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ORIGINAL PAPER

Transcriptional analysis of the two reverse gyrase encoding genes of *Sulfolobus solfataricus* P2 in relation to the growth phases and temperature conditions

Florence Garnier · Marc Nadal

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Abstract *Sulfolobus solfataricus,* a hyperthermophilic crenarchaeon, contains two genes encoding reverse gyrases, *topR1* and *topR2*. The steady-state level of their transcripts were quantified during the growth phases for cells maintained either at 72, or 80°C, and after temperature changes from one to the other temperature. The transcripts of both genes are weakly expressed, but the highest level is observed in actively dividing cells, and is almost undetectable in cells in decline phase. During the temperature shift experiments, there is no significant *topR2* variation. By contrast, there is a maximum 2.4-fold increase in *topR1* transcripts within 30 min after the downshift. After 1 h, the transcript level reaches the level characteristic of cells adapted to the new temperature. After an upward shift, the *topR1* expression pattern is inversely regulated with a transient decrease with the same time course. The *topR1* expression profile is completely different from that of *topR2* after temperature shift experiments; this suggests a different regulation process for the two reverse gyrase genes. The fine tuning of the *topR1* transcript expression within a short interval of time after a temperature shift illustrates a rapid adaptation response to temperature change.

Keywords Reverse gyrase · Topoisomerase · Archaea · Expression · Growth phase · Temperature

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F. Garnier · M. Nadal
Laboratoire de Génétique et Biologie Cellulaire,
Université de Versailles-Saint-Quentin-en-Yvelines,
CNRS UMR 8159, Bâtiment Buffon, 45 Avenue des Etats-Unis,
78035 Versailles cedex, France

Present Address:
F. Garnier · M. Nadal (✉)
Laboratoire de Biologie Moléculaire du Gène chez les
Extrémophiles, Université Paris Sud, Institut de Génétique et
Microbiologie, CNRS UMR 8621, Bâtiment 409,
91405 Orsay cedex, France
e-mail: marc.nadal@igmors.u-psud.fr

Introduction

DNA topoisomerases are ubiquitous enzymes present in all living organisms : Eucarya, Bacteria and Archaea. They act in all DNA metabolism processes such as replication, transcription, repair and recombination (Champoux 2001; Wang 2002; Corbett and Berger 2004). Based on their catalytic activities, two different types of DNA-topoisomerases are distinguished: type I- and type II-DNA topoisomerases, performing a transient single or double-strand break, respectively. Due to their transient double-strand breakage reaction allowing DNA decatenation, type II-DNA topoisomerases are essential during the chromosomal segregation process (Wang 2002). The role of type I-DNA topoisomerases during cellular processes is much more enigmatic. Indeed, the type I-topoisomerase group is composed of two subfamilies of topoisomerases different from mechanistic and phylogenetic properties. The type IB enzyme is present in all Eucarya, some bacteria and some viruses (Champoux 2001; Krogh and Shuman 2002; Forterre et al. 2007), while the type IA is present in all living cells. Topoisomerase IB relaxes both negatively and positively supercoiled DNA (Champoux and Dulbecco 1972), while topoisomerase IA can only relax the negatively supercoiled DNA (Wang 1971; Tse-Dinh 1998). A peculiar topoisomerase IA, named reverse gyrase, was first discovered in hyperthermophilic archaea (Kikuchi and Asai 1984; Nadal 2007), and later in hyperthermophilic bacteria as well (for a review see Nadal

2007). This topoisomerase introduces positive supercoils in DNA in an ATP- and magnesium-dependent manner (Kikuchi and Asai 1984; Forterre et al. 1985; Nadal et al. 1988). Finally, reverse gyrase is a chimeric enzyme; the carboxy-terminal part is a type IA-topoisomerase domain, whereas the amino-terminal part is related to the SF2 helicase family domain (Confalonieri et al. 1993; Nadal et al. 1994; Jaxel et al. 1996; Krah et al. 1996; Declais et al. 2000; Rodriguez and Stock 2002; Nadal 2007). Because the reverse gyrase encoding gene distribution is strictly restricted to hyperthermophilic organisms (Archaea or Bacteria), this protein has been considered the molecular marker of the thermophily (Forterre 2002). When there is no functional reverse gyrase gene—as shown in the hyperthermophilic Euryarchaeota *Thermococcus kodakaraensis* KOD1—there is a sharply decreased cell division rate at temperatures above 70°C (Atomi et al. 2004). Hyperthermophilic organisms are submitted at a permanent risk of DNA melting due to the high temperature of their environment; therefore, they require the reverse gyrase that is able to introduce positive supercoils in DNA to counterbalance DNA melting. Interestingly, the reverse gyrase activity was shown to increase with increasing temperature (Lopez-Garcia and Forterre 1999). All these data, namely the temperature-dependent reverse gyrase activity, the temperature-dependent cell division rate limitation without functional reverse gyrase, and the specific thermophilic species-gene distribution highlight a tight correlation between temperature, reverse gyrase and physiological state. In this context, it is important to know more about reverse gyrase regulation. To date, there are not many available data on the gene expression regulation of type-IA topoisomerases and even fewer data on reverse gyrase expression.

In *S. solfataricus,* genome analysis reveals that two genes encode two putative reverse gyrase proteins (She et al. 2001). These proteins are rather similar at the aminoacid level with an identity of 35%. The presence of two reverse gyrase encoding genes prompted us to compare their regulation in this organism. In this article, we study the reverse gyrase expression by monitoring the steady-state level of both *topR1* and *topR2* transcripts during the growth phases of *S. solfataricus* cells growing at two different temperature conditions (72 and 80°C) and also during the shift from one temperature to the other.

Materials and methods

Materials

RnaseA, Sarkosyl, agarose, phenol and DEPC from Sigma, [α-^{32}P]dATP (222TBq/mmol) from MP Biomedicals and Hyperfilm was from Amersham. Acrylamide and bis-acrylamide were purchased from Bio-Rad and other chemicals were from Carlo Erba. DnaseI-RNase free and Rnase inhibitor were from Boehringer (Mannheim, Germany) and proteinase K from Merck. DNA ligase and restriction enzymes were purchased from BioLabs.

Strains and culture conditions

S. solfataricus P2 (DSMZ 1617) culture was performed in shaking bath (New Brunswick Scientific Innova^{TM} 3100) using Erlenmeyer flasks with long neck. Cells were cultivated continuously either at 72 or 80°C in rich media as described by Zillig et al. (1994) in aerobic conditions and under vigorous shaking (200 rpm). To maintain the cells at a stable temperature, the temperature was measured inside the growth medium.

When monitoring the influence of the growth phase on the reverse gyrase expression, cell samples were collected at characteristic time points of the growth curve for cells incubated either at 72 or 80°C over an average period of 130 h. When monitoring the influence of the up and down shifts of temperature on the reverse gyrase expression, an early exponential phase culture grown ($OD_{600\ nm} = 0.5$) was first obtained at 72 or 80°C depending on the shift experiment. The 80°C preculture was divided into two parts, returning one to the original temperature and down-shifting the other part in another New Brunswick shaking bath pre-equilibrated at the new temperature. Cell samples were removed at various time points from the control (0, 4, 24 h) and shifted cultures (0, 5, 10, 15, 30 min, 1, 4, 24 h) for RNA extraction. In the same way, an aliquot of the 72°C early exponential preculture was up-shifted at 80°C while the remaining part was kept at 72°C. Cell samples were harvested in the same range of time points as was done earlier. It is important to notice that the time required for the re-equilibration of the new temperature after a down or up-shift is about 30 min.

DNA and RNA extraction

S. solfataricus cell samples were quickly cooled by adding an appropriate volume of frozen buffer containing 200 mM Tris-HCl pH 8.8 to reach a pH around 5.5. Cells were then centrifuged at 10,000 *g* for 10 min and resuspended either in lysis buffer as described previously (Jaxel et al. 1996), or in 50 mM of EDTA for DNA and RNA extraction, respectively. The subsequent steps for DNA extraction were performed as previously described (Jaxel et al. 1996). Aliquots of 0.5×10^{10} or 0.25×10^{10} cells were used for RNA extraction during growth phases and temperature shift experiments, respectively. Cells were disrupted by addition of SDS (final concentration: 0.25%) and RNAs extracted twice with 1 volume of acidic phenol (phenol saturated with

water), followed by an extraction with chloroform/isoamyl alcohol (24/1). The supernatant was neutralized by 60 mM Tris–HCl pH 8, 0.8M Ammonium-Acetate and then ethanol- or isopropanol-precipitated. The RNA pellet was dissolved with DEPC-treated water. The efficiency of RNA extraction was the same whatever the time point for cell harvesting and whatever the cell physiological state (about 42 μg of total RNA/0.25 × 10^{10} cells). The purity and concentration of RNA extracts were checked spectroscopically and electrophoretically on 1.2% agarose gel with TBE 1X buffer and then BET stained.

Northern blotting hybridization and mRNA quantification

The specific probes used in our northern blot hybridization experiments correspond to *S. solfataricus topR1*, *topR2*, *thsA* and 16S rDNA gene portions. They were amplified by PCR on *S. solfataricus* genomic DNA by using the following oligonucleotide primers : (forward 5′-GAGAGTA GCCCAAGATCTTTTCG-3′ and reverse 5′-GCGTACGT GCTCGGTCTTCC-3′ for *topR1*-probe); (forward 5′-AA GGAGTATCTTCAAAAGCAAGC-3′ and reverse 5′-GC TTAGAAATTGATATACTGAGATACC-3′ for *topR2*-probe); (forward 5′-GCATTTCTTTTGTTACATCTTTC GC-3′ and reverse 5′-ATCATTAGTTATGGTTACGTC ACCGAAGC-3′ for *thsA*-probe); (forward 5′-AGCCC GGGAAGCTTGTCTCTGAC-3′ and reverse 5′- GAACCG TTCCAGGACTCCTCGCCTATGGG-3′ for 16S rDNA-probe). The resulting PCR products were cloned into a pCR-Script plasmid previously restricted with *Srf*I as recommended by the manufacturer (Stratagene). The positive recombinant plasmids have been checked by sequencing and used as templates for specific PCR using the same couple of specific oligonucleotides as indicated above. The corresponding PCR products were used as probes and labeled with α-^{32}P-dATP (ICN) using the 'Random primed DNA labeling kit' (Roche). Different amounts of total RNA (1, 2.5 or 5 μg) were resolved in a formaldehyde-containing 1.2% agarose gel and fractionated by electrophoresis in 50 mM Hepes—1 mM EDTA—formaldehyde 16% containing buffer. The size-separated RNA were transferred to Nytran N + nylon membranes (Schleicher and Schuell) and hybridized with the specific probes overnight at 42°C. The resulting signal was recorded after exposure using an appropriate screen from the Bio-Rad Molecular Imager® System GS 505. Specific hybridized transcripts were quantified using the Bio-Rad Molecular Analyst® Software, version 1.5.

The transcripts of *topR1*, *topR2, thsA* and 16S in relation to the growth phase at 72 and 80°C, and to the temperature up and down shift experiments, were quantified from three independent kinetic experiments and from the three loaded different amounts of total RNA (1, 2.5 and 5 μg). The quantified *topR1*, *topR2* and *thsA* mRNA were normalized either to E1 or to time point 0 according to the growth phase or shift experiments, respectively (first level of normalization). The transcript steady-state levels of *topR1*, *topR2* and *thsA* were further normalized by performing a ratio transcript of interest/16S RNA (second level of normalization). Consequently this quantification method fixes the mRNA steady-state level of first point of the kinetics to 1 arbitrary unit, which is then considered a reference for comparison.

Results

For the study of the influence of temperature on the steady-state level of reverse gyrase transcripts, the choice of temperatures was particularly important. We wanted to obtain data at two steady-state growth temperatures, and compare the corresponding transcript level during both up and down shifts. To determine a reproducible division rate over a long period with replating in between, a temperature for which there is no sublethality either at the lower or upper temperature conditions was a prerequisite. Moreover, because the DNA helical repeat variation is directly and proportionally related to the temperature gap, it appears very important to keep the same gap in up and down temperature shifts, in order to observe a possible reversible and complementary in time and amplitude response in transcript steady-state level. We thus performed experiments at 72 and 80°C for continuous growth, and temperature up and down shifts with respect to a temperature gap of ±8°C, whatever the direction of the shift.

S. solfataricus cell growth at 72 or 80°C

Representative growth curves of *S. solfataricus* incubated at 72 or 80°C are shown in Fig. 1. Both growth curves have roughly the same shape with three main phases, an exponential phase, a stationary phase of variable length and a decline phase (Fig. 1). We arbitrarily subdivided the growth curve in successive phases, denoted E1, E2, S1, S2, S3, (S4, only for 72°C) D1, D2, for early- and late-exponential phases, early-, middle- and late-stationary phases, and early- and late-decline phases, respectively. In both temperature conditions, the early exponential phase E1, characterized by an optimal growth rate, is quite short and is about 6–7 h long. During this period, cells grown at 72°C have a doubling time of 9 h while cells cultivated at 80°C exhibit a shorter doubling time of 5.5 h. Beyond the E1 phase, the cell division rate decreases continuously as the culture aged (Fig. 1). When the cell density reaches the maximum OD, (average value of 4–5 or 5–6 for 80°C- and

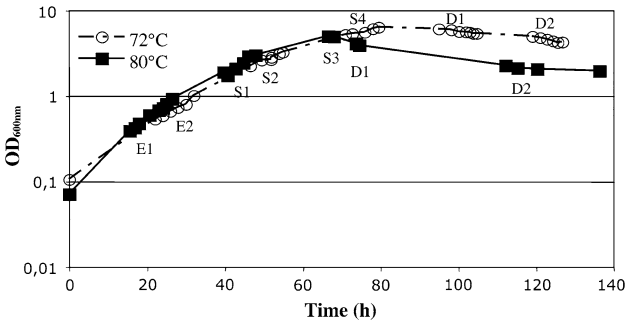

Fig. 1 Growth curves of *S. solfataricus* cells maintained either at 72°C (*open circle*) or 80°C (*filled square*). Cell growth was obtained by measuring the OD_{600} at different time points. The OD values are reported on a logarithmic scale. The different growth stages, arbitrarily defined, and described in Results, are indicated on the curves (E1, E2, S1, S2, S3 (S4, only for 72°C) D1 and D2)

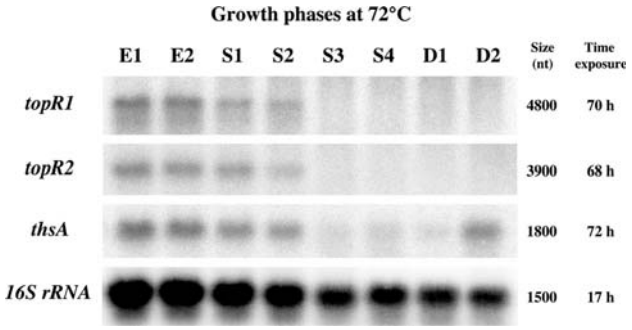

Fig. 2 Northern blot analysis of *topR1* and *topR2* in relation to the growth phase when *S. solfataricus* cells were grown at 72°C. The gene expressions of *thsA* and 16S rDNA genes monitored under the same conditions are also shown as controls of temperature and of loaded amount, respectively. The different cell harvesting time points, E1–E2, S1–S4 and D1–D2 correspond to early- and late exponential phase, early-, middle and late stationary phase, and early- and late decline phase, respectively. Total cellular *S. solfataricus* RNA was loaded in 5 µg amounts per lane. The respective transcript size was determined based on the standard molecular size of RNA markers from Promega and is indicated in nucleotides on the right. Time exposure in hours is also indicated on the right

72°C-adapted cells, respectively), the shape of the growth curve is different, depending on the temperature conditions. Cells maintained at 72°C present a well established stationary phase or plateau for a quite long period (about 20 h), including S3 and S4 phases. This stationary phase is followed by a decline phase occurring gradually (D1 and D2 phases, open circle curve, Fig. 1). By contrast, cells maintained at 80°C do not exhibit any plateau and as soon as the cells reach the maximum OD value, (S3 time point), they enter immediately a sharper decline phase (D1 and D2, filled square curve, Fig. 1). During the decline phase, irrespective of the temperature conditions, the cells probably lyse and/or their division ability is strongly inhibited due to a very low cell viability. Indeed, an aliquot of such cells replated into a fresh medium does not contain enough viable cells, if any, to resume growth (data not shown).

Steady-state level of reverse gyrase transcripts with changing growth phases at 72 or 80°C

Total RNA was isolated from cells grown either at 72 or 80°C and collected after different periods of growth, according to the landmarks indicated on the growth curves shown in Fig. 1 (E1, E2, S1, S2, S3, S4 [only for 72°C], D1 and D2). The transcript steady-state level of the two genes encoding a reverse gyrase was determined by northern blot hybridization by using *topR1* and *topR2* specific probes. In addition to the *topR1* and *topR2* probes, a *thsA* probe was used as a culture temperature control. The *thsA* gene (Sso0872) encodes for the alpha subunit of the rosettasome from hyperthermophiles (TF55). This macromolecular structure possesses a chaperon activity, particularly efficient under stress conditions such as temperature changes (Kagawa et al. 2003). An additional probe specific to 16S rRNA was also used for normalizing target transcript quantification. Each northern blot was sequentially hybridized with the different probes starting from the probe giving the weakest signal to the strongest one i.e. *topR1*, *topR2*, *thsA* and finally 16S rRNA successively. Whatever the culture temperature, only one transcript of about 4,800 or 3,900 nt was detected with the *topR1* and *topR2* probes, respectively (Fig. 2). The weak signal intensity obtained for both *topR1* and *topR2* mRNA indicates that both genes are transcribed at a low level in cells cultivated at 72 or 80°C.

The steady-state level of *topR1*, *topR2* and *thsA* mRNA in relation to the growth phase at 72 and 80°C is represented by histograms shown in Fig. 3. When *S. solfataricus* cells were grown at 72°C, the steady-state level of both *topR1* and *topR2* transcripts remained quite stable for early- (E1) and late-log (E2) growth phases (Fig. 3a, b). During the early stationary phase (S1), the *topR2* mRNA level slightly decreased (Fig. 3b), while the *topR1* mRNA level had decreased to around 55% of the reference E1 level (Fig. 3a). By the mid-stationary phase (S2), both steady-state mRNA levels continued to decrease and reached the same amount corresponding to approximately 40% of the E1 level (S2 phase: 38% for *topR1* (Fig. 3a), and 42% for *topR2* (Fig. 3b)). Throughout the rest of the growth (late stationary (S3) and decline (D1 and D2) phases), the *topR1* and *topR2* mRNA levels declined further to become nearly undetectable (Fig. 3a, b). The RNA quantification for S4 (not shown) is quite similar to S3 (see Fig. 2). In conclusion, the variations of *topR1* and *topR2* gene expression with changing growth phases at 72°C are quite similar.

When *S. solfataricus* cells were cultivated at 80°C, a significant increase of the *topR1* and *topR2* transcript levels

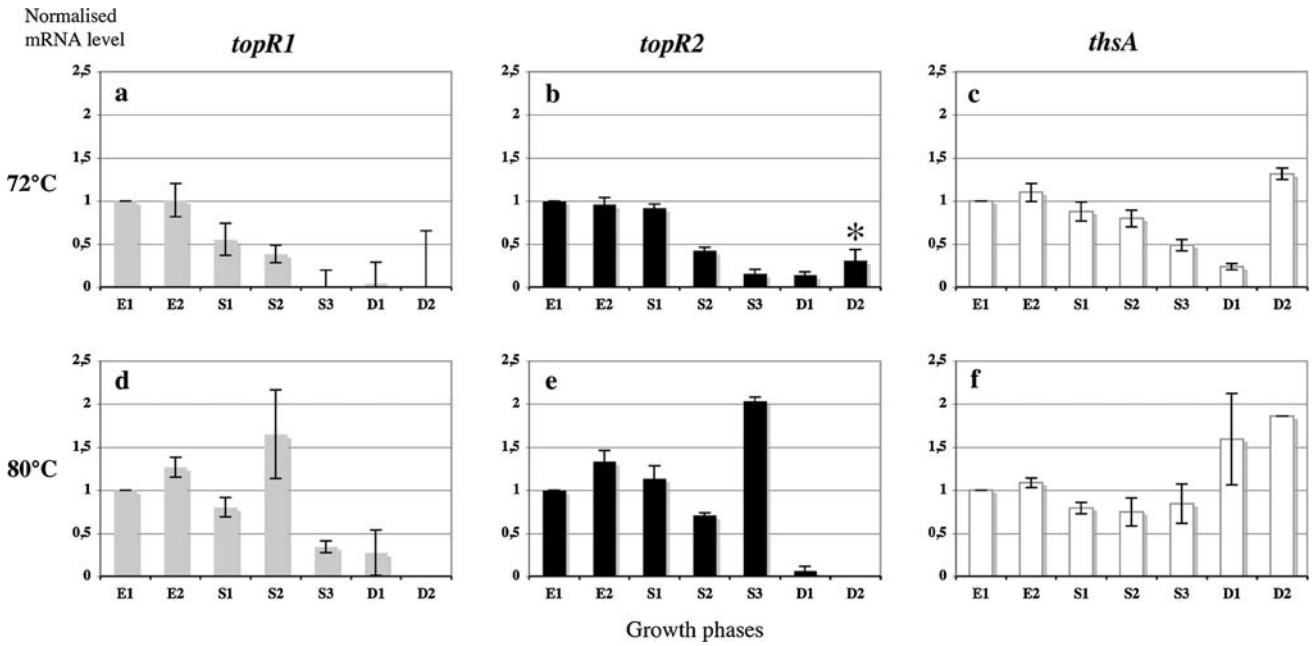

Fig. 3 Relative abundance of *S. solfataricus topR1* (**a** and **d**) and *topR2* (**b** and **e**) mRNA as a function of growth phases. Cells were grown from early exponential to decline phases in rich medium either at 72°C (a, b, c) or 80°C (d, e, f) and cell samples were regularly collected for RNA analysis. The steady-state level of *thsA* as a temperature control for the same time points is also shown in c and f for cells grown at 72 and 80°C, respectively. The relative levels of *topR1*, *topR2* and *thsA* throughout the growth phases are normalized to the corresponding level of 16S rRNA. The different quantification data represent the average of three independent experiments and of different loaded total RNA amounts for each kinetics experiment. *Due to a higher background and a resulting signal very close to the background, we considered that this value is not relevant

was observed for the E2 growth phase (27 and 33% increase respectively, compared with the E1 level, Fig. 3d, e). At the early stationary phase (S1), the *topR1* mRNA level had decreased to 80% of the reference E1 level before being notably enhanced at the S2 phase attaining a level nearly twice the S1 level (Fig. 3d). The *topR1* mRNA level then sharply diminished during the late stationary phase (S3), becoming almost undetectable in the D1 and D2 decline phases (Fig. 3d). The variations of *topR2* expression are roughly the same as for *topR1* except that it is shifted one growth phase forward (Fig. 3e). Indeed, the *topR2* decrease was more progressive, attaining in S2 approximately the same amount of mRNA (71% relative to the E1 level) as that observed for *topR1* (80%) but in S1. After the S2 decrease, the *topR2* mRNA level of S3 peaked to nearly three-times the S2 level. The *topR2* mRNA level further dropped off during the decline phase (both D1 and D2) (Fig. 3e). The characteristic transient increase in *topR* mRNA level for 80°C-adapted *S. solfataricus* cells in mid-stationary phase is not observed in cells grown at 72°C. This difference in transcriptional response could be related to a temperature-dependent physiological state. Indeed, cells grown at 72°C enter a stationary phase for approximately 20 h, due to a lower division rate, while cells growing more quickly at 80°C do not show any characteristic stationary phase.

The same northern blots were rehybridized with the specific *thsA* probe to compare the patterns of expression of *topR* and *thsA* genes throughout the growth phases of *S. solfataricus*. The overall steady-state level of *thsA* mRNA throughout the *S. solfataricus* growth at 72 or 80°C is shown in Fig. 3c. f, respectively. At 72°C, the *thsA* mRNA level is quite stable in exponential phase (E1 and E2), and then regularly decreases from S1 to D1 phase until it attains 23% of the mRNA level observed in E1 phase (Fig. 3c). During the D2 phase, the *thsA* mRNA level increased abruptly to more than five times the D1 level (Fig. 3c). At 80°C, the steady-state level of *thsA* mRNA is also quite stable in exponential phase (E1 and E2) and slightly decreases in S1 and S2 (Fig. 3f). Beyond the S2 phase, there is a gradual increase of *thsA* mRNA attaining in D1 and D2 values of 1.6- and 1.8-times the E1 level, respectively (Fig. 3f). In contrast to *thsA* mRNA that is always present at a high level in D2 phase whatever the culture temperature, *topR1* and *topR2* mRNA are mostly undetectable in the decline phase (Fig. 3).

S. solfataricus cell growth during up and down temperature shift experiments

When 72°C-adapted cells are up-shifted to 80°C, they decrease their doubling time from 9 to 5 h within 30 min

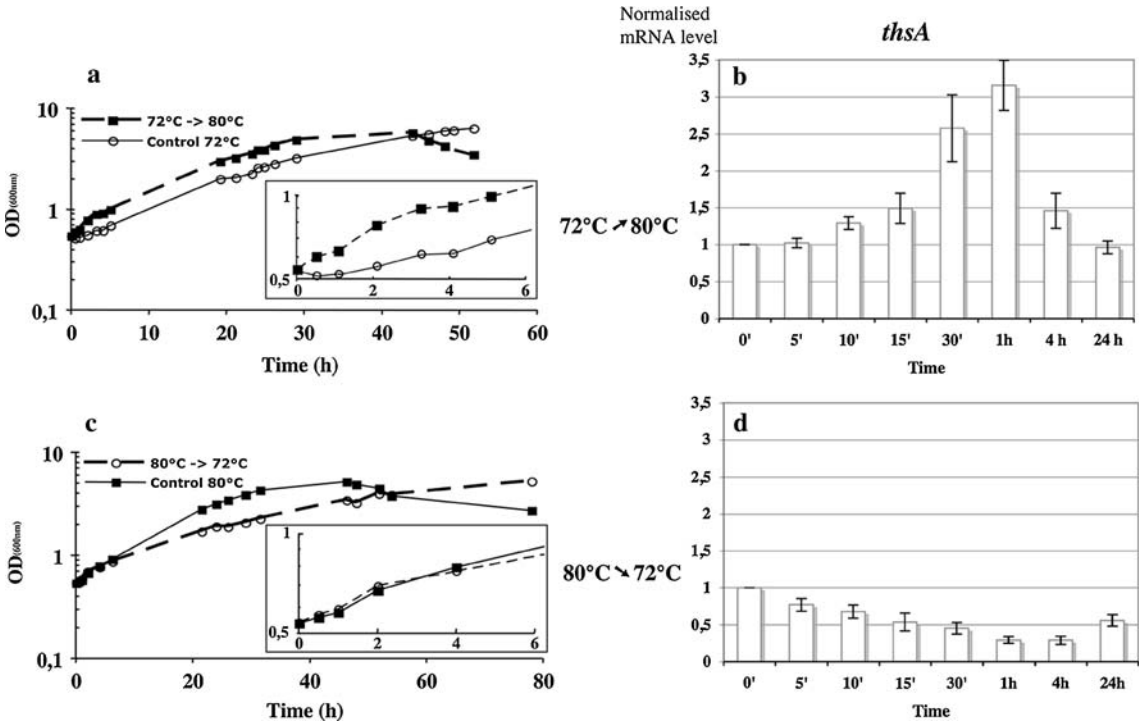

Fig. 4 Relative abundance of *thsA* mRNA isolated from *S. solfataricus* cells submitted to either up or down temperature shift experiments. Early-log phase cells grown at 72°C were shifted to 80°C (up shift) and reciprocally early-log cells grown at 80°C were transferred to 72°C (down shift). The time point 0 represent the state just before the shifting and is representative of the control cell state. After cell shifting, individual cell aliquots were harvested at increasing times and total cellular RNA was extracted (0, 5, 10, 15, 30 min, 1, 4, 24 h). An aliquot of control cells was maintained at the starting temperature to compare the cell doubling time and the steady-state level of *thsA* mRNA for control and shifted cells. For RNA analysis of control cells, cell aliquots were removed at 0, 4 and 24 h both at 72 and 80°C. a and c: Growth curves of control cells and shifted cells (72°C → 80°C: a; 80°C → 72°C: c). b and d: Time course of *thsA* mRNA level after up (72°C → 80°C : b) and down (80°C → 72°C: d) temperature shifts. The various time points from 0 to 30 are expressed in minutes and afterward in hours. The mRNA level of *thsA* is normalized to the corresponding 16S rRNA. The different quantification data represent the average of three independent experiments and of different loaded total RNA amounts for each kinetics experiment

and maintain this higher division rate for 4 h after the up shift (Fig. 4a). Beyond this period, the 80°C-shifted cells present a progressively increasing doubling time similar to that of cells continuously maintained at 80°C. Because of their rapid adaptation ability to higher temperature, the resulting growth curve exhibits the two main specific characteristics for 80°C-adapted cells: (1) a cell division arrest when the maximum cell density is reached; (2) an immediate entrance in decline phase with no plateau. During the same period, the control cells maintained at 72°C, continue to divide, albeit more slowly, and enter the stationary phase (S4 time point) (Fig. 4a). By contrast, when exponentially growing cells are down-shifted from 80 to 72°C, they conserve during the first 4 hours a division rate similar to that exhibited by control cells maintained at 80°C (Fig. 4c). It is only after this transitional period that the 72°C-shifted cells begin to decrease their division rate, reaching a doubling time of 16 h until 24 h after the down shift. This doubling time of 16 h is typical for cells in early stationary phase when continuously cultivated at 72°C. For comparison, 80°C control cells exhibit a faster doubling time of 9.5 h when estimated over the same period (between 4 and 24 h after T0). The rest of the growth curve monitored at 72°C (down-shift) (Fig. 4c) is similar to that obtained for control cells growing continuously at 72°C (Fig. 4a).

Steady-state level of reverse gyrase transcripts during up and down temperature shift experiments

We performed the transcriptional analyses for temperature up or down shifted cells as previously mentioned for growth phase experiments. The steady-state level of *topR1*, *topR2* mRNA in relation to the temperature shift is also represented by histograms, shown in Fig. 5 (*thsA* control in Fig. 4).

Whatever the temperature conditions, up- and down-shifted cells and corresponding control cells (data not shown), both collected at T0, 4 and 24 h, exhibit a *thsA*, *topR1* and *topR2* transcript level roughly similar to those

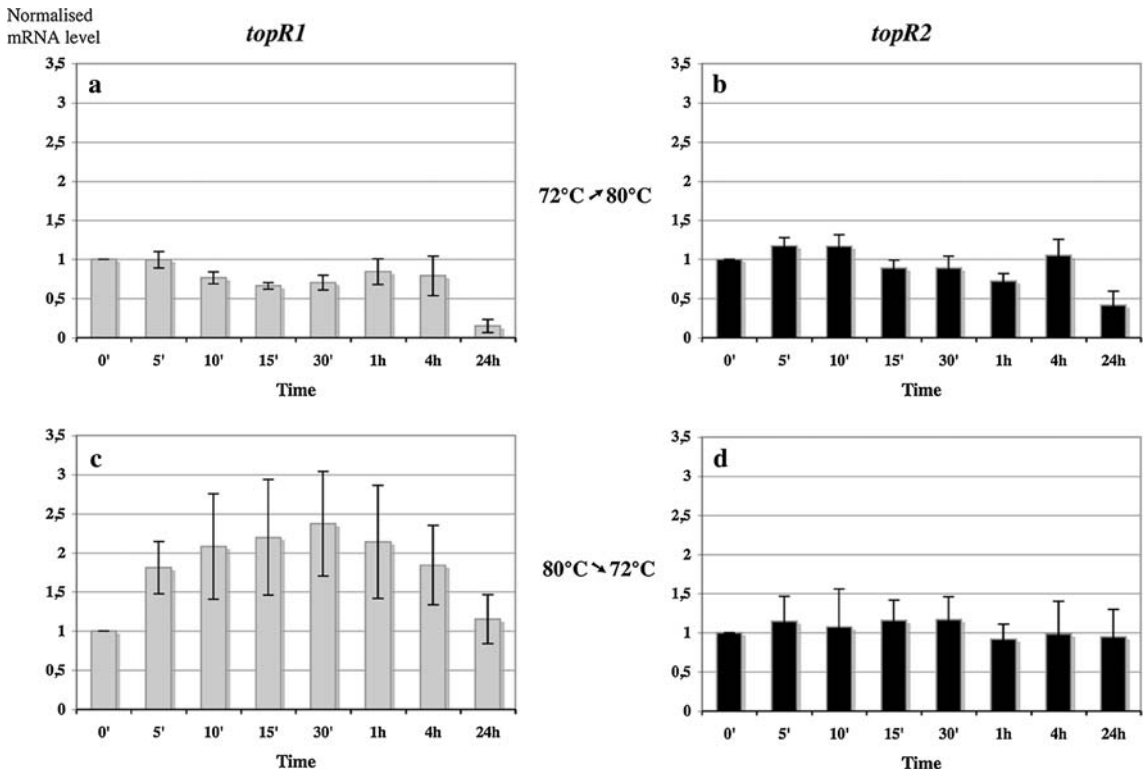

Fig. 5 Time course of *topR1* (**a** and **c**) and *topR2* (b and d) after temperature up (72°C → 80°C) and down (80°C → 72°C) shifts, respectively. The *topR1* and *topR2* mRNA analyses were performed with the same set of cell samples used for *thsA* mRNA analysis (see Fig. 4). The various time points from 0 to 30 are expressed in minutes and afterward in hours. The mRNA levels of *topR1* and *topR2* are normalized to the corresponding 16S rRNA. The different quantification data represent the average of three independent experiments and of different loaded total RNA amounts for each kinetics experiment

observed for 80 and 72°C-adapted cells in E1, E2 and S1 physiological states, respectively (Figs. 4, 5 compared with Fig. 3d–f). We can thus conclude that the cell response triggered by the temperature change itself might be observed within 1 h after the shift while up- and down-shifted cells collected beyond 1 h exhibit a cell response related to the physiological state dependent on the growth phase.

To control the temperature parameter change in temperature shift experiments, we monitored the *thsA* expression. Our results show a gradual transient increase or decrease of *thsA* in up- and down-shifted cells, respectively, the maximal variation being reached within 1 h in both temperature shifts (Fig. 4c, d). These results are in agreement with data previously reported studying the transcriptional analysis of *thsA* in *S. shibatae* after a heat- or cold-shock (Kagawa et al. 2003). Interestingly, the gradual increase or decrease of *thsA* already observed within the first 30 min after the up and down shifts, respectively (Fig. 4c, d), occurred during the 30 min required for the re-equilibration of the culture medium at the new temperature. This observation illustrates the ability of the cells to detect very early (i.e. within 10 min) the temperature change even prior to stabilization of the temperature. Monitoring *thsA* expression during our temperature up and down shift experiments is therefore a reliable indicator in controlling the temperature change.

Whatever the temperature shift experiment, the time-dependent expression of *topR2* remains quite constant throughout the kinetics (Fig. 5b, d), while a significant decrease of mRNA level is observed only at 24 h after the up shift (Fig. 5b). By contrast, when cells are shifted from 80 to 72°C, a 1.8-fold increase in the *topR1* transcript level is observed as early as 5 min later, and it continues to rise until 30 min by reaching a 2.4-fold increase, before gradually decreasing and returning at 24 h to the reference *topR1* mRNA level present before the down shift (Fig. 5c). When 72°C-adapted cells are transferred to 80°C, there is an inverse response for the *topR1* transcript level leading to a transient decrease (Fig. 5a). Indeed, the minimum level of detected *topR1* transcript is about 0.66, and is thus reduced by a factor of 1.5 within 15 min after the up shift (Fig. 5a), while the maxima are increased by a factor of 2.19 and 2.37 within 15 and 30 min, respectively, after the down shift (Fig. 5c). Between 1 and 4 h after the up shift, cells recover a higher *topR1* transcript level, around 80% of the T0 level, before decreasing again and reaching 15% of the T0 level within 24 h (Fig. 5a). Down and up shifting

cells produce a significant variation in the temporal expression of *topR1*. The *topR1* gene regulation occurring within short times during temperature shifts while the new temperature is still not re-equilibrated, might be an evidence for an important role of TopR1 in the response to environmental change.

Discussion

In the present work, we studied the regulation of both reverse gyrase encoding genes expression at the transcriptional level in relation to the temperature conditions and growth phases of *S. solfataricus*. Our results obtained by northern blots indicate that the two reverse gyrase genes of *S. solfataricus, topR1* and *topR2,* are both transcribed and that their corresponding transcripts are produced at a very low level as recently reported in a transcriptomic analysis (Lundgren and Bernander 2007). A very low abundance of transcripts encoding a type I-topoisomerase, albeit different from reverse gyrase, is not unique to Archaea and was also reported in Eucarya (Spellman et al. 1998; Tosh et al. 1999) and Bacteria (Richmond et al. 1999). In spite of the low abundance of reverse gyrase transcripts in *S. solfataricus*, we were able to detect significant quantitative variations with time in batch culture. We first studied the variations of *topR1* and *topR2* transcript levels depending on the growth phase of *S. solfataricus* when cultivated at 72°C and at 80°C. The corresponding quantifications of reverse gyrase mRNA indicate that these transcripts are present at either a very low or nearly undetectable level in resting cells or cells with a very low division rate. On the other hand, when the cells rapidly divide, the reverse gyrase-encoded mRNA reach their highest level (Fig. 3a, b, d, e). A global analysis of mRNA stability recently performed in *S. acidocaldarius* indicated an average mRNA half-life about 5 min (Andersson et al. 2006). When *S. acidocaldarius* genes display half-lives lower than the median value (5 min), the authors concluded that the transcriptional regulation of these genes is modulated by the frequency of transcription initiation. In view of these data, we can consider the same kind of transcriptional regulation for *topR1* and *topR2,* because they display a half-life of 3.77 and 4.16 min, respectively (Andersson et al. 2006). Therefore, the highest reverse gyrase mRNA level observed during the exponential phase of *S. solfataricus* at 72 and 80°C could result from an increased transcription initiation rate. Our results clearly establish an intimate link between the transcript abundance and the active cell division rate, whatever the doubling time. This observation is supported by the work of Atomi and Coll (Atomi et al. 2004) showing that the disruption of the unique gene encoding reverse gyrase in *T. kodakaraensis* KOD1 leads to a strong decrease in the cell division rate at temperatures above 70°C. It is thus tempting to attribute to reverse gyrase an essential role in controlling the cell division rate. A close association between high level of topoisomerase expression and active cell division is not unique to reverse gyrase of *S. solfataricus*, and has been reported not only for another type-IA topoisomerase (Spellman et al. 1998; Richmond et al. 1999; Tosh et al. 1999; Park et al. 2001) but also for a type-IB topoisomerase (Lee et al. 2001), and for type II-DNA topoisomerase (Wang 2002). When cells divide actively, their replication and transcription activities are highly enhanced leading to a constantly modified DNA topology with a higher dynamics in positive and negative supercoiling. Such a change in DNA topology in relation to the cell division ability has been reported on various plasmids isolated from hyperthermophilic archaea (Lopez-Garcia and Forterre 1997). The authors concluded from their study that the topological variations of plasmid DNA isolated from cells at different growth phases were species specific, both in terms of plasmid and of host cell. It is thus difficult to transpose a kind of response for a specific plasmid to the global scale of an entire chromosome. Given our results, and in agreement with the literature, we can claim that the regulation of reverse gyrase at the transcriptional level represents one of the fastest responses to changes of the physiological state of the cells. A higher transcript level might be necessarily associated with a need for a functional reverse gyrase, either quantitatively, or for renewing the pool of active proteins in order to counterbalance the various topological constraints caused by an active cell division rate.

When *S. solfataricus* cells go through stationary and decline phases, their *topR1* and *topR2* mRNA level decreases progressively, and becomes almost undetectable in resting and dying cells (Fig. 3a, b, d, e). In these physiological conditions, the reduced cell activity might involve only a relatively limited number of genes, thereby decreasing the global variations of DNA topology. However, progressive increase of Lk has been observed in a specific plasmid isolated from *Sulfolobus* sp. NZ59/2 in stationary phase (Lopez-Garcia and Forterre 1997). In any case, these two observations reinforce the idea that a smaller quantity of reverse gyrase is required in cells that are in stationary phase. Because we do not detect any significant *topR* transcript in resting cells, the pool of translatable mRNA is necessarily strongly reduced, with no renewing of the reverse gyrase pool. The amount of reverse gyrase resulting from this cell activity in that physiological environment must be stable enough and sufficient to assume its role in controlling the DNA topological state.

While the transcriptional response at 80°C is rather similar to that encountered at 72°C for cells in exponential,

early-stationary and decline phases, cells in mid- and late-stationary phase exibit a quite different, albeit transient, regulation on the *topR1* and *topR2* transcript level. Interestingly, we observed a transient increase of *topR1* transcripts in S2 (Fig. 3d) while the same phenomenon occurred for *topR2* transcripts but later in S3 (Fig. 3e). This time shift of the transient increase of the two *topR* transcripts could indicate that the function at first performed by TopR1 could be later assumed by TopR2 during the 80°C transition from mid- to late-stationary phase. This result illustrates a differential regulation of the two TopR encoding genes depending on cell physiological states (here, stationary phase combined with 80°C). In *S. acidocaldarius, a* differential regulation between *topR1* and *topR2* genes has also been observed, but only during the cell cycle. Indeed, *topR2* mRNA sharply increased at the G1/S transition while *topR1* mRNA remained constant during the whole cell cycle (Lundgren and Bernander 2007).

When we submitted *S. solfataricus* cells to up- and down-shifts between 72 and 80°C, the *topR1* and *topR2* genes displayed a significantly different regulation (Fig. 5). Whatever the temperature shift, the steady-state level of *topR2* transcripts remains quite constant throughout the kinetic experiment (Fig. 5b, d). Without any significant variation of the *topR2* mRNA level in response to temperature change, the regulation of *topR2* expression in *S. solfataricus* seems insensitive to the environmental temperature. However, we cannot exclude the possibility that, in order to maintain a constant *topR2* mRNA level under both growth temperatures, cells may tightly control either the efficiency of the promoter and/or the balance between the transcript production and degradation. By contrast, the *topR1* transcriptional analysis reveals a much more rapid adjustment of the corresponding transcript level, as only 5–10 min following the temperature shift (up or down) are required to produce quantitative modifications (Fig. 5a, c). Up-shifted *S. solfataricus* cells exhibit a transient decrease of *topR1* mRNA (Fig. 5a) while, in response to a down-shift, cells modulate their *topR1* transcript level by a transient 2.4-fold increase within 30 min (Fig. 5c). A relatively weak amplitude of the transcript rate variations is not restricted to the reverse gyrase encoding genes of *S. solfataricus,* but can be found in a number of topoisomerases encoding genes (Mudgil et al. 2002). It is interesting to notice that the minimum and maximum *topR1* transcript levels are both reached at the same time, 15–30 min after either up or down shift experiments, respectively. This reflects a reversible and complementary regulatory response depending on the temperature shift. However, while the growth medium takes 30 min to attain the target temperature, 5–10 min are sufficient to modulate significantly the *topR1* transcript level. The simplest hypothesis to explain these rapid variations is once again based on the modulation of transcription initiation frequency to allow a response as early as 5–10 min after a temperature change. This hypothesis is all the more plausible as it is in accordance with the link between the DNA topological state and the transcription ability, which depends on the temperature conditions. At low temperature, negatively supercoiled templates were shown to be more highly transcribed in an in vitro system (Bell et al. 1998). In addition, Lopez-Garcia and Forterre showed that following a heat shift, a sharp but transient increase of Lk was observed, while in response to a cold shift, Lk decreased significantly but once again transiently (Lopez-Garcia and Forterre 1997). In both cases the subsequent cell response adjusts the Lk in order to recover a DNA topology adapted to the final temperature, a linking number that is either above or below the initial Lk, corresponding to the initial lower and upper temperature, respectively. Given the transcriptional response of *topR1* to temperature shifts in our experiments, its promoter seems to be highly sensitive to the culture temperature and therefore highly reactive to the DNA superhelical density. It is tempting to associate this modulation of *topR1* transcript amount depending on temperature conditions to a concomitant variation at the protein level that restores an appropriate DNA topological state. The transient decrease of Lk upon cold shift leads to a highly negative DNA supercoil, and therefore might require more reverse gyrase to counterbalance this primary mechanistic response. This reverse gyrase requirement could essentially be triggered by an increase of corresponding transcripts. Conversely, the transient increase of Lk upon heat shift leads to highly positive DNA supercoiling, and therefore might require less reverse gyrase, which probably results from a partial inhibition of *topR1* transcription. A previous study performed with another strain, *S. islandicus*, showed that whatever the temperature shift, there was no significant change of reverse gyrase at the protein level (Lopez-Garcia and Forterre 1999). However, the absence of significant variation of the amount of reverse gyrase in *S. islandicus* was essentially estimated at the level of the corresponding proteolytic products (Lopez-Garcia and Forterre 1999). The reliability of a fine detection, even though delicate, is all the more important when the reverse gyrase proteolysis is considered as one of the regulation process of the global topoisomerase activity of the cell in particular conditions (Nadal et al. 1994; Declais et al. 2000; Valenti et al. 2006). An increase of reverse gyrase proteins could be expected from an increase of corresponding transcripts in our studies, and similarly a decrease of reverse gyrase proteins from a down regulated transcript. Our results at the transcriptional level are not inconsistent with an absence of variations at the protein level, and can even be complementary. Indeed, one can assume that, in our experiments,

the global reverse gyrase amount does not vary with temperature change as previously reported in *S. islandicus* (Lopez-Garcia and Forterre 1999), but on the other hand, the quantitative balance between TopR1 and TopR2 can be subjected to variations. This hypothesis can be elucidated only by using highly specific antibodies raised against *S. solfataricus* TopR1 and TopR2. When we compare the time course of events in the study of Lopez-Garcia and Forterre (1997) with that in our work, there are some strikingly overlapping observations: (1) the increase of *topR1* transcripts within 30 min following a cold shift (present study) coincides exactly with the time required for the maximal fall of Lk and for the beginning of DNA positive supercoiling recovery (Lopez-Garcia and Forterre 1997). (2) 23 h are necessary to restore a stable linking number corresponding to an appropriate positively supercoiled DNA at the final temperature (Lopez-Garcia and Forterre 1997); similarly, 24 h after a temperature change the *topR1* transcript level reaches the value normally associated with the target temperature (present study). Whatever the direction of the temperature shift, the first 30 min leading to the maximal topological change, correspond to the mechanic response of DNA (Duguet 1993). In the meantime, it also corresponds to the lapse of time necessary to modulate the transcript level in order to produce the adequate level of active proteins required during the subsequent steps beyond the first 30 min. The subsequent DNA supercoiling state progressively reached and adapted to the final temperature upon the following 24 h, is the result of the activity of the functional reverse gyrase pool present during that period.

DNA topoisomerases are involved in all the DNA metabolism pathways and, likely, TopR1 and TopR2 enzymes do not participate to the same extent in all of these processes, and thus might have different functions in the cell in relation to its physiological state and environmental parameters. As evidenced for topoisomerases of *E. coli* (Menzel and Gellert 1983; Tse-Dinh et al. 1997; Zechiedrich et al. 2000), the *S. solfataricus topR1* transcriptional analysis might reflect a regulation triggered by a homeostatic control of the superhelical density of the genome of *S. solfataricus* in response to the temperature conditions. In addition, a quite recent exhaustive genomic transcriptional study performed on *E. coli* leads the authors to propose that supercoiling might act as a second messenger that transmits information about the environmental change to many regulatory networks (Peter et al. 2004). It seems that TopR2 would be rather involved in cell cycle (Lundgren and Bernander 2007) while control of cell response to temperature conditions would be essentially triggered by TopR1 (present study). However, specific functions attributed to the two distinct reverse gyrases present in *S. solfataricus* remain to be more precisely identified and it would be very interesting to extend such a study to the reverse gyrases present in other hyperthermophilic organisms.

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