1 or TOP2 genes were expressed. Recombination in tandem arrays of repetitive nucleotide sequences, other than the rDNA cluster, appears to be unaffected by reducing the cellular level of DNA topoisomerase I or II (18). On the other hand, null top3 mutants show an increased recombination between a variety of sequence repeats (5), including subtelomeric sequences (20).

The mechanisms by which topoisomerases apparently suppress mitotic recombination remain to be explored. While studying the biological functions of DNA topoisomerases, using various mutants of the yeast S. cerevisiae, we found evidence of the involvement of DNA topoisomerases in genome stability. We observed that in the topoisomerase double mutant ∆top1 ∆top2-ts circular minichromosomes strongly multimerize. These multimeric forms consist of tandem copies of the monomeric rings, and their formation or resolution is readily affected by DNA topoisomerase I.

* This work was supported in part by Grants PB95-0131 and PB98-0487 from the Ministry of Science and Education of Spain (to J. R.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Recipient of a predoctoral training fellowship from the Ministry of Science and Education of Spain.
§ To whom correspondence should be addressed: IBMB, Consejo Superior de Investigaciones Científicas, Jordi Girona 18-26, 08034 Barcelona, Spain. Tel.: 34-93-4006178; Fax: 34-93-2045904; E-mail: jrbmcm@cid.csic.es.

1 The abbreviations used are: ts, temperature sensitive; bp, base pair(s); kb, kilobase(s).
**MATERIALS AND METHODS**

**Strains**—The yeast strains used in this study were kindly provided by R. Kim and J. C. Wang (Harvard University). All strains are derivatives of FY251 (MATa his3Δ200 leu2-Δ1 trp1-Δ63 ura3-Δ52). JCW 26 (top2–4), harboring a thermosensitive mutation in the TOP2 gene, was derived by one-step gene replacement (21). JCW27 (Δtop1) and JCW28 (Δtop1 top2–4) were derived from FY251 and JCW26, respectively, by the homologous-gene run method of gene replacement to create a null mutation in the TOP1 gene (22). JCW253 (Δtop2:TRP1) and JCW273 (Δtop2:TRP1 Δtop1) were derived by one-step gene replacement from JCW25 and JCW27, respectively, to create a null mutation in the TOP3 gene. Yeast cells were grown in synthetic selective media as described by Sherman (23). Cell transformation was carried out using the lithium acetate method described by Ito et al. (24).

**Plasmid and DNA Constructions**—Circular constructs Yp 0.8, Yp 1.4, Yp 1.5, and Yp 2, which did not contain DNA sequences to allow bacterial amplification, were constructed by self-ligation of linear DNA fragments obtained by the polymerase chain reaction or from bacterial plasmids. Yp 0.8 (855 bp) was constructed from the EcoRI yeast chromosomal fragment (1453 bp) containing TRP1 and ARS1. The following primer sequences: 5’-GCGGAAATCTTATTTCTTTACATCTTG-3’ and 5’-GCCGGAATTCTTATTTCTTTACATCTTG-3’ were used to amplify a segment of 869 bp having TRP1 and ARS1, which was then digested with EcoRI (primer-engineered EcoRI sites are shown in boldface) and self-ligated to obtain the 855-bp DNA ring. Yp 1.4 (1453 bp) was constructed by circularizing the 1453-bp EcoRI yeast chromosomal fragment containing TRP1 and ARS1. Yp 1.5 (1487 bp) was constructed by circularizing a DNA fragment, which was composed of a SacI-HindIII segment (1211 bp) containing yeast URA3, followed by a HindIII-SacI segment (276 bp) containing yeast ARS-HO. Yp 2 (2055 bp) was constructed by circularizing a DNA fragment, which was composed of an EcoRI-HindIII segment (1217 bp) containing yeast URA3, followed by a HindIII-EcoRI (833 bp) segment containing yeast ARS1. Yp 3.4 (3468 bp) was constructed by inserting the 1453-bp EcoRI yeast chromosomal fragment containing TRP1 and ARS1 into pHC624 (a 2015-bp plasmid derivative of pBR322). Yp 4.6 (4414 bp) was constructed by inserting the 1453-bp EcoRI yeast chromosomal fragment containing TRP1 and ARS1 into pBlueScript-KSII (a 2961-bp plasmid from Stratagene). DimERIC and trimERIC constructs of Yp 1.4 were constructed by self-ligation of the 1453-bp EcoRI yeast chromosomal fragment containing TRP1 and ARS1. Ligated products were treated with topoisomerase II to eliminate catenanes, and the resulting circular species containing two and three copies of the TRP1 ARS1 fragment were purified from agarose gels. Plasmids pRK-G1T1 (19) and YCp-GH1T1 (25) were kindly provided by R. Kim and J. C. Wang (Harvard University).

**DNA Isolation and Hybridization**—DNA from transformed yeast cells was prepared from yeast spheroplast as described by Sherman et al. (24). Blot hybridization was as described by Southern (27), using 32P-labeled probes obtained by random priming of gel-purified DNA sequences.

**Assay of Topoisomerase Unlinking Activity**—Yeast DNA topoisomerase II was purified from a S. cerevisiae strain BCY123 harboring a yeast DNA topoisomerase II expression clone YeTD2 by the method described by Liu et al. (29).

**RESULTS**

**DNA Minicircles Strongly Multimerize in Topoisomerase-deficient Yeast Cells**—DNA minicircles of different size containing an autonomous replicating sequence (ARS1 or ARS-HO) and a selective gene marker (TRP1 or URA3) were constructed to transform the S. cerevisiae strain FY251 and its derivative double mutant JCW 28 (Δtop1 top2–4), which carries a deletion in the TOP1 gene and a ts mutation in the TOP2 gene. Transformants of the FY251 and JCW 28 strains were obtained on selective media plates and then grown in liquid culture at 30°C, a permissive temperature for the top2–4 mutant. DNA samples from individual transformants were analyzed by agarose gel electrophoresis, and the DNA minicircle sequences were identified by blot-hybridization with 32P-labeled probes. Fig. 1a depicts the structure of the DNA minicircles constructed, and Fig. 1b shows the typical electrophoretic patterns obtained with these constructs following yeast transformation.

As DNA samples were analyzed by electrophoresis in TBE buffer containing calculated amounts of chloroquine, covalently closed DNA molecules produced characteristic ladders of topoisomers. In the FY251 strain, most of the constructs migrated as monomeric DNA circles (Fig. 1b, lane 1 in each panel). In contrast, in the JCW 28 (Δtop1 top2–4) strain, most of these DNA migrated as a variety of multimeric forms (Fig. 1b, lane 2 in each panel). The degree of multimerization was inversely proportional to the size of the rings. The smaller the monomeric rings, the stronger the multimerization. Constructs Yp 1.4, a 1.4-kb ring with the TRP1-ARS1 sequence, and Yp 1.5, a 1.5-kb ring with the URA3 ARS-HO sequence, similarly generate...
multimeric forms. We ruled out the possibility that the presence of multimeric forms could be due to their preferred uptake by the Δtop1 top2–4 cells at the time of transformation, thus identical results were obtained by transforming these cells with gel-purified monomeric forms of each construct. The multimers were invariably found in any single transfectant of the double mutant Δtop1 top2–4 strain, and the distribution of multimers did not change significantly in exponentially growing cells or in cell-saturated cultures.

**Multimers Are DNA Rings Containing Direct Tandem Repeats of the Monomeric Ring**—The ladder-type distribution of multimers generated in the Δtop1 top2–4 strain suggested that these DNA forms consisted of several complete copies of the monomeric sequence. This was confirmed by cutting them at restriction sites that were unique to the monomeric sequences. In the experiment depicted in Fig. 2b, DNA extracted from the Δtop1 top2–4 strain, which contained multimeric forms of the Yp 1.4 construct, was mixed with knotted DNA rings derived from P4-phage capsids. These mixtures were then treated with purified yeast topoisomerase II, and analyzed by agarose gel electrophoresis in the presence of ethidium (Fig. 2b, left panel). The knotted DNA rings derived from P4-phage capsids, which migrated as an smeary distribution (Fig. 2b, K), were converted to the unknotted form (Fig. 2b, U) in the reaction containing ATP and topoisomerase II. The same gel was then blotted and probed to visualize the multimeric forms of the Yp 1.4 construct (Fig. 2b, right panel). No alteration in the distribution of multimers was observed following topoisomerase II treatment in any of the reacted samples. This indicated, therefore, that the multimeric forms, rather than catenanes, were circular DNA molecules consisting of tandem repeats of the monomeric ring.

**TOP1 Is Essential to Avoid Multimerization**—To test whether the formation of multimers in the double mutant strain Δtop1 top2–4 was strictly related to the alteration of the TOP1 or TOP2 gene, we examined the stability of monomeric rings in the single mutant strains JCW26 (top2–4) and JCW27 (Δtop1). Fig. 3a shows the electrophoretic patterns of DNA rings extracted from strains FY251, JCW26, JCW27, and JCW28, transformed with either the Yp 1.4 construct (lanes 1–4) or Yp 1.5 construct (lanes 5–8). We observed that in the JCW26 (top2–4) strain the monomeric forms of these constructs were stable (lanes 2 and 6). No multimers were observed, even when this strain was grown at semipermissive temperature (30 °C) (data not shown). Multimeric forms of the constructs, however, were apparent in the single mutant strain JCW27 lacking topoisomerase I (lanes 3 and 7). The degree of multimerization in this strain was, however, much lower than in the double mutant Δtop1 top2–4 (lanes 4 and 8). We also examined the occurrence of multimerization in cells defective in topoisomerase III. The single mutant strain JCW253 (Δtop3) and the double mutant strain JCW273 (Δtop1 Δtop3) were transformed with the Yp 1.5 construct and the monomer stability of this ring was examined. As shown in Fig. 3a, lanes 9 and 10, the absence of topoisomerase III, either by itself or in conjunction with the absence of topoisomerase I, did not contribute to the generation of multimeric forms. The degree of multimerization in the double mutant Δtop1 Δtop3 (lane 10) was comparable to that observed in the single mutant Δtop1 (lane 7).

To corroborate that multimerization was strictly dependent of topoisomerase I deficiency, we examined minichromosome stability in the double mutant Δtop1 top2–4 containing the plasmid pRK-G1T1 (19). This plasmid derives from the single-copy yeast vector YCp50 and expresses the yeast TOP1 gene from an inducible yeast promoter pGAL1. As shown in the experiment depicted in Fig. 3b, multimerization did not occur in Δtop1 top2–4 cells expressing the plasmid-borne yeast TOP1 gene (lanes 1 and 2). An identical result was also obtained when expressing a plasmid-borne human TOP1 gene (lanes 3 and 4). We conclude, therefore, that topoisomerase I was necessary and sufficient to confer monomer stability of the circular minichromosomes. On the other hand, the alteration of topoisomerase II function as a result of the top2–4 mutation had no effect by itself, but it dramatically augmented multimerization when topoisomerase I was absent.

**Both Formation and Resolution of Multimers Take Place in Topoisomerase-deficient Yeast Cells**—For any given DNA con-
DNA Ring Multimerization in Topoisomerase-deficient Cells

Fig. 3. Minichromosome multimerization in yeast strains defective in one or several topoisomerases. a, DNA was extracted from the isogenic strains FY251, JCW26 (top2–4), JCW27 (∆top1), and JCW28 (∆top1 top2–4) harboring either the Yp 1.4 construct (lanes 1–4, respectively) or the Yp 1.5 construct (lanes 5–8, respectively). DNA was also extracted from the strains JCW253 (∆top3) and JCW273 (∆top1 ∆top3) harboring the Yp 1.5 construct (lanes 9 and 10, respectively). These DNA samples were examined by agarose gel electrophoresis as described in the legend to Fig. 1b. b, the double mutant strain JCW28 (∆top1 top2–4) was transformed with plasmid Ycp50, or one of its two derivatives pRK-G1T1 and Ycp-G1hT1, which carry the yeast TOP1 and the human TOP1 genes under the yeast pGAL1 promoter, respectively. These cells were grown in selective media containing 2% galactose to activate the expression of the TOP1 gene, and then transformed with the Yp 1.4 construct. DNA samples of these double transformants, which grew in selective media containing 2% galactose, were examined by gel electrophoresis as described in the legend to Fig. 1b. Lane 1, JCW28 containing Ycp50 and Yp 1.4. Lane 2, JCW28 containing pRK-G1T1 and Yp 1.4. Lane 3, JCW28 containing Ycp50 and Yp 1.4. Lane 4, JCW28 containing Ycp-hG1T1 and Yp 1.4.

struct, the relative amounts of monomers and multimers observed in topoisomerase-deficient yeast cells was very similar in all the transformants examined, and did not change in ongoing generations of the cells. This suggested that the distributions of multimers were the result of a steady-state equilibrium between generation and resolution pathways. To examine this hypothesis, we transformed the JCW28 (∆top1 top2–4) strain with gel-purified constructs that contained either 2 or 3 tandem repeats of the monomeric sequence of Yp 1.4. As shown in Fig. 4, in all the transformants examined, a broad distribution of multimers of the Yp 1.4 construct was observed (lanes 2 and 5). Such distributions contained more or fewer repeats than the initial dimeric or trimeric construct used to transform the cells (lanes 1 and 4), and were indistinguishable from the distributions previously obtained with the monomeric rings. Digestion of these multimers with the restriction enzyme EcoRI yielded a unique product of 1.4 kb (lanes 3 and 6), which confirmed that such multimers consisted of direct repeats of the monomeric sequence.

Multimerization Can Be Reverted by the Extrachromosomal Expression of Topoisomerase I—Experiments in the above section indicated that interconversion among different multimeric forms of minichromosomes takes place rapidly in topoisomerase-deficient cells. We hypothesized that the restoration of topoisomerase I expression, in cells already containing multimeric minichromosomes, would shift the distribution of multimers toward the monomeric form. In the experiment depicted in Fig. 5, the double mutant JCW28 (∆top1 top2–4), containing multimeric forms of the Yp 1.4 construct, was transformed with the plasmid pRK-G1T1. The distribution of multimers was then examined following the repression or the induction of the plasmid-borne TOP1 gene. As shown in Fig. 5a, multimers were present as long as the cells grew in media containing 2% dextrose, which represses the expression of TOP1 (lane 1). When cells were transferred for 50 generations to a medium containing 2% galactose, which induces the expression of TOP1, the distribution of multimers was shifted toward the monomeric form (lane 2). When the cells were returned to a medium containing 2% dextrose, the multimers reappeared (lane 3). These were again reverted to the monomer when the cells were grown in 2% galactose (lane 4). This effect of topoisomerase I expression on the directionality of multimer formation or resolution is similar to that described for the excision and integration of extrachromosomal rDNA rings, which also happens in ∆top1 top2–4 strains (19). Hence, in the same DNA samples analyzed above, we examined whether the formation and resolution of minichromosome multimers occurred in parallel to the excision and integration of rDNA rings. As shown in Fig. 5b, extrachromosomal rDNA rings were present in the ∆top1 top2–4 strain when the TOP1 gene was repressed (lanes 1 and 3). Upon induction of the TOP1 gene (lanes 2 and 4), the excised rDNA rings integrated back into the chromosome. The excision of rDNA rings and the multimerization of minichromosomes, therefore, take place concomitantly in the topoisomerase-deficient cells, and both processes are reversed upon expressing TOP1.
multimerization on topoisomerase I activity is ratified by showing that it does not occur in Δtop1 top2–4 cells expressing a plasmid-borne TOP1 gene.

The enhancing effect of the top2–4 mutation on the formation of multimers, in the top1 background, suggests that this phenomenon is related to the overall decreased capacity of these cells to remove DNA supercoils. Topoisomerase I and II provide the DNA swivel activity in yeast cells. In the single mutant Δtop1, topoisomerase II might closely compensate the absence of topoisomerase I. In the double mutant Δtop1 top2–4, however, DNA swivel activity must be severely reduced. The amount of topoisomerase II activity detected in lysates from top2–4 cells, grown at permissive temperature (26 °C), is at least 10-fold less than that detected in lysates from TOP2 cells. In that regard, as the main role of yeast topoisomerase II is to unlink the newly replicated DNA molecules, we suspected that in the Δtop1 top2–4 strain the increased multimerization was due to the incomplete unlinking of monomeric rings. We found, however, no trace of catenanes among the multimeric forms. This observation highlights a topoisomerase II function apart from DNA unlinking. The fact that Δtop3 strains does not contribute to the formation of multimers, further supports the hypothesis that multimerization is related to the reduced efficiency with which DNA supercoils are removed. Although Δtop3 strains are hyper-recombinogenic (5), topoisomerase III is the least abundant cellular topoisomerase and its ability to remove DNA supercoils is weaker than that of topoisomerase I or II (14).

The appearance of minichromosome multimers in the topoisomerase-deficient yeast cells could be due either to the high instability of monomeric forms, or to the increased stability of the oligomers. We show that DNA circles, consisting of two or three tandem copies of a given construct, are not stable when introduced in topoisomerase-deficient cells. Therefore, the distributions of multimers observed probably reflect a dynamic equilibrium of a bidirectional pathway, in which oligomeric constructs are continuously generated and then transformed back to simpler forms. In topoisomerase-deficient cells, both directions of the process could be enhanced, but the tendency to generate multimers surpasses the tendency to reduce them. In support of that, we show that pre-existing distributions of multimers can be efficiently reverted to the monomeric form just by turning on the expression of the TOP1 gene.

The multimerization of minichromosomes has several similarities with the instability of the rDNA gene cluster also observed in topoisomerase-deficient yeast cells. In a double mutant Δtop1 top2–4 strain, over half the rDNA genes are present as extrachromosomal rings containing one or more copies of the 9-kb rDNA unit. These excised rings integrate back into the rDNA locus when a plasmid borne TOP1 or TOP2 gene is expressed (19). Like minichromosome multimerization, the instability of the rDNA gene cluster is a bidirectional process, in which both the forward and reverse pathways are probably mediated by homologous recombination. We have verified that in Δtop1 top2–4 strains both cases of instability coexist and are analogously tuned. In topoisomerase-deficient cells, excision dominates over integration in the case of rDNA genes, and multimerization dominates over resolution in the case of minichromosomes. The expression of a plasmid-borne TOP1 gene readily eliminates the multimers as well as the excised rDNA rings.

The instability of circular minichromosomes and that of rDNA genes may be variations of the same process. If that is the case, a common threat is making circular minichromosomes and the rDNA genes particularly sensitive to topoi-

**DISCUSSION**

Multimerization of plasmids is mediated by factors involved in homologous recombination, and has frequently been observed in bacteria (30–33) and yeast (34). We report here that in topoisomerase-deficient yeast cells, circular minichromosomes generate broad distributions of multimeric forms, which consist of tandem copies of their monomeric sequences. By examining the presence of such minichromosome multimers in a set of isogenic yeast strains, with mutations in one or more of the three known topoisomerases, we find that the main determinant for multimerization is the absence of topoisomerase I. In TOP1 cells, the monomeric forms of minichromosomes remain stable regardless of additional mutations in the TOP2 or TOP3 genes. Conversely, incipient multimerization appears in Δtop1 or Δtop1 Δtop3 strains, and multimerization increases dramatically in the Δtop1 top2–4 strain. The dependence of multimerization on topoisomerase I activity is ratified by showing that it does not occur in Δtop1 top2–4 cells expressing a plasmid-borne TOP1 gene.

The enhancing effect of the top2–4 mutation on the formation of multimers, in the top1 background, suggests that this phenomenon is related to the overall decreased capacity of these cells to remove DNA supercoils. Topoisomerase I and II provide the DNA swivel activity in yeast cells. In the single mutant Δtop1, topoisomerase II might closely compensate the absence of topoisomerase I. In the double mutant Δtop1 top2–4, however, DNA swivel activity must be severely reduced. The amount of topoisomerase II activity detected in lysates from top2–4 cells, grown at permissive temperature (26 °C), is at least 10-fold less than that detected in lysates from TOP2 cells. In that regard, as the main role of yeast topoisomerase II is to unlink the newly replicated DNA molecules, we suspected that in the Δtop1 top2–4 strain the increased multimerization was due to the incomplete unlinking of monomeric rings. We found, however, no trace of catenanes among the multimeric forms. This observation highlights a topoisomerase II function apart from DNA unlinking. The fact that Δtop3 strains does not contribute to the formation of multimers, further supports the hypothesis that multimerization is related to the reduced efficiency with which DNA supercoils are removed. Although Δtop3 strains are hyper-recombinogenic (5), topoisomerase III is the least abundant cellular topoisomerase and its ability to remove DNA supercoils is weaker than that of topoisomerase I or II (14).

The appearance of minichromosome multimers in the topoisomerase-deficient yeast cells could be due either to the high instability of monomeric forms, or to the increased stability of the oligomers. We show that DNA circles, consisting of two or three tandem copies of a given construct, are not stable when introduced in topoisomerase-deficient cells. Therefore, the distributions of multimers observed probably reflect a dynamic equilibrium of a bidirectional pathway, in which oligomeric constructs are continuously generated and then transformed back to simpler forms. In topoisomerase-deficient cells, both directions of the process could be enhanced, but the tendency to generate multimers surpasses the tendency to reduce them. In support of that, we show that pre-existing distributions of multimers can be efficiently reverted to the monomeric form just by turning on the expression of the TOP1 gene.

The multimerization of minichromosomes has several similarities with the instability of the rDNA gene cluster also observed in topoisomerase-deficient yeast cells. In a double mutant Δtop1 top2–4 strain, over half the rDNA genes are present as extrachromosomal rings containing one or more copies of the 9-kb rDNA unit. These excised rings integrate back into the rDNA locus when a plasmid borne TOP1 or TOP2 gene is expressed (19). Like minichromosome multimerization, the instability of the rDNA gene cluster is a bidirectional process, in which both the forward and reverse pathways are probably mediated by homologous recombination. We have verified that in Δtop1 top2–4 strains both cases of instability coexist and are analogously tuned. In topoisomerase-deficient cells, excision dominates over integration in the case of rDNA genes, and multimerization dominates over resolution in the case of minichromosomes. The expression of a plasmid-borne TOP1 gene readily eliminates the multimers as well as the excised rDNA rings.

The instability of circular minichromosomes and that of rDNA genes may be variations of the same process. If that is the case, a common threat is making circular minichromosomes and the rDNA genes particularly sensitive to topoi-

---

*2 S. Trigueros and J. Roca, unpublished data.*
somerase deficiency. This thread does not alter, however, the stability of tandem copies of other DNA sequences located at the chromosomal level (18). The instability of rDNA genes has been attributed to their exceptionally high transcriptional rate (19). DNA supercoiling generated during their transcription would persist longer when the combined relaxation activity of the cellular topoisomerases is low. Supercoiling in general and negative supercoiling in particular may stimulate recombination by unwinding the DNA duplex or disrupting the chromatin structure. However, the rate of transcription in the circular constructs examined here (which harbor the yeast TRPI or URA3 genes) is not high. Nevertheless, we envisage that in circular minichromosomes any sporadic burst of DNA supercoiling might be more intense or persist longer than in chromosomal sequences, and that such an effect would be magnified in topoisomerase-deficient cells. One reason is that a burst of DNA supercoiling in a small domain cannot be diluted to the same extend as in a large one. Another reason is that the amount of topoisomerase activity required to modulate DNA supercoiling must correlate with the dimensions of the topological domains in which cellular DNA is organized, rather than with the total amount of cellular DNA. Therefore, the smaller the size of a topological domain, the more limited the diffusible topoisomerase activity that will reach it. Circular minichromosomes, hence, would become quickly affected when cellular levels of topoisomerase are low. This scheme would explain why the instability of the monomers is inversely proportional to their size; and also, why all the multimeric distributions are centered around circles of similar dimensions (about 5–10 kb, as seen in Fig. 1), regardless of the size of the monomeric construct that generated them. Taken together, these results suggest that topoisomerase dosage is finely adjusted by the cell for the removal of DNA supercoils. On one hand, cells might not be allowed to surpass a threshold amount of topoisomerase activity. Overexpression of topoisomerase I from multicopy plasmids carrying the TOP1 gene rapidly leads to cell death (35). On the other, subthreshold levels of topoisomerases compromise genome stability, which is promptly manifested as increased recombination in certain multicopy sequences (circular minichromosomes and the rDNA cluster). The reported findings will provide a suitable model to further study the interplay between DNA topology and genome stability, as well as mechanistic aspects of DNA recombination.

REFERENCES
1. Wang, J. C. (1996) Annu. Rev. Biochem. 65, 635–692
2. Goto, T., and Wang, J. C. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 7178–7182
3. Thrash, C., Bankier, A. T., Barrett, B. G., and Sterngrlanz, R. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4374–4378
4. Giaever, G., Lynn, R., Goto, T., and Wang, J. C. (1986) J. Biol. Chem. 261, 12448–12454
5. Wallasch, J. W., Chebret, G., Brodsky, G., Rolfe, M., and Rothstein, R. (1989) Cell 59, 469–419
6. Liu, L. F., and Wang, J. C. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 7024–7027
7. Brill, S. J., DiNardo, S., Voelkel-Meiman, K., and Sterngrlanz, R. (1987) NCI Monogr. 4, 11–15
8. Kim, R. A., and Wang, J. C. (1989) J. Mol. Biol. 208, 257–267
9. Saavedra, R. A., and Huberman, J. A. (1986) Cell 45, 65–70
10. Giaever, G. N., and Wang, J. C. (1988) Cell 55, 849–856
11. DiNardo, S., Voelkel, K., and Sterngrlanz, R. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 2016–2020
12. Uemura, T., Ohkura, H., Adachi, Y., Morino, K., Shiozaki, K., and Yanagida, M. (1987) Cell 50, 917–925
13. Rose, D., Thomas, W., and Holm, C. (1990) Cell 60, 1009–1017
14. Kim, R. A., and Wang, J. C. (1992) J. Biol. Chem. 267, 17178–17185
15. Gangloff, S., de Massy, B., Arthab, L., Rothstein, R., and Fabre, F. (1999) EMBO J. 18, 1701–1711
16. Wang, J. C., Caron, R. P., and Kim, R. A. (1990) Cell 62, 403–406
17. Wang, J. C. (1991) J. Biol. Chem. 266, 6659–6662
18. Christman, M. F., Dietrich, F. S., and Fink, G. R. (1988) Cell 55, 413–425
19. Kim, R. A., and Wang, J. C. (1989) Cell 57, 975–985
20. Kim, R. A., Caron, R. P., and Wang, J. C. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 2667–2671
21. Rothstein, R. J. (1983) Methods Enzymol. 101, 202–211
22. Roca, J., Gartenberg, M. R., Oshima, Y., and Wang, J. C. (1992) Nucleic Acids Res. 20, 4671–4672
23. Sherman, F. (1991) Methods Enzymol. 194, 3–21
24. Isit, H., Fukuada, Y., Murata, K., and Kimura, A. (1983) J. Bacteriol. 153, 163–168
25. Benedetti, P., Fiorani, P., Capana, L., and Wang, J. C. (1993) Cancer Res. 53, 4343–4348
26. Sherman, F, Fink, G. R., and Lawrence, C. (1983) Methods in Yeast Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
27. Southern, E. M. (1975) J. Mol. Biol. 98, 503–517
28. Worland, S. T., and Wang, J. C. (1989) J. Biol. Chem. 264, 4412–4416
29. Liu, L. F., Davis, J. L., and Calendar, R. (1981) Nucleic Acids Res. 9, 3979–3989
30. otter, H., and Dressler, D. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 4168–4172
31. Kolodner, R. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 4847–4851
32. Fishel, R. A., James, A. A., and Kolodner, R. (1981) Nature 294, 184–186
33. James, A. A., Morrison, P. T., and Kolodner, R. (1982) J. Mol. Biol. 160, 411–430
34. Harashima, S., Shimada, Y., Nakade, S., and Oshima, Y. (1989) Mol. Gen. Genet. 219, 405–498
35. Bjornsti, M. A., Benedetti, P., Viglianti, G. A., and Wang, J. C. (1989) Cancer Res. 49, 6318–6323
Circular Minichromosomes Become Highly Recombinogenic in Topoisomerase-deficient Yeast Cells
Sonia Trigueros and Joaquim Roca

J. Biol. Chem. 2001, 276:2243-2248.
doi: 10.1074/jbc.M008930200 originally published online October 27, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M008930200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 34 references, 15 of which can be accessed free at http://www.jbc.org/content/276/3/2243.full.html#ref-list-1