The RheA repressor is the thermosensor of the HSP18 heat shock response in *Streptomyces albus*

Pascale Servant, Cosette Grandvalet, and Philippe Mazodier*

Unité de Biochimie Microbienne, Institut Pasteur, 25, rue du Docteur Roux, 75724 Paris Cedex 15, France

Edited by Carol A. Gross, University of California, San Francisco, CA, and approved February 4, 2000 (received for review October 4, 1999)

Microorganisms have mechanisms to sense their environment and rapidly adapt to survive changes in conditions. In *Streptomyces albus*, various transcriptional repressors mediate the induction of heat shock genes. The RheA repressor regulates the synthesis of HSP18, a small heat shock protein, which plays a role in thermotolerance. The RheA protein was purified to determine how it responds rapidly to temperature. Gel retardation assays and footprinting experiments identified the specific target of RheA as an inverted repeat (TGTCATC 5N GATGACA) located in P*hsp18*, P*rheA* which is the common promoter region of the divergon. Gel retardation assays detected RheA-complexes formed with the *hsp18-rheA* promoters. The complexes did not form at higher temperature. *In vitro* transcription experiments showed that RheA is an autoregulatory protein and that its activity is inhibited by high temperature. The temperature-induced derepression by RheA is reversible. Dichroism circular spectroscopy revealed a reversible change of RheA conformation in relation with the temperature that could represent a transition between an active and an inactive form. Our experiments demonstrate that RheA acts as a cellular thermometer in *hsp18* regulation.

Cells exposed to an increase in temperature respond by rapidly inducing the expression of heat shock proteins (HSPs). Many HSPs are molecular chaperones or proteases, which ensure maintenance of protein integrity by acting on protein folding, secretion, repair, and degradation (1, 2). Although the induction of HSPs is a universal response, there are diverse regulatory mechanisms for controlling their synthesis in different organisms.

In *Escherichia coli*, heat shock gene transcription is regulated through control of the concentration and activity of the positive regulator σ^{32} (encoded by *rpoH*) (for reviews see refs. 3 and 4). In *Bacillus subtilis*, heat-inducible genes can be classified into at least four classes according to their regulatory mechanisms (5, 6). The thermoregulation of class I genes depends on a repressor (HrcA) that interacts with an inverted repeat motif called CIRCE (7, 8). Class II heat shock genes are positively regulated by the σ^{B} factor, whose synthesis and activity is increased under stress conditions (9). Class III genes are negatively regulated by CtsR, which recognizes a directly repeated heptanucleotide operator sequence (5). Class IV comprises heat shock genes of unknown regulation. In some bacteria, heat shock regulation mediated by HrcA and σ^{32} coexist (10, 11). In eukaryotes, a preexisting transcriptional activator, HSF, activates heat shock genes by binding to conserved upstream response elements. In unstressed cells, HSF is in a monomeric non-DNA-binding form. After heat shock, HSF is converted to the trimeric DNA-binding form (12–14).

At least four mechanisms have been described in thermoregulation in bacteria: (*i*) chaperone-mediated capture of regulators, (*ii*) changes in mRNA conformation, (*iii*) DNA supercoiling, and (*iv*) changes in protein conformation (for review, see ref. 15). The two first mechanisms concern the general system of heat shock response in bacteria. The others have been described for specialized functions such as expression of virulence genes. (*i*) The mechanisms that lead to the up-regulation of the transcriptional activity of heat shock genes differ among species, but the main chaperones (GroEL or DnaK) are involved in most cases. Denaturation of cellular polypeptides is the direct signal of temperature increase. In *E. coli*, a temperature up-shift leads to a rapid increase in the level of active σ^{32} caused by enhanced synthesis and stabilization. At 30°C, the DnaK chaperone system destabilizes σ^{32} (16, 17) and sequesters it in an inactive state that can facilitate its degradation by FtsH protease (18, 19). Heat shock denatures cellular polypeptides; the DnaK system binds these misfolded polypeptides and releases σ^{32}. An analogous feedback mechanism has been found in *B. subtilis*: the activity of HrcA is modulated by the GroE chaperonin system (20). (*ii*) The melting of the *rpoH* mRNA secondary structure at high temperature leads to enhanced ribosome entry and translation initiation without the involvement of other cellular components (21). (*iii*) Supercoiling has been implicated in thermosensing of virulence gene expression (22). Temperature may directly affect H-NS, altering its capacity for DNA binding (23–25). (*iv*) The TlpA autoregulator in *Salmonella typhimurium* changes its conformation according to the temperature (26).

In *Streptomyces albus*, a Gram-positive soil bacterium, there are at least three different regulatory networks that control the synthesis of different HSPs. Heat regulation of the *groEL* genes involves the HrcA repressor (27). The HspR repressor, which binds to an inverted repeat called HAIR, regulates the *dnaK* operon and the *clpB* gene (28, 29). A third regulatory mechanism represses the *hsp18* gene, which encodes a small HSP protein that plays a role in thermotolerance (30). The *rheA* gene (formerly *orfY*), 150 bp upstream from and in the opposite orientation to *hsp18*, encodes the repressor of *hsp18* (31). The mechanisms of thermoregulation of the RheA activity are unknown. Induction of HSP after temperature up-shift is usually transient. In the case of HSP18, a high level of expression is observed at high temperature for several hours. This last observation suggests that modulation of RheA activity is different from the feedback mechanisms described for σ^{32} and HrcA. To determine how variation of temperature can modify RheA activity, we purified the protein. RheA, in the absence of any other factors, was able to sense temperature changes and to respond immediately by regulating *hsp18* expression. Change of RheA conformation according to the temperature may be the basis of the transition between the active and inactive forms.

Materials and Methods

Bacterial Strains, Plasmids, and DNA Manipulation. *E. coli* K12 strain TG1 (32) was used as the general cloning host and *E. coli* strain BL21λDE3 (33) was used for overproduction of protein in the T7-promoter expression system. *E. coli* strains were grown in Luria–Bertani (LB) broth supplemented with kanamycin

This paper was submitted directly (Track II) to the PNAS office.

Abbreviation: HSP, heat shock protein.

*To whom reprint requests should be addressed. E-mail: mazodier@pasteur.fr.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.070426197. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.070426197

(25 μg/ml) as appropriate. Conventional $CaCl_2$ (34) or electroporation procedures were used for *E. coli* transformation. pET28a (Novagen) was used for the overproduction and purification of RheA from *E. coli*. Plasmid construction was carried out as described by Sambrook *et al.* (35). Plasmid DNA was extracted and purified from *E. coli* with the Qiagen plasmid kit.

Plasmid Construction. RheA was overproduced with pOLP12B. This plasmid was constructed by inserting a 674-bp *Bsp*HI/*Xho*I DNA fragment corresponding to the *rheA* coding sequence between the *Nco*I and *Xho*I sites of pET28a. The DNA fragment corresponding to the *rheA* sequence was generated by PCR using oligonucleotides OLP1 (5′-ATTCATGACCACCGCCGACCGCCC-3′) and OLP2 (5′-TACTCGAGGGACCGCCCGGACGAGGCC-3′), thus replacing the TGA stop codon by the *Xho*I restriction site. This allows the creation of a translational fusion adding six histidine residues to the carboxyl terminus of the protein and places expression of the gene under the control of a T7 promoter.

Purification of RheA. pOLP12B was used to transform BL21λDE3, in which the T7 RNA polymerase gene is under the control of the inducible *lacUV5* promoter. The resulting strain was grown in LB medium at 30°C until the mid-exponential phase (optical density at 600 nm approximately 0.9), isopropyl β-D-thiogalactoside (IPTG; 1 mM) was then added and incubation was continued for 6 h at room temperature. The cells were centrifuged at 2,600 × *g* for 15 min and resuspended in 1/50 of the culture volume of buffer I (50 mM sodium phosphate buffer, pH 8/300 mM NaCl/20 mM imidazole). The cells were disrupted by sonication, and cell debris was removed by centrifugation at 12,000 × *g* for 20 min. The resulting crude protein extracts were loaded on a 0.8-ml Ni-NTA-agarose (Qiagen; NTA is nitrilotriacetate) column previously equilibrated with buffer I. The column was washed with 20 ml of buffer I and 30 ml of buffer II (50 mM sodium phosphate buffer, pH 6/300 mM NaCl/30 mM imidazole]. RheA protein was then eluted with an imidazole gradient (30–500 mM), and analyzed by SDS/PAGE on a 15% acrylamide gel as described previously (36). Protein concentrations were determined by using the Bio-Rad protein assay (37) and UV absorbance (ε_{280} = 17,964 $M^{-1}\cdot cm^{-1}$).

Gel Retardation Assays. A 270-bp DNA fragment, containing the promoter region of *hsp18* and *rheA,* was amplified by PCR using oligonucleotides OP1 (5′-ATCAGGATCCAGGCCGGGTCGGGGGTGAAGG-3′) and OP2 (5′-ATCAGAATTCGCGGAAGGGGTCAGTGCGCA-3′). This fragment was cut with *Eco*RI and *Bam*HI and end-labeled radioactively with [α-^{32}P]dATP by using the Klenow fragment of DNA polymerase I. The labeled fragment was further purified by using the QIAquick PCR purification kit (Qiagen). RheA was bound to DNA in a 20-μl reaction mixture containing 10^4 cpm of ^{32}P-labeled DNA, 1 μg of calf thymus DNA, 100 mM NaCl, 10 mM Tris·HCl (pH 7.5), 1 mM DTT, 10 mM $MgCl_2$, 1 mM $CaCl_2$, and 10% (vol/vol) glycerol by incubation for 10 min at 30°C or 41°C. Samples were then loaded directly onto a 6% polyacrylamide gel (50 mM Tris·HCl, pH 8.5/400 mM glycine/1.73 mM EDTA/2.5% glycerol) for electrophoresis (14 $V\cdot cm^{-1}$), for 1 h at room temperature or 37°C (gel showing the loss of RheA DNA-binding capacity at high temperature). The gels were dried and autoradiographed. For competitor chase experiments, complementary oligonucleotides used were annealed to obtain double-stranded fragments.

DNase I Footprinting. DNA fragments used for DNase I footprinting were prepared by PCR, using the *Pfu* polymerase and 20 pmol of each primer, one of which was previously labeled by T4 polynucleotide kinase and [γ-^{32}P]dATP. Oligonucleotides OP2 and OP3 (5′-ATCAGGATCCGGTGGGGCCGGGGGTGG-3′) were used as primers. Labeled PCR products were purified by using the QIAquick PCR purification kit (Qiagen). RheA binding to DNA (50,000 cpm) was tested under the same conditions as described for the gel retardation assays with the addition of BSA (0.1 μg). Reactions were carried out as previously described (5) except that the quantity of DNase I added was 6.7 ng.

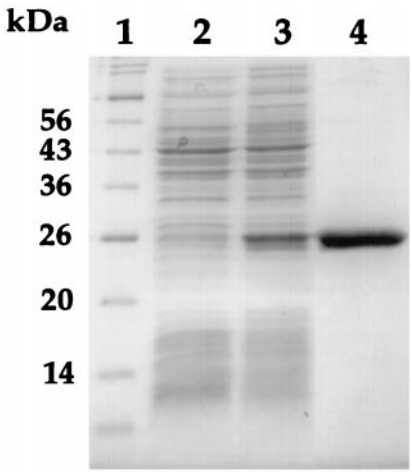

Fig. 1. Overproduction and purification of RheA. SDS/PAGE analysis of crude extracts from *E. coli* BL21λDE3 carrying pET28a (lane 2) or pOLP12B (lane 3) and purified RheA protein after Ni-NTA affinity chromatography (lane 4). Molecular mass standards (New England Biolabs) were loaded in lane 1.

***In Vitro* Run-off Transcription.** RNA polymerase, kindly supplied by A. M Duchêne (Institut Pasteur, Paris), was prepared from cultures of *Streptomyces coelicolor* A3(2) strain J1501 (38) grown 24 h at 30°C in YEME medium according to Holmes *et al.* (39) except omitting the final Superose 6 column. *In vitro* run-off transcription (39, 40) was performed with ≈0.2 pmol of DNA template and 5 μCi (1 μCi = 37 kBq) of [α-^{32}P]CTP. The 370-bp *Sal*I/*Mlu*I fragment of pPM1745 (41) containing the promoters P*hsp18* and P*rheA* was used as the template. The reaction products were then separated on a 5% polyacrylamide/8 M urea gel and visualized by autoradiography.

Circular Dichroism (CD) Spectroscopy. The CD spectra were obtained with a Jobin–Yvon CD6 dichrograph equipped with a thermostated cell holder. Each spectrum was the result of averaging at least four successive spectra. CD spectra of RheA (5 μM) were realized in 50 mM sodium phosphate buffer (pH 8) containing 100 mM NaCl. At each step we allowed a 10 min temperature equilibration time before recording the data.

Results

Overproduction and Purification of RheA. Overproduced RheA was the major protein band in SDS/PAGE of an extract of *E. coli* BL21λDE3 bearing pOLP12B (Fig. 1). After a one-step purification protocol that consisted of affinity chromatography of the His-tagged protein on a Ni-NTA-agarose column, electrophoretically pure (greater than 95%) RheA was obtained. Purified RheA migrated on SDS/PAGE as a band with an apparent molecular mass of 25 kDa, in agreement with the molecular mass of 23,953 Da calculated from the deduced amino acid sequence of the His-tagged protein. Purified RheA protein was soluble as shown by centrifugation. This allowed utilization of the protein in biochemical assays. Purified RheA was analyzed by gel filtration on a Superose 12 column. Two symmetrical peaks corresponding to monomeric and dimeric

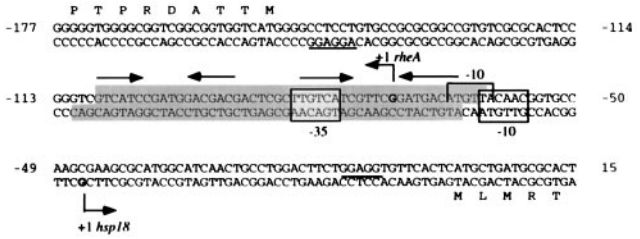

Fig. 2. Sequence of the *rheA* and *hsp18* promoters region. Positions are numbered relative to the translation initiation codon of HSP18. The transcription start sites are in boldface letters. The ribosome binding sites are underlined. The DNase-I-protected area is shaded, and the RheA inverted repeat recognition sequences are indicated by an arrow.

forms were detected at 22°C or 40°C (data not shown). The monomer–dimer equilibrium was not altered by the temperature of the chromatography.

Purified RheA Interacts with a Specific Inverted Repeat Motif. P*rheA* and P*hsp18* promoters are located in a divergent orientation within the noncoding intergenic region between the *rheA* and *hsp18* genes (Fig. 2). Purified RheA was used in gel mobility-shift DNA-binding assays with a 270-bp DNA fragment carrying the promoter regions of *rheA* and *hsp18*. RheA altered the mobility of this fragment in a concentration-dependent manner (Fig. 3*A*), indicating that RheA binding to the promoter region was specific. The location and sequence of the RheA binding site was determined by DNase I footprinting assays (Fig. 4). RheA protected a broad region on both strands. The protected area encompasses the −10 box of the RheA promoter, suggesting that RheA synthesis is negatively autoregulated. There are two inverted repeats within this region: a perfect inverted repeat (TGTCATCNNNNNGATGACA) overlaps the −35 sequence of *hsp18* and the −10 sequence of *rheA* (Fig. 2); and an imperfect inverted repeat (GTCATCNNNNNGACGAC). To confirm that those inverted repeats are involved in the *hsp18* regulation, unlabeled competitor chase experiments were performed by adding a series of amounts of different oligonucleotides containing the intact or mutated motif (Fig. 3). Oligonucleotides (OP45 and OP67) containing the perfect inverted repeat efficiently competed for the DNA-RheA complex (Fig. 3 *B* and *C*), whereas those carrying two mutations (OP101 and OP123) in this sequence had no effect on RheA binding to the labeled probe (Fig. 3*C*).

DNA Binding of RheA Is Temperature Dependent. We investigated the mechanism of thermoregulation of *hsp18* expression. It could involve a phosphorylation pathway leading to changes in gene expression. Although RheA contains a putative phosphorylation site (**RQAAERRY**) for a tyrosine kinase, no evidence for *in vivo* phosphorylation of RheA was obtained by incubating the *S. albus* wild type or *S. albus* pPS250, a strain overexpressing RheA (30), with [^{32}P]phosphate at various temperatures (results not shown). To test whether RheA was able to sense temperature without involvement of other cellular components, DNA mobility-shift assays were carried out with preincubation at various temperatures (Fig. 5*A*). The gel was at 37°C, an intermediate temperature, during electrophoresis. When the DNA/RheA sample was incubated at 30°C, the DNA fragment was shifted (Fig. 5*A*, lanes 2–4). This shift was almost completely lost when the preincubation was at 41°C (Fig. 5*A*, lanes 5–7). Thus RheA does not bind to the DNA at high temperature. The effect of the incubation at 41°C was reversible. Cooling the sample to 30°C for 1 min was associated with recovery of RheA binding (Fig. 5*A*, lanes 8 and 9). The temperature of the gel (37°C) affected the pattern of the

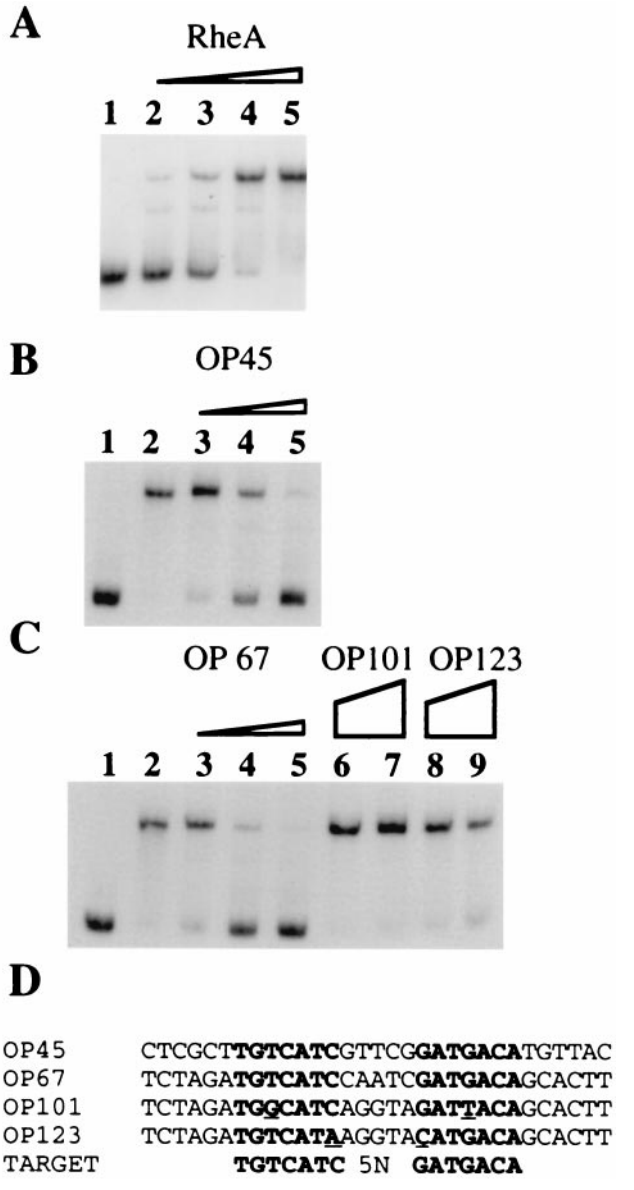

Fig. 3. *In vitro* analysis of RheA binding to a *S. albus* DNA fragment containing the *hsp18* and *rheA* promoters. Gel retardation assays were performed using a 270-bp radiolabeled DNA fragment corresponding to the *rheA-hsp18* promoters. Gels were run at room temperature. (*A*) ≈0.05 pmol of labeled DNA fragment was incubated with various amounts of purified RheA: lane 1, no protein; lane 2, 21.6 fmol; lane 3, 43.2 fmol; lane 4, 86.5 fmol; lane 5, 130 fmol. (*B*) Each lane contains ≈0.05 pmol of the labeled 270-bp fragment, 130 fmol of purified RheA (except lane 1), and one of a series of amounts of an unlabeled oligonucleotide competitor. Lane 1, no protein; lane 2, no competitor; lane 3, 0.06 pmol of OP45; lane 4, 0.12 pmol of OP45; lane 5, 0.24 pmol of OP45. (*C*) Each lane contains ≈0.05 pmol of the labeled 270-bp fragment, 130 fmol of purified RheA (except lane 1) and one of a series of amounts of an unlabeled oligonucleotide competitor. Lane 1, no protein; lane 2, no competitor; lane 3, 0.06 pmol of OP67; lane 4, 0.12 pmol of OP67; lane 5, 0.24 pmol of OP67; lane 6, 0.5 pmol of OP101; lane 7, 1.5 pmol of OP101; lane 8, 0.5 pmol of OP123; lane 9, 1.5 pmol of OP123. (*D*) Sequences of oligonucleotides used in the unlabeled competitor chase experiments.

migration of the DNA/RheA sample preincubated at 30°C (Fig. 5*A*, lanes 2–4). Indeed, when the gel was run at room temperature, 130 fmol of RheA totally shifted ≈0.05 pmol of DNA fragment (Fig. 3*A*). With the same quantities of DNA, 170 fmol of RheA was not sufficient to completely shift the DNA when the gel was run at 37°C. This result implies that part of the

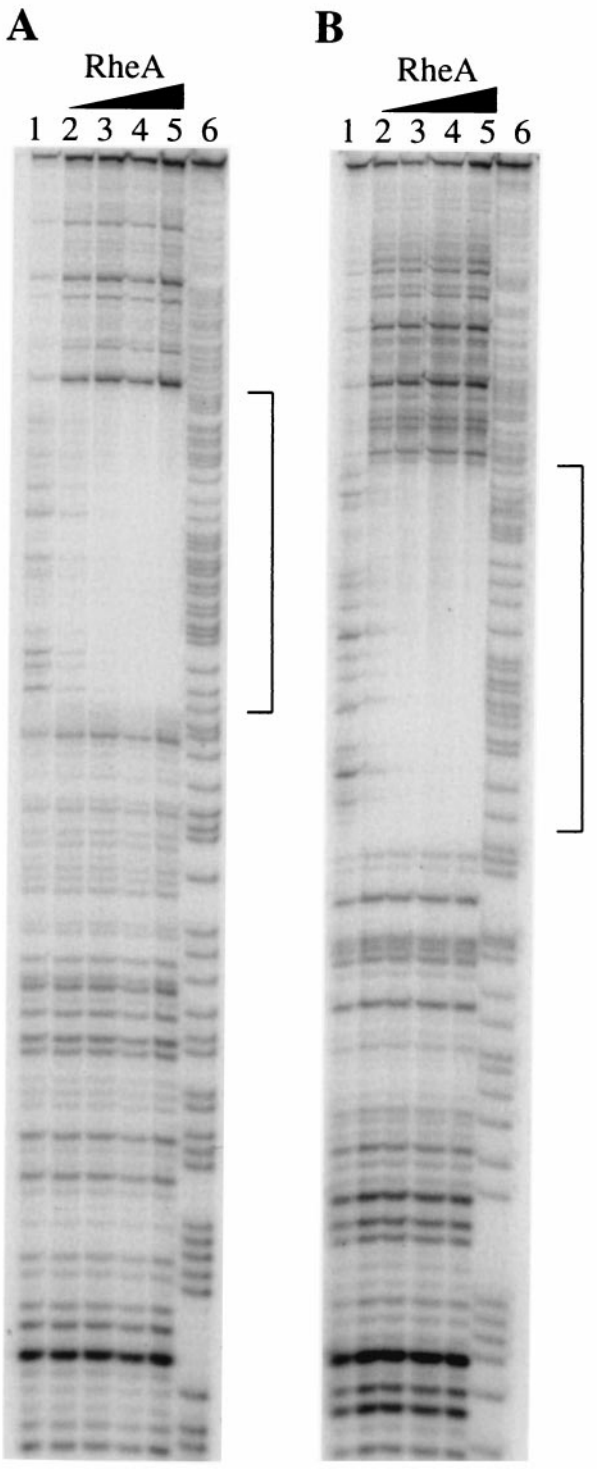

Fig. 4. DNase I footprinting analysis of RheA binding to the *hsp18* and *rheA* promoter regions. Lanes contain ≈0.12 pmol of labeled template strand (*A*) and nontemplate strand (*B*) of *hsp18*. Fragments were incubated with one of a series of amounts of purified RheA: lanes 1, no protein; lanes 2, 0.22 pmol; lanes 3, 0.44 pmol; lanes 4, 0.87 pmol; lanes 5, 2.6 pmol. A+G Maxam and Gilbert reaction products with the appropriate DNA fragments were loaded in lanes 6. Regions protected by RheA are indicated by brackets.

DNA-binding activity was lost during loading on the gel at 37°C. In conclusion, the kinetics for the loss of DNA-binding activity is extremely rapid (less than 1 min). The same kinetics was

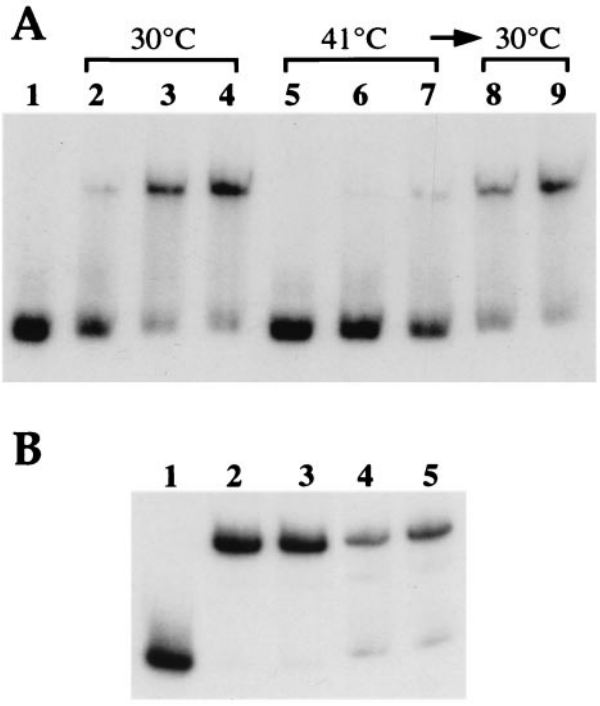

Fig. 5. Gel mobility-shift assays showing the loss of RheA DNA-binding capacity at high temperature and its recovery at low temperature. Gel retardation assays were performed with ≈0.05 pmol of the radiolabeled 270-bp DNA fragment corresponding to the *rheA* and *hsp18* promoters. (*A*) DNA-binding reactions were carried out at 30°C (lanes 2–4) or 41°C (lanes 5–7) or after a temperature shift-back from 41°C to 30°C (lanes 8 and 9). Quantities of RheA: lane 1, no protein; lanes 2 and 5, 43.2 fmol; lanes 3, 6, and 8, 130 fmol; lanes 4, 7, and 9, 170 fmol. Electrophoresis was at 37°C. (*B*) RheA was incubated 2 h at 30°C (lanes 2 and 4) or at 41°C (lanes 3 and 5) before a 10-min binding reaction at 30°C. Electrophoresis was at room temperature. Quantities of RheA: lane 1, no protein; lanes 2 and 3, 260 fmol; lanes 4 and 5, 86.5 fmol.

observed for recovery of DNA-binding activity when the temperature was increased (data not shown). RheA is not thermodenatured: it can be incubated at 41°C for at least 2 h and still recover fully its binding activity at 30°C (Fig. 5*B*).

Transcription Assays Show the Repression Action of RheA. To confirm that RheA represses *hsp18* gene expression and that it is an autoregulatory protein, *in vitro* transcription was performed at various temperatures (Fig. 6). A DNA fragment containing the *S. albus rheA* and *hsp18* promoters was used. Transcripts of 201 and 147 nt were observed for P*rheA* and P*hsp18,* respectively, in good agreement with the expected sizes of the run-off transcripts. In the presence of purified RheA, no mRNA synthesis was observed at 30°C. This result clearly demonstrates that RheA alone is sufficient to repress transcription at 30°C both from the *hsp18* promoter and from its own promoter. At 41°C, RheA, even at high concentrations, did not repress transcription. To show the reversibility of the reaction, RheA was heated at 41°C for 15 min and then cooled to 30°C. The results obtained showed that RheA fully recovered its activity as a repressor after temperature shift. No other factor was required, indicating that RheA is the cellular thermosensor for the *hsp18* gene expression.

RheA Conformational Change Induced by Temperature. The heat effect on RheA activity could be explained by a thermal transition between the active conformation, below 30°C, and an inactive state above 40°C. To test this possibility, the temperature effect on the overall conformation was explored by using

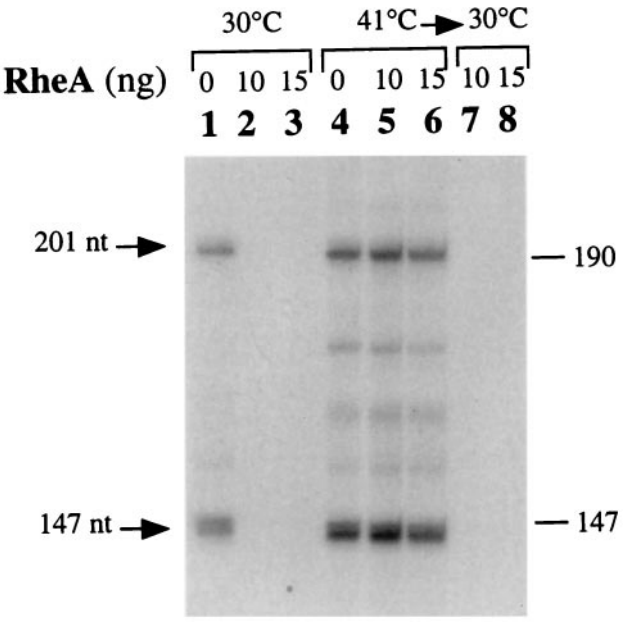

Fig. 6. *In vitro* transcriptional repression by RheA. Purified RheA was assayed for its ability to repress run-off transcription of the *hsp18* and *rheA* genes at various temperatures in the presence of the *S. coelicolor* RNA polymerase. The molecular weight markers (in nucleotides) correspond to *Hpa*II-digested pUC19. Transcripts of the expected size, 147 and 201 nt, were observed for P*hsp18* and P*rheA,* respectively.

CD spectroscopy. CD spectroscopy was performed on RheA between 195 and 260 nm at 30°C and after heating the sample to 41°C. The temperature increase led to a complete change of the profile (Fig. 7); this change was not caused by a trivial heat denaturation; indeed, after cooling the cell to 30°C, the spectrum obtained was similar to the initial spectrum recorded at 30°C. This result confirms the data obtained in the gel retardation and *in vitro* transcription assays, indicating stability of the protein in this range of temperature. This change of RheA conformation in relation to the temperature could represent a transition between an active and an inactive form.

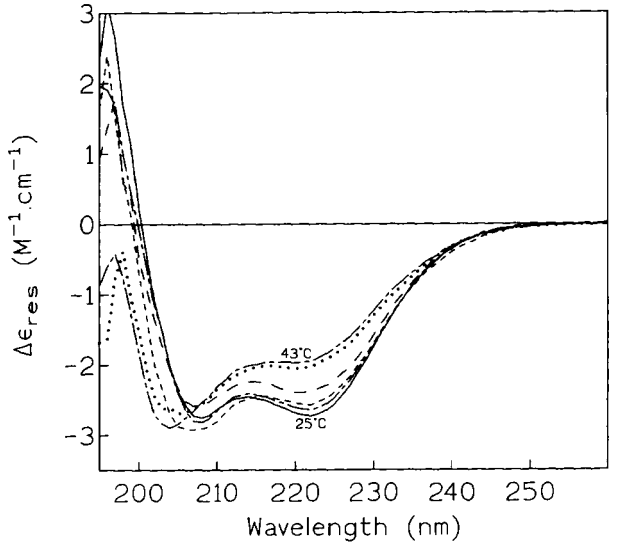

Fig. 7. Conformational change analyzed by CD spectroscopy. CD spectra of the purified RheA (5 μM). The spectra were recorded in the same cell at 25°C (—), 30°C (- —), 35°C (– –), 41°C (· · ·), and 43°C (- —) and after a temperature shift-back to 30°C (- - -).

Discussion

Temperature regulation of heat shock genes is critical for the survival of organisms in their environment. The involvement of HSPs in thermotolerance is well established. However the mechanism for the perception of the change in temperature that stimulates the expression of heat shock genes is still debated. In bacterial heat shock responses, repressor mechanisms appear more common than positive regulation, like that involving the sigma factor, σ^{32}. In *S. albus*, at least three negative regulators (HrcA, HspR, and RheA) control the synthesis of different HSPs (27, 28, 31). Two distinct regulatory patterns have been described for the major thermoinducible proteins in *S. albus*: constitutive synthesis of GroEL and HSP18 at high temperature and transient heat shock-induced synthesis of ClpB and DnaK (42). Our data are consistent with the following model of heat shock regulation. At low temperature, 30°C or below, RheA binds the target in the promoter region, thus preventing the expression of HSP18. When *S. albus* undergoes a heat shock, RheA is immediately inactivated. Our results indicate that elements residing in the primary amino acid sequence of RheA are sufficient both for maintenance of this repressor in the binding form at low temperature and for its heat-induced conversion to the non-DNA-binding form. RheA acts as a cellular thermometer which senses rises of temperature and derepresses the *hsp18* gene to synthesize HSP18, which allows the cell to survive at high temperature. Previously we have shown that HSP18 contributes to thermotolerance (41). The fact that RheA can directly sense the temperature enables the cell to respond rapidly. The constitutive synthesis of HSP18 (and RheA) for prolonged periods at high temperature is consistent with the idea that RheA is intrinsically temperature sensitive.

The mechanisms for the transduction of temperature effects into the signal pathway of the heat shock regulon remain to be determined for other repressors such as HrcA and HspR. In *B. subtilis*, the GroE chaperonin modulates the activity of the HrcA repressor (20). The interaction of GroEL with HrcA in this bacterium controls the availability of the active form of HrcA. Possibly a similar mechanism controls HrcA activity in *S. albus.* However this hypothesis apparently conflicts with our observation that GroEL is constitutively produced at high temperature in this species (41). Indeed, one of the predictions of the model proposing GroEL as the modulator of the CIRCE/HrcA regulon is that it explains the transient nature of *groEL* thermoinduction in *B. subtilis*. The HspR repressor does not appear to be temperature sensitive, and gel retardation assays show that HspR binds the HAIR (HspR-associated inverted repeat) motif at 30°C and 41°C (43), suggesting that *in vivo* another regulator modulates HspR activity. This regulator may be the DnaK chaperone system, which may activate HspR by facilitating its multimerization or its binding to the HAIR sequence. Previous data (our unpublished results and C. Smith, personal communication) indicate a direct interaction between DnaK/GrpE and HspR.

In this paper, we demonstrate that RheA acts as a cellular thermometer and that a thermoinduced change in the repressor leads either to an active or to an inactive form. To our knowledge only one bacterial transcriptional regulator capable of responding directly to high temperature has been previously described: TlpA, an autoregulatory repressor protein in *Salmonella typhimurium* (26). The role of TlpA in *Salmonella* is not well established, but it seems that it is a regulator of genes involved in virulence. A conformational change after a physiological temperature shift (from 25°C to 40°C) was reported by Hurme *et al.* (26) in their analysis of TlpA by CD. CD spectra acquired at 30°C and 41°C with RheA exhibit important differences that reflect a major structural change in the conformation of RheA. These structural changes

could explain the change in DNA-binding ability. TlpA contains an extended coiled-coil domain allowing the protein to switch between unfolded (monomer) and folded (coiled-coil oligomer) states. The probability that RheA could adopt a coiled-coil conformation was calculated with the COILS program (44). When a window of 14 and the MTIDK unweighted matrix were used, three regions (32–45, 65–78, 144–157) each with a significant probability for forming a coiled-coil structure were detected with the α-helical coiled-coil motifs as heptad amino acid sequences (a-b-c-d-e-f-g) that are repeated twice. Compared with the extensive coiled-coil domain of TlpA, RheA contains only short coiled-coil domains. The CD spectra permit estimating to 30% the percentage of α-helix in RheA. In addition, RheA contains a leucine zipper-like dimerization motif (at coordinates 191–212). RheA can form a dimer as indicated by the chromatography experiment. Thermoinduced dissociation of the dimer into monomers was not evidenced by gel chromatography at 22°C and 40°C.

The sequence specificity of RheA binding was clearly shown by DNase footprinting and gel mobility-shift assays. Binding was directed to an inverted repeat (TGTCATCNNNNNGATGACA) and was blocked only by competition with a fragment containing the same sequence. No "helix–turn–helix" DNA-binding type motif was found on RheA. A structure/function analysis of RheA should allow determination of the regions involved in "thermosensing" and DNA binding. In conclusion, thermoregulation of the activity of regulators through conformational change provides a simple and rapid mechanism that facilitates their survival under stress conditions.

We thank G. Rapoport, J. M. Betton, A. Chaffotte, J. Viala, I. Derré, H. Leh, and T. Msadek for their advice and constant interest throughout the work and A. Edelman for correcting this manuscript. We are indebted to Monique Monnot for her help with the CD experiments. This research was supported by the Pasteur Institute, the Centre National de la Recherche Scientifique, and Université Paris 7.

1. Hendrick, J. P. & Hartl, F.-U. (1993) *Annu. Rev. Biochem.* **62,** 349–384.
2. Georgopoulos, C. & Welch, W. J. (1993) *Annu. Rev. Mol. Biol.* **9,** 601–634.
3. Bukau, B. (1993) *Mol. Microbiol.* **9,** 671–680.
4. Yura, T., Nagai, H. & Mori, H. (1993) *Annu. Rev. Microbiol.* **47,** 321–350.
5. Derré, I., Rapoport, G. & Msadek, T. (1999) *Mol. Microbiol.* **31,** 117–131.
6. Hecker, M., Schumann, W. & Völker, U. (1996) *Mol. Microbiol.* **19,** 417–428.
7. Schulz, A. & Schumann, W. (1996) *J. Bacteriol.* **178,** 1088–1093.
8. Yuan, G. & Wong, S.-L. (1995) *J. Bacteriol.* **177,** 6462–6468.
9. Hecker, M. & Völker, U. (1998) *Mol. Microbiol.* **29,** 1129–1136.
10. Babst, M., Hennecke, H. & Fischer, H. M. (1996) *Mol. Microbiol.* **19,** 827–839.
11. Reisenauer, A., Mohr, C. D. & Shapiro, L. (1996) *J. Bacteriol.* **178,** 1919–1927.
12. Peteranderl, R. & Nelson, H. C. (1992) *Biochemistry* **31,** 12272–12276.
13. Rabindran, S. K., Haroun, R. I., Clos, J., Wisniewski, J. & Wu, C. (1993) *Science* **259,** 230–234.
14. Zhong, M., Orosz, A. & Wu, C. (1998) *Mol. Cell* **2,** 101–108.
15. Hurme, R. & Rhen, M. (1998) *Mol. Microbiol.* **30,** 1–6.
16. Liberek, K. & Georgopoulos, C. (1993) *Proc. Natl. Acad. Sci. USA.* **90,** 11019–11023.
17. Gamer, J., Multhaup, G., Tomoyasu, T., McCarty, J. S., Rudiger, S., Schonfeld, H. J., Schirra, C., Bujard, H. & Bukau, B. (1996) *EMBO J.* **15,** 607–617.
18. Tomoyasu, T., Gamer, J., Bukau, B., Kanemori, M., Mori, H., Rutman, A. J., Oppenheim, A. B., Yura, T., Yamanaka, K., Niki, H., *et al*. (1995) *EMBO J.* **14,** 2551–2560.
19. Herman, C., Thevenet, D., D'Ari, R. & Bouloc, P. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 3516–3520.
20. Mogk, A., Homuth, G., Scholz, C., Kim, L., Schmid, F. X. & Schumann, W. (1997) *EMBO J.* **16,** 4579–4590.
21. Morita, M. T., Tanaka, Y., Kodama, T. S., Kyogoku, Y., Yanagi, H. & Yura, T. (1999) *Genes Dev.* **13,** 655–665.
22. Dorman, C. J. (1991) *Infect. Immun.* **59,** 745–749.
23. Göransson, M., Sonden, B., Nilsson, P., Dagberg, B., Forsman, K., Emanuelsson, K. & Uhlin, B. E. (1990) *Nature (London)* **344,** 682–685.
24. Tobe, T., Yoshikawa, M., Mizuno, T. & Sasakawa, C. (1993) *J. Bacteriol.* **175,** 6142–6149.
25. White-Ziegler, C. A., Angus Hill, M. L., Braaten, B. A., van der Woude, M. W. & Low, D. A. (1998) *Mol. Microbiol.* **28,** 1121–1137.
26. Hurme, R., Berndt, K. D., Normark, S. J. & Rhen, M. (1997) *Cell* **90,** 55–64.
27. Grandvalet, C., Rapoport, G. & Mazodier, P. (1998) *J. Bacteriol.* **180,** 5129–5134.
28. Grandvalet, C., Servant, P. & Mazodier, P. (1997) *Mol. Microbiol.* **23,** 77–84.
29. Bucca, G., Hindle, Z. & Smith, C. P. (1997) *J. Bacteriol.* **179,** 5999–6004.
30. Servant, P. & Mazodier, P. (1996) *J. Bacteriol.* **178,** 7031–7036.
31. Servant, P., Rapoport, G. & Mazodier, P. (1999) *Microbiology* **145,** 2385–2391.
32. Gibson, T. J. (1984) Ph.D. thesis (Cambridge Univ., Cambridge, U.K.).
33. Studier, F. W. & Moffatt, B. A. (1986) *J. Mol. Biol.* **189,** 113–130.
34. Cohen, S. N., Chang, A. C. Y. & Hsu, L. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 2110–2114.
35. Sambrook, J., Frisch, E. F. & Maniatis, T. (1989) (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
36. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
37. Bradford, M. (1976) *Anal. Biochem.* **72,** 248–254.
38. Chater, K. F., Bruton, C. J., King, A. A. & Suarez, J. E. (1982) *Gene* **19,** 21–32.
39. Holmes, D. J., Caso, J. L. & Thompson, C. J. (1993) *EMBO J.* **12,** 3183–3191.
40. Buttner, M. J., Smith, A. M. & Bibb, M. J. (1988) *Cell* **52,** 599–607.
41. Servant, P. & Mazodier, P. (1995) *J. Bacteriol.* **177,** 2998–3003.
42. Mazodier, P., Guglielmi, G., Davies, J. & Thompson, C. J. (1991) *J. Bacteriol.* **173,** 7382–7386.
43. Grandvalet, C., de Crécy-Lagard, V. & Mazodier, P. (1999) *Mol. Microbiol.* **31,** 521–532.
44. Lupas, A., Van Dyke, M. & Stock, J. (1991) *Science* **252,** 1162–1164.