Biochemical and Biophysical Research Communications 386 (2009) 602–606

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Circularly permuted GTPase YqeH binds 30S ribosomal subunit: Implications for its role in ribosome assembly

Baskaran Anand, Parag Surana, Sagar Bhogaraju, Sushmita Pahari, Balaji Prakash *

*Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur 208016, India*

ARTICLE INFO

*Article history:*
Received 8 June 2009
Available online 18 June 2009

*Keywords:*
Ribosome assembly
cpGTPase
RNA chaperone
GTP hydrolysis
Circular permutation
Strand dissociation
Strand annealing
RNA folding
Ribosome binding
RNA binding

ABSTRACT

YqeH, a circularly permuted GTPase, is conserved among bacteria and eukaryotes including humans. It was shown to be essential for the assembly of small ribosomal (30S) subunit in bacteria. However, whether YqeH interacts with 30S ribosome and how it may participate in 30S assembly are not known. Here, using co-sedimentation experiments, we report that YqeH co-associates with 30S ribosome in the GTP-bound form. In order to probe whether YqeH functions as RNA chaperone in 30S assembly, we assayed for strand dissociation and annealing activity. While YqeH does not exhibit these activities, it binds a non-specific single and double-stranded RNA, which unlike the 30S binding is independent of GTP/GDP binding and does not affect intrinsic GTP hydrolysis rates. Further, S5, a ribosomal protein which participates during the initial stages of 30S assembly, was found to promote GTP hydrolysis and RNA binding activities of YqeH.



## Introduction

Circularly permuted GTPases (cpGTPases) represent a class of GTPases that display a curious circular permutation in the order of sequence motifs [1,2]. They represent various domains of life and are categorized into four subfamilies represented by proteins *Escherichia coli* YjeQ (RsgA), *Bacillus subtilis* YlqF (RbgA) and YqeH and *Saccharomyces cerevisiae* YawG [1,2]. In the past, efforts to elucidate the role of cpGTPases have shed light on their likely function in ribosome biogenesis [3–9]. YjeQ (RsgA) interacts with the 30S subunit of ribosome in presence of a GTP analog and it was shown that the GTPase activity is stimulated by 30S binding [3,5]. NOG2 and LSG1 are eukaryotic members of YawG subfamily and associate with the 60S pre-ribosomal particle [6,7]. YlqF (RbgA) participates in the late step of 50S subunit assembly in *B. subtilis* and its GTPase activity too was found to be stimulated by 50S binding [8,9].

YqeH, representing one of the four subfamilies of cpGTPases and distributed across bacterial and eukaryotic genomes including humans [1,2], was shown to participate in 30S biogenesis in *B. subtilis* [10,11]. An ortholog of YqeH in *Arabidopsis thaliana* was initially reported to be a plant Nitric Oxide Synthase (AtNOS1) [12]. It was later renamed as Nitric Oxide Associated protein (AtNOA1), since the NOS activity could not be reproduced [13–15]. Recently, it was confirmed to be a functional cpGTPase and not a Nitric Oxide Synthase [16]. In addition, studies on plastid encoded RIF1, an ortholog of YqeH in *A. thaliana*, shows that deletion of *rif1* gene impairs plastome encoded protein synthesis [17]. Interestingly, it was observed that YqeH from *B. subtilis* heterologously restores the mutant phenotype of *rif1*. These studies strengthen the view that YqeH performs a ribosome associated function. However, unlike other cpGTPase subfamilies such as YjeQ(RsgA) and YlqF(RbgA), direct association between YqeH and ribosomal subunits was not reported and the precise role of YqeH in ribosome assembly remains elusive. Here, we show that YqeH co-associates with the 30S ribosomal subunit and that this association is more stable in presence of GTP or its non-hydrolysable analog GDPNP. We also find that the N and C-terminal domains in this protein, mediate ribosome/RNA binding. Unlike YjeQ(RsgA) and YlqF(RbgA), a distinctive feature of YqeH is that the ribosome/RNA association does not influence its GTP hydrolysis rates. On the other hand, in line with 30S binding, we observe increased GTP hydrolysis rates in the presence of S5, a ribosomal protein known to participate in the early stages of 30S assembly. We also find that YqeH binds single and double-stranded RNA in a nucleotide independent fashion. We envisaged an RNA chaperone like activity for YqeH; however, we find that it does not exhibit RNA strand dissociation and annealing activities.

* Corresponding author. Fax: +91 512 2594010.
*E-mail address:* bprakash@iitk.ac.in (B. Prakash).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.06.078

## Materials and methods

*Co-sedimentation assay.* GST-tagged YqeH and its deletion constructs, GST-YlqF and His-S5 were cloned, overexpressed and purified to homogeneity. Crude ribosomes were purified from *B. subtilis*. Briefly, a reaction mixture, consisting of crude ribosomes ($A_{260}$ = 2), 500 nM protein (GST-YqeH or its derivatives; GST-YlqF; GST) and 1 mM nucleotide (GTP/GDP/GDPNP), and incubated at 37 °C for 30 min, was layered on a linear sucrose gradient (18–50%). Following centrifugation at 90,000*g*, samples were fractionated and resolved using SDS–PAGE. The presence of the proteins was detected using an anti-GST antibody in a Western blot. For details refer to Supplementary material.

*Nucleotide binding and GTP hydrolysis.* The nucleotide binding experiments were carried out with 5 μM YqeH (or GST) and 200 nM mant-GDP or mant-GDPNP (Jena Biosciences) as detailed in Supplementary material. After incubation, the fluorescent nucleotides were excited at 355 nm and emission (at 448 nm) was monitored between 400 and 600 nm using a spectrofluorimeter (Perkin-Elmer).

The hydrolysis of GTP into inorganic phosphate (Pi) was measured in a calorimetric Malachite green assay [18]. The reaction mixture (50 μl), consisting of 375 nM YqeH (or its derivatives) and 400 μM GTP was incubated for a given time and the absorbance at 630 nm was measured. To probe the influence of RNA and S5 on GTPase activity, the M assays additionally contained 2 μl of 1 mg/ml single or double-stranded RNA and 375 nM S5, respectively. For details refer to Supplementary material.

*Electrophoretic mobility shift assay.* EMSA was performed with 5 μM proteins (YqeH or its derivatives; GST; S5), 1 mM of nucleotides (GTP/GDP) and 1 μl of labeled (7000 cpm/μl) single or double-stranded RNA, incubated at 37 °C for 30 min before stopping the reaction. RNA was resolved in 12% native PAGE. The gel was dried and exposed to phosphor imager screen (Kodak) for visualization. For details, see Supplementary material. For annealing activity, the experiment was performed with complementary ssRNAs that were not pre-annealed. Equal amounts of both ssRNAs were added to increasing concentrations of YqeH (2.5, 5, 10 μM) and incubated for 2 h at 37 °C.

## Results

### *YqeH interacts with 30S ribosomal subunit*

For three of the four cpGTPase subfamilies, i.e., YjeQ (RsgA), YlqF (RbgA) and YawG, a direct interaction with the ribosomal subunits was shown [3–9]. However, similar studies have not been reported for YqeH, although it was shown to be involved in 30S assembly [10,11]. Further, domain assignments of YqeH predicted that the N-terminal region consists of treble-clef Zn-finger domain while the C-terminal domain was not characterized until lately [2]. A recent structural analysis of YqeH from *Geobacillus stearothermophilus* (gsYqeH), identifies the C-terminal domain to be a peptide/nucleotide recognition (PNR) domain [19]. This domain was found to be structurally homologous to tryptophan RNA-binding attenuation protein (TRAP) and the residues important for RNA binding in TRAP were found to be conserved in the PNR domain of YqeH as well [19]. Further, the N-terminal treble-clef Zn-finger domain is also present in ribosomal proteins S14 and L24E [2], further strengthening the view that YqeH may bind the ribosome. Therefore, using co-sedimentation experiments (see Materials and methods) we tested if YqeH would interact with ribosome. Further, to probe the effect of GTP/GDP binding on ribosome binding, experiments were conducted in the presence of GDP, GTP and GDPNP (non-hydrolysable GTP analog). To ascertain that the nucleotides do bind the protein, fluorescent nucleotide binding to YqeH was examined using mant-GDP, mant-GDPNP. An increased fluorescence of the mant nucleotides in presence of YqeH suggests their binding to the protein (Fig. 1B). Interestingly, a higher affinity for GDP than for GTP was suggested for gsYqeH and AtNOA1 [16,19]. Hence, nucleotide concentrations, in the co-sedimentation assays, were held in large excess over that of the protein, to ensure the desired nucleotide bound state of YqeH. We found that YqeH co-sediments with 30S stably in presence of GDPNP or GTP (Fig. 1E and F). On the contrary, in presence of GDP, the protein did not display a similarly strong interaction with 30S (Fig. 1G). Therefore, it appears that YqeH exhibits an apparent nucleotide (GTP/GDP) dependent 30S binding.

### *Domains neighbouring the CPG-domain are important for ribosomal interactions*

In an attempt to assess the role of N and C-terminal domains in ribosome binding, two deletion constructs – ΔN-YqeH lacking the N-terminal domain and ΔC-YqeH lacking the C-terminal domain, were created. Co-sedimentation experiments using these were conducted in the presence of GDPNP, as it promotes YqeH–30S interaction. In line with the RNA binding function assumed for the N-terminal domain [2], ΔN-YqeH failed to interact with 30S (Fig. 1I). This is consistent with the report, based on *in vivo* experiments, that disruption of $Zn^{2+}$ co-ordination in the treble-clef motif produces a phenotype identical to that observed in the YqeH null mutant [10]. Interestingly, 30S interaction was abolished for ΔC-YqeH as well (Fig. 1H). In line with this, complementation experiments conducted in plants with ΔC-AtNOA1 too failed to rescue the wild type phenotype [16]. These experiments suggest an importance for both N and C-terminal domains in 30S interaction. To further assess their ability to bind ribosomes, we performed co-sedimentation experiments with stand-alone N-terminal Zn-finger domain and C-terminal PNR domain. However, we found that both of these display poor binding, if any (Fig. 1J and K). The fact that exogenous addition of missing domains, i.e., PNR domain to ΔC-YqeH and Zn-finger domain to ΔN-YqeH, did not restore 30S binding (data not shown) suggests the need for these domains to be covalently linked to the CPG-domain, which upon binding GTP evokes a strong 30S interaction. That both ΔN-YqeH and ΔC-YqeH display reduced GTP hydrolysis rates when compared to wild type (Table 1) further underscores the importance of these domains.

### *YqeH binds RNA but does not display strand dissociation or annealing activity*

YqeH null mutant displays an altered ribosome profile lacking a mature 30S subunit and severely compromised 16S rRNA stability [10,11]. On the other hand, deletion of YjeQ/YloQ, another cpGTPase that also binds 30S, did not show a similarly altered ribosome profile [4,5]. This indicated that YqeH may possibly participate in the early stages of 30S assembly by assisting 16S rRNA folding into the native state. To investigate such a possibility, the following experiments were designed. As proteins known to promote the folding of RNA bind non-specific RNA and exhibit strand dissociation or annealing activity (reviewed in [20]), two complementary single-stranded RNA with an arbitrary sequence and of length 21 bp were synthesized (for details see Supplementary material). YqeH interaction with single (ssRNA) and double-stranded RNA (dsRNA) was assayed using Electrophoretic Mobility Shift Assay (EMSA) to examine its ability to dissociate dsRNA (strand dissociation activity) and to promote association of complementary ssRNAs (annealing activity). In order to probe the effect of the bound nucleotides on RNA binding, nucleotide-free