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Distinct Binding and Enzymatic Activities of Two Ribosome-dependent NTPases YchF and YihA*

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Abstract P-loop NTPases (GTPase and ATPase) are widely employed in both prokaryotes and eukaryotes to regulate various cellular processes. YchF and YihA are two highly conserved NTPases in bacteria, but their cellular roles remain elusive. Previous data revealed that the ribosome or ribosomal subunits are binding partners of these two NTPases. Here, we examined the binding preferences of *Escherichia coli* YchF and YihA to the 30S, 50S and 70S ribosomes in the presence of different nucleotides, and assayed whether these binding preferences were associated with the stimulation of their NTPase activities. Our data show that YchF and YihA display a strong preference for the 70S and 50S, respectively. While the 70S ribosome, but not the 50S or 30S, promotes both the ATPase and GTPase activities of YchF, YihA responds to both the 50S and 70S, with the moderate GTPase stimulation (~8.8 fold) seen in the presence of the 70S ribosome.

Key words YchF, YihA/YsxC, ATPase, GTPase, Ribosome assembly, GTPase-activating protein, translation regulation
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Small P-loop GTPases are molecular switches universally utilized in a variety of cellular regulatory processes in all domains of life[1-2]. There are nearly twenty GTPases identified in *Escherichia coli*, and most of them are essential for cell growth[1]. A majority of bacterial GTPases are implicated in ribosome associated processes, such as mRNA translation (IF2, EF-Tu, EF-G, EF4, RF3, SelB and BipA), ribosome biogenesis (RsgA, Era, YqeH, YlqF, ObgE, EngA and YihA), signal-peptide recognition (FtsY and Fth), and tRNA modification (TrmE)[1, 3] Comparative analyses indicated that they can be classified into four phylogenetical superfamilies, TrmE-Era-EngA-YihA-Septin-like, Obg-HflX-like, translational factor, and secretion factor[1,4-6]. While the superfamilies of translation and secretion GTPases have been well characterized, the molecular functions and mechanisms of many members from the other two superfamilies just began to emerge (for examples, see reference[7-10]). Genetic data suggest that most of the GTPases in these two superfamilies are involved in ribosome biogenesis (reviewed in reference[1]).

Among conserved bacterial GTPases, YihA and YchF are two subfamilies with unclear cellular function. YihA (also known as YsxC in *Bacillus subtilis*) is essential for growth[11-14]. Depletion of *ysxC* in *Staphylococus aureus* and *Bacillus subtilis* causes a decrease in mature 70S ribosomes with accumulation of free subunits, in immature forms, suggesting a possible role in ribosome assembly[13, 15]. In addition, association of YihA with the ribosome or cellular ribosome fractions have been confirmed in several bacterial species[13, 16-18], and a C-terminal helix of YihA is essential for its ribosome-binding activity[17]. Crystal structures of YihA indicated that it has a single typical GTPase domain with conserved G1-G5 motifs[19-20], but its basal GTPase level is extremely low[16, 21].

YchF subfamily proteins belong to the Obg family, however, unlike other family members, the

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function of YchF remains elusive. Crystal structure of *Hemophilus influenza* YchF revealed three domains, a typical P-loop GTPase domain at the N-terminus, a coiled-coil domain and a C-terminal TGS (ThrRS, GTPase, SpoT) RNA-binding domain[22]. Based on structural information and available data from YchF homologues in other bacteria and eukaryotes, it was tentatively suggested that YchF is involved in translation regulation [1, 22]. A unique biochemical property of YchF is that it is capable of hydrolyzing both GTP and ATP[15, 23–24].

Biochemical characterization of the binding and NTPase activities of YihA and YchF would generate essential clues for further dissection of their physiological roles *in vivo*. Although previous studies have provided evidence for their ribosome-dependent NTPase activities [16, 24], there is a large inconsistency among existing data regarding their ribosomal subunit binding preferences in the presence of different nucleotides, and it is unknown whether these subunit-specific bindings are associated with their NTPase stimulation. In the present study, we performed a series of *in vitro* binding and enzymatic measurements of YihA and YchF, using highly purified 30S, 50S and 70S ribosomes. With these minimal *in vitro* systems, we found that YihA and YchF preferentially bind to the 50S subunit and 70S ribosome, respectively, but the most apparent stimulation of their NTPase activities are seen in the presence of the 70S ribosome. These results and their functional implications are subsequently discussed in the context of their physiological roles.

1 Materials and methods

1.1 Gene cloning and protein purification

The gene of *ychF* was amplified from *Escherichia coli* DH-5α strain using forward (5′ CATGCCATGG-CGATGGGATTCAAATGCGGTATC 3′) and reverse (5′ CCCAAGCTTTTAGACGTTGAAAAGGAAGT-TC 3′) primers, respectively. The 1092-bp PCR products were digested by *Nco* Ⅰ and *Hin*d Ⅲ and ligated into the pET22b vector (Novagen). The expression plasmids were transformed into the *Escherichia coli* BL-21 (DE3) cells for overexpression. Cells were grown at 37℃ in 1 L Luria Bertani medium containing 100 mg/L ampicillin. Protein expression was induced at 30℃ for 5 h with 0.3 mmol/L Isopropyl β-D-1-thiogalactopyranoside (IPTG). Cells were harvested by centrifugation at 5 000 *g* (Avanti J-26 XP, JLA 10.500 rotor, Beckman Coulter) for 10 min, resuspended in 50 ml lysis buffer (50 mmol/L Tris-HCl, pH 7.5, 60 mmol/L NH_4Cl, 300 mmol/L KCl, 8 mmol/L $Mg(OAc)_2$, 20 mmol/L imidazole, 10% glycerol, 1 mmol/L PMSF), and lyzed by sonication (Ultrasonic cell crusher, Nanjing Xinchen Biotechnology). Clarified lysates were obtained by centrifugation at 13 500 r/min (Avanti J-26 XP, JLA 25.50 rotor, Beckman Coulter) for 45 min. The supernatants were transferred onto a Ni-NTA column (Ni Sepharose 6 Fast Flow, GE healthcare) pre-equilibrated with lysis buffer. Non-specific binding was washed with 10 column-volumes of wash buffer (50 mmol/L Tris-HCl, pH 7.5, 60 mmol/L NH_4Cl, 300 mmol/L KCl, 8 mmol/L $Mg(OAc)_2$, 40 mmol/L imidazole). YchF was eluted with 10 ml elution buffer (50 mmol/L Tris-HCl, pH 7.5, 60 mmol/L NH_4Cl, 300 mmol/L KCl, 8 mmol/L $Mg(OAc)_2$, 250 mmol/L imidazole). Fractions of His-tagged YchF were concentrated, subjected to a RESOURSE S column (GE healthcare), and eluted with a linear salt gradient from 10% buffer A (50 mmol/L Tris-HCl, pH 7.5, 100 mmol/L KCl, 8 mmol/L $Mg(OAc)_2$) to 100% buffer B (50 mmol/L Tris-HCl, pH 7.5, 1 000 mmol/L KCl, 8 mmol/L $Mg(OAc)_2$). Further purification was done using a gel filtration column (Superdex 200 column 10/300 GL, GE healthcare) in filtration buffer (50 mmol/L Tris-HCl, pH 7.5, 70 mmol/L NH_4Cl, 30 mmol/L KCl, 8 mmol/L $Mg(OAc)_2$, 1 mmol/L DTT). YchF factions were concentrated and stored at –80℃. The purify of the proteins was examined by SDS-PAGE.

The gene of *yihA* was cloned from the *E. coli* DH-5α genomic DNA (primers: 5′ CATGCCATGGC-GTTGACTAATTTGAATTATCAACAGA 3′ and 5′ CCCAAGCTTTTATTCGCCGTCCTGCGTTTCT 3′). PCR products were ligated into pET22b vector between *Nco* Ⅰ and *Hin*d Ⅲ restriction sites. Proteins were overexpressed in *E. coli* BL-21 (DE3) cells using 0.5 mmol/L IPTG at 16 ℃ overnight. Cells were collected in lysis buffer (20 mmol/L Tris-HCl, pH 8.0, 500 mmol/L NaCl, 6 mmol/L $Mg(OAc)_2$, 20 mmol/L imidazole, 1 mmol/L PMSF). Purification of YihA was similarly done as described above for YchF. The final purification of YihA was performed with size exclusion chromatography on a Superdex 75 (GE healthcare) in storage buffer (20 mmol/L Tris-HCl, pH 8.0, 500 mmol/L NaCl, 6 mmol/L $Mg(OAc)_2$, 1 mmol/L DTT).

1.2 Ribosome purification

Crude ribosomes were purified from the *Escherichia coli* DH-5α strain as previously described[7]. Crude ribosomes were layered over a 10% ～40% linear sucrose density gradient (15 mmol/L $Mg(OAc)_2$ and centrifuged for 8 h at 30 000 r/min using a SW 32 rotor (Beckman Coulter) at 4℃. Fractions containing the 70S peak were collected and concentrated. For 30S and 50S subunits, 70S ribosomes were subjected to a 10%～40% linear sucrose density gradient (2 mmol/L $Mg(OAc)_2$) for 8 h at 30 000 r/min using a SW 32 rotor. Fractions of the 30S and 50S subunits were collected and concentrated separately. The 30S, 50S and 70S were stored in a buffer containing 20 mmol/L Tris-HCl, pH 7.5, 100 mmol/L NH_4Cl, 10 mmol/L $Mg(OAc)_2$, and 1 mmol/L TCEP at –80℃ for further use.

1.3 Co-sedimentation assay

The reactions were started by mixing YchF (600 pmol) with ribosomal subunits (30 pmol, 30S, 50S or 70S) in the absence or presence of nucleotides (1 μmol GTP, GDP or GMppNp, ATP, ADP or AMppNp). After incubation for 15 min at 30℃ with binding buffer Ⅰ (20 mmol/L Tris-HCl, pH 7.5, 70 mmol/L NH_4Cl, 30 mmol/L KCl, 10 mmol/L $MgCl_2$), mixtures were centrifuged at 10 000 *g* for 10 min at 4℃ (to remove possible protein aggregates). The supernatants were gently transferred onto the top of 90 μl 33% sucrose cushion buffer(20 mmol/L Tris-HCl, pH 7.5, 70 mmol/L NH_4Cl, 30 mmol/L KCl, 10 mmol/L $MgCl_2$, 33% sucrose) and centrifuged at 95 000 r/min at 4℃ for 2 h using a TLA 100 rotor (Beckman Coulter).

Binding of YihA to the 30S, 50S and 70S was assayed similarly. YihA (600 pmol), and ribosomal subunits (30 pmol, 30S, 50S or 70S) were mixed in the absence of presence of nucleotides (1 μmol, GTP, GDP or GMppNp) in binding buffer Ⅱ (20 mmol/L Tris-HCl, pH 8.0, 150 mmol/L NH_4Cl, 10 mmol/L $MgCl_2$). The mixtures were carefully loaded onto a 90 μl 33% sucrose cushion buffer(20 mmol/L Tris-HCl, pH 8.0, 150 mmol/L NH_4Cl, 10 mmol/L $MgCl_2$, 33% sucrose) and centrifuged similarly as done for YchF.

After centrifugation, the supernatants were removed rapidly, and the pellets were resuspended in 15 μl binding buffer. 1/34 of total supernatants and 1/3 of total pellets were examined by 12% SDS-PAGE. Because YihA co-migrates with a certain 30S ribosomal protein, additional Western blotting experiments were performed (Figure 2d, f), using a mouse anti-His antibody (primary antibody) and a goat anti-mouse IgG as secondary antibody (coupled to horseradish peroxidase).

1.4 Enzymatic activity assay

Enzymatic activities of YchF and YihA were examined using a NADH-coupled spectrophotometric assay[9]. All reactions were measured at 37℃ in 200 μl reaction mixtures containing 50 mmol/L MOPS, 1 mmol/L phosphoenolpyruvate (Sigma, P0564), 0.5 mmol/L NADH(Sigma, N8129), 20 U/ml lactic dehydrogenase (Sigma, L7525) and 15 U/ml pyruvate kinase (Sigma, P1506). Based on different purposes, proteins of YchF and YihA were subjected to ultracentrifugation (Sartorius Vivaspin Turbo 15, VS15T02) for buffer change, and the experimental variables are as follows. The salt concentrations with different cations (300 mmol/L KCl, 300 mmol/L NH_4Cl or 300 mmol/L NaCl); different concentrations of $MgCl_2$ (1 mmol/L, 2 mmol/L or 10 mmol/L); 1 mmol/L ATP (Sigma, A2383) or 1 mmol/L GTP (Sigma, G8877); YchF (20 μmol/L) or YihA (10 μmol/L); 30S, 50S or 70S ribosomes (0.5 μmol/L). The process of the reaction was monitored continuously at 340 nm with the formation of NAD^+ using a multifunctional fluorescent analyzer (FLU-Ostar Omega, BMG LABTECH). The decline of the NADH-absorbance at 340 nm allows measuring the actual velocity of steady state ATP/GTP hydrolysis in real time. Three independent replicates were carried out for each reaction to estimate the average rates as well as the standard deviations.

2 Results and discussion

2.1 Different ion-dependences of YchF and YihA in their ATPase/GTPase activities

YchF was reported to be a K^+-dependent NTPase[23]. To optimize the conditions used for enzymatic measurements, we performed a continuous NADH-coupled enzyme assay to quantify the basal NTPase and GTPase activity of *Escherichia coli* YchF and YihA, respectively, in the presence of different cations. A substrate-regenerating system in the assay enables a linear range over a long period of time for accurate estimation of the apparent rate of ATP or GTP hydrolysis (Figure 1). Indeed, YchF hydrolyzes ATP more efficiently than GTP (Figure 1a), with apparent rates for its ATPase and GTPase activities being (0.342 ± 0.013) min^{-1} and (0.118 ± 0.007) min^{-1}, respectively. While Mg^{2+} is absolutely required for

YchF's ATPase activity as expected (Figure 1b), monovalent cations are seen to further stimulate the ATPase of YchF, and K^+ has the strongest stimulating effect (Figure 1d). In sharp contrast, the GTPase activity of YihA displays no preference for monovalent cations (Figure 1e). In summary, we confirmed that YihA and YchF possess slow, intrinsic ATPase/GTPase activity, and YchF displays a substrate preference for ATP and is more dependent on potassium (Figure 1f).

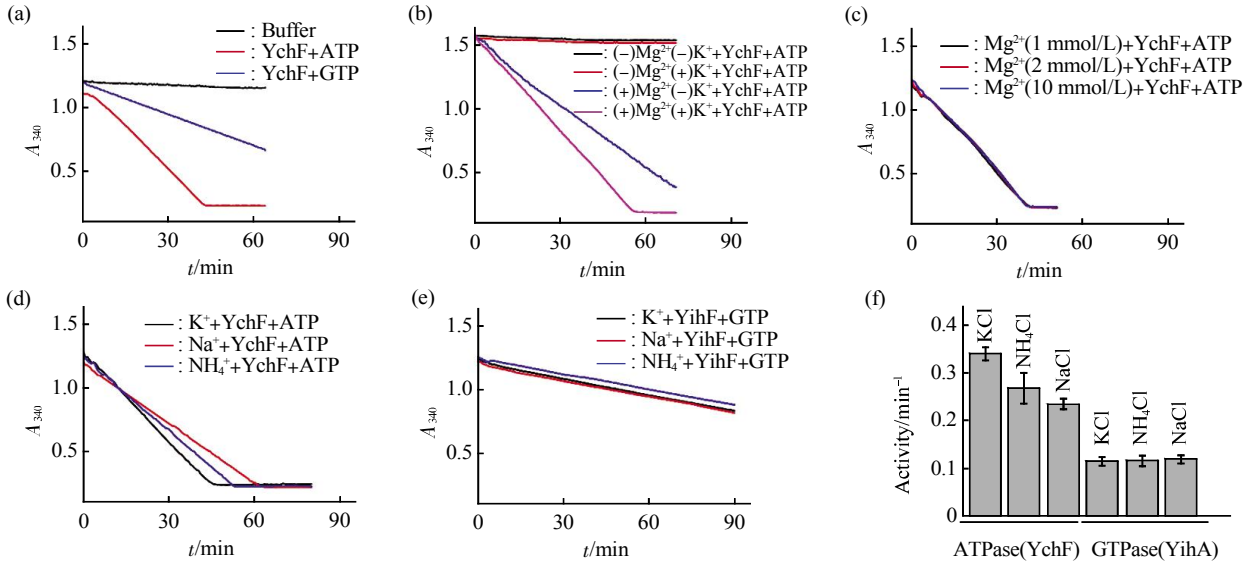

Fig. 1 YchF and YihA are two intrinsically slow NTPases

(a) NADH-coupled enzyme assays were carried out to measure YchF's ATP and GTP hydrolyzing activity. YchF hydrolyzes ATP 2.9 times faster than GTP under our experimental conditions. (b) Specific ATPase activity of YchF was measured in the absence or presence of 300 mmol/L KCl and 1 mmol/L Mg^{2+}. (c) Specific ATPase activity of YchF in the presence of different concentrations of Mg^{2+} (1 mmol/L, 2 mmol/L or 10 mmol/L). (d) Specific ATPase activity of YchF in the presence of 300 mmol/L KCl, NH_4Cl or NaCl. (e) Specific GTPase activity of YihA in the presence of 300 mmol/L KCl, NH_4Cl or NaCl. (f) Calculated the means and standard deviations for (d) and (e), from the left to the right, being (0.341± 0.014) min^{-1}, (0.269±0.032) min^{-1}, (0.235±0.011) min^{-1}, (0.117±0.007) min^{-1}, (0.118±0.009) min^{-1}, (0.121±0.006) min^{-1}, respectively. For clarification, only one reaction trace is shown.

2.2 YchF and YihA have distinct preferences for different ribosomal subunits

Next, we used co-sedimentation experiments to determine the binding affinity of YchF and YihA to highly purified ribosomal subunits, in the presence or absence of different nucleotides. In this assay, ribosome-bound proteins could be separated from unbound forms, by pelleting the reaction mixtures through a 33% sucrose cushion, to facilitate the qualitative comparison of binding affinities. As shown in Figure 2a～c, YchF has a strong preference for 70S ribosomes, and no binding was detected for the 30S and 50S subunits under current conditions. Furthermore, YchF binds to 70S ribosomes irrespective of the nucleotides, with the affinities following the order of AMppNp > ADP > ATP > APO (Figure 2c).

This is in sharp contrast to YihA, which, instead, binds strongly to the 50S subunit (Figure 2e), but not 30S (Figure 2d) or 70S ribosomes (Figure 2f). YihA band co-migrates with a certain ribosomal protein in the 30S subunit on SDS-PAGE, but our Western blotting using an anti-His antibody confirmed that YihA shows no binding to the 30S or 70S ribosomes (lower panels, Figure 2d and Figure 2f). This preference for the 50S subunit is in good agreement with previous data done with the *B. subtilis* homologue of YihA [16, 18]. Nevertheless, under the current experimental condition, no apparent difference in the binding of YihA to the 50S subunit in the presence of GTP, GDP, or GMppNp was detected. The fact that the stable binding of YihA could only be detected for the 50S subunit further suggests that YihA might bind to the inter subunit face of the 50S subunit, because one would expect to see its binding to both 50S and 70S if YihA binds to the solvent side of the 50S subunit.

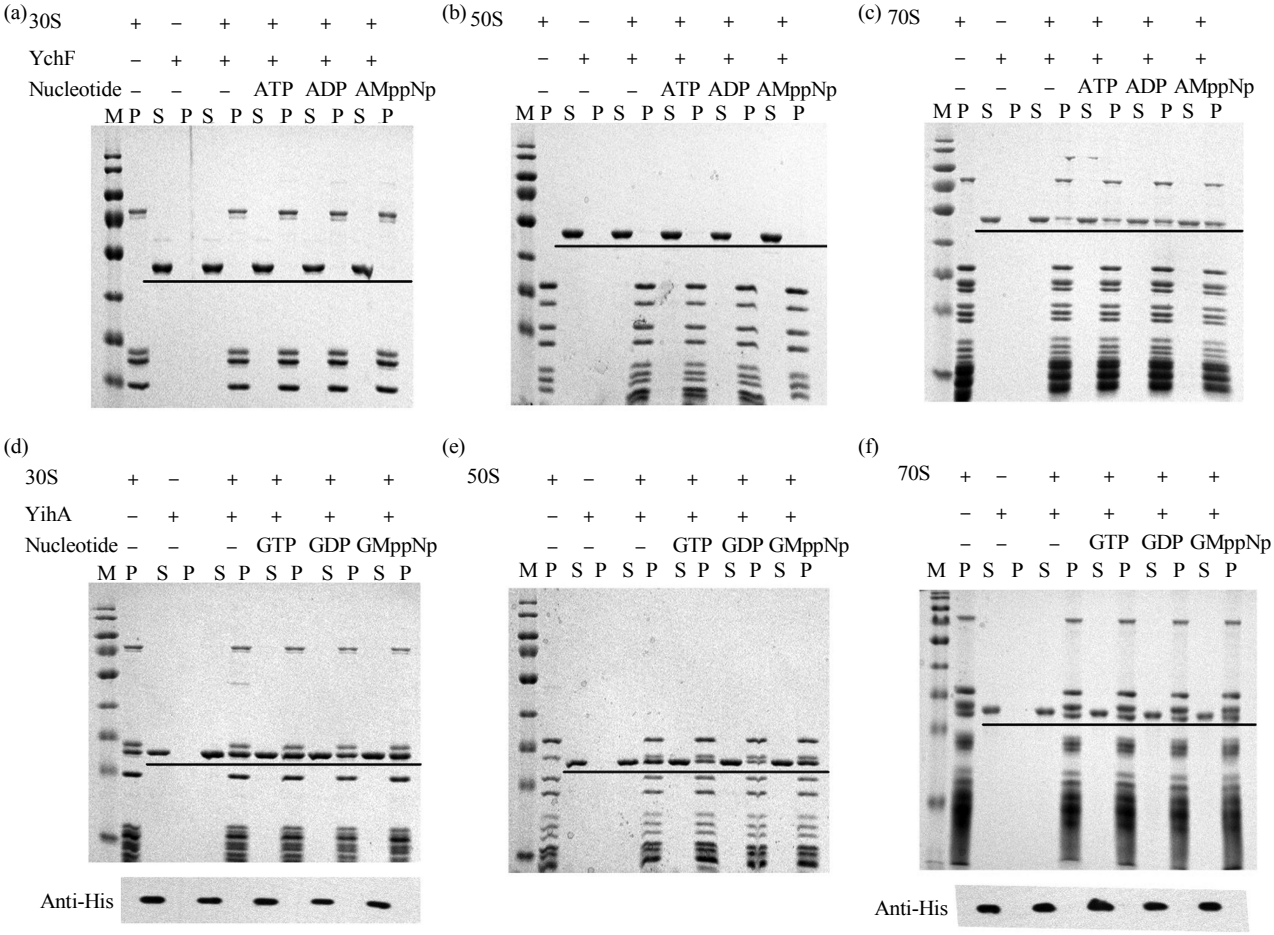

Fig. 2　In vitro binding of YchF and YihA to different ribosomal subunits

(a～c) Complexes were formed by incubating 30 pmol 30S (a), 50S (b), or 70S (c) and 600 pmol YchF in the absence or presence of nucleotides (ATP, ADP or AMppNp, 1 μmol) in a 100 μl binding system. Samples were subjected to a 33% sucrose cushion-based centrifugation, and ribosome-bound YchF were pelleted. The supernatant (S) and the pellet (P) were separated on a 12% SDS-PAGE. (d～f) Similar to (a～c), but for YihA. His-YihA was further detected using the anti-HIS antibody in panels (d) and (f).

2.3　Both ATPase and GTPase activities of YchF are stimulated by 70S, but not by 30S or 50S subunits

According to conditions optimized in Figure 1, we assayed which ribosomal fractions could best stimulate the NTPase activity of YchF. As shown in Figure 3a, the 30S subunit has no effect on YchF's ATPase activity. But the addition of 70S ribosomes

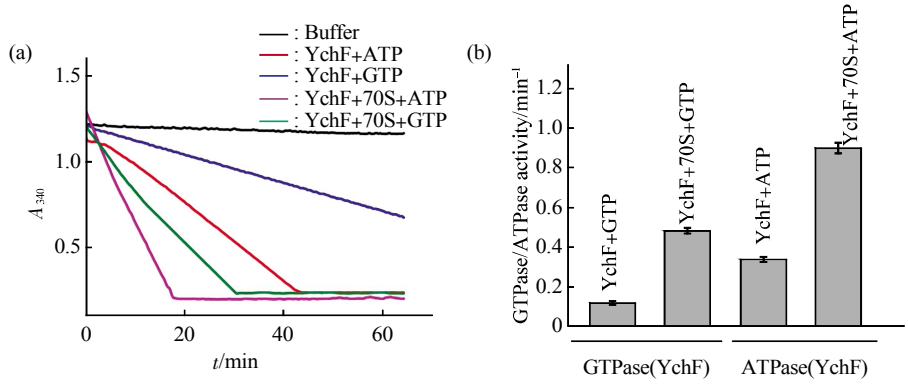

Fig. 3　The 70S ribosome stimulates both ATPase and GTPase activity of YchF

(a) The time-course of ATP or GTP hydrolysis by YchF (20 μmol/L) in the absence or presence of 70S (0.5 μmol/L). For clarification, only one trace for each reaction is shown. (b) Calculated specific ATPase or GTPase activity of YchF as shown in (a). The GTPase activity of YchF is stimulated from (0.118±0.007) min^{-1} to (0.483±0.014) min^{-1} upon the addition of the 70S ribosome. The ATPase activity of YchF increases from (0.343±0.013) min^{-1} to (0.903±0.029) min^{-1} upon the addition of the 70S ribosome.

resulted in 4.1-fold and 2.6-fold increases in its GTPase(Figure 3) and ATPase activities(Figure 4c, d), respectively, suggesting that the 70S ribosome is the NTPase-activating component of YchF. Very interestingly, in the presence of the 50S subunit, the reaction trace appears to have multiple phases(Figure 4b). The first ten-minute is associated with a slightly enhanced ATPase activity, followed by gradual decrease to reach a linear phase (after 15 min) with apparently inhibited ATPase activity. Notably, this is not due to the variation in experiment setup, because three repeat traces all display similar bi-phased curves (Figure 5). Altogether, in the presence of the 50S subunit, the overall rate of YchF ATPase (averaged

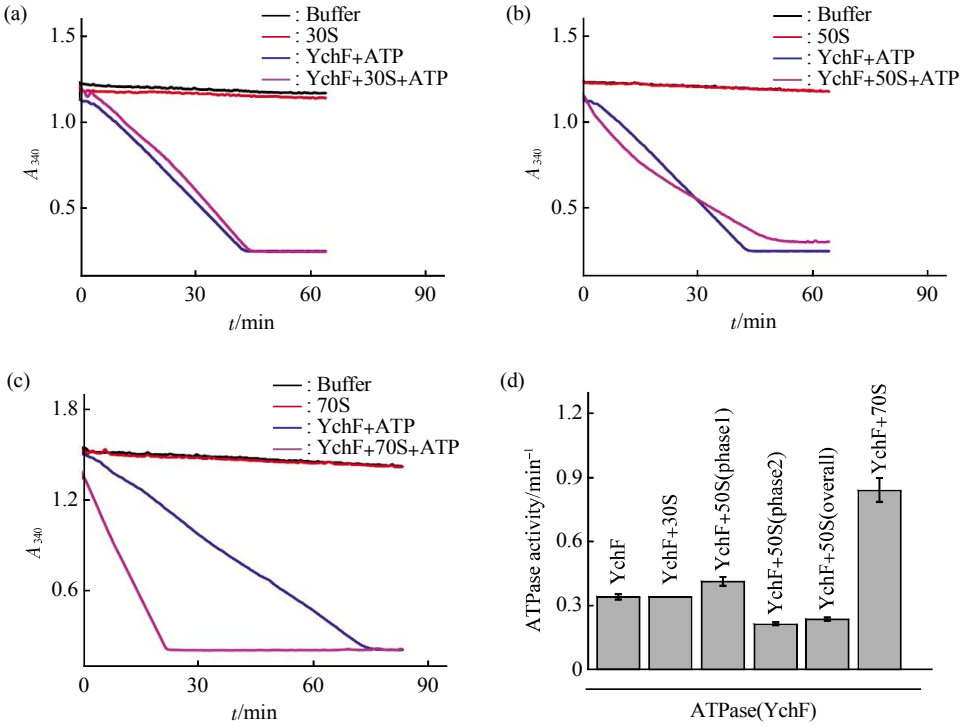

Fig. 4 The 70S ribosome, but not the 30S nor 50S, stimulates the ATPase activity of YchF

(a～c) The time-course of ATP hydrolysis by YchF (20 μmol/L) in the presence of 0.5 μmol/L 30S (a), 50S (b), or 70S (c). Reactions were carried out at 37 ℃ in a 200 μl system (see **Materials and methods**). For clarification, only one trace for each reaction is shown. (d) Calculated rates of ATP-hydrolysis by YchF as shown in (a～c). Specific ATPase activities are, from the left to the right, (0.343±0.013) min^{-1}, (0.334±0.002) min^{-1}, (0.414±0.017) min^{-1} (phase 1, 0～10 min), (0.215±0.007) min^{-1} (phase 2, after 15 min), and (0.840±0.056) min^{-1}, respectively.

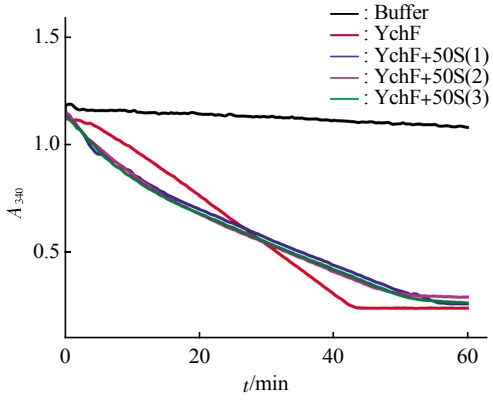

Fig. 5 Time-course of ATP hydrolysis by YchF in the presence of the 50S subunits

Confirming YchF's ATPase activity displays multiple phases with the addition of 50S subunit. As shown, all the three independent traces display similar multiple phases upon the addition of the 50S subunit.

over 45-min reaction time) is (0.238 ±0.006) min^{-1}, about 1.4 fold slower than its basal level.

The enzymatic data of YchF is consistent with its binding preference for the 70S ribosome. Since YchF has no detectable binding to the 30S and 50S subunit, it is highly likely that the strong association of YchF with the 70S ribosome(Figure 2c) involves cooperative interaction with both subunits in the 70S ribosome. Similar examples include translational GTPases, such as EF-Tu and EF-G, which bind to the inter subunit space within the 70S ribosome, directly contacting both subunits[25]. And for both EF-G and EF-Tu, their GTPase activities could be enhanced by several orders of magnitude by the 70S ribosome[26].

Therefore, our data are consistent with the putative suggestion that YchF is a translation factor[1, 22]. However, it is still unknown which substrate, ATP or GTP, is primarily used in its translation related functions.

2.4 GTPase activity of YihA is stimulated by both 50S and 70S ribosomes

Similarly, we applied NADH-coupled enzymatic assay to measure the apparent rates of GTP-hydrolysis by YihA in the presence of 30S, 50S and 70S ribosomes. Similar to YchF, the 30S subunit shows clearly no effect on YihA's GTPase activity. However, the rate of GTP-hydrolysis by YihA could be significantly enhanced by both the 50S (Figure 6b) and 70S ribosomes (Figure 6c). Particularly, in the presence of 70S ribosome, the rate has increased to ~8.8 fold of its basal level, whereas the 50S subunit only conferred a 2.1 fold increase.

The stimulation of YihA's GTPase activity by the 70S ribosome seems to be inconsistent with the binding data showing that YihA only stably associates with the 50S subunit (Figure 2d, f). However, the co-sedimentation assay is in principle a method that is more sensitive to the dissociation rate, and it is not measuring the actual dissociation constant. Therefore, YihA could still bind to the 70S ribosome, but with relatively large dissociation rate. Together, our data suggest that YihA might have two binding modes for the ribosome, one for the 50S subunit and the other for the 70S ribosome. While the 50S-binding activity is likely primarily required for its possible role in the assembly of the large ribosomal subunit[13, 15], its unexpected, gentle stimulated GTPase activity by the 70S ribosome further suggests that YihA might have another role in translation regulation.

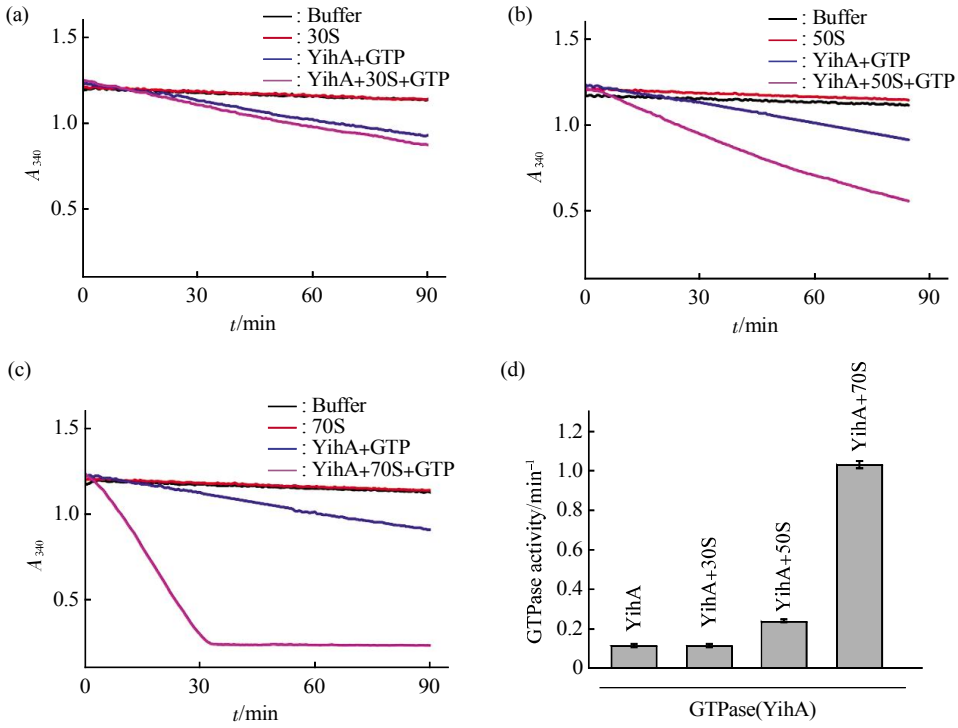

Fig. 6 Both the 50S and 70S ribosome stimulate the GTPase activity of YihA

(a~c) The time-course of GTP hydrolysis by YihA (10 μmol/L) in the presence of 0.5 μmol/L 30S (a), 50S (b), or 70S (c). Reactions were carried out at 37℃ in a 200 μl system (see **Materials and methods**). For clarification, only one trace for each reaction is shown. (d) Calculated rates of GTP-hydrolysis by YihA as shown in (a~c). Specific GTPase activities are, from the left to the right, (0.117±0.004) min^{-1}, (0.119±0.009) min^{-1}, (0.242±0.007) min^{-1}, and (1.031±0.019) min^{-1}, respectively.

In summary, we analyzed the binding preference of YchF and YihA for different ribosomal fractions using co-sedimentation assay and their enzymatic activities using a stable, multi-turnover, spectrometry-based method, which allowed reliable estimation and parallel comparison of stimulating effects of different

ribosomal subunits on their ATPase/GTPase activation. The measured relatively low NTPase activities of these two factors and moderate stimulation by the ribosome or ribosomal subunits are in line with previous data using different methods [15-16, 21, 23-24], and have recapitulated similar observations from other ribosome biogenesis GTPases with low intrinsic enzymatic activity[1, 3]. Together, these data not only complement the missing links but also help clarify inconsistency in existing literatures for these two conserved ATPase/GTPase.

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依赖于核糖体的 NTP 酶 YchF 和 YihA 的结合特性及酶活性研究 *

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摘要　P- 环 NTP 酶(GTP 酶和 ATP 酶)普遍存在于真核生物和原核生物中，参与调节不同的细胞进程．YchF 和 YihA 是细菌中两种高度保守的 NTP 酶，但其生理功能仍然不清楚．之前的研究表明这两种 NTP 酶可以与核糖体或者核糖体亚基结合．我们检测了在不同核苷酸存在的情况下，大肠杆菌 YchF 和 YihA 蛋白与核糖体 30S、50S、70S 颗粒的结合情况，同时也探究了核糖体亚基的结合是否与 NTP 酶活性的激活有关．数据表明 YchF 与 70S 结合，YihA 与 50S 结合．70S 核糖体能同时激活 YchF 的 ATP 酶和 GTP 酶活性．然而 YihA 的 GTP 酶活性可以分别被 50S 和 70S 激活，并且 70S 呈现了 8.8 倍的激活效应．这些数据为进一步研究这两种保守的 NTPase 的生理功能奠定了基础．

关键词　YchF，YihA/YsxC，ATP 酶，GTP 酶，核糖体组装，GTP 酶激活蛋白，翻译调控
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