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**Modulation of the RNA polymerase activity by AtcB, a protein associated with a DnaK chaperone network in *Shewanella oneidensis***

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## **Abstract**

Bacteria possess several molecular pathways to adapt to changing environments and to stress conditions. One of these pathways involves a complex network of chaperone proteins that together control proteostasis. In the aquatic bacterium *Shewanella oneidensis*, we have recently identified a previously unknown co-chaperone of the DnaK/Hsp70 chaperone system, AtcJ, that is essential for adaptation to low temperatures. AtcJ is encoded in the *atcJABC* operon, whose products, together with DnaK, form a protein network allowing growth at low temperature. However, how these proteins allow cold adaptation is unknown. Here, we found that AtcB directly interacts with the RNA polymerase and decreases its activity. In addition, AtcB overproduction prevents bacterial growth due to RNA polymerase inhibition. Together, these results suggest that the Atc proteins could direct the DnaK chaperone to the RNA polymerase to sustain life at low temperatures.

## **Keywords**

Bacterial adaptation / DnaK chaperone system / J-domain proteins (JDP) / RNA polymerase / Cold stress / Stress response

## Introduction

To colonize and survive in many ecological niches, bacteria have the astonishing ability to adapt to a large number of stress conditions and to changing environments. For example, in response to stresses, bacteria can reorganize gene expression using complex regulatory mechanisms to adjust their metabolism to the disappearance of some nutrients [1]. In other conditions including heat or oxidative stresses that are known to alter protein folding and trigger protein aggregation, all living cells use a class of proteins called chaperones that prevent such deleterious effects on the proteins [2–4]. Together, chaperone networks ensure that protein homeostasis (proteostasis) is maintained.

DnaK (also called Hsp70 in eukaryotes) is an ATP-dependent chaperone that is present in most organisms [5–9]. It participates in the folding (and unfolding) of a large range of substrate proteins, and as a consequence, DnaK is virtually involved in all cellular functions, from metabolism to DNA replication and cell division. The mechanism of action of DnaK implies the contribution of two co-chaperones, one belonging to the J-domain protein (JDP) family which was named after the *E. coli* DnaJ protein, and a nucleotide exchange factor (NEF). The JDPs interact with substrate proteins, address them to ATP-bound DnaK and stimulate ATP hydrolysis by DnaK, inducing a conformational change of DnaK [10,11]. ADP-bound DnaK interacts tightly with the substrate, and substrate release depends on the NEF co-chaperone that allows the exchange of ADP to ATP. In addition to DnaJ, many other JDPs are usually present in a given organism to enlarge the functions and the panel of substrate proteins that DnaK can take care of [9–11].

In the aquatic bacterium *Shewanella oneidensis*, a model for stress adaptation [12–15], we have recently identified a previously unknown JDP, AtcJ, that is essential for

growth at low temperature (Fig. 1) [16]. We have found that AtcJ is encoded in a four-gene operon, *atcJABC*. Interestingly, we have demonstrated that AtcC and AtcB are also essential at low temperatures. In addition to its interaction with DnaK, AtcJ interacts with AtcC, and AtcC binds AtcB (Fig. 1) [16]. However, it is still unknown how these proteins together allow bacterial growth at low temperatures.

Here, we found that AtcB interacts with the RNA polymerase and modulates its activity. These results represent an important step forward to understand the physiological functions of the Atc proteins and understand how they can allow cold adaptation.

## Materials and Methods

**Strains and growth conditions.** Strains were grown aerobically with shaking in rich LB medium at the temperatures indicated in the figures. When necessary, chloramphenicol (25 µg/mL), kanamycin (25 µg/mL), or ampicillin (50 µg/mL) was added. The wild-type *S. oneidensis* used in this study is MR1-R [17], and the *E. coli* strains used in this study are MG1655 [18] and BL21 (DE3) (Novagen). The  $\Delta atcB$  strain was constructed as described earlier [16]. Growth of *S. oneidensis* and *E. coli* strains was measured in liquid cultures or on LB-agar plates supplemented with chloramphenicol and with 0.2% (w/v) L-arabinose when indicated, as described [16].

**Plasmids.** Plasmids pET-RpoA<sub>S<sub>0</sub></sub>, pET-RpoB<sub>S<sub>0</sub></sub> and pET-RpoC<sub>S<sub>0</sub></sub>, allowing the overproduction of RpoA<sub>6His</sub>, RpoB<sub>6His</sub>, and RpoC<sub>6His</sub>, and the plasmid pBad24-CBP-AtcC used in co-purification experiments were constructed as described in Supplementary Materials and Methods. The plasmids p/*acZ*, pBad33-AtcB, pBad33-

AtcC, pBad24-CBP-AtcB, pET-AtcB, pET*hsp90*<sub>S0</sub>, pET52-AtcB, and pET52-HtpG<sub>S0</sub> were constructed as described [13,16].

**Protein purification.** To produce proteins, BL21 strains containing pET-RpoA<sub>S0</sub>, pET-RpoB<sub>S0</sub>, pET-RpoC<sub>S0</sub>, pET-AtcB, pET*hsp90*<sub>S0</sub>, pET52-AtcB, and pET52-HtpG<sub>S0</sub> were grown at 37°C until OD<sub>600</sub> reached 0.8, then 1 mM IPTG was added for 3 hours at 28°C. To purify the RpoA<sub>6His</sub>, AtcB<sub>6His</sub>, HtpG<sub>6His</sub>, *Strep*AtcB and *Strep*HtpG proteins, the same procedures as described earlier were performed [13,16].

**Polymerase complex reconstitution.** To purify and reconstitute the core RNA polymerase composed of  $\alpha_2\beta\beta'$  (RpoA<sub>2</sub>, RpoB, RpoC), cells that produced RpoB<sub>6His</sub> and RpoC<sub>6His</sub> were collected, resuspended in buffer A (40 mM sodium phosphate buffer pH 7.4, 500 mM NaCl, 10% glycerol), and lysed by French Press. Following the protocol described by Fujita and Ishihama [19], lysates were centrifuged 10 min at 10 000 rpm, and the pellets were washed 2 times with buffer B (50 mM Tris-HCl pH 8.0, 10 mM EDTA, 100 mM NaCl, 0.5% Triton X-100). Then, the pellets were resuspended in buffer C (50 mM Tris-HCl pH 8.0, 1 mM EDTA, 10 mM DTT, 200 mM KCl, 10 mM MgCl<sub>2</sub>, 20% glycerol, 6 M urea). After 30 min on ice, resuspensions containing RpoB<sub>6His</sub> and RpoC<sub>6His</sub> were mixed with purified RpoA<sub>6His</sub>, according to a molar ratio of 4RpoA:1RpoB:1RpoC. This mixture was dialyzed overnight at 4°C in buffer D (50 mM Tris-HCl pH 8.0, 0.1 mM EDTA, 1 mM DTT, 0.3 M KCl, 10% glycerol), and centrifuged 20 min at 14 000 rpm. Protein concentration of the supernatant corresponding to the core RNA polymerase was measured by Bradford assay and stored at -80°C.

**In vitro transcription-translation.** The “*E. coli* S30 Extract System for Linear Templates” kit (Promega) was used to measure the efficiency of transcription-translation in vitro. Assays were performed according to the manufacturer instruction

with slight modification as follows. Reaction mixtures (50  $\mu$ L) contained 10 ng/ $\mu$ L of pBest-Luc vector, the S30 extract, and purified proteins or buffer as indicated in the figure. After 3 hours at 37°C, luciferase activity was measured from 20  $\mu$ L, using buffer F (25 mM HEPES pH 7.5, 0.5 mM ATP, 10 mM MgCl<sub>2</sub>, 200  $\mu$ M D(-)-Luciferin) and a Tecan Spark Microplate reader. Luciferase activity was expressed as percent of the level measured in the absence of AtcB.

**Co-purification assays.** In vivo co-purifications were performed from MR1  $\Delta$ atcB strain containing the pbad33 vector or pBad33-AtcB (coding for AtcB<sub>6His</sub>). Cells were grown at 28°C until OD<sub>600</sub> reached 0.8 and then expression from the vector was induced with the addition of 0.2% (w/v) of L-arabinose during 2 hours. Cells were collected by centrifugation 10 min at 10 000 rpm, resuspended in buffer A supplemented with 5 mM imidazole, and lysed by French Press. Lysates were centrifuged 10 min at 14 000 rpm, and supernatants were loaded on HisPur™ Ni-NTA resin (Thermo) equilibrated in buffer A. After 1 hour at 4°C, beads were washed successively with buffer A supplemented with increasing concentration of imidazole (20 mM to 100 mM). Proteins were eluted with buffer A supplemented with 500 mM imidazole, samples were heat denatured and separated by SDS-PAGE subsequently stained with Coomassie Blue. When indicated, proteins were cut out from the gel for identification by mass spectrometry analysis.

Co-purification experiments from *E. coli* were performed with the MG1655 strain containing either the pBad24-CBP vector or the pBad24-CBP-AtcB plasmid. Strains were grown at 37°C. At OD<sub>600</sub>=0.5, 0.05% arabinose was added and cells were collected 1 hour later. Equivalent amount of cells were resuspended in buffer G (10mM Tris-Hcl pH = 8, 150mM NaCl, 1 mM Mg acetate, 1mM imidazole, 2mM CaCl<sub>2</sub>, 0.1% Triton X-100, 0.1% Tween-20, 20mM  $\beta$ -mercaptoethanol), lysed with

French press and centrifuged 10 min at 8000 rpm. Co-purification was performed as described [13]

For in vitro co-purifications using purified proteins, the core RNA polymerase (36  $\mu$ g), AtcB-strep (20  $\mu$ g) and HtpG-strep (20  $\mu$ g) were mixed as indicated in the figure in buffer H (100 mM Tris-HCl pH = 8.0, 150 mM NaCl, 1 mM EDTA, 0.05% Triton X-100) before the addition of 50  $\mu$ L Strep-Tactin beads (IBA Lifesciences). Samples were then treated and analyzed as described [16].

**Blue Native PAGE.** After an overnight culture at 28°C, strains as indicated in the figure were diluted to OD<sub>600</sub>=0.05, grown at 28°C, and 0.2 % arabinose was added at OD<sub>600</sub>=0.8 to induce expression from the plasmids. Cells were collected two hours later. Pellets were resuspended in 2 mL of 50 mM Tris-HCl pH 7.5, 10% glycerol, 1 mM DTT and lysed with French press. Extracts were centrifuged 10' at 10 000 rpm, and supernatants were centrifuged again 20' at 14 000 rpm. Protein concentrations of the soluble extracts were determined by Bradford assays and equivalent amount of proteins were separated on BN-PAGE (3-12%) as described earlier [20]. Gels were stained with 0.2% Coomassie blue R250, 0.05% Coomassie blue G250, 45% ethanol, 10% acetic acid. For Western blot analysis, proteins were transferred onto PVDF membranes.

**Mass spectrometry analyses.** To identify proteins, LC-MS/MS experiments were performed by the Proteomics core facility of the IMM as described [21].

**$\beta$ -galactosidase activity.** MG1655/*placZ* *E. coli* strains containing either pBad24-CBP, pBad24-CBP-AtcB, or pBad24-CBP-AtcC as indicated in the figure were grown at 28°C. To follow growth, OD<sub>600</sub> was measured every 40 min, and during exponential growth phase, expression from vector was induced with the addition of

0.2% (w/v) L-arabinose. For  $\beta$ -galactosidase activity measurement, same amount of cells were harvested and stored at  $-20^{\circ}\text{C}$  for subsequent  $\beta$ -galactosidase assay following the same procedure as described [16].

**Western Blot.** The level of the AtcB<sub>6His</sub> and AtcC<sub>6His</sub> proteins were observed as described in Supplementary Materials and Methods.

## Results and discussion

**AtcB overproduction significantly reduces bacterial growth.** We have previously shown that deletion of the genes coding for the AtcJ, AtcB and AtcC proteins strongly reduces *S. oneidensis* growth at low temperature ( $7^{\circ}\text{C}$ ), but not at permissive temperature ( $28^{\circ}\text{C}$ ) [16]. As expected, we have demonstrated that these growth defects were complemented by producing the proteins in trans from a plasmid. Surprisingly, we found that overproduction of AtcB in a wild type *S. oneidensis* strain significantly reduces growth on plates and in liquid cultures at  $28^{\circ}\text{C}$  (Fig. 2A and S1). This result is specific of the overproduction of AtcB since overproduction of AtcC did not affect bacterial growth (Fig. 2A). To make sure that the toxicity observed with AtcB compared to AtcC overproduction was not due to a difference in protein level, we confirmed that similar levels of AtcB and AtcC were produced in the two strains by Western blot on total protein extracts (Fig. 2B).

Strikingly, overproduction of AtcB – but not of AtcC – was also toxic in an *E. coli* wild-type strain (Fig. 2C). Note that *E. coli* does not possess any homolog of the Atc proteins. Therefore, these results suggest that overproduction of AtcB could target and impact a general and essential pathway that is conserved between *S. oneidensis* and *E. coli*.

**AtcB interacts with the RpoB/RpoC subunits of the RNA polymerase.** In an attempt to understand why AtcB overproduction is toxic, we aimed to identify its partner proteins. To do that, we set up a pull-down approach. In these experiments, a 6His-tagged AtcB was produced in *S. oneidensis* grown at 28°C and purified from the cell lysate on Ni-beads. As a control, the same protocol was performed with a strain that did not produce AtcB. As expected, we observed on SDS-PAGE a band corresponding to AtcB that migrates with a relative molecular weight (MW) of about 30 kDa (Fig. 3A). Western blot analysis with an anti-His antibody confirmed that this band corresponds to AtcB (Fig. 3A). Interestingly, an intense additional band of high MW was observed when AtcB was purified. Mass spectrometry analysis revealed that this band contains the proteins RpoB (150 kDa) and RpoC (155 kDa), the  $\beta$  and  $\beta'$  subunits of the RNA polymerase, respectively (Fig. S2). This result was also confirmed by Western blot analysis with an anti-RpoB antibody (Fig. 3A). Bacterial RNA polymerase (RNAP), a large protein complex, is the key enzyme for transcription [22]. It is composed of a core enzyme ( $\alpha_2\beta\beta'\omega$ ) made of 2  $\alpha$  subunits (RpoA), a  $\beta$  (RpoB) and  $\beta'$  (RpoC) subunits, and a small  $\omega$  subunit (RpoZ). To initiate transcription, a sigma factor transiently interacts with the core enzyme forming the holoenzyme [23].

Since the RpoB and RpoC proteins are highly conserved between *E. coli* and *S. oneidensis* with more than 80% sequence identity, we hypothesized that AtcB could also interact with RpoB/RpoC in *E. coli*. Therefore, AtcB with a CBP-tag was heterologously produced in *E. coli*, and a pull-down experiment was performed. When AtcB was purified on calmodulin resin, we observed a doublet of bands corresponding to high MW proteins identified by mass spectrometry as RpoB and

RpoC, as well as a band just above the AtcB band corresponding to the  $\alpha$  subunit of the RNA polymerase, RpoA (Fig. 3B and Fig. S3).

To confirm that AtcB interacts with the RNA polymerase complex and to show that the interaction is direct, we performed a pull-down experiment with purified proteins. As already described, the RpoB and RpoC proteins are not stable when purified alone. However, they are stabilized by the RpoA protein [19]. Therefore, the RpoA, RpoB and RpoC proteins were purified as a complex following a previously published procedure [19] (Fig. S4). AtcB with a Strep-tag was incubated with or without the RpoA-RpoB-RpoC complex, and AtcB was pulled-down on a Strep-Tactin resin. We observed that the RpoB and RpoC proteins were co-purified with AtcB (Fig. 3C). As a control, we checked that the RNA polymerase subunits were not retained on the resin in the absence of AtcB or in the presence of a non-related protein, HtpG with a Strep-tag (Fig. 3C).

Finally, we wanted to directly detect the native complex made of the RNA polymerase and AtcB without any step of purification. To do that, wild-type and  $\Delta atcB$  *S. oneidensis* strains containing an empty vector or a plasmid with the *atcB* gene were grown at 28°C, and AtcB production was induced by adding arabinose for 2 hours. Soluble protein extracts from these strains were separated on blue native PAGE (BN-PAGE), a technic that allows the separation of protein complexes in native conditions. Proteins were directly visualized after Coomassie blue staining (Fig. 3D, upper panel) or by Western blot with an anti-RpoB antibody (Fig. 3D, lower panel). No difference was noticed between the soluble extracts of the WT and  $\Delta atcB$  strains. Western blot directed against RpoB and a subsequent mass spectrometry analysis revealed that the core RNA polymerase complex corresponds to the intense band that migrates slightly above the 480 kDa marker (Fig. 3D and Fig. S5). As already

described, the core RNA polymerase migrates on BN-PAGE with an apparent MW around 480 kDa, slightly above its expected MW of about 400 kDa [24]. However, when AtcB was overproduced, we observed an additional band after Coomassie blue staining that was also detected with the anti-RpoB antibody, indicating that AtcB overproduction modifies RNAP migration. Surprisingly, Western blot and mass spectrometry analysis indicated that AtcB was present in the two bands (Fig. 3D and S5). This experiment confirms that AtcB interacts with the core RNA polymerase.

**AtcB overproduction reduces the level of transcription.** Since AtcB interacts with the RNA polymerase, we hypothesized that the toxicity observed upon AtcB overproduction could be due to an inhibition of the transcription. To test this hypothesis, a plasmid coding for AtcB or AtcC was introduced into an *E. coli* strain containing a *lacZ* reporter gene encoded on another plasmid. Note that the expression of *lacZ* and *atcB* or *atcC* are under the control of a pBad promoter, inducible by arabinose. We assumed that a decrease in transcription would result in a decrease in the level of  $\beta$ -galactosidase activity. In our experiments, strains were first grown without inducer for three hours. Then, arabinose was added to induce gene expression from the plasmids. Bacterial growth and  $\beta$ -galactosidase activity were measured with time (Fig. 4A). Interestingly, the level of  $\beta$ -galactosidase activity – that was normalized by the number of bacteria – was significantly reduced in the strain producing AtcB, reaching 54 % of the level measured with the wild-type strain at 200 minutes after induction. This result suggests that an excess of AtcB leads to a reduction of transcription in vivo.

To confirm this result, we set up an in vitro assay to test the effect of AtcB on transcription. We used a commercially available semi-purified S30 extract containing

all the proteins needed for the transcription and the translation of a DNA matrix (coding for the luciferase), including the RNA polymerase. When the DNA matrix was incubated with the S30 extract, a basal level of luciferase activity was measured (set to 100%) (Fig. 4B). Interestingly, we observed that when the S30 extract was incubated with the DNA matrix and increasing concentrations of purified AtcB (from 0.2 to 1  $\mu$ M), luciferase activity was strongly decreased. Indeed, 1  $\mu$ M AtcB led to a reduction of more than 75% of the level of activity measured in the absence of AtcB. We show that this result is specific of AtcB since adding 1  $\mu$ M of purified HtpG did not affect the level of luciferase activity. Altogether, these experiments strongly suggest that AtcB interacts with the RNA polymerase and inhibits its activity.

In summary, our experiments demonstrate that AtcB from *S. oneidensis* directly interacts both in vivo and in vitro with the RNA polymerase, and most probably with the RpoB and/or RpoC catalytic subunits. In addition, an excess of AtcB inhibits the activity of the RNA polymerase. Although the phenotype of toxicity observed upon AtcB overproduction both in *E. coli* and in *S. oneidensis* is not physiologically relevant, it could reveal an unanticipated role of the Atc proteins to explain their essentiality at low temperatures. Indeed, low temperature is known to alter transcription by decreasing the processivity of the RNA polymerase, which can lead to early transcription termination [25,26]. In addition, low temperature could also alter the folding and stability of the RNA polymerase. Interestingly, DnaK was shown to protect the RNA polymerase from heat denaturation and participate to its reactivation [27]. Therefore, we hypothesize that AtcB, through its interaction with AtcC that itself contacts AtcJ, could target the DnaK chaperone to the RNA polymerase. Together, the Atc proteins and DnaK could collaborate by a still unknown mechanism to remodel the RNA polymerase, allowing efficient transcription at low temperature.

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## Figure Legends

**Figure 1. The Atc proteins from *Shewanella oneidensis* allow cold adaptation by an unknown mechanism.** The Atc proteins are encoded by the *atcJABC* operon. In the absence of *atcJ*, *atcB* or *atcC*, growth of *S. oneidensis* is drastically affected at low temperatures. AtcJ interacts with AtcC, and AtcC interacts with AtcB. AtcJ is a J-domain co-chaperone of DnaK. The mechanism allowing cold adaptation that involves the Atc proteins and DnaK has not been determined yet.

**Figure 2. AtcB overproduction is toxic both in *S. oneidensis* and in *E. coli*.** (A) Growth of *S. oneidensis* strains overproducing AtcB. Cultures of wild type *S. oneidensis* strains containing a vector (p), or a plasmid allowing the overproduction of AtcB<sub>6His</sub> (pAtcB) or AtcC<sub>6His</sub> (pAtcC) were normalized and serial-diluted. 2 µL of each dilution was spotted on LB-agar plates with or without 0.2% arabinose. Plates were incubated at 28°C. (B) Same strains as in A were grown in the presence of arabinose. Total protein extracts were analyzed by a Western blot revealed with HisProbe-HRP. (C) Same plasmids as in A were introduced in a wild-type *E. coli*

MG1655 strain to heterologously overproduce AtcB or AtcC. Same procedure as in A was performed. Plates with or without 0.2% arabinose were incubated at 28°C.

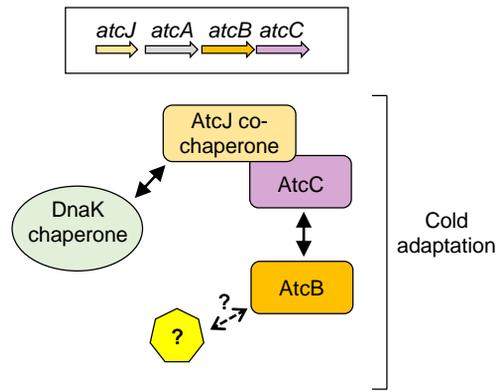
Results shown on the plates and on the Western blot are representative of at least three independent experiments.

**Figure 3. AtcB interacts with the RNA polymerase.** (A) In vivo co-purification of AtcB and the RNA polymerase in *S. oneidensis*. *S. oneidensis*  $\Delta atcB$  strains containing a vector (p) or a plasmid allowing the production of AtcB<sub>6His</sub> were grown at 28°C and 0.2% arabinose was added to induce the production of AtcB<sub>6His</sub>. Protein extracts from these strains were incubated on Ni-NTA resin. Proteins eluted were heat-denatured and separated by SDS-PAGE. Proteins were either stained with Coomassie blue, or transferred by Western blot subsequently probed with anti-RpoB or anti-6his antibodies. (B) In vivo co-purification of AtcB and the RNA polymerase in *E. coli*. MG1655 strains containing a vector (p) or a plasmid allowing the overproduction of AtcB with a CBP-tag were grown at 37°C before 0.05% arabinose was added. CBPAtcB was purified on calmodulin beads. CBPAtcB and the proteins co-purified were separated by SDS-PAGE, and stained with Coomassie blue. (C) In vitro interaction between AtcB and RpoB-RpoC. Purified RpoA, RpoB, and RpoC were incubated with <sub>strep</sub>AtcB, purified on Strep-tactin resin and proteins were separated by SDS-PAGE. Proteins were stained with Coomassie blue. (D) Detection of the native complexes AtcB-RNA polymerase by blue-native PAGE (BN-PAGE). *S. oneidensis* strains as indicated in the figure were grown at 28°C, and arabinose was added to produce AtcB. Soluble protein extracts (30 µg) from these strains were separated on a BN-PAGE. Proteins were either stained with Coomassie blue or transferred by Western blot probed with an anti-RpoB antibody. Gels shown in A-D are

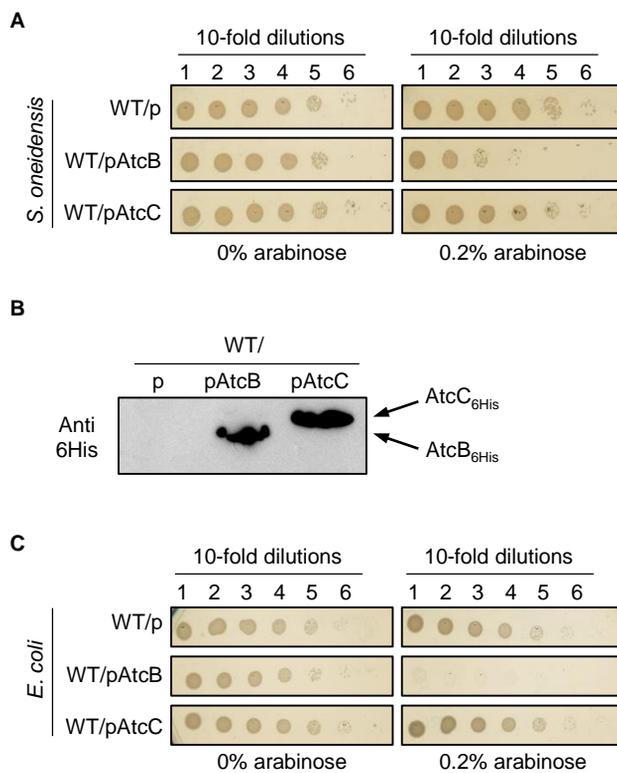
representative of at least three independent experiments. In A, B and D, mass spectrometry analyses were performed to identify each protein of interest.

**Figure 4. AtcB inhibits the activity of the RNA polymerase.** (A) AtcB overproduction reduces transcription using a *lacZ* reporter. *E. coli* MG1655 strain containing a plasmid with a *lacZ* gene under the pBad promoter was co-transformed with a vector or with plasmids allowing the production of AtcC (pAtcC) or AtcB (pAtcB) also under the control of a pBad promoter. After 3h, arabinose was added, and growth (inset) and  $\beta$ -galactosidase activity were measured with time using a Tecan SparkControl microplate reader. Note that  $\beta$ -galactosidase activity was normalized by the amount of bacterial cells. (B) In vitro transcription/translation assays. Commercial S30 extracts (Promega) allowing in vitro transcription/translation were incubated with a plasmid coding for the luciferase and with increasing concentrations (0 to 1  $\mu$ M) of purified AtcB or with 1  $\mu$ M HtpG. After three hours of incubation, luciferase activity was measured using a Tecan Spark microplate reader. In A and B, data from at least three replicates are presented as mean +/- SD.

Figure 1



**Figure 2**



**Figure 3**

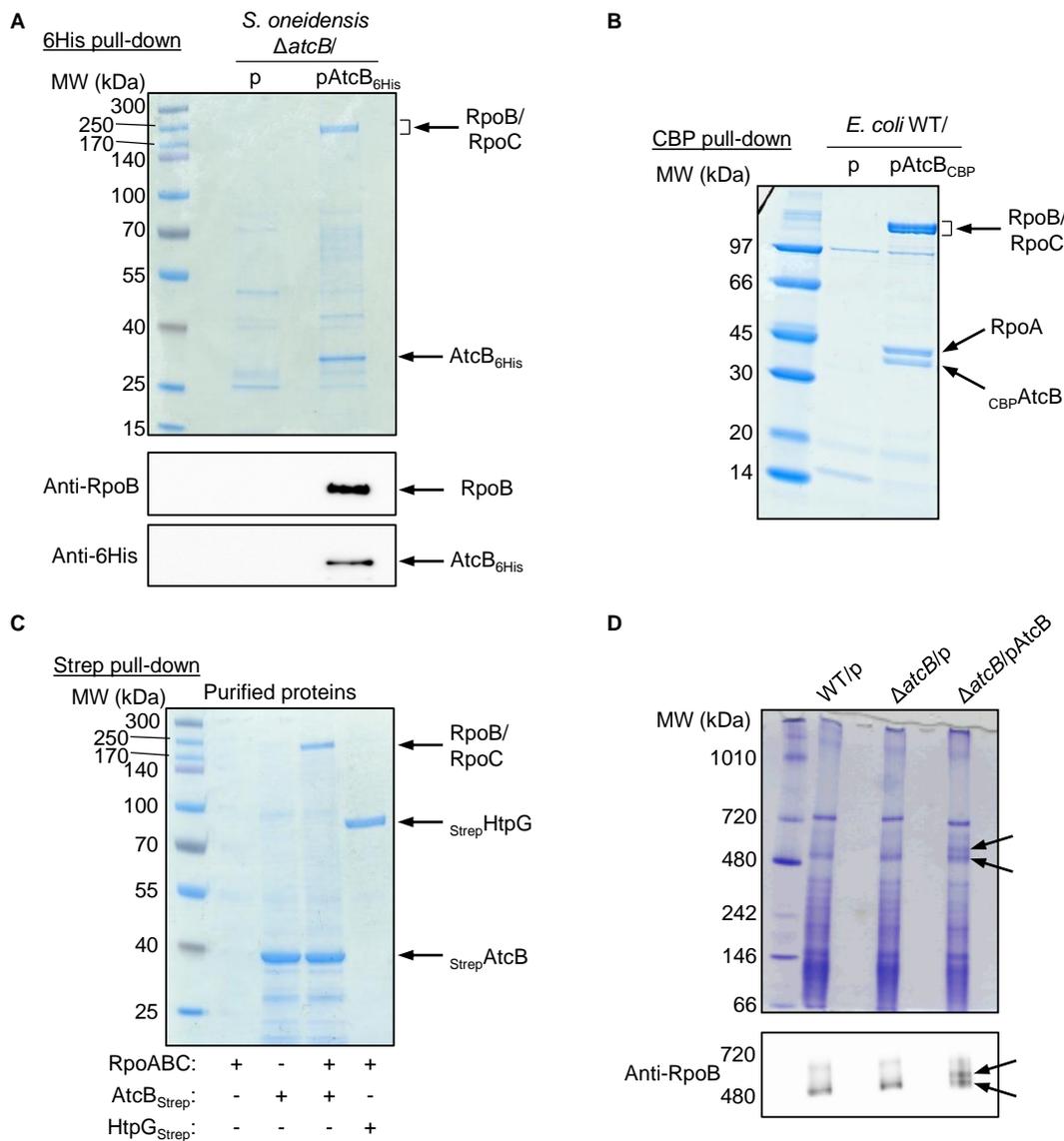
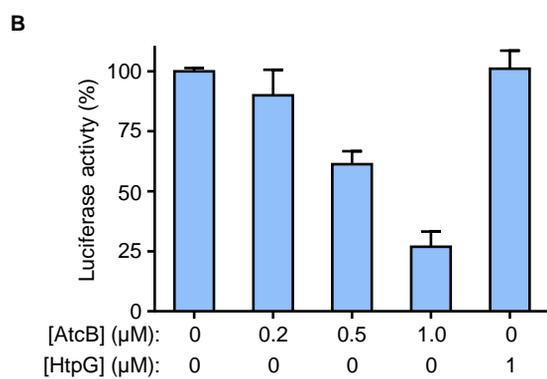
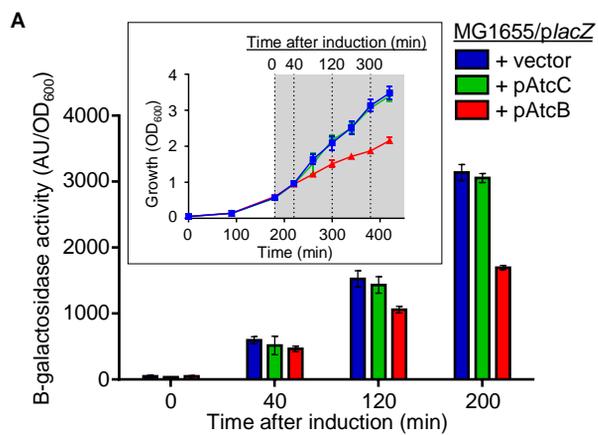


Figure 4



## Supplementary material

### Supplementary Materials and Methods

**Plasmid construction.** To construct pET-RpoA<sub>So</sub>, pET-RpoB<sub>So</sub> and pET-RpoC<sub>So</sub>, allowing the overproduction of RpoA<sub>6His</sub>, RpoB<sub>6His</sub> and RpoC<sub>6His</sub>, respectively, genes were PCR-amplified from the chromosome of MR1 [1] using forward oligonucleotides with the NdeI restriction site, and reverse nucleotides with the SacI restriction site and a sequence coding for a 6His tag (6 x CAC). After digestion, the DNA fragments were inserted by ligation into the pET24b vector (Novagen) digested with the corresponding restriction enzymes. The pBad24-CBP-AtcC plasmid was constructed by digesting pT25-AtcC [2] with XbaI and XhoI enzymes, and the digestion product was cloned into the pBad24-CBP vector [3] previously digested with the same enzymes.

**Western Blot.** To check in the wild-type MR1-R strain the production of AtcB<sub>6His</sub> and AtcC<sub>6His</sub> from pBad33-AtcB and pBad33-AtcC, respectively, cells were grown at 28°C, expression was induced with 0.2% (w/v) L-arabinose at OD<sub>600</sub>=0.8, and after 2 hours cells were collected. Total protein extracts were heat-denatured, separated by SDS-PAGE, and transferred to nitrocellulose membrane. Proteins were probed using anti-RpoB (Biolegend) or anti-6His (Thermo) antibodies, or with HisProbe-HRP (Thermo).

### Supplementary References

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## Supplementary Figure Legends

### Figure S1. AtcB overproduction in *S. oneidensis* is also toxic in liquid cultures

(related to Fig. 2A). *S. oneidensis* strains with a vector (p), or a plasmid allowing the overproduction of AtcB<sub>6His</sub> (pAtcB) were inoculated at OD<sub>600</sub>=0.02 with 25 µg/mL chloramphenicol and 0.2% arabinose. Strains were incubated at 28°C and OD<sub>600</sub> was measured with time.

### Figure S2. Mass spectrometry analyses (related to Fig. 3A). The band that was

cut out of the gel as indicated was analyzed by mass spectrometry. Indicated are the most abundant proteins that were identified. Coverage: percentage of the protein sequence covered by identified peptides. #PSMs: number of peptide spectrum matches. # Peptides: total number of distinct peptide sequences identified in the protein group. # Unique Peptides: number of peptide sequences that are unique to a protein group. MW: molecular weight (kDa). The β and β' subunits of the RNA polymerase are written in red.

**Figure S3. Mass spectrometry analyses (related to Fig. 3B).** Bands that were cut out of the gel as indicated were analyzed by mass spectrometry. Indicated are the most abundant proteins that were identified. Coverage: percentage of the protein sequence covered by identified peptides. #PSMs: number of peptide spectrum matches. # Peptides: total number of distinct peptide sequences identified in the protein group. # Unique Peptides: number of peptide sequences that are unique to a protein group. MW: molecular weight (kDa). The  $\beta$  and  $\beta'$  subunits of the RNA polymerase are written in red, the  $\alpha$  subunit in green, and AtcB in blue.

**Figure S4. Purified proteins used in the co-purification experiment in Fig. 3C.** The purified proteins AtcB (2  $\mu$ g), HtpG (2  $\mu$ g), and the complex RpoA/RpoB/RpoC (6  $\mu$ g total) were visualized on SDS-PAGE stained by Coomassie blue.

**Figure S5. Mass spectrometry analyses (related to Fig. 3D).** Bands that were cut out of the gel as indicated were analyzed by mass spectrometry. Indicated are the most abundant proteins that were identified. Coverage: percentage of the protein sequence covered by identified peptides. #PSMs: number of peptide spectrum matches. # Peptides: total number of distinct peptide sequences identified in the protein group. # Unique Peptides: number of peptide sequences that are unique to a protein group. MW: molecular weight (kDa). The  $\beta$  and  $\beta'$  subunits of the RNA polymerase are written in red, the  $\alpha$  subunit in green, and AtcB in blue.

Figure S1

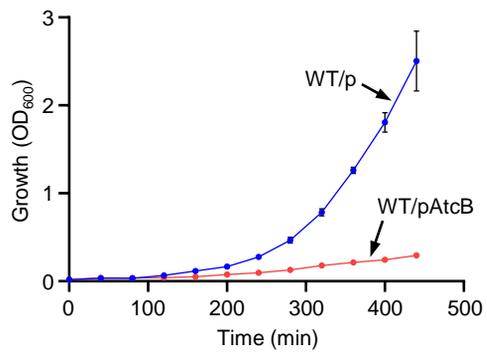
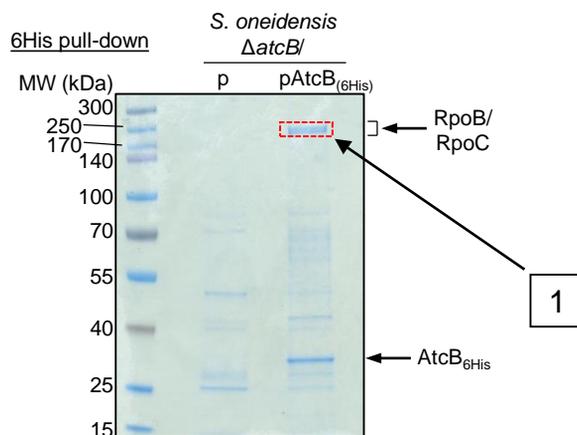
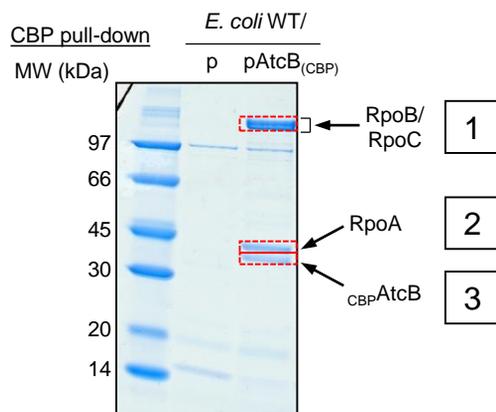


Figure S2



1	Description	Coverage	# PSMs	# Peptides	# Unique Peptides	MW [kDa]
	DNA-directed RNA polymerase beta' subunit RpoC [Shewanella oneidensis MR-1]	87	886	121	121	155,422
	DNA-directed RNA polymerase beta subunit RpoB [Shewanella oneidensis MR-1]	87	733	140	140	150,201
	putative surface lipoprotein (plasmid) [Shewanella oneidensis MR-1]	26	19	17	7	145,527
	tandem-95 repeat protein [Shewanella oneidensis]	15	17	14	4	145,837
	ATP-grasp domain-containing protein [Shewanella oneidensis MR-1]	15	15	15	15	167,518
	NAD-glutamate dehydrogenase [Shewanella oneidensis]	9	10	10	10	182,219

Figure S3



1

Description	Coverage	# PSMs	# Peptides	# Unique Peptides	MW [kDa]
RNA polymerase, beta prime subunit [OS=Escherichia coli str. K-12 substr. MG1655]	89	1769	147	147	155,063
RNA polymerase, beta subunit [OS=Escherichia coli str. K-12 substr. MG1655]	90	1438	151	123	150,538

2

Description	Coverage	# PSMs	# Peptides	# Unique Peptides	MW [kDa]
RNA polymerase, alpha subunit [OS=Escherichia coli str. K-12 substr. MG1655]	96	409	41	41	36,489
RNA polymerase, beta prime subunit [OS=Escherichia coli str. K-12 substr. MG1655]	17	22	14	14	155,063
RNA polymerase, beta subunit [OS=Escherichia coli str. K-12 substr. MG1655]	14	15	10	10	150,538

3

Description	Coverage	# PSMs	# Peptides	# Unique Peptides	MW [kDa]
Uncharacterized protein OS=Shewanella oneidensis (strain MR-1) GN=SO_1848 PE=4 SV=1	58	185	12	12	29,01
RNA polymerase, alpha subunit [OS=Escherichia coli str. K-12 substr. MG1655]	73	58	22	22	36,489

**Figure S4**

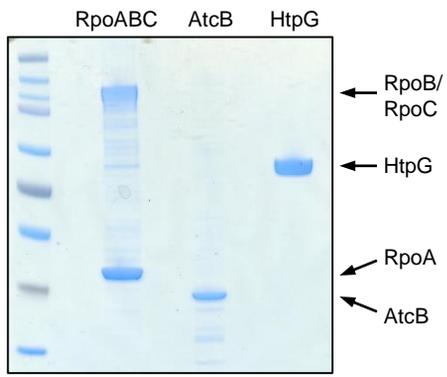
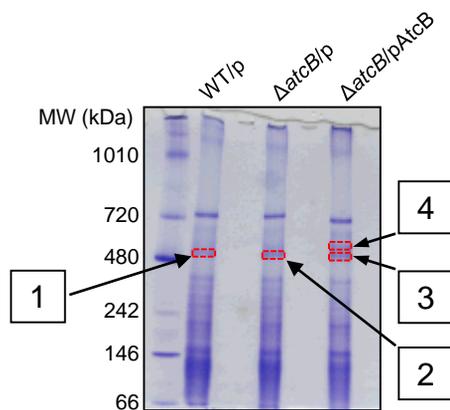


Figure S5



1	Description	Coverage	# PSMs	# Peptides	# Unique Peptides	MW [kDa]
	DNA-directed RNA polymerase subunit beta OS=Shewanella oneidensis (strain MR-1) GN=rpoB PE=3 SV=1	80	436	108	108	150,201
	DNA-directed RNA polymerase subunit beta' OS=Shewanella oneidensis (strain MR-1) GN=rpoC PE=3 SV=1	80	436	95	95	155,422
	DNA-directed RNA polymerase subunit alpha OS=Shewanella oneidensis (strain MR-1) GN=rpoA PE=3 SV=1	84	148	27	27	36,138
	Periplasmic metalloprotease OS=Shewanella oneidensis (strain MR-1) GN=SO_0578 PE=4 SV=1	48	53	29	29	88,218
	Probable cytosol aminopeptidase 2 OS=Shewanella oneidensis (strain MR-1) GN=pepA2 PE=3 SV=1	52	37	19	19	54,688
	Probable cytosol aminopeptidase 1 OS=Shewanella oneidensis (strain MR-1) GN=pepA1 PE=3 SV=2	59	37	23	23	52,995
	Glutamate dehydrogenase (NAD+) Gdh OS=Shewanella oneidensis (strain MR-1) GN=gdh PE=4 SV=1	26	36	31	31	182,219
	Elongation factor Tu 1 OS=Shewanella oneidensis (strain MR-1) GN=tuf1 PE=3 SV=1	51	32	13	1	43,32
	Elongation factor Tu 2 OS=Shewanella oneidensis (strain MR-1) GN=tuf2 PE=3 SV=1	52	30	13	1	43,257
	Phosphate acetyltransferase OS=Shewanella oneidensis (strain MR-1) GN=pta PE=3 SV=1	39	28	20	20	77,803

2	Description	Coverage	# PSMs	# Peptides	# Unique Peptides	MW [kDa]
	DNA-directed RNA polymerase subunit beta OS=Shewanella oneidensis (strain MR-1) GN=rpoB PE=3 SV=1	82	494	115	115	150,201
	DNA-directed RNA polymerase subunit beta' OS=Shewanella oneidensis (strain MR-1) GN=rpoC PE=3 SV=1	82	476	98	98	155,422
	DNA-directed RNA polymerase subunit alpha OS=Shewanella oneidensis (strain MR-1) GN=rpoA PE=3 SV=1	94	184	31	31	36,138
	Probable cytosol aminopeptidase 2 OS=Shewanella oneidensis (strain MR-1) GN=pepA2 PE=3 SV=1	52	39	19	19	54,688
	Phosphate acetyltransferase OS=Shewanella oneidensis GN=DI594_10830 PE=3 SV=1	42	37	22	22	77,833
	Elongation factor Tu 2 OS=Shewanella oneidensis (strain MR-1) GN=tuf2 PE=3 SV=1	56	28	14	1	43,257
	Inosine-5'-monophosphate dehydrogenase OS=Shewanella oneidensis (strain MR-1) GN=guaB PE=3 SV=1	52	24	15	15	51,652
	Elongation factor Tu 1 OS=Shewanella oneidensis (strain MR-1) GN=tuf1 PE=3 SV=1	55	26	14	1	43,32
	Periplasmic metalloprotease OS=Shewanella oneidensis (strain MR-1) GN=SO_0578 PE=4 SV=1	38	29	21	21	88,218
	Glutamate dehydrogenase (NAD+) Gdh OS=Shewanella oneidensis (strain MR-1) GN=gdh PE=4 SV=1	18	28	22	22	182,219

Figure S5 (continued)

3	Description	Coverage	# PSMs	# Peptides	# Unique Peptides	MW [kDa]
	DNA-directed RNA polymerase subunit beta' OS=Shewanella oneidensis (strain MR-1) GN=rpoC PE=3 SV=1	76	539	88	88	155,422
	DNA-directed RNA polymerase subunit beta OS=Shewanella oneidensis (strain MR-1) GN=rpoB PE=3 SV=1	75	487	90	90	150,201
	DNA-directed RNA polymerase subunit alpha OS=Shewanella oneidensis (strain MR-1) GN=rpoA PE=3 SV=1	81	231	24	24	36,138
	Probable cytosol aminopeptidase 2 OS=Shewanella oneidensis (strain MR-1) GN=pepA2 PE=3 SV=1	76	54	25	25	54,688
	Periplasmic metalloprotease OS=Shewanella oneidensis (strain MR-1) GN=SO_0578 PE=4 SV=1	62	52	32	32	88,218
	Glutamate dehydrogenase (NAD+) Gdh OS=Shewanella oneidensis (strain MR-1) GN=gdh PE=4 SV=1	39	51	43	43	182,219
	Elongation factor Tu 2 OS=Shewanella oneidensis (strain MR-1) GN=tuf2 PE=3 SV=1	57	34	14	1	43,257
	Inosine-5'-monophosphate dehydrogenase OS=Shewanella oneidensis (strain MR-1) GN=guaB PE=3 SV=1	56	28	16	16	51,652
	Phosphate acetyltransferase OS=Shewanella oneidensis GN=DI594_10830 PE=3 SV=1	53	31	24	24	77,833
	Elongation factor Tu 1 OS=Shewanella oneidensis (strain MR-1) GN=tuf1 PE=3 SV=1	57	32	14	1	43,32
	...					
	Uncharacterized protein OS=Shewanella oneidensis (strain MR-1) GN=SO_1848 PE=4 SV=1	59	21	10	10	29,01

4	Description	Coverage	# PSMs	# Peptides	# Unique Peptides	MW [kDa]
	DNA-directed RNA polymerase subunit beta' OS=Shewanella oneidensis (strain MR-1) GN=rpoC PE=3 SV=1	81	391	95	95	155,422
	DNA-directed RNA polymerase subunit beta OS=Shewanella oneidensis (strain MR-1) GN=rpoB PE=3 SV=1	83	391	111	111	150,201
	DNA-directed RNA polymerase subunit alpha OS=Shewanella oneidensis (strain MR-1) GN=rpoA PE=3 SV=1	94	132	29	29	36,138
	Phosphate acetyltransferase OS=Shewanella oneidensis GN=DI594_10830 PE=3 SV=1	35	31	18	18	77,833
	Glutamine synthetase OS=Shewanella oneidensis (strain MR-1) GN=glnA PE=3 SV=1	50	25	16	16	51,741
	Uncharacterized protein OS=Shewanella oneidensis (strain MR-1) GN=SO_1848 PE=4 SV=1	31	16	7	7	29,01
	Inosine-5'-monophosphate dehydrogenase OS=Shewanella oneidensis (strain MR-1) GN=guaB PE=3 SV=1	48	17	13	13	51,652
	Glutamate dehydrogenase (NAD+) Gdh OS=Shewanella oneidensis (strain MR-1) GN=gdh PE=4 SV=1	14	17	15	15	182,219
	Sulfate adenylyltransferase subunit 1 OS=Shewanella oneidensis (strain MR-1) GN=cysN PE=3 SV=2	28	10	10	10	52,902
	Elongation factor Tu 1 OS=Shewanella oneidensis (strain MR-1) GN=tuf1 PE=3 SV=1	34	11	8	1	43,32
	Elongation factor Tu 2 OS=Shewanella oneidensis (strain MR-1) GN=tuf2 PE=3 SV=1	34	11	8	1	43,257