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Deletion of the topoisomerase III gene in the hyperthermophilic archaeon *Sulfolobus islandicus* results in slow growth and defects in cell cycle control

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Abstract

Topoisomerase III (topo III), a type IA topoisomerase, is widespread in hyperthermophilic archaea. In order to interrogate the *in vivo* role of archaeal topo III, we constructed and characterized a topo III gene deletion mutant of *Sulfolobus islandicus*. The mutant was viable but grew more slowly than the wild-type strain, especially in a nutrient-poor medium. Flow cytometry analysis revealed changes of the mutant in growth cycle characteristics including an increase in proportion of cells containing either more than two genome equivalents or less than one genome equivalent in exponentially-growing cultures. As shown by fluorescence microscopy, a fraction of mutant cells in the cultures were drastically enlarged, and at least some of the enlarged cells were apparently capable of resuming cell division. The mutant also shows a different transcriptional profile from that of the wild-type strain. Our results suggest that the enzyme may serve roles in chromosomal segregation and control of the level of supercoiling in the cell.

Keywords: Hyperthermophilic archaea; *Sulfolobus islandicus*; Topoisomerase III; *topA* deletion; Phenotype

1. Introduction

DNA topoisomerases are ubiquitous enzymes that catalyze topological changes in DNA and essential for DNA transactions involving the opening of the double helix, such as DNA replication, transcription, repair and recombination (Champoux, 2001; Wang, 2002). All organisms possess at least one type IA DNA topoisomerase, which relaxes negatively supercoiled DNA by transiently breaking one strand of the DNA helix to allow the passage of the other strand (Champoux, 2001; Wang, 2002). The wide distribution of type IA topoisomerases suggests that these enzymes serve a critical function, which remains to be fully understood. There are two type IA topoisomerases in *Escherichia coli*: topoisomerases I and III (Champoux, 2001). Topoisomerase I is efficient in relaxing negatively supercoiled DNA, whereas topoisomerase III is an efficient decatenating enzyme (Champoux, 2001; Nurse et al., 2003). *E. coli* cells that lack both type IA topoisomerase activities filament extensively and do not segregate chromosomal DNA (Zhu et al., 2001).

Hyperthermophilic archaea, which live optimally above 80°C, are unique in DNA topology. Native plasmids isolated from these organisms are relaxed to slightly positively supercoiled, while those from bacteria are negatively supercoiled (Saffi et al., 2000). Almost all hyperthermophilic archaea whose genomes have been sequenced possess two type IA enzymes (reverse gyrase and topoisomerase III) and a type IIB enzyme (topoisomerase VI) (Champoux, 2001). Topoisomerase VI appears to exist only in Archaea (Bergerat et al., 1997; Champoux, 2001). The enzyme relaxes both positive and negative supercoils, and is likely involved in the decatenation of replication intermediates and/or regulation of the level of supercoiling. Reverse gyrase is the signature

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protein of hyperthermophiles including both archaeal and bacterial members, and probably represents an adaptation of these organisms to growth at high temperature. This contention was challenged by the observation that a reverse gyrase knockout mutant of *Thermococcus kodakaraensis* KOD1, a hyperthermophilic archaeon, was able to grow at 90°C (Atomi et al., 2004). However, the mutant did show an increasingly slower growth rate than the wild type with increasing temperature.

The other type IA enzyme (encoded by *topA*) in thermophilic archaea has been designated as topoisomerase III based on sequence and cleavage site analyses (Dai et al., 2003). A topoisomerase III from the hyperthermophilic archaeon *Sulfolobus solfataricus* (*Sso* topo III) has been biochemically characterized (Dai et al., 2003; Chen and Huang, 2006). The enzyme is capable of efficiently relaxing negatively supercoiled DNA at 75°C. The function of topoisomerase III in thermophilic archaea remains obscure. Here we report the first genetic analysis of archaeal topo III. Taking advantage of the recent availability of the genetic system in *Sulfolobus islandicus* (Deng et al., 2009), we constructed a topo III deletion mutant in this organism. The mutant was viable but showed a slow-growth phenotype and altered flow cytometry characteristics. Our results suggest that topoisomerase III may function in chromosomal segregation and control of DNA supercoiling in the cell.

2. Materials and methods

2.1. Strains and growth conditions

Sulfolobus islandicus Rey15A was isolated from an enrichment culture generated from a sample collected from a solfataric field in Iceland (Contursi et al., 2006). *S. islandicus* E233 (Δ*pyrEF*) is a spontaneous uracil-auxotrophic mutant derived from *S. islandicus* Rey15A (Deng et al., 2009). The organisms were grown at 75°C with shaking in either TY-rich or SCVy-basic medium (Peng et al., 2009). Growth was measured at OD_{600}.

2.2. Deletion of the topA gene

A knockout vector was constructed by inserting a knockout cassette, which contained the *pyrEF* gene from *S. solfataricus* P2, flanked by the upstream and downstream 1-kb neighboring sequences of the topoisomerase III gene (*topA*) of *S. islandicus* Rey15A, into plasmid pUC18. The *pyrEF* gene was amplified by PCR from the genomic DNA of *S. solfataricus* using the following primer pair: 5′-CGCCGGATCCCATCAAACTTATGTCTCATT/5′-CCGCGCATGCTATTCCTCGTGTAGATTTTC (*Bam*H I and *Sph* I sites are underlined). The resulting 1.71-kb fragment was cloned at the *Bam*H I/*Sph* I sites of pUC18, yielding plasmid pURA. Two primer pairs, 5′-GACGAATTCACAGTAGCGTTAGTTCATAATGAG/5′-TCATGGATCCTAACTCTTTCTAAGAGATAATAA (*Eco*R I and *Bam*H I sites are underlined) and 5′-GATGCATGCCATGCCAATCTGAAAAGATGAGG/5′-GAGCATGCTCCTTTATCCAGAATCAGTAGCGAG (*Sph* I sites are underlined), were used for PCR amplification of a 1.04-kb fragment upstream and a 1.04-kb fragment downstream, respectively, of the *topA* gene from *S. islandicus* Rey15A. The resulting fragments were inserted into the *Eco*R I/*Bam*H I sites and the *Sph* I site, respectively, of pURA. The sequence and orientation of the inserts were verified by DNA sequencing. The knockout plasmid was propagated in *E. coli*, isolated, linearized and transformed into *S. islandicus* E233, as described previously (Contursi et al., 2006). Transformed cells were plated on 0.8% Gelrite plates. Colonies were picked and inoculated in liquid medium. After growth to late-exponential phase at 75°C, cells were harvested, and the total DNA was extracted as described previously (Xiang et al., 2003). The deletion mutant (*ΔtopA*) was identified by PCR using a pair of primers (5′-TAATTCCCGGTCCAGTCAATGTTC/5′-CTAGATGCTGCGTCAGTAATGTCAAAG) targeting the *topA* gene. The sizes of the PCR products amplified from the wild-type *S. islandicus* DNA and the *ΔtopA* mutant DNA, in which the *topA* gene was replaced by the *pyrEF* gene, were 4087 bp and 3765 bp, respectively.

2.3. Southern hybridization

A sample (10 μg) of genomic DNA from the wild-type *S. islandicus* or the *ΔtopA* strain was digested with either *Hin*d III or *Pst* I. The restriction fragments were subjected to electrophoresis in 0.8% agarose and electrophoretically transferred onto a Hybond N^+ nylon membrane (Amersham Pharmacia Biotech, USA). Oligonucleotide probes for *pyrEF* (5′-CATCGCCATAACTTCCACCTTGACTACCCATAC) and *topA* (5′-CTTCCCTTGTAACCCAAAAAGATGTCCTGCCGC) were labeled at the 5′-end with [γ-^{32}P] ATP using T4 polynucleotide kinase. Southern hybridization was performed as described (Sambrook et al., 1989).

2.4. Immunoblotting

Polyclonal antiserum against topo III was raised in rabbit by using purified recombinant protein as the antigen. For immunoblotting, crude extracts (~200 μg of protein) from *S. islandicus* E233 and the *ΔtopA* mutant were loaded onto a 10% SDS polyacrylamide gel. Immunoblotting was carried out using anti-topo III antibodies, as described previously (Guo et al., 2003).

2.5. Growth curves

The wild-type strain and the *ΔtopA* mutant were grown at 75°C with shaking to the early exponential growth phase (~OD_{600} of 0.2) in either rich or basic medium. The cultures were then diluted by ~40 folds into the same medium. During the subsequent incubation, samples were taken at specified time intervals, and cell density was measured.

2.6. Flow cytometry

The wild-type strain or the *ΔtopA* strain was grown at 75°C in either rich or basic medium. Samples (0.3 mL) were taken