
Conserved P-loop GTPases of Unknown Function in Bacteria: An Emerging and Vital Ensemble in Bacterial Physiology

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Abstract

Establishing the roles of conserved gene products in bacteria is of fundamental importance to our understanding of the core protein complement to sustain cellular life. P-loop GTPases and related ATPases represent an abundant and remarkable group of proteins in bacteria that in many cases have evaded characterization. Here, efforts aimed at understanding the cellular function of a group of eight conserved, poorly characterized genes encoding P-loop GTPases, *era*, *obg*, *trmE*, *yjeQ*, *engA*, *yihA*, *hflX*, *yehF*, and a related ATPase, *yjeE*, are reviewed in considerable detail. While concrete cellular roles remain elusive for all of these genes, and considerable pleiotropy has plagued their study, experiments to date have frequently implicated the ribosome. In the cases of *era*, *obg*, *yjeQ* and *engA*, the evidence is most consistent with roles in ribosome biogenesis, though the prediction is necessarily putative. While the protein encoded in *trmE* clearly has a catalytic function in tRNA modification, the participation of its GTPase domain remains obscure as do the functions of the remaining proteins. A full understanding of the cellular functions of all of these important proteins remains the goal of on-going studies of cellular phenotype and protein biochemistry.

Introduction

While genomics has provided staggering amounts of sequence information, it has simultaneously expanded the sphere of the uncharted. Since the completion of the first genome sequence for a free-living organism (*Haemophilus influenzae*) in 1995 bacteriologists have been faced with the dilemma

that about one third of the genes in any microbe typically encode uncharacterized proteins (Tatusov et al. 2000). Estimates for the human genome have been similar in magnitude (Lander et al. 2001; Venter et al. 2001) and there is now an emerging consensus that a key hurdle facing life scientists is the assignment of function of uncharacterized genes. While we might have expected the uncharacterized fraction of genomes to encode auxiliary functions, it has become clear from microbial genomics that many uncharacterized proteins are highly conserved and carry out critical roles. In particular, the P-loop GTPases and related ATPases form a relatively large group of conserved and often indispensable proteins in bacteria where there is a paucity of functional characterization. Indeed, this group of proteins has become the subject of intensive study by several groups and has been the subject of several reviews (Caldon and March 2003; Caldón et al. 2001; Leipe et al. 2002; Mittenhuber 2001; Morimoto et al. 2002). The often essential nature and broad conservation of these uncharacterized proteins suggests that they play central roles in bacterial physiology. This review, while reasonably comprehensive across the most relevant work, focuses on the careful genetic and biochemical studies that are steadily improving our understanding of the functions of conserved, uncharacterized GTPases in bacteria.

P-loop GTPases and related ATPases in bacteria

GTPases function as crucial molecular switches in a broad variety of biochemical processes. The majority of GTPases are part of a vast class of

homologous proteins known as P-loop NTPases that share a mononucleotide-binding fold and catalyze hydrolysis of the β - γ phosphate ester bond of the nucleotide. Of all the nucleotide binding folds, the P-loop fold is by far the most abundant; it has been estimated that 10-18% of predicted gene products are P-loop NTPases (Koonin et al. 2000). Structurally, P-loop NTPases are α/β proteins that are characterized by an N-terminal Walker A motif consisting of a flexible loop spanning a β -strand and helix with the signature GxxxxGK[ST], where the function of this loop is to position the triphosphate moiety of the nucleotide (Walker et al. 1982). A Walker B motif is distal and contains a conserved carboxylate-containing residue, aspartate or glutamate. The conserved acidic residue is located at the end of a typically hydrophobic β -strand and has the role of coordinating a Mg^{2+} cation that also has interactions with the β and γ phosphates (Walker et al. 1982).

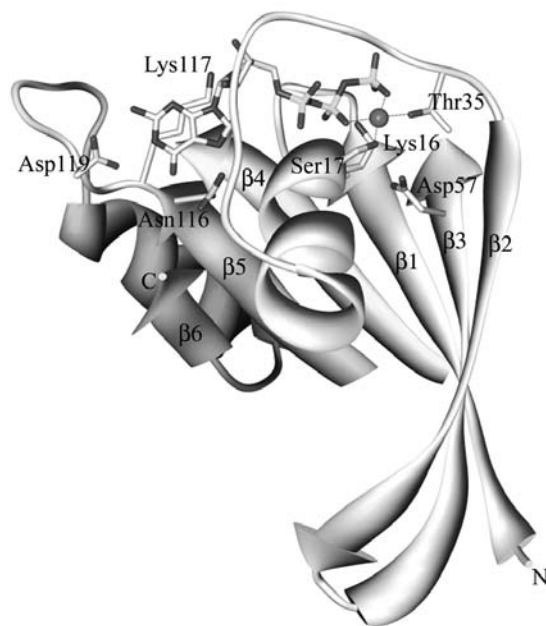


Figure 1. Structural core of β -strands and associated loops in the GTPase domain of p21 Ras (PDB identifier ICTQ). The strand topology and Walker motifs characteristic of P-loop GTPases ($\beta 1$ through $\beta 6$) of the TRAFAC class are emphasized through the removal of extraneous loops and helices in the structure. Of note is the antiparallel nature of the strand $\beta 2$ adjacent to the Walker B strand $\beta 3$. Highlighted are the bound non-hydrolyzable GTP analogue 5'-guanylimidodiphosphate (GMP-PNP), Walker A (GxxxxGK[ST]) residues K16 and S17, Walker B (hhhDxxG) residue D57, GTPase specificity ([NT]KxD) residues N116 and D119, and the TRAFAC class-specific residue T35. Also shown is a bound Mg^{2+} molecule (magenta sphere).

The P-loop GTPases share a common structural core having an arrangement of strands and loops in the GTPase domain characterized by the prototype Ras protein (Figure 1). Koonin and colleagues developed a phylogenetic classification of P-loop GTPases and related ATPases by dividing some 60 distinct, ancient groups into two large classes (Leipe et al. 2002). The first is the TRAFAC (translation factor-related) class that includes classic GTPases, such as translation factors and the extended Ras family, as well as some ATPases such as kinesin and myosin. The second class was called SIMBI after its three largest subgroups, the signal recognition GTPases, the MinD superfamily and the BioD superfamily. The TRAFAC class encompasses the proteins discussed in this review and is characterized by a conserved β -strand topology where strand 3, adjacent to the Walker B strand, is uniquely anti-parallel and there is a conserved threonine or serine with a key role in Mg^{2+} coordination in the loop preceding β -strand 3 (Bourne et al. 1991; Leipe et al. 2002). Other features of P-loop GTPases include a specific form of the Walker B (hhhDxxG), where h is a hydrophobic amino acid, and the glycine amide interacts with the γ -phosphate, and a distal [NT]KxD, not found in other P-loop NTPases, that imparts specificity for guanine over other nucleotide bases.

Table 1 highlights a group of eight conserved bacterial P-loop GTPases and a related ATPase. These encompass TRAFAC class P-loop NTPases, present in the model bacterium *E. coli*, that are most broadly conserved and remain poorly characterized for physiological function in bacteria. Indeed, apart from well-characterized translation factors such as IF2, EF-tu and EF-G, the vast majority of bacterial P-loop NTPases in this class remain functionally obscure (Leipe et al. 2002). The table details the conservation of orthologues for this group of eight in 43 microbes spanning 30 major phylogenetic lineages as determined by Koonin and colleagues in the clusters of orthologous groups (COG) database (<http://www.ncbi.nlm.nih.gov/COG/old/>). The patterns of conservation reveal that, while broadly conserved among eubacteria, these genes are absent in archaea, apart from *hflX*, *yihA* and *ychF*. The latter, *ychF*, is present in all 30 phyloge-

Table 1.
Conserved, bacterial, P-loop GTPases (and a related ATPase) of unknown function.

Gene ¹	Conservation ²	Phenotype	Biochemistry	Proposed role ³
<i>era</i> <i>bex</i>	-----QVDRLB CEFGHSNUJX-TW	essential in <i>E. coli</i> ; cell division defect; chromosome segregation defect; altered ribosome profile; genetic interactions with <i>ksgA</i> , <i>dnaG</i> and <i>rbfA</i> ; slow growth and sporulation defect in <i>B. subtilis</i>	slow GTPase; binds 16S rRNA, cell membrane and MazG; KH domain-mediated RNA binding; GTPase stimulated by RNA; x-ray structure; cryo-em co-structure with 30S	ribosome biogenesis
<i>obg</i> <i>yhbZ</i> <i>obgE</i> <i>cgtA</i>	-----YQVDRLB CEFGHSNUJXITW	essential; chromosome segregation defect; genetic interactions with <i>rrmJ</i> , <i>recA</i> and <i>recB</i> ; chemical- genetic interactions with replication inhibitors	slow GTPase; binds 50S and 30S ribosome; co-fractionation with ribosomal proteins and RNA; x-ray structure; novel N-terminal Obg-fold	ribosome biogenesis
<i>trmE</i> <i>mnmE</i> <i>thdF</i>	-----YQVD-LB CEFGHSNUJXITW	deficiency in 5-methyl-laminomethyl-2-uridine (U34) of tRNAs; synthetic lethality with unknown mutation(s)	fast GTPase; t-RNA modification; x-ray structure; N-terminal formyl-tetrahydrofolate binding, C-terminal G-domain	t-RNA modification
<i>yjeQ</i> <i>rsgA</i> <i>yloQ</i>	-----QV-RLB CEFGHSN-J--TW	slow growth; filamentous; chemical-genetic interactions with translation inhibitors; altered ribosome profile	slow GTPase; binds 30S ribosome; GTPase stimulated by 30S; x-ray structure; central circularly permuted G-domain; N-terminal OB-fold; C-terminal Zn finger	ribosome biogenesis
<i>engA</i> <i>der</i> <i>yfgK</i> <i>yphC</i>	-----QVDRLB CEFGHSNUJXITW	essential; filamentous; chromosome segregation defect; genetic interaction with <i>rrmJ</i>	slow GTPase; x-ray structure; two adjacent G-domains; C-terminal KH-like domain	ribosome biogenesis
<i>yihA</i> <i>ysxC</i>	AOM-K-YQV--LB -EFGHSNUJX--W	essential; filamentous; septation defect	binds GTP and GDP	-----
<i>hflX</i> <i>ynbA</i>	-OM-KZ-QVDRLB CEFGHSN-J-I--	high frequency of lysogenization (<i>hfl</i>) locus	-----	-----
<i>ychF</i> <i>yyaF</i>	AOMPZYQVDRLB CEFGHSNUJXITW	-----	binds GTP and nucleic acid; x-ray structure	-----
<i>yjeE</i> <i>ydiB</i>	-----QVDRLB CEFGHSNUJXIT-	essential	slow ATPase; binds ADP and YjeF; x-ray structure	-----

¹ The *E. coli* gene name is listed along with synonyms, including that for the orthologue from *B. subtilis*.

² Conservation has been reported for 43 genomes as documented in the COG database (<http://www.ncbi.nlm.nih.gov/COG/old/>) according to the following legend: A, *Archaeoglobus fulgidus*; O, *Halobacterium sp. NRC-1*; M, *Methanococcus jannaschii*, *Methanobacterium thermoautotrophicum*; P, *Thermoplasma acidophilum*, *Thermoplasma volcanium*; K, *Pyrococcus horikoshii*, *Pyrococcus abyssi*; Z, *Aeropyrum pernix*; Y, *Saccharomyces cerevisiae*; Q, *Aquifex aeolicus*; V, *Thermotoga maritime*; D, *Deinococcus radiodurans*; R, *Mycobacterium tuberculosis*, *Mycobacterium leprae*; L, *Lactococcus lactis*, *Streptococcus pyogenes*; B, *Bacillus subtilis*, *Bacillus halodurans*; C, *Synechocystis*; E, *Escherichia coli K12*, *Escherichia coli O157*, *Buchnera sp. APS*; F, *Pseudomonas aeruginosa*; G, *Vibrio cholerae*; H, *Haemophilus influenzae*, *Pasteurella multocida*; S, *Xylella fastidiosa*; N, *Neisseria meningitidis MC58*; *Neisseria meningitidis Z2491*; U, *Helicobacter pylori*, *Helicobacter pylori J99*, *Campylobacter jejuni*; J, *Mesorhizobium loti*, *Caulobacter crescentus*; X, *Rickettsia prowazekii*; I, *Chlamydia trachomatis*, *Chlamydia pneumoniae*; T, *Treponema pallidum*, *Borrelia burgdorferi*; W, *Ureaplasma urealyticum*, *Mycoplasma pneumoniae*, *Mycoplasma genitalium*.

³ Proposed roles, where applicable, are based on the balance of experimental evidence to date.

netic lineages. Interestingly only four of these widely conserved genes, *ychF*, *obg*, *trmE* and *yihA*, are also found in the eukaryote, *S. cerevisiae*. This finding is consistent with the outlook to date that, while eukaryotic G-proteins are celebrated for their roles in transmembrane receptor-mediated cell signaling, prokaryotic GTPases appear not be involved in analogous processes in bacteria. Table 1 also summarizes information pertaining to the phenotype of the null mutant or, in the case of essential genes, the phenotype associated with depletion of the gene product in a conditional mutant. Indicated in addition, are salient findings from biochemical studies, often with recombinant proteins *in vitro*. Where reasonable, I have proposed a physiological function for each of these proteins that, in my view, best sums the data published to date. Below, I present that analysis supported by phenotypic and biochemical characterization of these loci with an emphasis on recent publications.

Studies of phenotype and biochemistry of conserved bacterial P-loop NTPases

Era, the first discovered bacterial GTPase, was named for its Ras-like GTPase domain (Ahnn et al. 1986) and has been extensively studied. Only recently has a resolution regarding its physiological function begun to develop. Era was shown to be essential in *E. coli* more than a decade ago (Gollop and March 1991; Lerner and Inouye 1991) and subsequent studies have shown cross-species complementation by orthologues from several bacteria (Pillutla et al. 1995; Zuber et al. 1997). The *B. subtilis* orthologue Bex was shown to be dispensable in that organism but led to a slow growth phenotype and was vital to sporulation (Minkovsky et al. 2002). *E. coli* Era has been proposed to regulate cell division (Britton et al. 1998; Johnstone et al. 1999; Lu and Inouye 1998). Depletion of Era led to filamentous cells having normal DNA replication and nucleoid segregation but apparently blocked for cell division. In *B. subtilis*, on the other hand, depletion of Bex led to diffuse, unsegregated chromosome in elongated cells, suggesting a role, prior to division, in nucleoid segregation (Minkovsky et al. 2002). Interestingly, a mutant in

E. coli era was isolated as a suppressor of a temperature sensitive *dnaG* mutant encoding DNA primase (Britton et al. 1997). Microarray and other studies have linked era function with energy metabolism (Inoue et al. 2002; Pillutla et al. 1996; Powell et al. 1995). Much phenotypic work on era has suggested a role in ribosome function. Depletion of Era leads to an increase in dissociated 30S and 50S subunits (Sayed et al. 1999) and to an accumulation of 17S rRNA, an unprocessed precursor of 16S rRNA (Inoue et al. 2003). Relevant genetic interactions noted for era include the gene *ksgA* (Lu and Inouye 1998), coding for a 16S rRNA dimethyltransferase and *rbfA* (Inoue et al. 2003), encoding a cold shock protein that specifically associates with 30S ribosomal subunits.

Biochemical studies of Era have focused on its GTPase activity, protein structure and interactions. Purified recombinant Era has a slow GTPase activity ($k_{\text{cat}} \sim 1 \text{ h}^{-1}$) that is stimulated many fold by interaction with RNA (Meier et al. 2000; Sullivan et al. 2000). X-ray crystallographic analysis revealed a two lobe structure for Era with an N-terminal GTPase region and a C-terminus that contains a signature KH RNA binding domain (Chen et al. 1999). Indeed, this C-terminal domain has been repeatedly implicated in the 16S RNA binding activity of Era (Hang and Zhao; Hang et al. 2001; Inoue et al. 2003; Johnstone et al. 1999; Meier et al. 1999; Meier et al. 2000) and in its interaction with the 30S ribosomal subunit (Sayed et al. 1999). Most recently a co-structure of Era in complex with the 30S ribosomal subunit validated much of the biochemical and phenotypic data that pointed to a *bona fide* interaction with the ribosome (Sharma et al. 2005). The co-structure shows Era in complex with the 3' region of 16S rRNA in a cleft between the head and platform of the 30S subunit, locking it in a conformation that is unfavorable for association with the 50S subunit. Interestingly, Era is in the S1 protein binding site. The co-structure of Era with the 30S subunit is compelling in itself and ties together many of the biochemical and phenotypic studies of this bacterial GTPase. Thus Era appears to have a role in the assembly of the 30S subunit, perhaps by chaperoning the 16S rRNA. Presumably the

assembly process would be complete with Era dissociation and S1 incorporation.

The next best studied bacterial P-loop GTPase is Obg. The *obg* gene was first discovered in *B. subtilis* (Trach and Hoch 1998). It has been studied in a wide variety of organisms including *E. coli*, *B. subtilis*, *Streptomyces coelicolor* and *Caulobacter crescentus*, and shown to be essential for cell growth (Arigoni et al. 1998; Kok et al. 1994; Maddock et al. 1997; Morimoto et al. 2002), sporulation (Vidwans et al. 1995) and morphological differentiation (Okamoto and Ochi 1998). Pleiotropy has characterized the consequences of Obg depletion in cells just as it has for Era. Cell filamentation, defective chromosome partitioning and altered DNA replication have been reported (Foti et al. 2005; Kobayashi et al. 2001; Slominska et al. 2002). Depletion of the Obg orthologue in *C. crescentus* resulted in slow growth and reduced levels of 50S ribosomal subunit (Datta et al. 2004). Genetic interactions discovered so far for *obg* have pointed to ribosome function and to DNA replication. The *obg* gene was selected from a random genomic multicopy library for suppressors of an *E. coli* mutant in *rrmJ*, encoding a 23S rRNA methyltransferase (Tan et al. 2002). Most recently, a transposon insertion mutant in the 3' end of *obg* was isolated in a search for mutants sensitive to DNA replication inhibitors (Foti et al. 2005). In the same work, similar chemical-genetic interactions were noted in a dominant negative mutant directed at the GTPase function of Obg and synergism was evident between *obg* mutants and null mutants in DNA repair genes *recA* and *recB*.

The Obg protein has a very slow intrinsic GTPase activity with turnover recorded for pure recombinant proteins from *B. subtilis* and *C. crescentus* on the order of 1 h⁻¹ (Buglino et al. 2002; Lin et al. 1999; Welsh et al. 1994). High resolution structural details for Obg to date have included both apo and nucleotide-bound forms of the proteins from *B. subtilis* and *Thermus thermophilus* (Buglino et al. 2002; Kukimoto-Niino et al. 2004). Co-structural information came from studies of the *B. subtilis* protein where ppGpp nucleotide, an effector molecule of the stringent response, was discovered at the active site of the crystallized protein (Buglino

et al. 2002), however, the physiological relevance of the ligand remains obscure. Obg is a two domain protein with a unique N-terminal glycine rich region, nicknamed the Obg domain, and a C-terminal GTP-binding domain. These domains share a significant interaction interface that is mediated in part by the conserved GTPase switch elements. Indeed, some significant conformational changes were noted in the nucleotide bound and apo forms suggesting the likelihood of nucleotide dependent signaling between the two folds. A number of biochemical studies have suggested that Obg has an affinity for ribosomes, in particular the 50S subunit (Lin et al. 1999; Sato et al. 2005; Wout et al. 2004; Zhang and Haldenwang 2004). Interestingly, "pull-down assays" with the *E. coli* protein have revealed association of Obg with 16S and 23S ribosomal RNAs as well as with several ribosomal proteins, including RNA helicase CsdA and chaperone ClpA. Particularly interesting, in light of the unanticipated co-structure Obg with ppGpp, was finding of Wout and coworkers that recombinant *E. coli* Obg copurified with SpoT, the ribosome-associated (p)ppGpp hydrolase/synthetase enzyme with a role in the stress response. The simplest interpretation of the phenotypic and biochemical evidence available to date is that Obg has a role in ribosome biogenesis.

Gene *trmE* was identified more than twenty years ago in the isolation of mutants that were deficient for the synthesis of 5-methylaminomethyl-2-thiouridine based on a phenotype of reduced read through of UAG codons (Elseviers et al. 1984). Many years later it was found to be allelic with *thdF*, a gene previously shown to be involved in thiophene and furan oxidation (Alam and Clark 1991), and was shown to be a GTPase that was essential for viability in some genetic backgrounds, presumably due to synthetic lethal interaction(s) (Cabedo et al. 1999; Yim et al. 2003). TrmE is thought to be a key enzyme in the multi-step modification of the wobble position uridine (U34) in tRNAs. The altered base is capable of base pairing with G and A but not with C or U, a feature that is important for mixed codon families and influences frameshifting during translation (Brierley et al. 1997; Urbonavicius et al. 2003). Compared to

most bacterial GTPases, TrmE has a high intrinsic GTPase activity, with a turnover of more than 500 h⁻¹ (Yamanaka et al. 2000). A recent x-ray crystallographic study of the protein revealed an N-terminal formyl-tetrahydrofolate binding domain, a central helical domain with a conserved cysteine-containing motif, and a C-terminal Ras-like fold (Scrima et al. 2005). These investigators proposed that TrmE catalyzes the first step of 5-methylaminomethyl-2-thiouridine synthesis with formylation of position 5 of the uridine, a step that is probably activated by a covalent adduct with the conserved cysteine (Yim et al. 2003). While the chemical role of TrmE in t-RNA modification is becoming apparent, the importance of the GTP binding and hydrolysis functions of TrmE remain unclear. It seems likely, nevertheless, that the GTPase function of TrmE has a role in the t-RNA modification steps. It was noted by Scrima and coworkers that significant conformational rearrangements would be necessary to accomplish the proposed chemical steps (Scrima et al. 2005). The G-domain of TrmE may therefore have a role in transducing the energies of binding and hydrolysis of GTP into conformational changes which make the chemistry possible.

While the first accounts of investigations on *era*, *obg* and *trmE* are now decades old, studies of the balance of genes in Table 1, including *yjeQ*, have been post-genomic. Despite initial reports of indispensability (Arigoni et al. 1998; Kobayashi et al. 2003) gene *yjeQ* has been shown to be expendable in *E. coli* and *B. subtilis* (Campbell et al. 2005; Freiberg et al. 2001; Himeno et al. 2004). The early conclusions of an essential phenotype are probably derivative of a slow growth defect that has been recently demonstrated for the *B. subtilis* and *E. coli* mutants (Campbell et al. 2005; Himeno et al. 2004). Depletion of the *yjeQ* orthologue in *B. subtilis* resulted in the accumulation of 30S and 50S ribosomal subunits and sensitivity to antibiotics that bind at the peptide channel or peptidyl-transferase centre of the ribosome (Campbell et al. 2005). That work also demonstrated a profound filamentous phenotype in the *B. subtilis* mutant. Pure, recombinant *E. coli* YjeQ protein showed a low intrinsic GTPase (~ 10 h⁻¹), characterized by

burst kinetics where GTP hydrolysis was shown to exceed catalytic turnover by some 45,000-fold (Daigle et al. 2002). That work documented an extraordinary disconnection between fast chemical steps of GTP hydrolysis and slow release of products GDP and/or phosphate. Such a disconnection is, of course, paradigmatic of the capacity of GTPases to store and transduce the energy of GTP binding and hydrolysis into a signal imparted to a partner protein. In the case of YjeQ and its orthologues, it's now clear that the partner is the ribosome (Campbell et al. 2005; Daigle and Brown 2004; Himeno et al. 2004). Cell fractionation studies revealed that YjeQ was in low copy in *E. coli* and bound entirely to ribosomes (1:200, YjeQ:ribosomes) (Daigle and Brown 2004). Recombinant YjeQ bound stoichiometrically and tightly to the 30S subunit of the ribosome in the presence of a non-hydrolyzable GTP analogue and the GTPase activity of YjeQ was shown to be stimulated many fold by the 30S subunit of the ribosome (Daigle and Brown 2004; Himeno et al. 2004). Two x-ray studies have revealed the structural details of YjeQ and its orthologues (Levdiko et al.; Shin et al. 2004). The protein is characterized by an unusual connectivity where the G-protein motifs are circularly permuted. This central permuted GTPase domain is flanked by an N-terminal oligonucleotide binding (OB) fold and a C-terminal Zinc-binding domain. The OB-fold was found to be critical to both 30S binding and ribosome stimulated GTPase activity (Daigle and Brown 2004). At present it appears very likely that YjeQ has a role in ribosome function where its stoichiometry with ribosomes would be most consistent with a catalytic role in ribosome biogenesis.

The gene *engA* has been shown to be essential in *Neisseria gonorrhoeae* (Mehr et al. 2000), *E. coli*, (Hwange and Inouye 2001) and *B. subtilis* (Morimoto et al. 2002). The depletion of EngA was shown to result in filamentous cells with defective chromosomal segregation in *E. coli* (Hwange and Inouye 2001) and in curved elongated cells with condensed nucleoids in *B. subtilis* (Morimoto et al. 2002). Like *obg*, *engA* at high copy was shown to rescue the growth defects of a null mutation in the gene coding for the heat-

induced rRNA methyltransferase, *rrmJ* (Tan et al. 2002). X-ray crystallographic studies have highlighted a unique structural feature of the EngA protein, namely, tandem GTPase domains (Robinson et al. 2002). These adjacent N-terminal GTPase domains are followed by a C-terminal domain that is analogous to KH domains, but lacks structural features that are indicative of the RNA-binding capacity of such protein folds. While there's clearly a paucity of data to propose a role for EngA, ribosome biogenesis seems a likely function given the phenotype and structure of this conserved GTPase.

Several studies of the dispensability of gene *yihA* point to an essential role for the encoded protein in both *E. coli* and *B. subtilis* (Arigoni et al. 1998; Wang and Kuramitsu 2003). Depletion of YihA in *E. coli* led to filamentation and a block in cell division steps beyond nucleoid segregation (Dassain et al. 1999). Recombinant *E. coli* YihA protein was purified and shown to bind GDP with micromolar affinity (Lehoux et al. 2003). Because of its potential as a therapeutic target for new antibacterial drugs, YihA was recently the subject of an assay development effort where affinity capillary electrophoresis was employed to look for small molecules interacting with the protein (Lewis et al. 2004). The latter work highlights a remarkable bit of irony in the field. The conserved bacterial GTPases are regarded as exciting targets for new antibacterial drugs, and yet these proteins lack the functional characterization necessary for conventional target-based drug discovery efforts.

The *E. coli hflX* gene is present in a locus that governs the lysis-lysogeny decision and has been implicated in controlling the proteolysis of the λ phage cII repressor (Noble et al. 1993). Thus far, the dispensability of *hflX* appears unaddressed. HflX protein is the founding member of a family within the Obg-HflX superfamily of conserved GTPases and, to date, is completely uncharacterized (Leipe et al. 2003).

YchF is in a subfamily of Obg-like proteins about which much is unknown, including its dispensability. The crystal structure of YchF revealed an N-terminal P-loop GTPase domain, a central coiled

coil domain and a C-terminal half β -barrel (Teplyakov et al. 2003). In addition to the structural work, these researchers used fluorescence microscopy to demonstrate that the pure recombinant protein could bind both GTP and nucleic acid. The latter experiments were motivated by the observation of a deep and positively charged cleft among the three domains. This line of investigation highlights the capacity of structural studies to provide testable hypotheses for biochemical studies aimed at understanding the function of uncharacterized proteins.

The last of the conserved P-loop proteins considered here is YjeE, an essential (Allali-Hassani et al. 2004; Freiberg et al. 2001) ATPase that has been somewhat enigmatic to structural classification. The structure of the orthologue from *Haemophilus influenzae* was solved as part of a structural genomics initiative and while its fold is reminiscent of that of the TRAFAC class of P-loop NTPases, it also has some significant structural similarity to P-loop kinases (Teplyakov et al. 2003). The protein has thus been proposed to fill a "topological niche." Allali-Hassani and coworkers (Allali-Hassani et al. 2004) showed that depletion of YjeE led to slow growth and that proposed Walker mutants, K41A, T42A and D80Q, were impaired for complementation of the growth defect. Indeed, while the former two variants were clearly in the Walker A motif, D80 appears to have been wrongly assigned to the Walker B motif. This is due to the presence in YjeE of an additional parallel β -strand (strand 4) between strands 1 and 3 of the typical TRAFAC fold depicted in Figure 1 (Leipe D.D, personal communication, 2005). This would make E108 the actual conserved Walker B carboxylate-containing residue with D80 playing an important but nevertheless mysterious role. A very low ATPase activity (1 h^{-1}) was characterized for the recombinant protein, as well as a micromolar affinity for ADP using fluorescence resonance energy transfer from a conserved active site tryptophan to fluorescently labeled ADP (Allali-Hassani et al. 2004). YjeE has been proposed to function in cell wall biosynthesis based on phylogenetic pattern, its presence in bacterial genomes is coincident with known cell wall

enzymes, and on genome context, it is often found near the cell wall amidase *amiB*. In any case, phenotypic or biochemical experiments that support this proposal have yet to be reported.

Conclusions

It is clear that the celebrated role of eukaryotic GTPases in receptor-mediated cell signaling is a paradigm that is not applicable to bacteria. Even so, bacterial GTPases have on balance proven comparatively refractory to functional understanding. Despite decades of study, for example of *era*, *obg*, and *trmE*, there remains much to learn still regarding the cellular function of these conserved bacterial GTPases. Pleiotropic effects associated with lesions in these particular loci has been especially confounding, though recent investigations have come to focus on what is likely their true cellular role, ribosome function. Examinations of *yjeQ*, *engA*, *yihA*, *hflX*, *ychF* and *yjeE*, on the other hand, have been relatively recent and post-genomic. Here too, the evidence gathered to date appears to point to the ribosome, at least for *yjeQ* and *engA*. In sum, the progress of research into this important group of proteins indicates that, in contrast to the high throughput genomic studies that generated their sequences, functional understanding will be advanced one gene/protein at a time through concerted investigations of cellular phenotype and *in vitro* biochemistry.

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