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Genetic Interaction Screens with Ordered Overexpression and Deletion Clone Sets Implicate the *Escherichia coli* GTPase YjeQ in Late Ribosome Biogenesis▽†

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The *Escherichia coli* protein YjeQ is a circularly permuted GTPase that is broadly conserved in bacteria. An emerging body of evidence, including cofractionation and in vitro binding to the ribosome, altered polysome profiles after YjeQ depletion, and stimulation of GTPase activity by ribosomes, suggests that YjeQ is involved in ribosome function. The growth of strains lacking YjeQ in culture is severely compromised. Here, we probed the cellular function of YjeQ with genetic screens of ordered *E. coli* genomic libraries for suppressors and enhancers of the slow-growth phenotype of a Δ*yjeQ* strain. Screening for suppressors using an ordered library of 374 clones overexpressing essential genes and genes associated with ribosome function revealed that two GTPases, Era and initiation factor 2, ameliorated the growth and polysome defects of the Δ*yjeQ* strain. In addition, seven bona fide enhancers of slow growth were identified (Δ*tgt*, Δ*ksgA*, Δ*ssrA*, Δ*rimM*, Δ*rluD*, Δ*trmE*/*mnmE*, and Δ*trmU*/*mnmA*) among 39 deletions (in genes associated with ribosome function) that we constructed in the Δ*yjeQ* genetic background. Taken in context, our work is most consistent with the hypothesis that YjeQ has a role in late 30S subunit biogenesis.

GTPases are molecular switch proteins that perform a diverse array of cellular functions, including directing protein synthesis, mediating transmembrane signaling, translocating proteins, and controlling cell proliferation and differentiation (7). The YjeQ protein of *Escherichia coli* represents a subfamily of GTPases in the YlqF/YawG family, whose defining structural feature is circular permutation of the GTPase domain (29). The YjeQ subfamily has a unique domain architecture with an N-terminal oligonucleotide/oligosaccharide fold (OB-fold) domain, a central circularly permuted (G4-G1-G2-G3) GTPase domain that adopts the classical P-loop fold, and a C-terminal TAZ2-like zinc finger domain (30, 41). Despite the circular permutation, YjeQ catalyzes the rapid hydrolysis of GTP (100 s^{-1}) with a low steady-state turnover rate, 9.4 h^{-1} (15). Low intrinsic GTPase activity is not uncommon among GTPases, and this suggests that a binding partner is required for maximal activity (6, 7). Cell fractionation studies revealed that the number of copies of YjeQ in *E. coli* is low and that YjeQ is bound entirely to ribosomes (ratio of YjeQ to ribosomes, 1:200) (14). Indeed, recombinant YjeQ has affinity for both subunits of the ribosome but binds stoichiometrically and tightly to the 30S subunit in the presence of a nonhydrolyzable GTP analogue (14). Likewise, the GTPase activity of YjeQ is stimulated manyfold by the 30S subunit of the ribosome (14). The binding and GTPase stimulation of YjeQ by ribosomes are mediated by the OB-fold domain (14). Protein initiation factor 1 possesses a similar domain and binds in the A site of the 30S subunit (13). The precise binding site for YjeQ on ribosomes has not been determined, but aminoglycoside antibiotics which bind near the A site are capable of inhibiting the ribosome-stimulated GTPase activity, although they do not directly compete for the same binding site (11, 22).

YjeQ is broadly conserved in bacteria and has been shown to be dispensable for growth but critically important for the overall fitness of *E. coli*, *Bacillus subtilis*, and *Staphylococcus aureus* (11, 12, 22). The reduced virulence of an *S. aureus yjeQ* deletion strain in mouse models (12) implicates YjeQ as a valid antibacterial target, and thus a complete understanding of its in vivo function would be a great asset. To probe the in vivo function of YjeQ, chemical synthetic lethality was examined using a *B. subtilis* deletion strain, and the results highlighted a connection between YjeQ and peptide channel or peptidyl transferase inhibitors (11). Isolation of ribosomal subunits from strains lacking *yjeQ* revealed accumulation of free 30S and 50S subunits and a decreased level of 70S ribosomes (11, 22). Additionally, a subset of the accumulated 30S subunits are nonfunctional and have been found to contain 17S rRNA, a precursor of the mature 16S rRNA (22). Despite this wealth of biochemical data, the cellular function of YjeQ is unknown.

Bacterial GTPases function in all steps of the translation pathway, initiation, elongation, termination, and recycling. There is also growing evidence for the involvement of GTPases in the stage preceding translation, ribosome biogenesis (8). Ribosome biogenesis is a well-coordinated process that requires more than 170 nonribosomal accessory proteins in eukaryotes (21). To date, only a few accessory proteins have been identified in prokaryotes, suggesting that a number of factors

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involved in this process have not been discovered. The *B. subtilis* GTPase, YlqF, has recently been shown to be involved in the late stages of 50S subunit assembly, with the L16 and L27 proteins missing from 50S subunits isolated from cells with YlqF depleted (33, 44). Similarly, the GTPases Obg and EngA have also been implicated in the late stages of 50S subunit assembly, and depletion of these proteins results in pre-50S particles and precursors of both the 16S and 23S rRNAs (5, 23, 27, 37, 39). A well-studied *E. coli* GTPase, Era, is believed to be involved in 30S subunit assembly as cells in which Era is depleted show accumulation of unassociated subunits (38) and of 17S rRNA, (25). Cells lacking YjeQ share phenotypes with cells depleted of these GTPases, and YjeQ may also play a role in ribosome biogenesis.

Recently, there has been a breakthrough in the genetic tools for investigating *E. coli.* Mori and coworkers created two extremely useful clone sets: a deletion set with all of the nonessential genes in *E. coli* deleted (4) and a set of clones expressing high copy numbers of all *E. coli* genes from plasmid pCA24N containing a T5 promoter (28). Bacterial geneticists have long exploited the effects of increasing gene dosage to identify genetic interactions with mutants under study. We report here our screening efforts, using one clone at a time, to detect suppressors among an ordered set of 374 clones overexpressing largely essential genes and genes associated with ribosome function. Interestingly, two GTPases involved in protein translation, Era and initiation factor 2 (IF2), were identified as high-copy-number suppressors of the slow-growth and polysome defects observed in the *yjeQ* deletion strain. Like high-copy-number suppression, gene deletion is a well-known route for identifying genetic interactions, and the deletion clone set of Mori and coworkers provides a ready template for moving deletions into any genetic background using established methods (16). In the work reported here, we identified 47 dispensable genes with a role in ribosome function and moved deletions in these genes into the Δ*yjeQ* background. Deletions in *tgt*, *ksgA*, *ssrA*, *rimM*, *rluD*, *trmE*/*mnmE*, and *trmU*/*mnmA* were found to enhance the slow growth of the Δ*yjeQ* parent strain, and the results obtained suggest that there is a functional connection between YjeQ and the gene products. Together, these genetic interaction screens with ordered overexpression and deletion clone sets and the increasing amount of information about YjeQ point to a role for YjeQ in 30S ribosome biogenesis and subunit association.

MATERIALS AND METHODS

***yjeQ* deletion strain.** The *yjeQ* deletion strain used in this study was obtained from the single-gene deletion library of all nonessential genes in *E. coli* K-12 (4). The parent strain of this deletion strain is *E. coli* BW25113 ($lacI^q$ $rrnB_{T14}$ $\Delta lacZ_{WJ16}$ *hsdR514* $\Delta araBAD_{AH33}$ $\Delta rhaBAD_{LD78}$), a derivative of *E. coli* K-12 (16). The FRT-flanked kanamycin resistance cassette was removed from the *yjeQ* locus using plasmid pCP20, coding for the FLP recombinase, leaving a sequence encoding a small 34-amino-acid in-frame scar peptide in place of *yjeQ* (4, 16).

Suppressor screen. Overexpression plasmids for all essential and ribosome function-related genes were mini-prepped from the ASKA library complete set of open reading frame clones of *E. coli* (28). Plasmid pCA24N is a high-copy-number plasmid with a chloramphenicol resistance cassette for selection in *E. coli* and the isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible promoter $P_{T5\text{-}lac}$ controlling the expression of an N-terminally histidine-tagged protein (28). Each overexpression plasmid was transformed into the *yjeQ* deletion strain using standard protocols (36) and frozen in a 96-well format. The following controls were used for each plate; well A1, wild-type BW25113; well B1, Δ*yjeQ*; well G12, Δ*yjeQ* plus pCA24N-*yjeQ*; and well H12, Δ*yjeQ* plus pCA24N. The frozen stocks were used to inoculate overnight cultures in Luria-Bertani (LB) medium in 96-well microtiter plates (200 μl medium per well), and in the morning each overnight culture was diluted 1,000-fold in fresh medium. The samples were incubated at 37°C with shaking at 250 rpm, and the optical density at 600 nm of each well was recorded. To confirm potential suppressors, plasmids were isolated from each clone and transformed into wild-type and Δ*yjeQ E. coli*, and growth curves were obtained and compared to those of strains with the parent plasmid pCA24N.

Polysome profiles. Strains were grown overnight in LB medium at 37°C, and in the morning the cultures were diluted 1,000-fold in 1 liter of LB medium to obtain an initial optical density at 600 nm of approximately 0.005. Suppressor strains were grown at 37°C with shaking at 250 rpm to an optical density at 600 nm of 0.180 (in the absence or presence of 1 mM IPTG), while double-deletion strains were grown at room temperature with no shaking to an optical density at 600 nm of 0.200. Appropriate controls were prepared under both conditions. The cells were pelleted by centrifugation at 15,000 × *g* for 20 min and resuspended in 5.5 ml of buffer A (20 mM Tris [pH 7.5 at 4°C], 10.5 mM magnesium acetate, 300 mM NH_4Cl, 0.5 mM EDTA, 3 mM β-mercaptoethanol) containing DNase. Cells were lysed by three passages through a French press at 10,000 to 12,000 lb/in^2, and the lysates were clarified by centrifugation at 31,000 × *g* for 45 min. Three milliliters of each clarified lysate was layered onto an equal volume of a 35% sucrose cushion in buffer A, which was followed by ultracentrifugation at 104,000 × *g* using an MLA 80 rotor in a Beckman ultracentrifuge at 4°C for 16 h. The ribosomal pellets were resuspended in 750 μl of buffer A, and a total of 75 U of absorbance at 260 nm was loaded into a Beckman XL-I analytical ultracentrifuge interference cell. Ribosome profiles were created from interference data collected during 1 h of centrifugation at 30,000 rpm at 20°C, using Micrococal Origin 6.0 software and sedimentation time derivative analysis (Beckman Coulter, Inc., Palo Alto, CA).

Deletion of dispensable translation proteins in the *yjeQ* deletion strain. Chromosomal DNA was prepared from a deletion strain for each gene of interest, using the Keio collection of *E. coli* single-gene deletions (4). Primers that amplified 500 to 1,000 bp up- and downstream of the deletion region were designed, and PCR products were PCR purified using a Qiagen PCR purification kit. Each PCR product was transformed into the *yjeQ* deletion strain containing pK03-*yjeQ* (*yjeQ* open reading frame, including 200 bp upstream of the start and 30 bp downstream of the stop cloned into the NotI and SalI sites of pK03 [31]) and pKD46 (16) with selection on LB medium containing kanamycin (15 μg/ml) and chloramphenicol (20 μg/ml). Following transformation, colonies were streaked on LB medium containing kanamycin and sucrose (5%, wt/vol) at 30°C to promote loss of pK03-*yjeQ*, and subsequent patching on LB medium containing kanamycin and LB medium containing kanamycin and chloramphenicol was used to confirm plasmid loss. Strains were then screened by PCR to confirm that both *yjeQ* and the target gene were deleted. Growth curves for double-deletion strains, each single-deletion parent strain, and wild-type *E. coli* strain BW25113 were prepared by inoculating 200 μl of LB medium with a 1,000-fold dilution of an overnight culture and growing the culture at room temperature with no shaking. The optical density at 600 nm was monitored using an Envision multilabel plate reader (Perkin Elmer, Woodbridge, Ontario, Canada). Each sample was examined in triplicate multiple times to confirm the growth phenotypes.

RESULTS

Suppressors of slow growth identified from an ordered *E. coli* overexpression library. Although *yjeQ* is dispensable for growth in culture, cells in which *yjeQ* is deleted exhibit a dramatic reduction in the growth rate. Using the reduced growth rate as our screening phenotype, we set out to identify suppressors in an ordered overexpression library recently made available by Mori and coworkers (28). Figure 1A clearly illustrates the slow-growth phenotype of the *yjeQ* deletion strain, which is characterized by an increased lag phase and a decreased exponential growth rate. This defect could be restored to the wild type by reintroducing *yjeQ* on a plasmid (Fig. 1A), which eliminated the possibility of polar effects on downstream genes. To identify suppressors of the slow growth, we introduced plasmids for all of the essential genes in *E. coli*, as well as a number of dispensable genes that are known to be in-

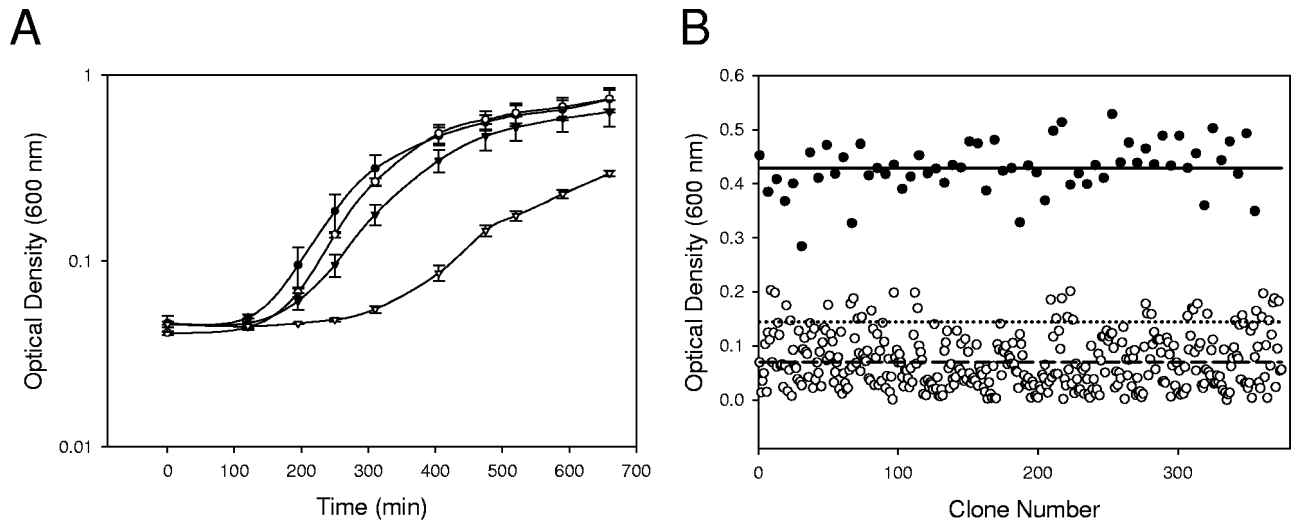

FIG. 1. Growth phenotype and suppressor screening for correction of the slow growth of the Δ*yjeQ* strain. (A) Growth curves for the Δ*yjeQ* strain and wild-type *E. coli*. Symbols: ●, wild type plus pCA24N-*yjeQ*; ○, wild type plus pCA24N; ▼, Δ*yjeQ* plus pCA24N-*yjeQ*; ▽, Δ*yjeQ* plus pCA24N. (B) Primary screen for suppressors of Δ*yjeQ* slow growth. The time point used correlates to 340 min in panel A. The filled circles show the results for the high-copy-number control (Δ*yjeQ* plus pCA24N-*yjeQ*), and the open circles show the growth data for each individual clone screened. The solid line indicates the mean of the high-copy-number control, the dashed line indicates the sample mean, and the dotted line indicates 1.5 standard deviations above the sample mean.

volved in ribosome function (see Table S1 in the supplemental material). The latter group was included in this study due to the growing evidence that YjeQ, like many uncharacterized bacterial GTPases, may have a role in ribosome function (8). The library clones were on the high-copy-number plasmid pCA24N under the control of the IPTG-inducible T5 promoter (28). In the absence of inducer, the leak in YjeQ expression was sufficient to correct the slow-growth phenotype (Fig. 1A), and consequently we decided to perform the genetic screen in the absence of IPTG to avoid extremely high levels of protein overexpression, which may have been toxic to the cells. A limitation to this method is the possibility that some genes may not be sufficiently expressed to provide suppression, and as a result, overexpression was primarily due to leaky gene expression and the gene copy number in the cells.

The reduced growth rate of the Δ*yjeQ* strain is easily distinguishable in liquid media, and a comparison of the growth of the fully complemented deletion strain (Δ*yjeQ* plus pCA24N-*yjeQ*) with that of the empty-vector control (Δ*yjeQ* plus pCA24N) allowed selection of a suitable time point (340 min) that provided the greatest difference between the strains (Fig. 1A). This time point occurs in mid-exponential phase, and Fig. 1B shows the optical density of each overexpression clone compared to that of the fully complemented deletion strain at 340 min. The optical density values are shown along with the clone descriptions in Table S1 in the supplemental material. As Fig. 1B shows, none of the overexpressed genes were able to restore growth to wild-type levels. A number of clones, however, were able to significantly improve growth compared to the growth of the slow-growth control. To further investigate some of these clones, a cutoff value of 1.5 standard deviations above the mean of the sample data was used. Use of this initial cutoff resulted in identification of 36 genes as potential suppressors of the slow growth of the *yjeQ* deletion strain.

To further investigate the high-copy-number suppressor phenotype for these 36 genes, each overexpression plasmid was retransformed into the *yjeQ* deletion strain and into wild-type *E. coli*. The former procedure was used to eliminate chromosomal suppressors, and the latter was used to determine if the genes provided a competitive growth advantage that was not linked to the function of *yjeQ*. The initial screening results were not replicated with nine genes, and these genes did not suppress the slow growth of Δ*yjeQ*. For an additional 21 genes there was a growth advantage relative to the wild type (see Table S2 in the supplemental material), suggesting that the copy number of the gene products is rate limiting for growth in *E. coli*. The remaining six genes were found to be bona fide suppressors of the slow-growth phenotype of the Δ*yjeQ* mutant. These genes and their functions are listed in Table 1. Of these six genes, the products of three are involved in translation (*infB*, *rplE*, and *era*), one encodes a protein with an unknown function (*yabQ*), and the remaining two are both dispensable genes involved in cellular metabolism and stress

TABLE 1. Suppressors of Δ*yjeQ* mutant slow growth

Clone	Gene	Function
Suppressors of slow growth and polysome defect		
114	*infB*	Protein chain IF2
223	*era*/*rbaA*	GTPase
Suppressors of slow growth		
12	*rplE*	50S ribosomal subunit protein L5
81	*oxyR*	Bifunctional regulatory protein sensor for oxidative stress
360	*yabQ*	Unknown
365	*csrA*/*zfiA*	Regulatory gene inhibiting glycogen biosynthesis

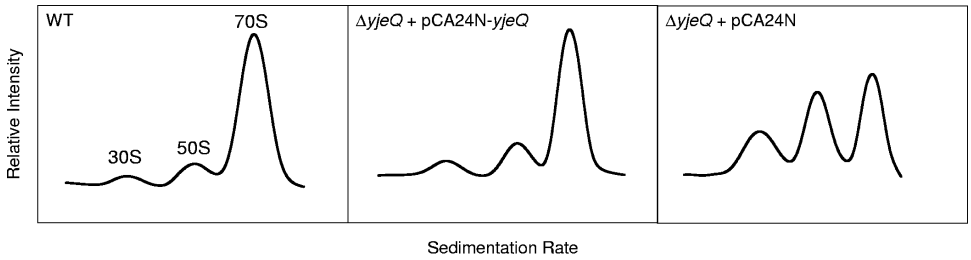

FIG. 2. Ribosome profiles for wild-type and Δ*yjeQ E. coli*. The strains used are indicated. The profiles were prepared using analytical ultracentrifugation. WT, wild type.

response (*csrA* and *oxyR*). We next set out to further characterize these suppressors.

High-copy-number suppression of the polysome defect in the Δ*yjeQ* mutant with *infB* and *era*. It has previously been observed that strains lacking YjeQ accumulate 30S and 50S ribosomal subunits and that there is a concomitant decrease in the number of 70S ribosomes (Fig. 2) (11, 22). This polysome defect can be restored to the wild-type phenotype by complementation with pCA24N-*yjeQ* (Fig. 2). Using analytical ultracentrifugation, we were able to quantify the ribosomal subunit distributions for the six partial suppressors of the *yjeQ* deletion strain. The suppression of the Δ*yjeQ* strain slow growth by overexpression of four genes, *rplE*, *yabQ*, *csrA*, and *oxyR*, was moderate but significant, as shown by the growth curves in Fig. S1 in the supplemental material. Despite the improved growth rate the ribosome distribution pattern of these suppressor strains did not differ from that of the *yjeQ* deletion strain (data not shown).

The remaining two suppressors, *infB* and *era*, encode GTPases with a role in ribosome function. Overexpression of either of these genes in the Δ*yjeQ* background increased the levels of 70S ribosomes and decreased the levels of free 30S and 50S subunits. The magnitude of the change correlated well with the expression levels of these two genes. The top panel in Fig. 3A shows that overexpression of *infB* in a Δ*yjeQ* background partially restored the growth rate to the wild-type growth rate by decreasing an extended lag period in the mutant, as well as by modestly increasing the exponential growth rate (the doubling time was decreased by 12 min). Concomitantly, the ratios of free 30S and 50S subunits decreased with an increase in the level of 70S ribosomes (compare Fig. 2 and Fig. 3A, bottom panel). Furthermore, we showed that this altered ribosome subunit distribution was related to the level of *infB* expression. The addition of IPTG to induce expression of *infB* resulted in a further increase in the level of 70S ribosomes, bringing the total concentration closer to wild-type concentrations (Table 2). Very similar results were obtained when *era* was overexpressed, which resulted in a comparable improvement in the growth rate and ribosome subunit distribution (Fig. 3B) (the doubling time was decreased by 22 min). As observed with *infB*, the shift in subunit distribution toward that of the wild type was mediated by the level of expression of *era*, and there was an additional improvement when IPTG was added to the medium (Table 2). From these data we concluded that overexpression of two GTPases that function on the ribosome, IF2 and Era, is able to partially suppress the slow growth of cells lacking *yjeQ* and that this suppression is correlated with increasing the amount of 70S ribosomes in the cell.

Identifying genetic interactions using double deletions. As a complementary method to the high-copy-number suppression screen, we also screened for genetic interactions and enhancement or suppression of the growth phenotype with a large number of targeted gene deletions and the Δ*yjeQ* mutant. Given the results of our suppressor screen, we felt that a focus on genes involved in ribosome function was justified. A total of 39 dispensable genes involved in ribosome function (see Table S3 in the supplemental material) were the focus of an effort to create double-deletion strains with Δ*yjeQ* and each of the genes on the short list mentioned above. Each of the 39 gene deletions was moved from a comprehensive library of deletion strains (4) into the Δ*yjeQ* background in the presence of a plasmid copy of *yjeQ*. The parent strain initially contained a plasmid copy of *yjeQ* to allow us to detect synthetic lethal genetic interactions, and the plasmid was subsequently removed in all cases to create viable double-deletion strains. All strain constructions were confirmed by checking for drug sensitivity, as well as by PCR using *yjeQ* primers.

To detect genetic interactions, we compared the growth rates of the Δ*yjeQ* single mutant, mutants with single mutations in each of the other genes, mutants with each of the double deletions, and the wild-type parent *E. coli*. In the majority of cases, the growth rate was the same as that of the slowest-growing single-deletion strain, and we interpreted this as no effect (no enhancement or suppression). None of the double-deletion strains had an improved growth rate compared with that of either of the single-deletion strains. Seven of the double deletions resulted in a more severe slow-growth phenotype (enhancement). Table 3 shows these seven enhancing genes and their functions, and growth curves for the seven double-deletion strains are shown in Fig. S2 in the supplemental material. Six of the seven genes are involved in RNA modification, and the seventh encodes a small RNA that rescues stalled ribosomes.

The enhanced slow-growth phenotypes of these seven double-deletion strains fall into two subgroups. In the first subgroup *rimM* and *rluD* deletion strains had slow-growth phenotypes, similar to that of the *yjeQ* deletion strain (see Fig. S2 in the supplemental material). When the mutation was in the Δ*yjeQ* background, there was a more severe growth defect than there was in either of the single-deletion strains. Members of the second subgroup, which included mutants with double deletions of *yjeQ* and *ksgA*, *ssrA*, *tgt*, *trmU*, or *trmE*, had a syner-

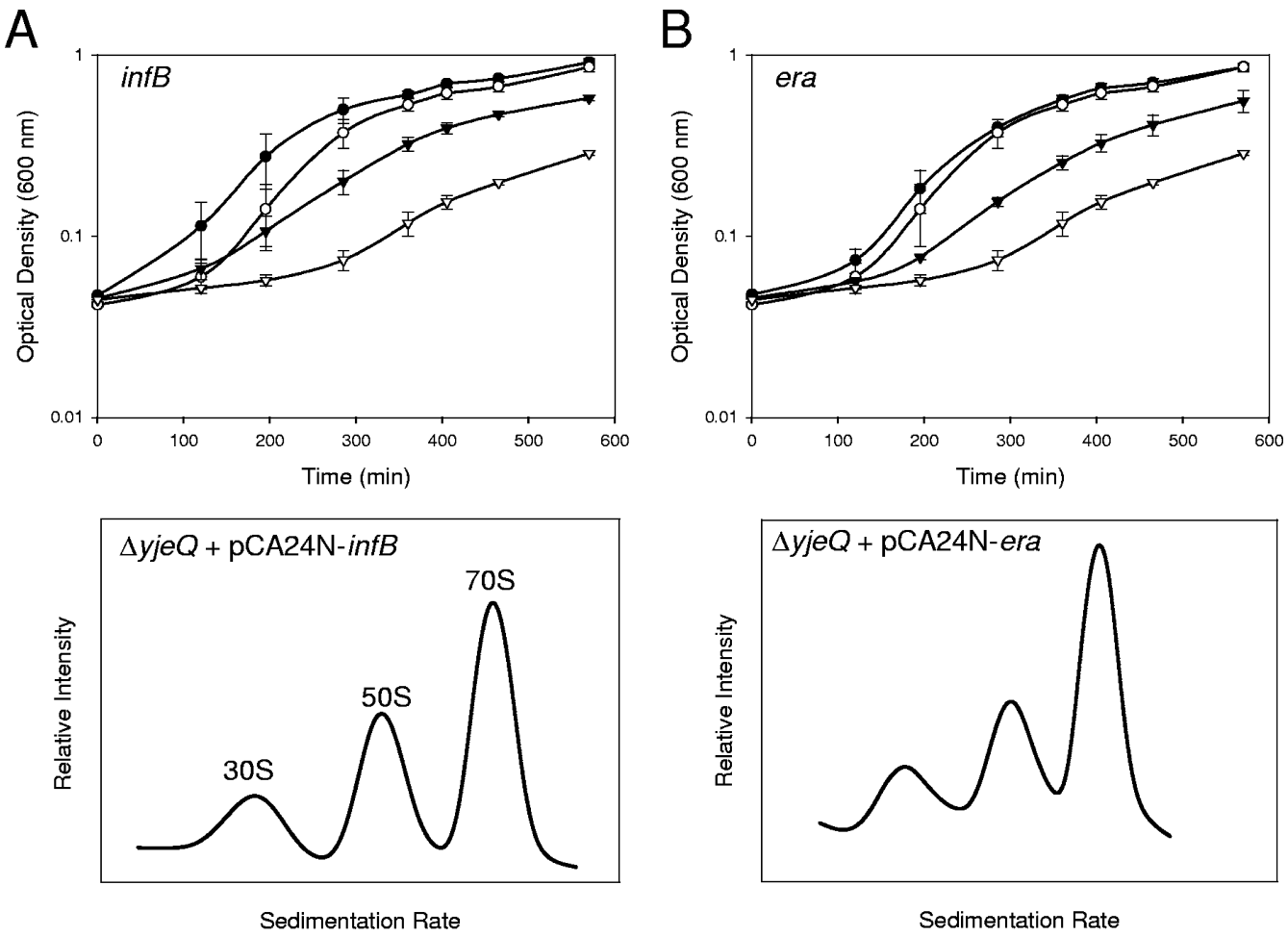

FIG. 3. Suppression of the Δ*yjeQ* slow growth by correction of the defective ribosome profile. In the growth curves circles indicate wild-type *E. coli* and inverted triangles indicate Δ*yjeQ E. coli*; filled symbols indicate that the pCA24N plasmid with the gene indicated was overexpressed, and open symbols indicate that pCA24N was is present. Ribosome profiles were prepared using analytical ultracentrifugation. (A) Suppression by overexpression of *infB*. (B) Suppression by overexpression of *era*.

gistic phenotype. *ksgA*, *ssrA*, *tgt*, *trmU*, and *trmE* deletions ahd little effect on growth, and the mutants closely resembled wild-type *E. coli*. Deletion of these genes in the Δ*yjeQ* background, however, resulted in enhancement of the Δ*yjeQ* growth defect (see Fig. S2 in the supplemental material). The enhancement of the slow-growth phenotype seen in all of the double-deletion mutants suggests that there is a functional link between the products of these genes and that of *yjeQ*.

Ribosome profiles for strains with slow-growth-enhancing double deletions. To further characterize the double-deletion strains, the ribosome profile of each single mutant was com-

TABLE 2. Ribosomal subunit distributions of wild-type, Δ*yjeQ*, and Δ*yjeQ* suppressor strains

Strain	% of total		
	30S subunit	50S subunit	70S subunit
Wild type	4.6	12.7	82.7
Δ*yjeQ* + pCA24N-*yjeQ*	9.4	18.8	71.8
Δ*yjeQ* + pCA24N	28.5	33.3	38.2
Δ*yjeQ* + pCA24N-*infB*	20.6	31.7	47.6
Δ*yjeQ* + pCA24N-*infB* + IPTG[a]	15.9	20.8	63.3
Δ*yjeQ* + pCA24N-*era*	17.3	31.8	50.9
Δ*yjeQ* + pCA24N-*era* + IPTG[a]	15.0	27.4	57.6

[a] The concentration of IPTG was 1 mM.

TABLE 3. Double deletions that enhance the slow-growth phenotype

Gene	Function
ksgA/*rsmA*	*S*-Adenosylmethionine-6-*N*′,N′-adenosyl (rRNA) dimethyltransferase
ssrA	tmRNA; rescues stalled ribosomes
tgt	tRNA-guanine transglycosylase
rimM	16S rRNA processing protein
rluD/*sfhB*	23S rRNA pseudouridine synthase (pseudouridines at positions 1911, 1915, and 1917)
trmE/*thdF*/*mnmE*	tRNA modification GTPase
trmU/*asuE*/*mnmA*	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase

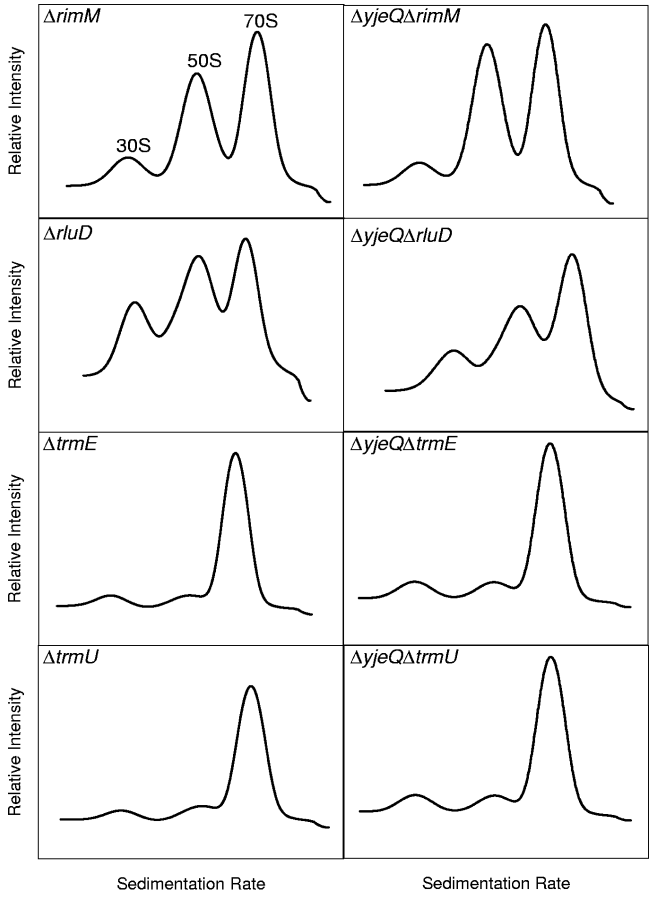

FIG. 4. Ribosome profiles of double-deletion strains. Each double-deletion ribosome profile resembles the ribosome profile of the parent single-deletion strain. The strains used are indicated. The ribosome profiles were prepared using analytical ultracentrifugation.

pared to the ribosome profiles of the double mutants. As mentioned above, *ksgA*, *ssrA*, and *tgt* deletion strains grow like wild-type *E. coli*. Correspondingly, the ribosome profiles of these three deletion strains also resemble that of the wild type (see Fig. S3 and Table S4 in the supplemental material). Deletion of these genes in the Δ*yjeQ* background yielded strains with ribosome profiles that were similar to that of the *yjeQ* deletion strain (see Fig. S3 in the supplemental material). Together, double deletions of *yjeQ* and *ksgA*, *ssrA*, or *tgt* resulted in a more severe growth defect than the defect in the *yjeQ* deletion strain, but this was not a result of alteration of the levels of free ribosomal subunits or intact ribosomes.

Mutants with double deletions of *yjeQ* and *rimM*, *rluD*, *trmE*, or *trmU* all had ribosome profiles that were different from the ribosome profile of the *yjeQ* deletion strain, and indeed for each of these strains the ribosome profile resembled the Δ*rimM*, Δ*rluD*, Δ*trmE*, or Δ*trmU* parent profile (Fig. 4; see Table S4 in the supplemental material). A *rimM* deletion strain had a severe growth defect, and this was reflected in its ribosome profile, which showed a slightly elevated level of 30S subunits and a large increase in the level of 50S subunits compared with the wild type. The ribosome profile for a *rimM yjeQ* double-deletion strain also showed a slightly increased amount of free 30S subunits and a dramatic increase in the amount of 50S subunits (Fig. 4), a profile that is similar to that of the *rimM* mutant. Similarly, the growth rate of the *rluD* deletion strain was severely altered, which was reflected in the dramatic accumulation of free 30S and 50S subunits and the concomitant decrease in the level of 70S subunits (Fig. 4). This phenotype is similar to but more pronounced than that of the *yjeQ* deletion strain. The *rluD yjeQ* double-deletion strain had a ribosome profile that closely resembled that of the Δ*rluD* strain (Fig. 4). The finding that double deletions of *yjeQ* and *rimM* or *rluD* enhanced slow growth suggests that RimM and RluD function in the same pathway as YjeQ.

The final and perhaps most surprising ribosome profiles were those of strains with double deletions of *yjeQ* and *trmE* or *trmU*. Both Δ*trmE* and Δ*trmU* strains had ribosome profiles similar to that of the wild type, despite the fact that the Δ*trmU* strain had a moderate slow-growth phenotype. Interestingly, deletion of either *trmE* or *trmU* in the Δ*yjeQ* background restored the ribosome subunit levels to the wild-type levels (Fig. 4; see Table S4 in the supplemental material). This result is surprising considering the dramatic reduction in growth rate associated with these double-deletion strains.

Taken together, the results showing enhancement of slow growth for all seven double-deletion strains provide a functional link between the seven genes examined and *yjeQ*. The ribosome profiles highlight how two deletions interact with one another with respect to ribosome subunit distributions and may indicate which deletion results in a more dominant phenotype.

DISCUSSION

A considerable body of biochemical data is available for the YjeQ protein; however, the cellular function of YjeQ remains unknown. In the work described here, we performed the first genetic interaction studies for *yjeQ* and obtained evidence linking *yjeQ* to a number of other genes with established roles in ribosome function. Screening for suppressors in an ordered library of 374 clones overexpressing largely essential genes and genes associated with ribosome function revealed that two GTPases, Era and InfB (IF2), ameliorated the growth and polysome defects of the Δ*yjeQ* strain. In addition, seven bona fide enhancers of slow growth were identified (Δ*tgt*, Δ*ksgA*, Δ*ssrA*, Δ*rimM*, Δ*rluD*, Δ*trmE*/*mnmE*, and Δ*trmU*/*mnmA*) among 39 deletions (in genes associated with ribosome function) that we constructed in the Δ*yjeQ* genetic background. Taken in context, our work is most consistent with the hypothesis that YjeQ has a role in ribosome function, likely in the late steps of 30S subunit biogenesis.

High-copy-number suppression is a powerful method for exploring genetic interactions and learning more about cellular function. Increasing the gene copy number and consequently the protein copy number can lead to a gain of function for a high-copy-number protein such that it compensates for the mutation. The new function is often analogous to that already characterized for the suppressing protein, and there is potential to learn more about the mutant. Typical suppressor screens use random genomic libraries to identify genes of interest using strong selection pressure or through onerous screening of necessarily redundant clones. The subtle growth phenotype

resulting from the *yjeQ* deletion made selection an impractical approach. Indeed, this growth phenotype also presented a considerable challenge for a large genetic screen of thousands of redundant clones. Instead, we screened a targeted, ordered library to detect suppressors among an overexpression clone set recently made available to the scientific community (28). Indeed, the ordered clone set has considerable advantages over the conventional random genomic library, including nonredundancy, better control over gene expression, knowledge of the genome coverage, ready identification of suppressors, and flexibility to focus on a subset of genes of interest.

All six genes that were capable of high-copy-number suppression (*rplE*, *infB*, *era*, *yabQ*, *csrA*, and *oxyR*) decreased the extended lag phase of the Δ*yjeQ* strain, while both *infB* and *era* also decreased the doubling time during exponential growth. For the four genes that decreased the lag phase without altering the exponential growth rate (*rplE*, *yabQ*, *csrA*, and *oxyR*) this suggests that there was an indirect suppression effect, where overexpression of a particular gene was able to start growth and did not directly correct for the ribosome defect associated with *yjeQ* depletion. These four high-copy-number suppressors were capable of suppressing the slow-growth phenotype resulting from the *yjeQ* deletion but not the polysome defect. Their functions include an unknown function (*yabQ*), a 50S subunit protein (*rplE*), and two proteins (*csrA* and *oxyR*) that function in diverse areas of metabolism. Interestingly, *csrA* and *oxyR* encode proteins that are involved in regulatory networks and respond to changes in the cellular environment and stress (18, 19, 43). This raises the possibility that YjeQ may be involved in mediating stress signals and translation.

The two remaining high-copy-number suppressors, *infB* and *era*, were able to partially correct the ribosome profile defect with an induction-dependent increase in 70S ribosomes, in addition to ameliorating the slow growth. The *infB* gene encodes the protein IF2, an essential GTPase that is involved in the first stage of translation. One function of IF2 is to bring the 30S and 50S subunits together in the presence of initiator tRNA (2, 3). In this work we observed that overexpression of IF2 in a Δ*yjeQ* strain partially corrected the slow growth and ribosome profile defect. This was likely due to an increase in free subunit association mediated by increased levels of IF2. Overexpression of IF2 alone could not completely correct either the slow growth or ribosome defect, suggesting that the function of YjeQ is not limited to subunit association.

The Era protein is a GTPase that is believed to be involved in 30S subunit biogenesis, and depletion of this protein results in phenotypes strikingly similar to the phenotypes observed after loss of YjeQ (26, 38). Era has previously been found to correct the defective polysome profile of an *rbfA* deletion mutant, and it has been proposed that Era and RbfA (ribosome binding factor A) have overlapping functions in ribosome biogenesis (25, 26). While both *era* and *rbfA* were tested in this study, only *era* was a high-copy-number suppressor of the Δ*yjeQ* slow-growth phenotype. These findings suggest a function distinct from that of RbfA and likely related to the GTPase activity of Era. Together, the abilities of high levels of Era and IF2 to suppress the Δ*yjeQ* slow-growth and ribosome profile defects suggest that YjeQ has a role in late 30S subunit biogenesis and/or subunit association.

As a complement to the high-copy-number suppression screen for genetic interactions with *yjeQ*, we also used gene deletion to look for synthetic interactions with *yjeQ*. Typically, genetic interaction studies use random mutagenesis to identify genes of interest. Given evidence to date linking YjeQ to ribosome function and the isolation in this work of high-copy-number suppressors in Era and InfB, we felt justified in focusing here on deletions in genes with known roles in ribosome function. In a screen of 39 gene deletions in the Δ*yjeQ* background, the approach yielded seven enhancers (Δ*rimM*, Δ*rluD*, Δ*ksgA*, Δ*tgt*, Δ*ssrA*, Δ*trmE*, and Δ*trmU*) of the slow-growth phenotype. The results for three of the double-deletion strains (*rimM*, *rluD*, and *ksgA*) again indicate that YjeQ has a role in 30S subunit biogenesis and/or perhaps subunit association.

RimM (ribosome maturation factor M) is a protein that has been implicated in the maturation of 30S subunits. Mutants with deletions of *rimM* have a number of similarities with a *yjeQ* deletion mutant. They have a fivefold-lower growth rate in rich media and a polysome profile defect and accumulate 17S rRNA (9). Suppression of the slow growth and translational deficiency is mediated by overexpression of RbfA, a protein that along with RimM is important for the processing of pre-16S rRNA (10). The phenotypic similarities between *yjeQ* and *rimM* deletion mutants and the identification of a genetic interaction are consistent with the idea that YjeQ may function in 30S biogenesis. Indeed, we posit that RimM may function before YjeQ in this process since the ribosome profile of the double-deletion strain resembles the ribosome profile of the *rimM* deletion.

E. coli rRNA has 10 pseudouridines, 9 of which are in the 50S subunit RNA and 1 of which is in the 30S subunit (35). All of the *E. coli* pseudouridine synthases are dispensable for cell viability, and the only one whose loss results in a growth defect is RluD, a protein that is responsible for the synthesis of pseudouridine at the highly conserved nucleotides 1911, 1915, and 1917 of helix 69 in 23S rRNA (35). These nucleotides form part of the universally conserved intersubunit bridge B2a, which influences subunit association (1). A deletion strain lacking *rluD* has defects in ribosome assembly, biogenesis, and function, and all of these phenotypes can be accounted for by loss of the pseudouridine residues (20). Thus, the genetic interaction between *yjeQ* and *rluD* may well be a reflection of the role that YjeQ plays in subunit association. Of course, such a conclusion is reinforced by the finding in this work that high-copy-number InfB (IF2) suppressed the slow-growth and polysome defects of the Δ*yjeQ* strain. The *yjeQ rluD* double-deletion ribosome profile more closely resembles the *rluD* deletion profile, suggesting that the function of RluD is either dominant over or occurs prior to that of YjeQ.

KsgA is responsible for one of only two rRNA modifications that have been conserved throughout evolution. It catalyzes the dimethylation of A1518 and A1519 in helix 45 near the 3′ end of 16S rRNA (34). The *ksgA* gene is dispensable in *E. coli*, and deletion results in slower growth and reduced translational fidelity (24). The dimethylation occurs at an intermediate point in the assembly of the 30S subunit, indicating that KsgA is a ribosome biogenesis factor (17). Through an unknown mechanism, the *ksgA* gene is a multicopy suppressor of a cold-sensitive Era mutant (32). The genetic interaction between *yjeQ* and *ksgA* is consistent with the hypothesis that YjeQ has a role in 30S biogenesis.

RimM, RluD, and KsgA are all involved in the biogenesis of the 30S subunit or subunit association. Five genetic interactions also place the function of YjeQ at this stage of the translation process. Furthermore, a *yjeQ ksgA* double deletion enhances slow growth; *ksgA* is a multicopy suppressor of an Era mutant; *era* is a multicopy suppressor of an *yjeQ* deletion; *era* is also a multicopy suppressor of an *rbfA* deletion mutant; RbfA overexpression suppresses an *rimM* deletion; and a *yjeQ rimM* double deletion enhances slow growth.

Three additional enhancers of the slow-growth phenotype resulting from the *yjeQ* deletion, Δ*trmU*, Δ*trmE*, and Δ*tgt*, represent deletions in genes that encode tRNA-modifying enzymes. The functional impact of tRNA modification is still unknown in most cases; however, it likely influences the translational properties or supports the stability of tRNAs (42). It is interesting that individual deletions of the *trmU*, *trmE*, and *tgt* genes have little impact, but when they are combined in the Δ*yjeQ* background, there are severe growth defects. In addition, the ribosome profiles closely resemble the wild-type ribosome profile, suggesting that defective ribosome assembly is not the dominant factor contributing to the growth defect in these strains. Rather, these data imply that YjeQ has a function that compensates for tRNA modification defects. One might hypothesize that when YjeQ and a tRNA-modifying enzyme are both absent, the translation efficiency is diminished and the ribosomes become locked in the 70S form and are not able to perform proper translation. This suggests a putative role for YjeQ in translational fidelity or proper tRNA movement on the ribosome. Alternatively, it is possible that if tRNA modification helps translation function more efficiently, in the *yjeQ* deletion strain, where the pool of intact 70S ribosomes is limited, these modifications become even more critical.

The final genetic interaction with *yjeQ* seen was the interaction with the *ssrA* gene. The *ssrA* gene codes for a small RNA known as transfer-messenger RNA or 10Sa RNA. This small RNA relieves ribosome stalling in a process called *trans*-translation (45). As seen in the ribosome profiles, there is a reduced pool of 70S ribosomes in cells lacking YjeQ. Removing the *ssrA* gene causes a reduction in the pool of functional ribosomes by allowing a buildup of stalled ribosomes, and this is likely responsible for the observed enhancement of the slow-growth phenotype.

The genetic interactions seen here for *yjeQ* are summarized in Fig. 5, where they are put in the context of previously reported interactions (for example, the interaction of *rfbA* with *era* and *rimM*). In aggregate, these interactions provide fresh evidence that *yjeQ* has a role in ribosome function. Particularly intriguing is the preponderance of interactions with emerging factors in 30S subunit biogenesis, namely, *era*, *rimM*, and *ksgA*. Suppression by high-copy-number *era* of both the growth and polysome defects is especially compelling given the recent cryo-electron microscopy costructure showing Era in the S1 protein binding site of the 30S subunit (40). Era appears to have a role in the assembly of the 30S subunit, where the process would conclude with Era dissociation and S1 incorporation. Thus, we propose here that YjeQ functions late in the 30S biogenesis pathway and that in its absence nearly mature 30S subunits accumulate. YjeQ may recognize maturing 30S subunits in part based on modifications to 16S rRNA, as suggested by the genetic interactions with *rimM* and *ksgA*. To overcome the delayed entry into translation in the absence of YjeQ, overexpression of the late ribosome biogenesis factor Era or the translation initiation factor IF2 may help to promote progression into translation initiation. Regardless, this work provides additional insights into the genetic interactions of *yjeQ* and further evidence that the YjeQ protein has a role in ribosome function, either in the late stages of 30S subunit biogenesis or in early steps in ribosome subunit association. In addition, we describe genetic interaction screens performed using a novel approach involving ordered overexpression and deletion clone sets recently made available to the *E. coli* community (4, 28), which should be generally applicable to studies of uncharacterized genes.

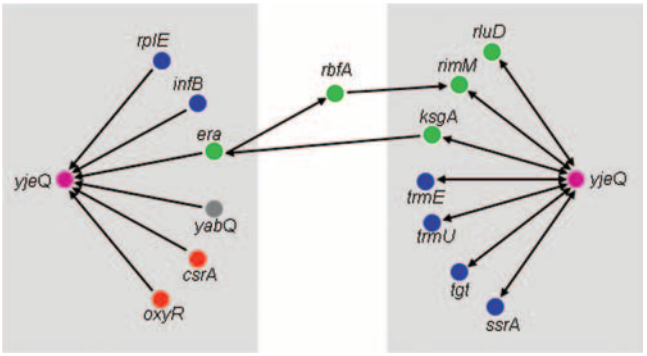

FIG. 5. Genetic interaction network for *yjeQ*. Genes interacting with *yjeQ* on the left are multicopy suppressors of the *yjeQ* deletion mutant slow growth. Deletions of the genes interacting with *yjeQ* on the right are slow-growth enhancers. The overlap between the two gene sets is shown in the center. Gene functions are color coded; blue indicates genes that code for proteins involved in translation, green indicates genes that encode ribosome biogenesis factors, orange indicates genes that are general metabolism genes, and gray indicates a gene that encodes a protein with an unknown function.

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