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Interaction between *Bacillus subtilis* YsxC and ribosomes (or rRNAs)

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ABSTRACT

YsxC is an essential P-loop GTPase, that binds to the 50S ribosomal subunit, and is required for the proper assembly of the ribosome. The aim of this study was to characterize YsxC ribosome interactions.

The stoichiometry of YsxC ribosome subunit complex was evaluated. We showed that YsxC binding to the 50S ribosomal subunit is not affected by GTP, but in the presence of GDP the stoichiometry of YsxC-ribosome is decreased. YsxC GTPase activity was stimulated upon 50S ribosomal subunit binding. In addition, it is shown for the first time that YsxC binds both 16S and 23S ribosomal RNAs.

1. Introduction

Ribosomes are cellular organelles composed of approximately 60% ribosomal RNA (rRNA) and 40% protein that catalyze protein synthesis in the cell. The bacterial 70S ribosome contains about 55 ribosomal proteins (depending on the species) and 3 ribosomal RNAs, organized into two subunits: the small (30S) and large (50S) ribosomal subunits. Formation of the ribosomal particle involves a complex series of processes, *i.e.*, synthesis, processing and modification of both rRNA [1,2] and ribosomal proteins [3,4], and assembly of the components. Recently, by using quantitative mass spectrometry, Chen and Williamson [5] were able to provide a clear picture of in vivo 30S and 50S assembly. A variety of non-ribosomal factors are involved in the ribosome biogenesis process. Among them, several phosphate-binding loop (P-loop) GTPases (YlqF/RbgA [6,7], CgtAE/ObgE [8,9], YphC [10] and YsxC [10,11]) have been suggested to be necessary for bacterial ribosome assembly because dissociated ribosomal subunits accumulate in cells depleted in these GTPases.

YsxC is an essential P-loop GTPase in in *Escherichia coli* [12,13], *Bacillus subtilis* [14] and *Staphylococcus aureus* [11]. The protein from *B. subtilis* [15,16] was crystallized as a single domain protein of 22kDa. The protein undergoes conformational changes during nucleotide binding [16]. YsxC associates primarily with the 50S subunit of the ribosome [11,17] and, when expressed in *E. coli*, co-purifies with the ribosomal fraction [17]. In this study, we thoroughly characterized YsxC association with the ribosome, by examining the role of nucleotides, quantifying the number of YsxC molecules bound to the ribosomal subunits, and investigating YsxC binding to the ribosomal RNAs.

2. Materials and methods

2.1. Preparation of recombinant and wild-type YsxC proteins

Recombinant *B. subtilis* $(His)_6$YsxC was cloned, expressed and purified to homogeneity, as described [17], and stored in 50 mM NaPO4 buffer, pH 8, containing 15% (v/v) glycerol and either 0.75 M NaCl or 0.5 M KCl.

Mutants in the P-loop motif were generated by the Quickchange protocol (Stratagene) with the pET15b-*ysxC* [17] used as a template, and the oligonucleotides indicated (Supplementary Table 1). The constructs were confirmed by DNA sequencing (Genome express). Standard genetic techniques were used [18]. Expressions were done at 18 °C. Purifications of the mutants were the same as for the wild-type protein.

Abbreviations: P-loop, phosphate-binding loop; GMPPNP, guanylyl-imidodiphosphate; rRNA, ribosomal RNA; Tm, melting temperature; TSA, thermal shift assay; DTT, dithiothreitol

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2.2. Assay of GTPase activity

GTPase activity was followed by spectrophotometric recording of NADH oxidation in the presence of a GTP-regenerating system, at 37 °C. Enzyme (20 μg) was added to the assay medium (50 mM $NaPO_4$, pH 8.0, 0.15 M NaCl, 30 mM KCl, 4 mM phosphoenolpyruvate, 0.4 mM NADH, 8 μg of pyruvate kinase, 4 μg of lactate dehydrogenase, 2 mM $MgCl_2$, and 1 mM GTP, 200 μl final volume), as detailed [19]. GTP was regenerated from GDP by pyruvate kinase, with a concomitant production of pyruvate from phosphoenolpyruvate. Pyruvate was then converted to lactate by lactate dehydrogenase, with, concomitantly, NADH oxidation to NAD^+, which was followed by the change in absorbance at 340 nm for 10 min ($\varepsilon NADH_{340nm}$ = 6220 (mol^{-1} cm^{-1}). The experiments were performed with a Safas UVmc^2 spectrophotometer. Control experiments were systematically performed in the absence of nucleotides (protein alone) or in the absence of proteins (nucleotides alone) and the rates of NADH disappearance in all cases were negligible. The activities were expressed as μmol GTP hydrolyzed/min/mg protein.

2.3. Ribosome purification and preparation of 30S and 50S subunits

Ribosomes from *B. subtilis* (strain 168) were prepared following the detailed protocol of Fechter et al. [20] and separated on a 5–20% sucrose gradient into 30S and 50S subunits, as described [17]. 30S and 50S subunits fractions were stored at −80 °C (final concentration: 12 μM).

2.4. Binding of YsxC to ribosome

A filtration-based binding assay was used [21]. YsxC was incubated with 0.2 μM ribosome in 80 μl of 30 mM Tris–HCl, pH 7.5, 10 mM Mg acetate, 60 mM NH_4Cl, 60 mM KCl, 2.5 mM dithiothreitol (DTT) at 30 °C for 10 min. The mixture was applied onto Centricon YM-100 (Millipore) which was centrifuged for 5 min at 3000*g* to retain the ribosome-bound YsxC, and washed twice with 100 μl of the same buffer. 40 μl of buffer was applied onto the filter for 1 min and the ribosome-bound YsxC was collected from the inverted filter by centrifugation (3000*g* for 1 min). The recovered YsxC was detected by Western blotting using an India™ HisProbe-HRP (Pierce) and quantified. Control experiments without ribosomes were done.

2.5. In vitro synthesis of 5S, 16S, and 23S RNA

5S, 16S, and 23S DNAs were amplified from *B. subtilis* genomic DNA by PCR using the primers depicted in Supplementary Table 2. The cloning into the pET21 vector (Novagen) of the amplified products (116, 1470, and 2926 bp for 5S, 16S, and 23S DNA, respectively) were done according established methods [18]. All constructs were checked by sequencing.

In vitro transcription reactions were realized using the RiboMAX™ Large scale RNA Production Systems kit (Promega). DNA templates were linearized by *EcoRI* (5S DNA) or *XhoI*, (16S and 23S RNA), prior to in vitro transcription at 37 °C for 4 h. In vitro transcripts were further purified using the MEGAclear™ kit (Ambion). The quality of in vitro transcripts was examined by denaturing gel electrophoresis (1% agarose for 16S and 23S RNA) or 6% acrylamide gel electrophoresis for 5S RNA). RNA concentration was determined by absorbance at 260 nm.

2.6. 5′-end rRNA biotinylation

rRNAs were dephosphorylated by calf intestine phosphatase (CIP, 0.01 unit/pmole of 5′ end of RNA) at 37 °C for 1 h. CIP was removed by phenol extraction. After ethanol precipitation, RNAs were phosphorylated at 37 °C for 1 h by T4 polynucleotide kinase using UTP-biotin (10 units polynucleotide kinase/100 pmol RNA, 25 pmol Biotin-11-UTP (Fermentas)). Biotinylated 16S and 23S rRNAs were further purified using the MEGAclear™ kit (Ambion). 5S rRNA was purified by ammonium acetate precipitation.

2.7. rRNA blot overlay assay

rRNA blot overlay assays were performed following the protocol described by Palaniyandi et al. [22]. In brief, after YsxC migration on a 14% SDS–PAGE and electrotransfer to nitrocellulose, the membranes were washed three times 10 min and incubated in binding buffer (50 mM Tris–HCl, pH 8.0, 150 mM KCl, 0.5 mM DTT, 1 mM EDTA, 10 μg/ml *E. coli* tRNA) in the presence of biotinylated rRNA at 4 °C for 16 h. The blots were washed three times with binding buffer at room temperature, 5 min per wash. Biotin-labeled RNA was detected using the Biotin Chromogenic Detection Kit (Fermentas).

2.8. Thermal shift assay (TSA)

The assay used a real-time PCR machine (CFX Manager), the iCycler iQ Real-Time Detection System (Bio-Rad), which monitors fluorescence changes of sypro orange dye (excitation/emission: 490/575 nm) as it interacts with the protein undergoing thermal unfolding, in thin-walled 96-well PCR plates. Each well (25 μl) contained 2 μg protein and 2 μl of the fluorescent Sypro orange dye solution (Molecular Probes, 500× in DMSO, diluted 5 times in water), in 50 mM $NaPO_4$, pH8.0, 0.15 M NaCl and was heated from 20 to 100 °C in 0.2 °C steps.

3. Results and discussion

3.1. The stoichiometry of YsxC-ribosome

We had shown by sucrose density gradient that YsxC associated mainly with the free 50S ribosomal subunit in the absence of exogenous nucleotides [17]. However, the amount of bound YsxC had not been quantified previously. To better characterize YsxC-ribosome associations, the stoichiometry of the complex was evaluated, using a filtration-based binding assay [21].

The number of YsxC molecules bound per 70S, 50S or 30S particle was determined at different YsxC-particle ratios in the binding assay (up to 10-fold YsxC excess) in buffer 1 (Fig. 1a) (30 mM Tris–HCl, pH 7.5, 10 mM Mg acetate, 60 mM NH_4Cl, 60 mM KCl, 2.5 mM DTT). Control experiments without ribosomes were done to check that there was no non-specific interaction between YsxC and the filter membrane (Fig. 1a). The stoichiometry of the YsxC-50S complex was determined to be 1 and a high excess of YsxC did not significantly affect this ratio (see Table 1 and Ref. [23] for establishing the amount of recovered protein and ribosome). By contrast, there was no YsxC binding to 30S, and a faint binding to 70S ribosome (perhaps due to partial ribosome dissociation) (Fig. 1a).

We also investigated a buffer we used in a former study (buffer 2: 10 mM Tris–HCl, pH 7.4, 10 mM Mg acetate, 30 mM KCl, [17]), and the buffer used by Nakano et al. [21], (buffer 3: 10 mM Tris–HCl, pH 7.4, 8.2 mM Mg acetate, 50 mM NH_4Cl, 1 mM DTT, 0.3 mM EDTA), for measuring YlqF binding to the 50S subunit by the same filtering technique (Fig. 1b and 1c and Table 1). The ionic strength of buffer 2 was lower than that of buffer 1, resulting in more YsxC binding to all ribosomal particles (Table 1). YsxC had a strong tendency to stick to the ribosomal subunits in buffer 3: the more YsxC we added, the more binding we observed (Fig. 1c). As the ionic strengths of buffers 1 and 3 were comparable,

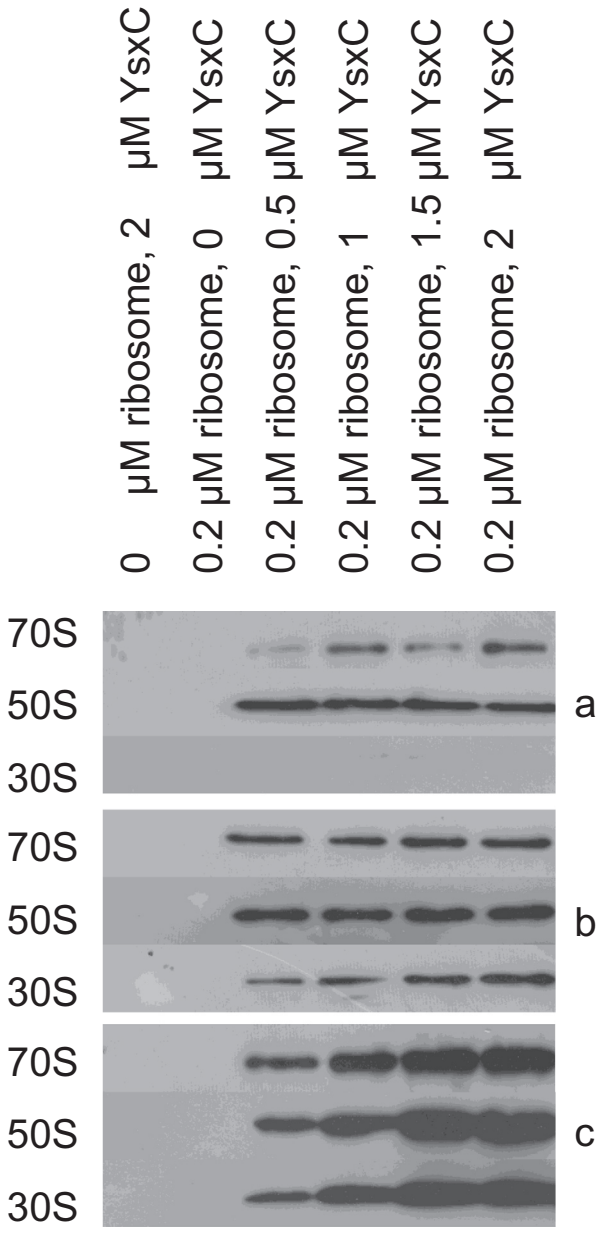

Fig. 1. Binding of YsxC to 70S ribosome or each subunit in three different buffers. A filtering technique was used. YsxC in the range of 0–2 μM was incubated in the presence (0.2 μM) or absence of ribosome at 30 °C for 10 min in different buffers, (a) buffer 1: 30 mM Tris–HCl, pH 7.5, 10 mM Mg acetate, 60 mM NH_4Cl, 60 mM KCl, 2.5 mM DTT, (b) buffer 2: 10 mM Tris–HCl, pH 7.4, 10 mM Mg acetate, 30 mM KCl [17], (c) buffer 3: 10 mM Tris–HCl, pH 7.4, 8.2 mM Mg acetate, 50 mM NH_4Cl, 1 mM DTT, 0.3 mM EDTA [21]. The mixture was applied to a Centricon YM-100 filter (Millipore). After centrifugation and washing of the filter, ribosome-bound YsxC was recovered from the inverted filter by centrifugation. Binding to ribosome was analyzed by Western blotting.

we presumed that KCl might protect YsxC against denaturation, thus preventing YsxC to "stick" to the 50S particle. Association of another GTPase (the CgtAC protein from *Caulobacter crescentus*) with the 50S ribosomal particle was also shown to be dependent on both the salt concentration and the magnesium counter ion used in the assay [24].

In order to investigate KCl putative role to protect YsxC against denaturation, thermal shift assay (TSA) experiments were performed in the presence of increasing concentrations of NaCl or KCl (Fig. 2). The melting temperature (Tm) of YsxC in the presence of 0.15 M (final concentration) NaCl was 40.8 °C. When KCl was used instead of NaCl, the observed Tm was 53.7 °C (Table 2), indicating that KCl has a more stabilizing effect on YsxC than NaCl at this concentration. This protective effect of KCl is consistent with its intracellular concentration found in bacteria: 200 mM in *E. coli* [25] and 400 mM in *B. subtilis* [26]. The stabilizing effect of these two salts further increased as the salt concentration raised (Fig. 2 and Table 2). Higher concentrations of either KCl or NaCl (from 0.25 M onwards) turned out to be equally beneficial for the protein stability. In preliminary experiments, we noticed that we did not obtain TSA curves of satisfactory quality when the protein was prepared and stored in a 0.15 M NaCl buffer. Consequently, the protein was subsequently stored at −80 °C in phosphate buffer either in the presence of 0.75 M NaCl, or 0.5 M KCl, and was diluted just before the experiments allowing us to obtain reproducible data for the denaturation curves. Upon dilution of YsxC at a final concentration of either 0.15 M NaCl or KCl, the protein was found to be stable at 4 °C for at least 1 week, as checked by thermal denaturation curves (data not shown).

YsxC is a so-called HAS (Hydrophobic Amino acid Substituted for catalytic glutamine)-GTPase [27], because a hydrophobic amino acid (Y 79) is found instead of a catalytic glutamine otherwise present in many GTPases (e.g. Q61 in Ras) [28]. Interactions with potassium have been reported to enhance the GTPase activity of some of these GTPases, such as EngA [19,29], YqeH [30], RbgA [31], and MnmE [32,33]. However, we were unable to measure any increase in the GTPase activity of YsxC in the presence of KCl concentration up to 400 mM. Potassium displays a stabilizing effect on YsxC, but has no effect on the GTPase activity of the protein.

3.2. Influence of exogenous nucleotides on the YsxC-50S subunit binding

Whereas most GTPases bind to the ribosome in their active GTP-bound state, YsxC is able to interact with the 50S subunit in its nucleotide-free state [17]. Only a few exceptions including Era, which associates with the 30S ribosomal subunit, and HflX, which binds to the 50S ribosomal subunit, are known to be able to interact with the ribosome in the absence of nucleotide [34,35].

Table 1
Influence of the buffer composition on the stoichiometry of the YsxC bound per particle. The number of YsxC molecules bound per ribosome was estimated by analysis of in vitro binding of a 5-fold excess of YsxC to the 50S subunit. YsxC (1 μM) was preincubated with 0.2 μM ribosomal particle at 30 °C for 10 min, applied to Microcon 100 (Millipore), and centrifuged. The 50S-bound YsxC complex was recovered by centrifugation of the inverted column. To establish the stoichiometry, we first measured the absorbance of the recovered ribosome-YsxC at 260 nm with a nanodrop apparatus. The absorbance of a 0.2 μM 30S, 50S, and 70S ribosome solution at 260 nm for a path length of 1 cm is 2.9, 5.8, and 8.7, respectively [23] and the one of a 0.2 μM YsxC solution is 0.007 at 260 nm. YsxC absorbance being negligible, ribosome concentration can be determined by measuring the absorbance at 260 nm. Then, each concentration of YsxC was determined by separate quantitative Westerns using known amounts of YsxC to generate standard curves and the amount was analyzed by Western blotting. The experiments were done in triplicate and the mean value ± the standard deviation is indicated.

Buffer	YsxC bound per particle		
	70S	50S	30S
Buffer 1	0.3 ± 0.2	1 ± 0.3	0
Buffer 2	2 ± 0.6	3 ± 1	1 ± 0.3
Buffer 3	4 ± 0.7	8 ± 2	4 ± 1

Buffer 1 (this study): 30 mM Tris–HCl, pH 7.5, 10 mM Mg acetate, 60 mM NH_4Cl, 60 mM KCl, 2.5 mM DTT. Buffer 2 [17]: 10 mM Tris–HCl, pH 7.4, 10 mM Mg acetate, 30 mM KCl. Buffer 3 [21]: 10 mM Tris–HCl, pH 7.4, 8.2 mM Mg acetate, 50 mM NH_4Cl, 1 mM DTT, 0.3 mM EDTA.

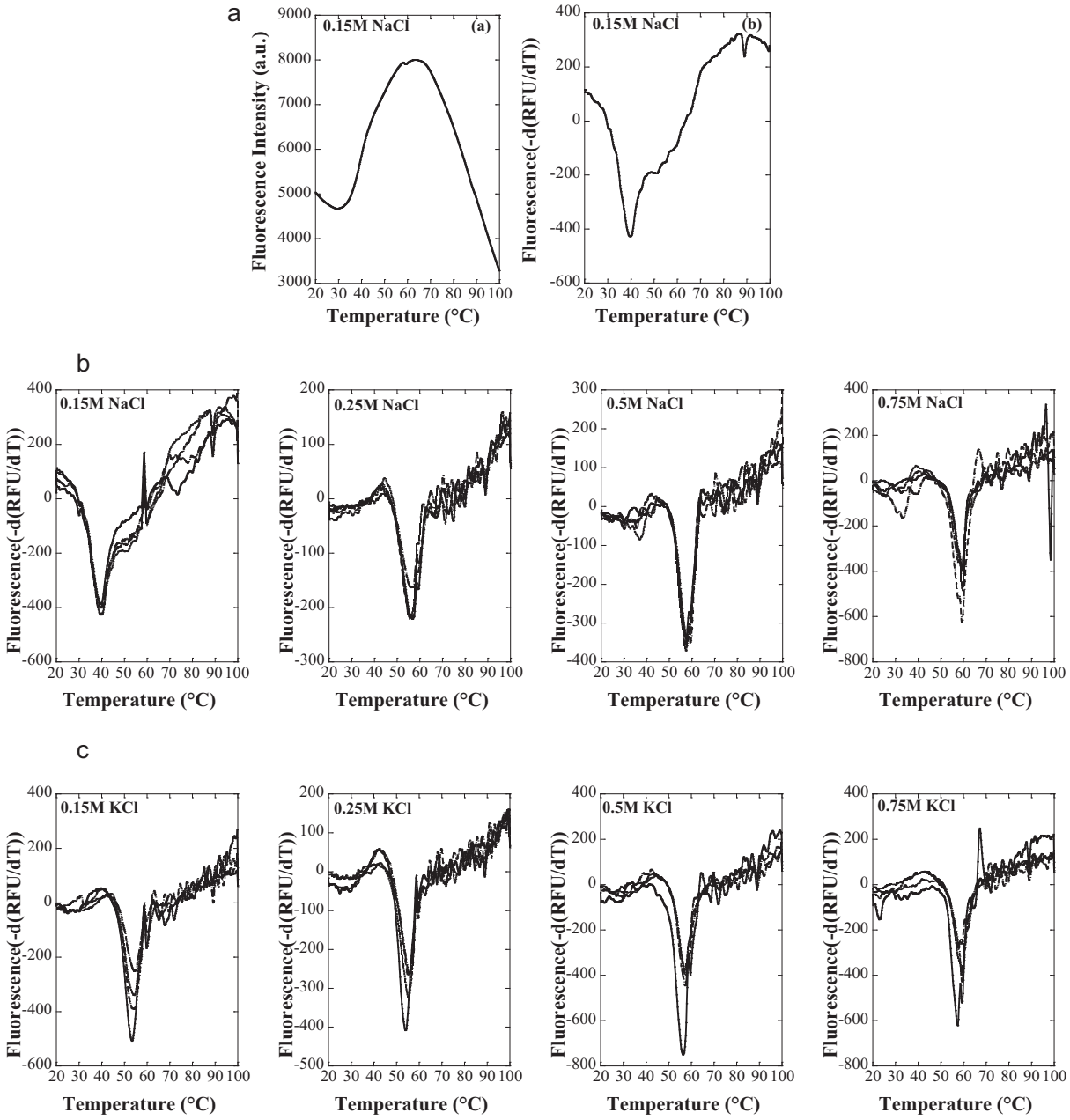

Fig. 2. YsxC thermal shift assays (TSA). (a) Example thermal shift data of YsxC showing the raw data curve alongside the negative first derivative curve. Protein melting profiles were monitored (see Section 2). The assay includes the presence of sypro orange dye, the fluorescence of which is exacerbated upon binding to hydrophobic patches of unfolded protein. As the temperature gradually increases, and above a certain threshold, YsxC starts to unfold and exposes its hydrophobic regions, resulting in dye binding and an increased fluorescence signal of the dye. At higher temperature, the protein is completely unfolded and begins to aggregate, protein aggregates accumulate and clump together. The dye is quenched, resulting in a decrease in fluorescence. The melting temperature of the protein (Tm) is obtained at the midpoint of the melting curve (figure (a) on the left). This value corresponds to the minimum of the negative derivative curve of the thermal denaturation data (figure (b) on the right). The melting temperature is an indicator of protein stability and can be used to optimize conditions that minimize protein denaturation. (b) Influence of increasing concentrations of NaCl (0.15–0.75 M) on YsxC melting temperature. (c) Influence of increasing concentrations of KCl (0.15–0.75 M) on YsxC melting temperature.

Table 2
Thermal stability of YsxC in the presence of increasing concentrations of KCl and NaCl. Tm values were displayed by the software (iCycler iQ Real-Time Detection System, Bio-Rad) from the raw data curves and the negative derivative curves. All TSA experiments were done in quadruplicate, in 50 mM $NaPO_4$, pH8.

KCl (final concentration)	0.15 M	0.25 M	0.5 M	0.75 M
Tm (°C)	53.7 ± 0.3	55 ± 0.7	57.1 ± 0.3	59.3 ± 0.08
NaCl (final concentration)	0.15 M	0.25 M	0.5 M	0.75 M
Tm (°C)	40.8 ± 0.2	55.8 ± 0.6	57.6 ± 0.07	59.3 ± 0.15

The effect of the absence or the presence in the binding buffer of excess (200 μM) GDP, GTP, or guanylyl-imidodiphosphate (GMPPNP) (a non-hydrolysable GTP analog) on the stoichiometry of YsxC-50S ribosomal subunit complex in buffer 1 was evaluated, using the same filtration-based binding assay (see Section 2). The stoichiometry of 1 mol YsxC/mole 50S ribosomal subunit was independent of the absence or the presence of GTP or GMPPNP in the binding buffer (Fig. 3). However, GDP-YsxC had a reduced binding ability to 50S particle, since the ratio of bound YsxC/50S particle never exceeded 0.5 (Fig. 3).

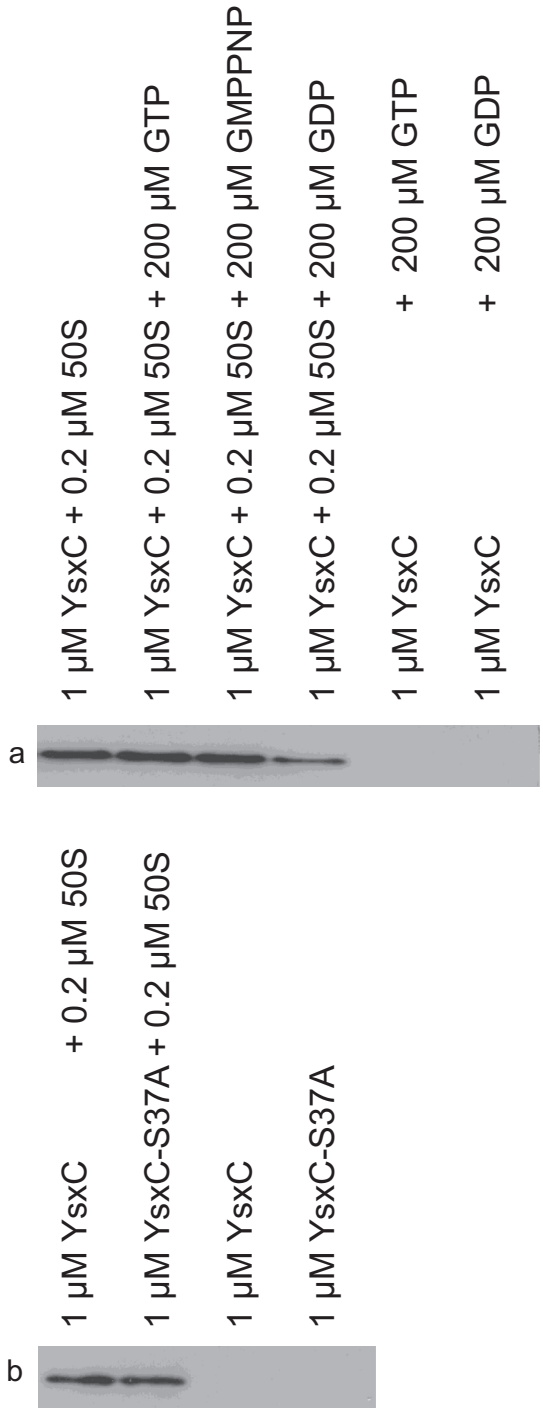

Fig. 3. Binding of YsxC to the 50S ribosomal subunit, as influenced by addition of nucleotides or by YsxC GTPase activity. (a) Influence of exogenous nucleotides on the binding of YsxC bound to the 50S particle. YsxC (1 μM) was preincubated with 0.2 μM ribosomal 50S particle at 30 °C for 10 min, in the absence or presence of either 200 μM GTP, GMPPNP, or GDP in buffer 1 (30 mM Tris–HCl, pH 7.5, 10 mM Mg acetate, 60 mM NH_4Cl, 60 mM KCl, 2.5 mM DTT), applied to Microcon 100 (Millipore), and centrifuged. Nucleotides (200 μM, final concentration) were added in the washing and elution buffers. The 50S-bound YsxC complexes were recovered by centrifugation of the inverted column. Binding to ribosome was analyzed by Western blotting. Control experiments without ribosomes (1 μM YsxC + 200 μM GTP and 1 μM YsxC + 200 μM GDP) are shown. (b) Binding of an GTPase inactive mutant of YsxC to the 50S particle. YsxC or YsxC-S37A were preincubated with 0.2 μM ribosomal 50S particle at 30 °C for 10 min in buffer 1 and binding to the 50S subunit was analyzed by filtering experiment, as explained above. Control experiments without ribosome (1 μM YsxC and 1 μM YsxC-S37A) are shown.

We reported in an earlier study [17] that YsxC binding to the 50S ribosomal subunit of *B. subtilis* was stronger in the presence of the non-hydrolysable GTP analogue GMPPNP.

In that study, another technique had been used, as YsxC-50S complex had been layered on a sucrose gradient and centrifuged at 200000*g* for 190 min. This lapse of time might allow YsxC to slowly hydrolyse GTP into GDP, resulting in a possible dissociation of YsxC from the 50S particle during the ultracentrifugation step, consistent with the presence of YsxC throughout many of the early fractions [17].

3.3. Ribosome binding of a Walker A mutant of YsxC

YsxC mutants that targeted conserved residues within the Walker A (YsxC-K36A, YsxC-S37A) or the conserved Asp of the DXXG motif (YsxC-D75A) were created. These mutations are supposed to alter YsxC GTPase activity. We were unable to obtain soluble proteins for the YsxC-K36A and YsxC-D75A mutants, whereas YsxC-S37A mutant was obtained in a soluble form.

YsxC-S37A binding to the 50S ribosomal subunit was performed with no added exogenous nucleotide and measured by the filtering technique, as described in Section 2. This mutation had no effect on the protein ability to bind to 50S particles (Fig. 3b).

The fact that the protein, following the mutation of a conserved residue in the Walker A motif is still able to bind the 50S ribosomal particle corroborates the independence of YsxC-ribosome interaction on the presence of exogenous nucleotides.

3.4. GTP-hydrolyzing activity of YsxC is stimulated by the 50S ribosomal particle

The intrinsic GTPase activity of YsxC is very low (0.004 μmol min^{-1} mg^{-1}, or 0.09 mmol min^{-1} $mmol^{-1}$), but in the same range as the one of Era from *E. coli* (0.01–0.02 mmol min^{-1} $mmol^{-1}$ [36]), CpgA from *B. subtilis* (0.05 mmol min^{-1} $mmol^{-1}$ [37]), or ras p21 (0.005–0.006 mmol min^{-1} $mmol^{-1}$, [38]). When 1 μM 50S particles was added, YsxC activity raised by a 3.7- to 9-fold factor (0.33–0.83 mmol min^{-1} $mmol^{-1}$). The stimulation of YsxC GTPase activity is in the same order of magnitude than the stimulation observed for HflX upon binding to the 50S (8-fold increase for the hydrolysis of GTP [35]) but lower than that observed for 70S-bound CpgA (50-fold [37]). By contrast, no enhancement in YsxC activity could be detected in the presence of 30S particles.

As expected, the Walker A mutant (S37A) displayed no measurable GTPase activity, either in the presence or in the absence of 50S particles. This is consistent with the fact that mutation of the equivalent residue in other GTPases has been found to significantly alter the hydrolytic activity [39]. The lack of GTPase activity in YsxC Walker A mutant strongly supports the conclusion that the measured activity detected in the wild-type YsxC preparation is indeed borne by this protein and not due to some contaminant(s), YsxC-S37A mutant being purified to the same degree of homogeneity than the wild-type enzyme (Supplementary Fig. 1).

3.5. YsxC binds 16S and 23S rRNAs

To examine whether YsxC associates with the rRNA, YsxC, bound to a nitrocellulose membrane, was incubated in binding buffer containing biotinylated ribosomal RNAs. After an extensive wash of the membrane, the presence of bound rRNA was detected by specific biotin chromogenic detection. The results revealed that YsxC wild-type is able to bind to both the 16S and 23S rRNA, but not to the 5S RNA (Fig. 4). The Walker A YsxC mutant behaves simi-

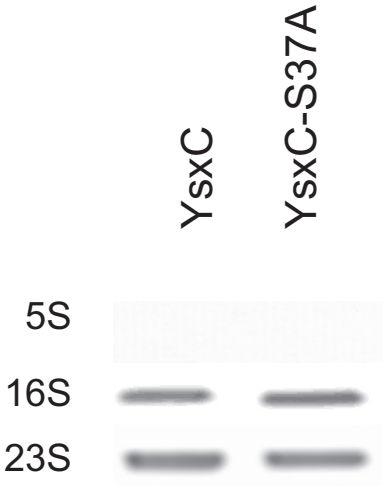

Fig. 4. YsxC or YsxC-S37A was electrotransferred to a nitrocellulose membrane, washed three times with binding buffer containing yeast tRNA to avoid unspecific binding (10 min per wash) and incubated with biotin-labeled RNA transcripts, as described in Section 2. Biotin-labeled RNA was detected using the biotin chromogenic detection kit from Fermentas.

larly as the wild-type protein (Fig. 4). Again, YsxC shows similarities with Hflx, since this protein binds both the 16S and 23S rRNA [35] while it specifically associates with the 50S ribosomal particles [35,40]. Another GTPase, Obg/CgtA, that binds to the 50S subunit [24,41] and to the 30S subunit [8], has also been reported to bind both the 16S and 23S rRNA [8].

The binding of YsxC to 23S rRNA is consistent with the interaction of the protein with the 50S ribosomal subunit. The protein probably has motives or domains that are responsible for this binding. The fact that YsxC is also able to bind the 16S RNA can suggest that the protein recognizes secondary structures in naked 23S or 16S rRNA rather than a specific sequence, although YsxC associates also with the small ribosomal subunit under non-stringent conditions (Fig. 1b). YsxC interaction with ribosomal RNA strongly supports a role for this protein in the assembly of both ribosomal subunits. Earlier YsxC depletion experiments resulting in the accumulation of ribosomal large subunit intermediates sedimenting slightly slower than 45S [10] are also in favor of YsxC requirement for 50S ribosome assembly.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2015.03.001.

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