***Rickettsia prowazekii* sigma factor σ^{73} can be overexpressed in *Escherichia coli* and promotes RNA polymerase binding and transcription**

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The sigma factor σ^{73} of the obligate intracytoplasmic bacterium *Rickettsia prowazekii* was overexpressed and purified from *Escherichia coli*. The rickettsial *rpoD* gene encoding σ^{73} was cloned into a *NdeI*–*BamHI*-cleaved pET-15b vector under control of T7 transcription and translation signals. The recombinant plasmid encoded a 75 kDa fusion protein that was overproduced in *E. coli* BL21(DE3) and purified from inclusion bodies after solubilization with guanidine hydrochloride and using His.Bind metal chelation resin. The N-terminal His.Tag sequence of the 75 kDa fusion protein was removed by thrombin treatment to obtain *R. prowazekii* σ^{73T} . The *R. prowazekii* σ^{73T} as well as the 75 kDa fusion protein had the ability to bind to core DNA-dependent RNA polymerase of both *R. prowazekii* and *E. coli* and to stimulate their interaction with a rickettsial promoter.

Keywords: *Rickettsia prowazekii*, sigma factor, RNA polymerase, overexpression

INTRODUCTION

The aetiological agent of epidemic typhus, *Rickettsia prowazekii*, is an obligate intracellular parasite. This Gram-negative bacterium grows within the cytoplasm of a eukaryotic cell with a generation time of about 10 h (reviewed by Winkler, 1990). *R. prowazekii* has a structurally classic prokaryotic DNA-dependent RNA polymerase (RNAP) with a subunit composition of $\beta'\beta\alpha_2\sigma$ (Ding & Winkler, 1990). The core enzyme ($\beta'\beta\alpha_2$) is capable of synthesizing RNA, but does not initiate transcription at specific promoter sites on DNA. The RNAP holoenzyme consists of the core plus the σ subunit, which is able to dissociate and is required for specific initiation of transcription. The major sigma factor of *R. prowazekii* has a calculated molecular mass of 73 kDa (Marks *et al.*, 1992) and migrates anomalously during SDS-PAGE at approximately 85 kDa (Ding & Winkler, 1990) (anomalous migration is also characteristic of *Escherichia coli* σ^{70} , the *E. coli* major sigma factor). Based on the deduced amino acid sequence, the *R. prowazekii* σ^{73} has 45 % amino acid identity with that of *E. coli* σ^{70} , which increases to 80 % in the C-terminal half of the molecule

(Marks *et al.*, 1992). Both *R. prowazekii* σ^{73} and *E. coli* σ^{70} and the subunits of *R. prowazekii* and *E. coli* core RNAP exhibit antigenic cross-reactivity (Ding & Winkler, 1990).

Nevertheless, the rickettsial RNAP holoenzyme differs significantly from the major *E. coli* RNAP, σ^{70} . Relative to *E. coli* σ^{70} , the rickettsial RNAP is a much weaker DNA-melting protein (Ding & Winkler, 1993). Although the rickettsial RNAP can form closed complexes on a linear template, formation of open complexes and specific transcripts is not observed on a linear template and requires a supercoiled template. Recently, another difference was found between *R. prowazekii* and *E. coli* RNAPs (Aniskovitch & Winkler, 1995). At the four promoters tested, the *R. prowazekii* RNAP holoenzyme forms transcriptionally active complexes that are unstable, remain dissociable, and are susceptible to challenge with heparin under conditions where the corresponding *E. coli* complexes are not. In this work, we overexpressed and purified the *R. prowazekii* σ^{73T} and demonstrated its activity in transcriptional stimulation of *R. prowazekii* RNAP. Herein, we have also shown the possibility of forming transcriptionally active hybrid RNA polymerase composed of *E. coli* core RNAP and *R. prowazekii* σ^{73} . The results of this work will allow further structure/function studies of *R. prowazekii* RNAP in comparison with *E. coli* σ^{70} .

Abbreviations: GuHCl, guanidine hydrochloride; RNAP, DNA-dependent RNA polymerase; σ^{73T} , the thrombin-cleaved protein (σ^{73} fused with His.Tag).

METHODS

Construction of the plasmid for overproduction of the 75 kDa fusion protein. The plasmid pHW61, designed for overproduction of the 75 kDa fusion protein, was constructed as shown in Fig. 1 by inserting the *rpoD* gene between the *NdeI* and *BamHI* sites in the pET-15b vector (Novagen). The *R. prowazekii rpoD* gene was derived from plasmid SP25 (Marks *et al.*, 1992). To create an *NdeI* site overlapping the initiation methionine codon of the gene, PCR-based, oligonucleotide-directed mutagenesis was carried out with the GeneAmp kit (Perkin Elmer Cetus). A pair of 20-residue oligonucleotide primers (5'-GGTATCATATGAGCAATAGC-3' and 5'-CATCCATTCCGATATCAAGC-3') was used to amplify (30 cycles) a *rpoD* fragment of 240 bp containing a new *NdeI* site and an existing *HindIII* site. The protocol employed *Taq* DNA polymerase and consisted of a denaturation step at 94 °C for 1 min, a primer annealing step at 40 °C for 1 min, an extension

step at 72 °C for 3 min, and a final elongation step at 72 °C for 7 min. The PCR product was gel-purified using Gene Clean (Bio 101) and cloned into the pCR II vector (Invitrogen) with the insert orientation *EcoRI-HindIII-NdeI-EcoRI-XhoI* (*E. coli* MOB802/pCR-L1). At this step, DNA sequencing was carried out by the dideoxynucleotide chain-termination method of Sanger *et al.* (1977) using modified T7 DNA polymerase to confirm that the correct mutation had been constructed.

To recreate the *R. prowazekii rpoD* gene, the purified 4.5 kb *HindIII-XhoI* fragment of SP25 was ligated to the 0.3 kb *HindIII-XhoI* fragment from pCR-L1. This plasmid, SPL1, was transformed into *E. coli* XL1-Blue host strain (Stratagene). The plasmid pHW61 was created by cloning the purified 2.0 kb *NdeI-BamHI* fragment of SPL1 into *NdeI-BamHI*-cleaved pET-15b. The plasmid pHW61 was transformed into *E. coli* BL21(DE3) that contains an inducible T7 RNA polymerase. The plasmid pHW60 was constructed for overproduction of σ^{73}

Fig. 1. Construction of the plasmid pHW61 that overexpresses the 75 kDa fusion protein.

by cloning the same *rpoD* *NdeI*-*Bam*HI fragment present in pHW61 into the pET-11a plasmid, which has no His.Tag sequence.

Restriction, ligation and agarose electrophoresis were performed by standard procedures (Maniatis *et al.*, 1982). Preparation of competent cells and transformation were done by the method of Nishimura *et al.* (1990).

Growth of the 75 kDa fusion-protein-overproducing cells. A few microlitres of *E. coli* BL21(DE3)(pHW61) from a glycerol stock were inoculated into 5 ml M9 medium plus 1% (w/v) Bacto-tryptone and 100 µg ampicillin ml⁻¹ and the culture was grown at 37 °C to an OD₆₀₀ of 0.4 (Beckman DU-50 spectrophotometer). The cells were collected by centrifugation, resuspended in 500 ml fresh medium, and incubated at 37 °C. When the OD₆₀₀ reached 0.4, IPTG was added to a final concentration of 0.4 mM. After 60 min, rifampicin was added to a final concentration of 100 µg ml⁻¹. The cells were harvested after overnight incubation at 37 °C.

Purification of the 75 kDa fusion protein. The pET His.Tag System was used to purify the 75 kDa fusion protein. Purification was done at 4 °C. Cells from 500 ml culture (about 0.8 g wet weight) were suspended in 40 ml binding buffer (20 mM Tris/HCl, pH 7.9, 500 mM NaCl, 5 mM imidazole), passed through a French pressure cell at 5000 p.s.i. (34.5 MPa), and centrifuged at 20000 *g* for 15 min to sediment inclusion bodies. The pellet was suspended in 20 ml binding buffer and again passed through a French pressure cell and centrifuged. The pellet from the final centrifugation was resuspended in 20 ml binding buffer containing 6 M guanidine hydrochloride (GuHCl). After incubation on ice for 1 h, insoluble material was removed by centrifugation at 39000 *g* for 30 min. The supernatant was filtered through a 0.45 µm membrane and loaded on 2.5 ml His.Bind metal chelation resin (Novagen) previously charged with 50 mM NiSO₄ and equilibrated with binding buffer containing 6 M GuHCl. The column was washed at a flow rate of 13 ml h⁻¹ with 25 ml binding buffer containing 6 M GuHCl and then with 15 ml of the same buffer containing 20 mM imidazole. The 75 kDa fusion protein was eluted at a flow rate of 13 ml h⁻¹ with 15 ml binding buffer containing 6 M GuHCl buffer and 40 mM imidazole.

To renature the 75 kDa fusion protein, the eluate from the His.Bind resin column (15 ml at 20–40 µg protein ml⁻¹) was first dialysed against storage buffer (10 mM Tris/HCl, pH 7.9; 0.1 mM EDTA; 0.1 mM DTT; 5%, v/v, glycerol; 500 mM NaCl) containing 2 M GuHCl and then against three changes of the same buffer without denaturant. The material was then either cleaved with thrombin to obtain σ^{73T}, or, when the 75 kDa fusion protein was the final product, dialysed against storage buffer.

Obtaining σ^{73T} from the 75 kDa fusion protein by proteolytic cleavage. The N-terminal His.Tag sequence of the 75 kDa fusion protein was removed, leaving the tripeptide (GSH) on the N-terminus of the sigma factor, by incubating 150 µg of the 75 kDa fusion protein in 3.8 ml buffer, containing 10 mM Tris/HCl (pH 7.9), 0.1 mM EDTA, 0.1 mM DTT, 5% (v/v) glycerol, 500 mM NaCl, and 0.65 µg thrombin, for 2 h at 8 °C. The reaction was stopped by adding PMSF to a final concentration of 1 mM. Cleavage of the His.Tag leader was monitored by native PAGE (8–25% gel gradient). The σ^{73T} obtained was dialysed against storage buffer.

In vitro transcription assay. *R. prowazekii* Madrid E was cultivated in the yolk sacs of antibiotic-free, embryonated hens' eggs and purified as described previously by Winkler (1976). The RNAP from *R. prowazekii* was purified by the method of

Ding & Winkler (1990) and had 10% saturation with σ⁷³ (Ding & Winkler, 1994). *E. coli* RNAP core enzyme was purified from the B strain (Lowe *et al.*, 1979). The 75 kDa fusion protein or σ^{73T} (from 0 to 200 ng) was mixed with 0.5 µg of the poorly sigma-saturated *R. prowazekii* RNAP or *E. coli* core RNAP and incubated for 30 min in an ice-bath before using in *in vitro* transcription assays.

Plasmids pHW23 and pHW12 were used for *in vitro* transcription assays (Ding & Winkler, 1993). In addition to vector genes, plasmid pHW23 contains a portion of the *R. prowazekii* citrate synthase gene (the *gltA* gene) and pHW12 contains a portion of the *R. prowazekii* ATP/ADP translocase gene (*tlc*) under control of their own promoters and terminated by a *trpA* terminator sequence. Supercoiled plasmid was purified by two rounds of CsCl density centrifugation.

Transcription reactions were performed in buffer (final volume of 50 µl) containing 40 mM Tris/HCl (pH 7.9), 10 mM MgCl₂, 1 mM DTT, 50 mM NaCl, 100 µg BSA ml⁻¹ and 50 units RNasin ribonuclease inhibitor. Supercoiled plasmid template (1.5 µg) was preincubated with reconstituted RNAP for 20 min at 37 °C to allow open complex formation. Single-round transcription was initiated by the addition of four nucleotides, 1 mM ATP, 0.5 mM CTP and GTP, 0.1 mM UTP, 10 µCi (370 kBq) [α-³²P]UTP, together with heparin (200 µg ml⁻¹). After 30 min of elongation at 37 °C, transcription was stopped by adding an equal volume of a solution containing 10 mM EDTA (pH 8.0), 450 mM NaCl, 1% (w/v) SDS and 400 µg proteinase K ml⁻¹. After further incubation for 30 min at 37 °C, the transcripts were recovered by ethanol-precipitation with yeast tRNA (100 µg ml⁻¹) as a carrier, and resuspended in 10 µl gel loading buffer (Ding & Winkler, 1993). Gel electrophoresis and autoradiography were as described elsewhere (Ding & Winkler, 1993). In some cases, the radioactivity, as determined by scintillation counting, in a gel piece without any band (background) was subtracted from the radioactivity of the same-size gel piece containing a transcript band to quantify the amount of specific transcript.

RESULTS AND DISCUSSION

Overexpression of the 75 kDa fusion protein

In developing a system for cloning and overexpression of *R. prowazekii* σ⁷³, two factors were important. First, since there was a potentially toxic interaction of σ⁷³ with the *E. coli* RNAP core, we reduced basal σ⁷³ gene expression by placing the *rpoD* gene under the control of the T7 promoter and by using an inducible T7 RNAP. Indeed, insertion and expression of the rickettsial *rpoD* in the pFLAG system (under the control of the *lac* promoter) was toxic. Second, to provide a fast and effective purification of σ⁷³, we constructed a His-tagged fusion protein so that *R. prowazekii* σ⁷³ could be separated from similar *E. coli* sigma factors. *R. prowazekii* *rpoD* was cloned into *NdeI*-*Bam*HI-cleaved pET-15b under the control of strong bacteriophage T7 transcription and translation signals (Fig. 1). The resultant recombinant plasmid pHW61 encoded a fusion protein of molecular mass 75 kDa. A stretch of six consecutive histidine residues (an affinity tag for one-step purification by metal chelation chromatography) and a thrombin cleavage site (a means for removal of the oligohistidine domain) were expressed at the N-terminal end of *R. prowazekii* σ⁷³. The

Fig. 2. Overexpression and purification of the 75 kDa fusion protein. Samples were separated by gradient SDS-PAGE (10–15% gel) and stained with Coomassie blue. Lanes: 1, molecular mass markers (Sigma); 2 and 3, cells of *E. coli* BL21(DE3)(pET-15b) and *E. coli* BL21(DE3)(pHW61), respectively, grown as described in Methods; 4–7, the supernatant (lanes 4 and 5) and pellet (lanes 6 and 7) obtained after centrifugation of the broken *E. coli* BL21(DE3)(pET-15b) cells (lanes 4 and 6) or *E. coli* BL21(DE3)(pHW61) cells (lanes 5 and 7); 8, the overproduced 75 kDa fusion protein after purification.

Fig. 3. Accumulation of the 75 kDa fusion protein in *E. coli* BL21(DE3)(pHW61) cells. The samples are the whole cells (lanes 1–3) or pellets (lanes 4–6) obtained after centrifugation of the broken cells. Lanes: 1 and 4, samples before induction; 2 and 5, samples after induction and 2 h incubation with rifampicin; 3 and 6, samples after induction and overnight incubation with rifampicin.

recombinant plasmid pHW61 was transferred to *E. coli* BL21(DE3) containing a chromosomal copy of the gene for T7 RNA polymerase under the control of the *lac* operator. This strain was used as the host for the 75 kDa fusion protein gene expression because it is deficient in the *lon* and *ompT* proteases that can degrade protein during purification. In addition, since BL21 is sensitive to rifampicin, transcription by the host RNAP could be inhibited.

Densitometer scanning revealed that the overproduced 75 kDa fusion protein made up approximately 5% of the total cellular protein (Fig. 2, lane 3). Several changes in

growth and induction parameters were tried without success to obtain an increase of the 75 kDa fusion protein expression. A possible reason for this modest level of expression in *E. coli* BL21(DE3)(pHW61) (as compared to a nonricketsial protein expressed in the same system; Chang & Doi, 1990) may be that the pools of some tRNAs in the recipient cells are inadequate due to the frequency and clustering of rare *E. coli* codons in the A+T-rich *R. prowazekii* *rpoD* gene (Winkler & Wood, 1988). This may also explain the continued accumulation of the 75 kDa fusion protein in the presence of rifampicin, which would increase the levels of tRNA available (Fig. 3).

Purification of the 75 kDa fusion protein

The overproduced 75 kDa fusion protein was found to be in the form of inclusion bodies that sedimented with the cell debris in the broken cells (Fig. 2, lane 7). Formation of inclusion bodies stipulated that the purification must be carried out under denaturing conditions. GuHCl was chosen as the denaturing agent because it has been used successfully to extract *E. coli* σ^{70} from inclusion bodies and to renature this protein (Lowe *et al.*, 1979; Gribskov & Burgess, 1983). The 75 kDa fusion protein from solubilized inclusion bodies was purified by adsorption to His.Bind metal chelation resin and elution with buffer containing 40 mM imidazole. *E. coli* σ^{70} could be adsorbed to this resin in the absence of imidazole and could be eluted with 5 mM imidazole. Thus, the 75 kDa fusion protein could be separated from *E. coli* proteins, including *E. coli* σ^{70} , by metal chelation chromatography. A gradual removal of 6 M GuHCl by dialysis was necessary to reconstitute an active 75 kDa fusion protein. As determined by native PAGE, approximately 90% of the preparation was in the monomeric form. Using this scheme, we were able to purify 0.6–1 mg of the 75 kDa fusion protein from 500 ml culture. The protein purity was above 95%, as determined by densitometric analysis (Fig. 2, lane 8).

Fig. 4. Transcriptional stimulation of *R. prowazekii* (Rp) RNAP by the 75 kDa fusion protein and σ^{73T} . *R. prowazekii* RNAP (500 ng), which had about 10% of the stoichiometric amount of σ^{73} , was mixed with the 75 kDa fusion protein or σ^{73T} and incubated for 30 min at 0 °C before using in *in vitro* single-round transcription reactions. Lanes: 1 and 2, *R. prowazekii* RNAP plus 100 and 200 ng 75 kDa fusion protein, respectively; 3, *R. prowazekii* RNAP alone; 4 and 5, *R. prowazekii* RNAP plus 200 and 100 ng σ^{73T} , respectively; 6, 200 ng σ^{73T} alone; 7, 200 ng 75 kDa fusion protein alone.

Characterization of the 75 kDa fusion protein

The purified 75 kDa fusion protein, like the native *R. prowazekii* σ^{73} subunit (Ding & Winkler, 1990), cross-reacted on immunoblots with antisera to the *E. coli* σ^{70} subunit (data not shown). The ability of the 75 kDa fusion protein to bind to *R. prowazekii* core polymerase and stimulate its interaction with the *gltA* promoter was demonstrated in single-round transcription assays (Fig. 4). The *R. prowazekii* RNAP, which had about 10% of the stoichiometric amount of σ^{73} based on SDS-PAGE profiles, was used as a substitute for the rickettsial core enzyme. This *R. prowazekii* RNAP produced detectable transcript from the *gltA* promoter (1072 c.p.m., lane 3). The 75 kDa fusion protein alone had no detectable transcriptional activity (lane 7). Addition of 0.1 μ g 75 kDa fusion protein to RNAP increased transcription from the *gltA* promoter to 2421 c.p.m. (lane 1). Increasing the level of the 75 kDa fusion protein led to the subsequent enhancement of RNA polymerase transcription activity (3011 c.p.m., lane 2). Thus, we demonstrated that the renatured, overproduced 75 kDa fusion protein functions as a sigma factor. However, since we have not been able to determine the number of active core molecules in the rickettsial RNAP preparation (a difficult task since it is not active on linear templates), the corresponding number is also unknown for the sigma factor preparation.

Formation of *R. prowazekii* σ^{73T}

Although the 75 kDa fusion protein was found to be active, the presence of the His.Tag sequence may affect the activity of this protein as compared to the native σ^{73} . For purification of σ^{73} from the overproduction system BL21(DE3)(pHW60), the method of Gribskov & Burgess (1983), which has been used successfully in our laboratory for purification of σ^{70} (Ding & Winkler, 1993), was used. The preparation was inactive and, as determined by native PAGE, consisted of multimers and aggregates of σ^{73} protein. To obtain monomeric σ^{73} , GuHCl (6 M) was used to denature and renature this material as described for the 75 kDa fusion protein. Although *in vitro* transcription assays demonstrated that this monomeric σ^{73} was active, it was contaminated with *E. coli* σ^{70} and complete chromatographic separation of these two sigma factors was unsuccessful. Therefore, we removed the N-

Fig. 5. Native PAGE migration of rickettsial sigma factor preparations. Lanes: 1(a, b), native PAGE (8–25% gel gradient) of σ^{73} purified from the overproduction system BL21(DE3)(pHW60); 2(a, b), σ^{73T} obtained from the cleavage of the 75 kDa fusion protein; 3(a, b), 75 kDa fusion protein purified from the overproduction system BL21(DE3)(pHW61). Samples were stained with silver.

terminal His.Tag sequence of the 75 kDa fusion protein by thrombin treatment to form σ^{73T} . Cleavage of the His.Tag leader was monitored by native PAGE (8–25% gel gradient) (Fig. 5). As shown in Fig. 5, σ^{73T} obtained from the 75 kDa fusion protein comigrates with σ^{73} purified from the overproduction system BL21(DE3)(pHW60).

Characterization of *R. prowazekii* σ^{73T}

The stimulatory effect of the 75 kDa fusion protein was compared with that of σ^{73T} in a single-round *in vitro* transcription assay (Fig. 4). Alone, *R. prowazekii* σ^{73T} as well as the 75 kDa fusion protein had no detectable transcriptional activity (lanes 6 and 7). Addition of σ^{73T} (0.1 μ g, lane 5) to RNAP increased transcription from the *gltA* promoter to 2635 c.p.m., a level nearly equal to that observed after the addition of the same amount of fusion protein (2421 c.p.m., lane 1). Similar results were obtained when 0.2 μ g of these proteins was used (3011 and 2802 c.p.m. for lanes 2 and 4). Since the activity of the 75 kDa fusion protein was approximately equal to that of σ^{73T} , the presence of the His.Tag sequence did not appear to significantly affect the activity of the molecule.

Stimulation of *E. coli* core RNAP by the 75 kDa fusion protein

The 75 kDa fusion protein also had the ability to stimulate transcription from the rickettsial *lfc* promoter when added to *E. coli* core RNAP (Fig. 6). *E. coli* core RNAP alone

Fig. 6. Stimulation of interaction between the *E. coli* core RNAP and the *tlc* promoter by the 75 kDa fusion protein. *E. coli* core RNAP (500 ng), which had no detectable σ^{70} , was mixed with the 75 kDa fusion protein, incubated for 30 min at 0 °C and used in the *in vitro* transcription assay. Lanes: 1, *E. coli* core RNAP alone; 2, 3, 4 and 5, *E. coli* core RNAP plus 30, 60, 100 and 140 ng *R. prowazekii* 75 kDa fusion protein, respectively.

had no detectable transcription activity (lane 1). However, when increasing amounts of the 75 kDa fusion protein were added to the *E. coli* core RNAP, transcription was observed (lanes 2–5). The ability to bind to *E. coli* core RNAP and stimulate its interaction with the *tlc* promoter was also seen using σ^{73T} (data not shown). Therefore, the interactive domains of *R. prowazekii* core RNAP and σ^{73} must be sufficiently similar to their *E. coli* counterparts to yield a transcriptionally competent heterologous enzyme containing core RNAP from one bacterium and sigma subunit from another.

In conclusion, we have developed a strategy for over-producing and purifying enzymically active σ^{73} of *R. prowazekii* in *E. coli*. In addition, we showed that the rickettsial sigma factor obtained could form transcriptionally active hybrid RNAP with the *E. coli* core enzyme. This interaction may explain the toxicity seen in other attempts at overexpression. This work provides a key element for structure/function studies of the *R. prowazekii* sigma factor and RNAP holoenzyme.

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REFERENCES

- Aniskovitch, L. P. & Winkler, H. H. (1995). Instability of *Rickettsia prowazekii* RNA polymerase-promoter complexes. *J Bacteriol* **177**, 6301–6303.
- Chang, B.-Y. & Doi, R. H. (1990). Overproduction, purification, and characterization of *Bacillus subtilis* RNA polymerase σ^A factor. *J Bacteriol* **172**, 3257–3263.
- Ding, H.-F. & Winkler, H. H. (1990). Purification and partial characterization of the DNA-dependent RNA polymerase from *Rickettsia prowazekii*. *J Bacteriol* **172**, 5624–5630.
- Ding, H.-F. & Winkler, H. H. (1993). Characterization of the DNA-melting function of the *Rickettsia prowazekii* RNA polymerase. *J Biol Chem* **268**, 3897–3902.
- Ding, H.-F. & Winkler, H. H. (1994). The molar ratio of σ^{73} to core polymerase in the obligate intracellular bacterium, *Rickettsia prowazekii*. *Mol Microbiol* **11**, 869–873.
- Gribskov, M. & Burgess, R. R. (1983). Overexpression and purification of the sigma subunit of *Escherichia coli* RNA polymerase. *Gene* **26**, 109–118.
- Lowe, P. A., Hager, D. A. & Burgess, R. R. (1979). Purification and properties of the σ subunit of *Escherichia coli* DNA-dependent RNA polymerase. *Biochemistry* **18**, 1344–1352.
- Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982). *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Marks, G. L., Winkler, H. H. & Wood, D. O. (1992). Isolation and characterization of the gene coding for the major sigma factor of *Rickettsia prowazekii* DNA-dependent RNA polymerase. *Gene* **121**, 155–160.
- Nishimura, A., Morita, M., Nishimura, Y. & Sugino, Y. (1990). A rapid and highly efficient method for preparation of competent *E. coli* cells. *Nucleic Acids Res* **18**, 6169.
- Sanger, F., Nicklen, S. & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* **74**, 5463–5467.
- Winkler, H. H. (1976). Rickettsial permeability: an ADP-ATP transport system. *J Biol Chem* **251**, 389–396.
- Winkler, H. H. (1990). *Rickettsia* species (as organisms). *Annu Rev Microbiol* **44**, 131–153.
- Winkler, H. H. & Wood, D. O. (1988). Codon usage in selected AT-rich bacteria. *Biochimie* **70**, 977–986.

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