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REVIEW ARTICLE

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The structure–function analysis of Obg-like GTPase proteins along the evolutionary tree from bacteria to humans

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Abstract

Obg proteins belong to P-loop guanine triphosphatase (GTPase) that are conserved from bacteria to humans. Like other GTPases, Obg cycles between guanine triphosphate (GTP) bound "on" state and guanine diphosphate (GDP)-bound "off" state, thereby controlling various cellular processes. Different members of this group have unique structural characteristics; a conserved glycine-rich N-terminal domain known as obg fold, a central conserved nucleotide binding domain, and a less conserved C-terminal domain of other functions. Obg is a ribosome dependent GTPase helps in ribosome maturation by interacting with several proteins of the 50S subunit of the ribosome. Obg proteins have been widely considered as a regulator of cellular functions, helping in DNA replication, cell division. Apart from that, this protein also takes part in various stress adaptation pathways like a stringent response, sporulation, and general stress response. In this particular review, the structural features of ObgE have been highlighted and how the structure plays important role in interacting with regulators like GTP, ppGpp that are crucial for executing biological function has been orchestrated. In particular, we believe that Obg-like proteins can provide a link between different global pathways that are necessary for fine-tuning cellular processes to maintain the cellular energy status.

KEYWORDS

GTP, Obg, ppGpp, ribosome, TRAFAC GTPase

1 | INTRODUCTION

G-Proteins have been found significant for their role in various biological processes (Yoshino et al., 2018). These protein-coupled receptors influence so many important downstream events during signal transduction (Yoshino et al., 2018). In the absence of signal, G proteins are bound to GDP and remain inactive. Upon the arrival of the signal, GDP bound to G-protein is released and subsequent binding of GTP to this protein occurs, triggering conformational changes of the protein. As a result, GTPases get activated by guanine nucleotide exchange factors GTPase activity then hydrolyzes the bound GTP to GDP and Pi (inorganic phosphate), thus converting back the active form to the inactive one. GTPase

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activating proteins (GAPs) further aid this GTPase activity by hydrolysis of bound GTP to GDP, hence inactivating the G-Protein (Wennerberg & Rossman, 2005). GTPases are known to play an important role not only in signal transduction response, but also in translation, regulation of cell division, differentiation and movement and protein translocation through membranes.

Based on structural analogy and sequence similarity, GTPases are divided into two major categories: the TRAFAC class (translation factors), comprises proteins involved in translation, signal transduction, cellular physiology, and stress response. The other category SIMIBI class (signal recognition particle, MinD, and BioD) contains the signal-recognition- associated GTPases, different MinD-like ATPases (Maracci & Rodnina, 2016) and several other proteins having kinase or phosphate transferase activity. Based on sequence, structure, and domain architecture, these major groups are subdivided into different families and subfamilies contain a pool of universally conserved GTPases found in either bacteria or eukaryotes. TRAFAC GTPases are functionally different from SIMIBI family and largely fall under the class of RA-GTPases, a term used to refer to subclass of GTPase involved in ribosome functions such as assembly, maturation, and maintenance. This RA class of GTPase has a high affinity for GTP, enabling them to respond during the fluctuation of the cellular energy level and in pursuit of regulation of energy mediated processes such as protein translation, ribosome assembly (Kubarenko et al. 2005).

TRAFAC GTPases are functionally versatile, acting as ribosome assembly checkpoints that bind to the immature parts in the GTP-bound state and prohibit association of r-proteins any further until the maturation takes place (Bennison et al., 2019). A detailed analysis of genome sequence reported that TRAFAC (translation factor) class GTPases is divided into five superfamilies as shown in Figure 2. The most prominent ones are the Obg-HflX-like (Group- E) and TrmE-Era-EngA class of superfamilies (Group-D) mentioned in the figure (Suwastika et al., 2014). HflX family is characterized by the presence of a distinct conserved domain having a glycine-rich segment which is N-terminal to the GTP binding domain (Leipe et al., 2002; Dutta et al. 2009). According to phylogenetic analysis, it can be assumed that HflX was acquired by the eukaryotes from α-proteobacteria via the mitochondrial route (Leipe et al., 2002). Another theory postulates that HflX emerged in bacteria and the vast phyletic distribution of the current HflX family came up secondarily via the method of multiple horizontal transfers (Leipe et al., 2002). HflX is known to portray both GTPase and ATPase activities and is involved in manganese homeostasis, splitting of 70S ribosome binding, and the unwinding of the secondary structure of RNA (Bennison et al., 2019). This particular GTPase has also been reported as a heat shock protein in *Escherichia coli*. (Dutta et al. 2009). Several other GTPases are belonging to the ancient transcription factor sub-families, one of them is TypA (Tyrosine phosphorylated protein A), and also known as BipA which is an outcome of duplication of EF-G gene present in bacteria (Leipe et al., 2002). TypA is seen to be associated with environmental and stress response in bacteria along with virulence in pathogenic bacteria (Atkinson et al., 2015). The molecular details are yet to be known for TypA but it was proposed that it assists in transcript selective translational control (Margus et al. 2007). LepA is another product of EF-G gene duplication, following an early transfer, possibly from pro-mitochondrial endosymbiont into the eukaryotic lineage (Leipe et al., 2002). It was originally found to be associated with the cell membrane fraction and exhibits similarity to a large extent with the translation factor GTPases (Margus et al., 2007). LepA has a unique feature of back-translocating post- translocational ribosomes. As per results suggest, it recognizes ribosomes after a defective reaction, inducing a back translocation that gives EF-G a second chance to translocate tRNAs correctly (Margus et al., 2007). RsgA (ribosome small-subunit dependent GTPase A) is a distinct GTP hydrolytic protein, found broadly in bacteria and plants which gets activated with the help of a small ribosomal subunit (Kimura et al., 2008).

An extensive study of RsgA in both *Bacillus subtilis* and *E. coli* showed that it is involved in the assembly of 30S subunit and/or joining of the subunit (Britton 2009). According to the suggestions of Serror and coworkers, RsgA being a translation factor is distinctively involved in the translation of key cell wall-building proteins. Although evidence of such a model is not established, hence needs further study (Britton 2009). EngA (a Group-D member mentioned in Figure 2) family of GTPases belong to TRAFAC class and is universally present in all bacteria and *Arabidopsis sp*. but devoid in other eukaryotes or archaea. Perhaps this signifies that this family has probably originated from bacteria and the plant members obtained it from the pro-chloroplast symbiont via horizontal transfer (Leipe et al., 2002). EngA and its orthologs are known to consist of two GTPase domains (Leipe et al., 2002; Agarwal et al., 2012). The family of EngA has been named after essential Neisserial GTP-binding protein A since it was first discovered in *Neisseria gonorrhoeae* (Agarwal et al., 2012). Era (*E. coli* Ras-like protein) is widely conserved across all forms of life, was initially identified as a bacterial protein, and serves as an essential GTPase in *E. coli*. A homolog of Era (ERAL1) has also been determined in the human genome

that is involved in the process of apoptosis (Britton 2009; Akiyama et al., 2001). Era is a versatile GTPase concerned with cell division and energy metabolizing activities. Apart from this characteristic feature of Era, it also binds to the ribosomal subunit interface and stops the joining of 30S and 50S, hence acting as a checkpoint for 16S maturity before 70S assembly which in turn regulates the cell cycle (Sharma et al. 2005). Depleted levels of Era in bacteria result in unprocessed 16S precursor build-up and increased levels of intracellular unassociated 30S and 50S subunits (Sharma et al. 2005).

In this review, we focused on Obg class of TRAFAC GTPases that are widely conserved from bacteria to humans. Obg is an essential protein for the growth of different bacteria by controlling the basic cellular processes such as DNA replication, cell division, and general stress response mechanism. ObgE, a homolog of *E. coli*, was originally identified as a potential DNA replication protein in *B. subtilis* situated downstream of the sporulation gene Spo0B (Spo0B-associated GTPase; Britton 2009). It was observed that the growth rate of *E. coli* depends on the concentration of cellular Obg, implying the fact that Obg plays a major role in its growth (Kint et al., 2014). In *B. subtilis* and *Streptomyces sp*, the initiation of sporulation is dependent on the changes in cellular GTP-GDP pools (Lopez et al., 1981; Ochi, 1986; Ochi et al., 1981; Ochi et al., 1982; Wout et al., 2004) and it was proposed that the Obg proteins act as a sensor for these pools (Lin et al., 1999; Okamoto & Ochi, 1998; Wout et al., 2004). DRG (Developmentally regulated GTP-binding protein) belongs to Obg superfamily which is found in eukaryotes and archaea. DRG1 and DRG2 are the two highly conserved paralogs of DRG. They play important role in cell proliferation and regulation of microtubules (Westrip et al., 2021). Therefore, the importance of Obg protein on the bacterial life cycle is multifarious and needs to be analyzed for a better understanding of its physiology. In the present article, we are trying to highlight a few key issues of Obg-like proteins and how they are correlated with the cellular functions of different bacteria.

1.1 | Analysis of G domain of Obg proteins

All the translational GTPases (trGTPases) are an ancient superfamily of proteins that took multiple changes during the course of evolution. The evolution of the existing trGTPases started with the joining of the OB-fold (oligonucleotide/oligosaccharide binding fold, which is five/six-stranded closed beta-barrel formed by 70–80 amino acid residues) to a Ras-like GTPase that carried out the role of transporting aminoacyl tRNAs of ancestral origin to the membrane associated self-folding PTC RNA (Feng et al., 2014). Four families of trGTPases, EF1, EF2, IF2, and SelB, a specialized EF1-like factor that delivers selenocystyl tRNA to the ribosome, were identified and were observed to be present in all forms of life indicating their existence in the last universal common ancestor on earth (LUCA; Leipe et al., 2002; Atkinson et al., 2015). During evolution, these four "core set" factors have diversified by means of gene duplication, horizontal gene transfer, and sub-functionalization leading to other factors of various taxonomic ranges (Atkinson et al., 2015). In the distant relatives of trGTPases, such as ObgE, the function of ribosome biogenesis has been seen rather than actively participating in the translational process (Atkinson et al., 2015). Although there are differences in overall function between different GTPases, the structure-function relationship of the G domain which promotes their GTP binding and hydrolysis remain well conserved as evident from the domain architecture of different members. The characteristic features of this TRAFAC family of GTPases are the presence of α/β sheet and the central β sheet consists of no less than 6 β chains (mostly parallel) surrounded by α helices on both sides (Verstraeten et al. 2011). Basically, the domain consists of 5 different G motifs G1, G2, G3, G4, G5 and two switches; switch I and switch II facilitates the interaction between guanosine nucleotides. G1 motif is also known as walker A or P loop motif (Verstraeten et al. 2011) which is responsible for binding with GTP, GDP, and other purine nucleotides such as ATP (Verstraeten et al. 2011). The G2 motif is featured by a conserved threonine and interacts with the effector molecule and is therefore liable for coordinating and Mg^{2+} ion which then binds to the β and γ phosphates (Verstraeten et al. 2011). The G3 region is also referred to as the Walker B motif. It contains a typical DX2G motif that is concerned with Mg^{2+} coordination and binding to the γ-phosphate (Verstraeten et al. 2011). The G3, just Like G2 indicates massive conformational modifications between the GDP and GTP-bound state and is therefore referred to as the Switch II region. The G4 motif determines nucleotide specificity by forming hydrogen bonds exclusively with guanine rings (Verstraeten et al. 2011). The G5 motif is not strictly conserved by GTPase since protein nucleotide contacts usually solely affect backbone atoms (Verstraeten et al. 2011). These features are common to most TRAFAC GTPases with little variations.

The Obg protein of the bacteria mainly consists of the two highly conserved domains and a non-conserved domain in the C-terminal region (Kint et al. 2013) as shown in Figure 3. The glycine-rich N-terminal domain that has a unique fold (cannot be seen in other classes of proteins), is known as the "Obg fold" (Kint et al. 2013).

This domain comprises six left- handed type II helices and an eight-stranded beta-barrel structure (Kint et al. 2013) as seen in *B. subtilis*. In the N-terminal domain, glycine residues are conserved across bacteria or eukaryotes (Morimoto et al. 2002) and are responsible for the functioning and correct folding of the Obg protein. The C-terminal domain is not much conserved and it takes part in nucleotide-binding apart from its role in bacterial physiology. This domain participates in stress response by interaction with SpoT proteins, the producer of alarmone ppGpp that regulates the expression of numerous genes under nutrient-depleted conditions. In various bacteria, the crystal structure of the C terminal varies in case of apo form and that one which is bound to GDP and ppGpp. The GTP-binding domain is conserved in all G proteins, and the conserved sequence promotes the binding of GTP to GDP and even GTP hydrolysis as highlighted in Figure 3 (Kint et al. 2013). The G domain of Obg proteins shows Ras-like folds; contains five α-helices and six-stranded β-sheets and is conformationally similar across different bacteria except for the case of switch regions. This particular domain consists of five conserved motifs (G1-G2) responsible for GDP and GTP recognition and hydrolytic functioning as it can be seen for other TRAFAC GTPases. G1 is responsible for alpha and beta interaction for guanine nucleotide binding whereas G2 is for binding and coordinating Mg^{2+}. The G3 and G4 motif of ObgE performs a canonical function such as hydrolytic activity and G5 acts as a recognition site of guanine nucleotides. G domain in Obg proteins functions in energy stabilization and is associated with switching mechanism and conformational changes of the protein aided by the presence of switch elements. There are two putative switch elements present in the G2/G3 region of ObgE in *B. subtilis*. Switch I and switch II are responsible for conformational changes in GTPase from "off" state (GDP bound) to "on" state (GTP bound) that allows reversal interaction with other components and processing of the downstream signal. GDP bound state is open in conformation and similar across different bacteria. However, the conformation of the switch region (particularly switch II) differs considerably upon binding with nucleotide (GTP/ppGpp) as the partial unwinding of the α-helices takes place in the structure (Dutta et al., 2009).

1.2 | Interaction with the bacterial ribosome

ObgE binds to its cellular ribosomal partner, the 50S subunit, and acts as an anti-association factor blocking 70S ribosome formation, hence inhibiting translation initiation. Since the anti- association and binding activities of ObgE are controlled by the levels of guanine nucleotides and (p)ppGpp, it was proposed that ObgE, responsible for 50S subunit assembly is a checkpoint protein that is capable of sensing cellular energy stress by the levels of (p)ppGpp and links it to several pathways of growth control (Gkekas et al., 2017). A study of the structural data informed that the N-terminal domain (NTD) of ObgE mimics the structure of A-site of tRNA, showing specific interactions with the center of ribosomal peptidyl-transferase (Feng et al., 2014). ppGpp has demonstrated the ability to increase the binding of ObgE to the 50S subunit and therefore assist in the disassociation of the 70S ribosome. Although it's been seen that in the apostate, the binding affinity of ObgE to the 50S subunit is weak, a significant enhancement of the binding of ObgE to the 50S subunit was observed when guanine nucleotides were added. As compared to the apostate, the occupancy of ObgE on the 50S subunit increased by over 5-fold in the presence of ppGpp. The presence of GTP or GMPPP shows a higher affinity binding of ObgE to 50S subunit than the presence of GDP. This marked effect of ppGpp and the presence of different nucleotides affect the binding ability of ObgE to the 50S subunit and suggest that ObgE might adjust its behavior according to the changes in the nucleotide pool during different growth phases (Feng et al., 2014). A question was raised whether ObgE could bind with 70S ribosome in vitro and an unexpected result came out since ObgE was found to be incompatible with 70S ribosome when present in excess and later resulted in disassembled subunits. ObgE remained associated with the 50S subunit that got separated from the dissociation of the 70S ribosome. This splitting activity of the 70S was also promoted by GDP and ppGpp, thus requires no energy input is from GTP- hydrolysis (Feng et al., 2014).

When there is plenty of GTP in the middle of the log phase, the primary function of ObgE is to act as a 50S assembly factor to promote the maturation of the 50S subunit. In comparison to this, when the cells are in a stationary phase or are facing a nutrient deficient state, there is a sharp rise of intracellular ppGpp that influences Obg. ObgE-ppGpp complex acts as an effector which over-stays on the 50S subunit, resulting in down-regulation of the subunits (Feng et al., 2014). When ObgE binds to the 50S subunit it restricts the association of the naked 30S subunit along with programmed 30S-preIC to the 50S subunit. Structural reports revealed that conformational changes at several inter-subunit bridging contacts on the 50S subunit that also includes B1a, B2a, and B4 are restricted by ObgE from binding to 30S. This not only prevents the maturation of 50S subunit but also restricts a large number of 50S subunits to participate in

the translation, thereby reducing the levels of active 70S ribosomes and thus controlling the rate of protein synthesis during stressed conditions (Feng et al., 2014). The cryo-EM structure of the 50S subunit is bound with ObgE-GMPPNP is shown in Figure 1 by Feng B et.al. Their analysis suggests that ObgE is bound to the 50S inter-subunit part at a position where translational GTPases attach frequently. The complex formation of the 50S subunit and ObgE brings about conformational changes in both of them (Feng et al., 2014). Noticeable changes in the conformation of the 50S subunit side are observed on the uL1 stalk, bL12 stalk, helix 38, helix 34, and helix 58 along with helix 69 of the 23S rRNA. All of them are situated in the inter-subunit face of the 50S subunit (Feng et al., 2014). The binding of ObgE-NTD with the 50S subunit takes place primarily in rRNA helices that also include helix 89, helix 90, helix 91, helix 93 and the A-loop (Feng et al., 2014). Mutations in a different area of ObgE that interacts with 50S subunit leads to 50S maturation defect, 23S rRNA processing, and reduced binding of several 50S proteins such as bL33, bL34, and impaired uL16.

1.3 | Regulation of Obg

The conserved and essential ObgE-GTPase binds to the ribosome and affects its assembly. The overall regulation of this conserved GTPase has still remained enigmatic. Perhaps it is supposed to be connected with the regulation of general stress response factor sigma factor B (σB). σB transcription factor in *B. subtilis* is a group of approximately 22 operons, the products of which are known to deal with general stress response in the bacterium (Zhang & Scott, 2001). This regulon is induced when the bacterium undergoes depletion in the cell's energy charge, or any environmental stress such as acid, salt, heat, or ethanol, and thus activates σB (Zhang & Scott, 2001). σB is present in an inactive state in *B. subtilis* under unstressed conditions because of its association with an anti-σB protein called regulator of Sigma B-W (RsbW). This association breaks upon exposure to stress; discharging σB from RsbW and a release factor RsbV binds to RsbW in place of σB. It has been indicated that the ribosomal protein L11 and the essential GTP-binding protein Obg was required for stress activation of SigB (Scott & Haldenwang, 1999). Alternatively, in vitro analysis showed that Obg co- fractionated with ribosomal subunits and the stressosome components RsbT and RsbS respectively (Scott et al. 1999). RsbT is an essential component in this stress activation pathway (Woodbury et al., 2004; Zhang & Scott, 2001) RsbT is required for activation of SigB to establish genetic regulation under stress conditions by inhibiting the action of RsbS. Even though the physiological role of Obg on SigB-mediated stress regulatory cascade is unknown, it indicates the possibility of a link between the protein synthesis machinery, Obg, and different factors associated with the stressosome. In another analysis with *E. coli* done by Maouche et al., it was demonstrated that an operon that encodes two ribosomal proteins, is also responsible for expressing ObgE and the expression of this operon changes according to the growth phase of the bacterium and in turn depend on ppGpp and DksA which are the transcription regulators (Maouche et al., 2016). Their analysis indicates that the transcription of ribosomal genes can be correlated with ObgE expression as the expression varies from time to time; during exponential growth, the expression was highest, which decreased at the time of entry into the stationary phase and later

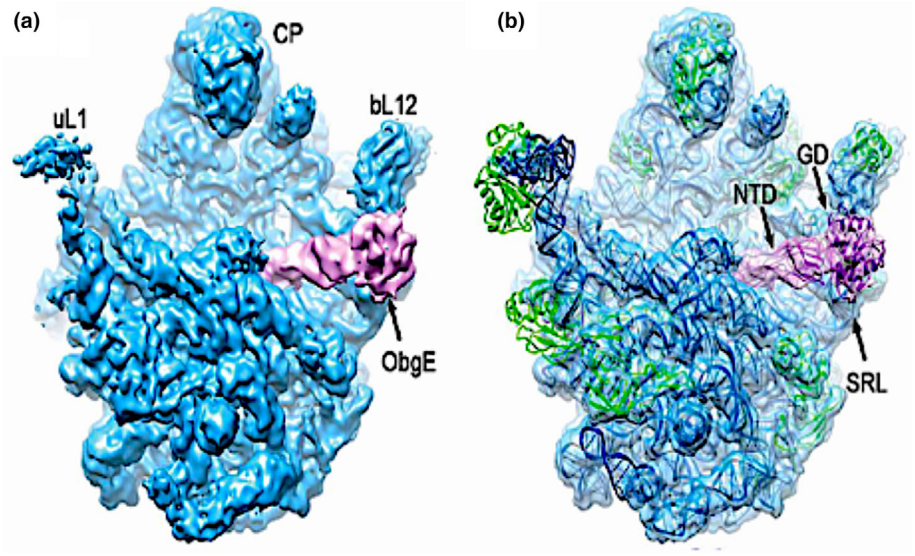

FIGURE 1 Cryo-EM structure of the 50S.ObgE-.GMPPNP complex. (a) the cryo-EM map of the 50S.ObgE-. GMPPNP complex is displayed in surface representation, with the 50S subunit and ObgE colored blue and pink, respectively. (b) the atom model of the 50S.ObgE-. GMPPNP complex is displayed in cartoon representation, and superimposed with the density map. Ribosomal RNA is colored as blue, ribosomal proteins are colored as green, and ObgE is colored as purple, CP, central protuberance; relative arrangements of uL1, uL1 stalk; bL12, bL12 stalk are shown. (adopted from Feng et al., 2014)

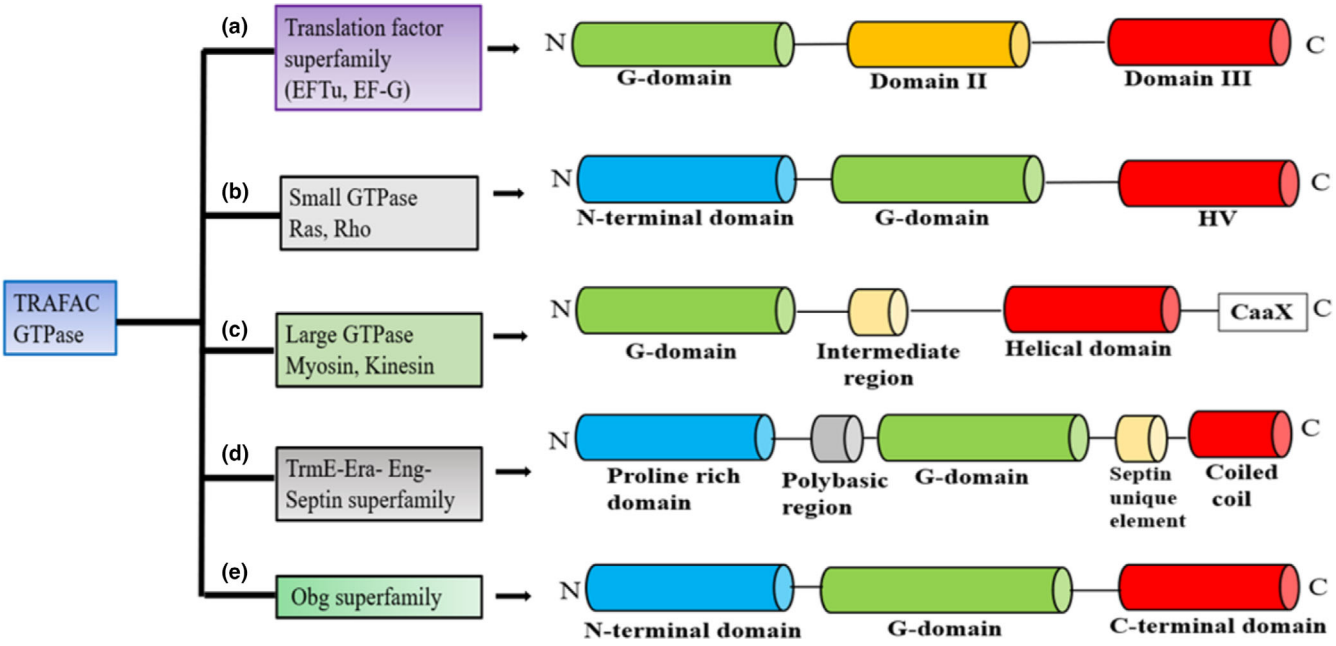

FIGURE 2 Flow diagram of TRAFAC GTPase superfamilies. (a) The translation factor superfamily is mainly composed of three domains (domain I, II and III), where GTP binding domain is present within the N terminal end (domain I). (b) The small GTPase superfamily comprises an N-terminal domain, a GTP binding domain and a C- terminal domain containing a hypervariable (HV) domain which functions as a membrane-binding domain. (c) Like translation factor superfamily, the N- terminal domain of large GTPase superfamily also have an N-terminal GTP-binding domain and a C-terminal helical domain, which is important for dimerization and formation of GMP, followed by C aa x motifs (where, C = cysteine, *A* = aliphatic amino acid, *X* = terminal residue). Two domains are linked by intermediate region. (d) TrmE-Era-Eng-Septin superfamily includes protein structures consisting of proline-rich N-terminal domain that interacts with other septins followed by GTP bind domain via a polybasic region that helps the GTP binding domain and a C-terminal domain correspondingseptin unique element (SUE). (e) Obg superfamily consists of glycine rich N-terminal domain, GTP binding domain and C terminal domain

became untraceable (Maouche et al., 2016). The correlation of expression between ribosomal protein and ObgE may be evocative of its ribosomal function. In mutants where ppGpp or DksA were knocked out, the expression of the operon was reduced. That indicates that Obg and these ribosomal genes are being strictly regulated by transcription factor DksA and alarmone ppGpp (Table 1).

1.4 | Different faces of Obg function

Obg is a multifaceted protein that takes part in a wide range of biological functions in bacteria. The functional aspects of Obg are involved in the process of sporulation initiation, DNA replication in *Bacillus sp*. and *Caulobacter sp*. along with the function of ribosome assembly, cell division, chromosome segregation, and stress response. There are several pieces of evidence that suggest the functioning of Obg GTPases in ribosome maturation in eubacteria, yeast mitochondria, and also in human nuclei (Datta et al., 2005; Hirano et al., 2006; Sato et al., 2005; Suwastika et al., 2014). The human homolog of Obg, ObgH1 is localized in the mitochondria of HeLa cells (Hirano et al., 2006; Suwastika et al., 2014).

1.5 | Persistence

Obg acts as a regulator in *Streptomyces coelicolor* for the initiation activity of cellular differentiation which is dependent on its GTP binding ability (Caldon Pauline Yoong & March, et al., 2001). It has also been observed that Obg has a central role to play in the modulation of bacterial persistence under the condition of nutrient starvation (Gkekas et al., 2017). This Obg-mediated persistence relies on an alarmone (p)ppGpp dependent pathway that acts by inducing the expression of the hokB toxin (Gkekas et al., 2017). A type I toxin-antitoxin module encodes the HokB peptide that disrupts the membrane potential, thus finally leading to persistence (Verstraeten et al., 2015). Obg also has the ability to control the persistence in *Pseudomonas aeruginosa* that constitutes a conserved regulator of antibiotic tolerance (Verstraeten et al., 2015). A property that is commonly present in all Obg bacterial proteins is their ability to

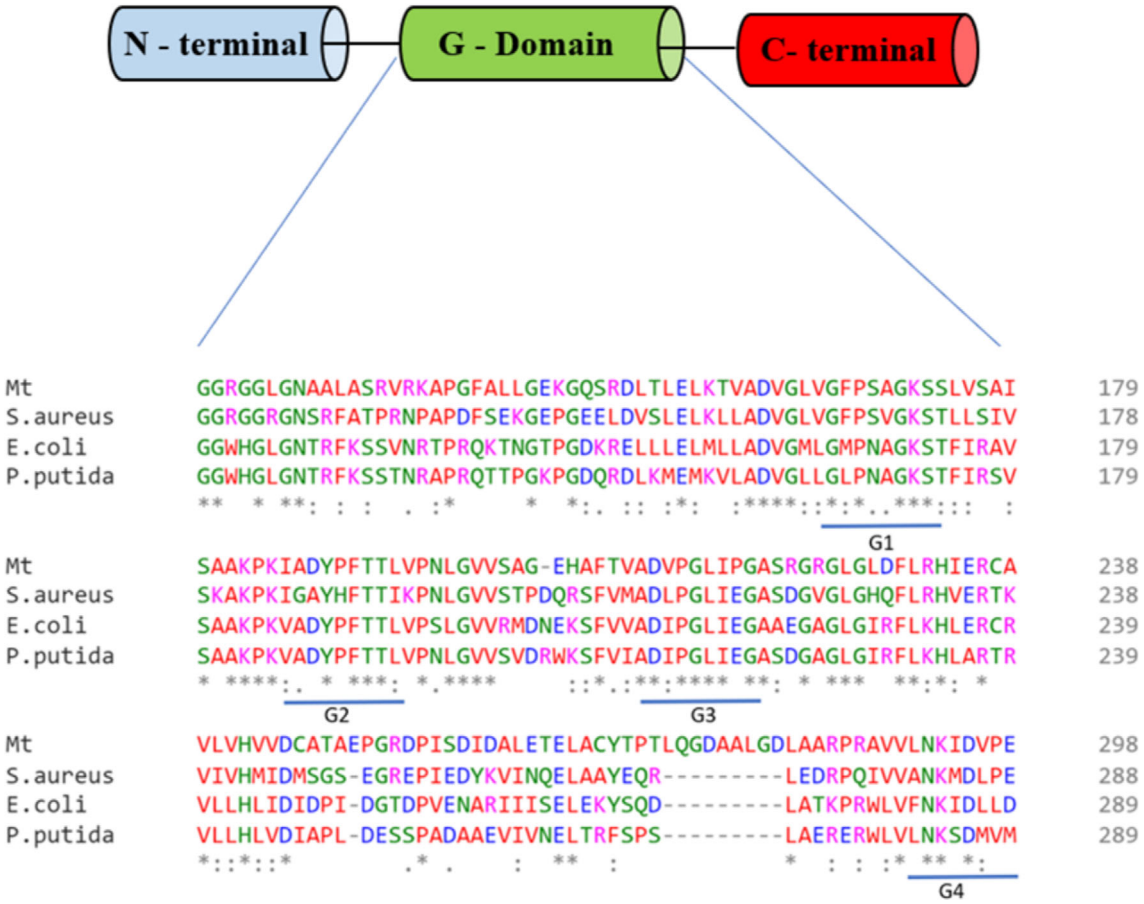

FIGURE 3 Structural diagram of conserved GTPase Obg: Upper panel shows domain structure of Obg class of proteins found in different bacteria. GTP binding domain has been analyzed and Clustal omega multiple sequence alignment showing similarities and differences in the G-domain sequences across four bacterial species (Mt- *mycobacterium tuberculosis, S.aureus-Staphylococcus aureus, E.coli-Escherichia coli, P.putida- pseudomonas putida*. (*) denotes fully conserved residues, (:) denotes strongly similar residues and (.) indicates weakly similar residues

TABLE 1 Examples of Obg-like subfamilies across the three domains of life

Domains of life	Examples		GTPase	Functions
Prokaryotes	Bacteria	*Escherichia coli*	ObgE	• Obg helps in replication fork stress elimination. • It is involved in the regulation of bacterial cell division and chromosome segregation • It helps in ribosome maturation and fitness • It prevents premature translation by acting as an anti-association factor on 50S ribosomal subunit • Regulation of general stress response factor (Sigma factor B) • It acts as a sensor for GTP-GDP pool and plays a major role in bacterial sporulation • It actsasconserved regulator of antibiotic tolerance in various bacteria
		Bacillus subtilis	BsObg	
		Vibrio harveyi	Cgt A	
		Neisseria gonorrhoeae	Eng A	
Archea	*H.archaeon, N.archaeon, C.archaeon*		DRG	• Involves in cell proliferation and translation • Regulation of microtubules
Eukaryotes	Plant	*Arabidopsis thaliana*	AtObgC	It plays important role in chloroplast‘‘s protein synthesis at the early embryogenesis
	Human		hOLA1	• Cellular stress response • Oncogenesis • Protein Synthesis

interact with ribosomes and the functional aspect of this interaction between Obg and ribosome was led by mainly two observations: the first was in *E. coli*, where a defective cell unable to undergo the essential step of 50S ribosomal subunit modification and the second was ObgE overproduction prevents the ribosomal defects. In another example, *Caulobacter crescentus* CtgA/Obg was shown to be associated in the assembly or stability of ribosome since its depletion caused disturbance in the polysome profile (Michel 2005). A similar observation was found in *E. coli* where ObgE was shown to be co-transcribed with a couple of ribosomal proteins. This information may indicate the possible role of ObgE in ribosome biogenesis. It has been recently proposed that highly conserved small GTPase Obg protein (YhbZ, CgtA) can be used as a molecular target to develop new therapeutic interventions to act against bacteria that are resistant to drugs. Since the structure of bacterial Obg proteins is very unique by virtue of having a highly conserved glycine-rich N-terminal domain, a variable C-terminal domain, and a conserved GTP-binding domain (Bonventre et al., 2016; Zielke et al., 2015) makes it an interesting candidate for potential drug targeting. The GTPase domain can be targeted for screening of the efficacy of the inhibitors acting against Obg (Bonventre et al., 2016). Considering the ever-growing cases of antibiotic resistance in bacteria, the drug therapy against this conserved GTPase can be an alternative method of preventing bacterial growth (Bonventre et al., 2016).

1.6 | Cell division

Obg is an essential protein in the regulation of key biological processes in bacteria. A mutant isoform of ObgE, that is, ObgEK268I, also denoted as ObgE*, triggers a programmed cell death (PCD) mechanism in *E. coli*. Obg* associated pathway has a fundamental difference from that of the bacterial PCD pathway, hence emerging as a novel pathway in bacteria. ObgE principally acts as a checkpoint protein in a cell cycle and can withhold the process of cell cycle division. (Dewachter et al., 2016). When encountered with certain physiological conditions, ObgE acts in a similar way to that of the regulators of the eukaryotic cell cycle. Upon defective proceeding of the cell cycle, ObgE can efficiently lead to inhibition of cell division or cause PCD. Thus, just like eukaryotic cell cycle regulator proteins, ObgE plays an indispensable role to correct the defects of the cell cycle in bacteria (Dewachter et al., 2016).

1.7 | Obg and replication

Obg plays a vital role in reliving from replication stress in *E. coli*. ObgE transposon mutant shows hypersensitivity to hydroxyurea (HU), the replication inhibitor (Kint et al., 2012). The HU inhibits the class I ribonucleotide reductase which decreases the cellular pool of triphosphonucleotide (ATP, GTP, CTP, and TTP), resulting in the arrest of the replication fork. It was proposed based on these observations that ObgE aids in the survival of the cell at the time of DNA replication when replication forks are halted (Foti et al., 2005; Kint et al., 2012). The wild type Obg ensures the correct way of replication initiation by sensing the decreased level of tri-phosphonucleotides, thereby stabilizing the replication fork. Another study in *Vibrio cholerae* revealed that the bacterium requires a basal cellular level of Obg protein to overcome the inhibition of replication caused by the treatment of HU (Kint et al., 2012; Shah et al., 2008).

The cgtA gene product of *Vibrio harveyi* falls under the small GTP-binding protein subfamily, known as Obg-like proteins (Słomińska et al., 2002). An insertional mutation in the cgtA gene of a viable *V. harveyi* has recently been studied which shows a unique chromosomal arrangement. These mutant cells lead to the formation of long filaments in chromosomes that are expanded, non-partitioned, or rarely partitioned. This kind of phenotype indicates damage in the chromosome partition mechanism (Słomińska et al., 2002). Studies with flow cytometry also revealed that mutation of cgtA causes disturbance in the synchronization of initiation of chromosome replication. Along with this, in mutant bacterial cells, the number of large cells is increased significantly due to inhibition of chromosome replication and/or cell division, hence suggesting the involvement of cgtA gene product in cell growth to replication of chromosome and cell division. Thus, it can be stated that CgtA, a homolog of Obg-like GTP binding protein is essential in the functioning of chromosome regulation (Słomińska et al., 2002).

1.8 | Ribosome biogenesis

As ribosome is the protein-synthesizing machinery of the cell, proper ribosomal fitness is required for the production of proteins inside the cellular environment. The fitness of this macromolecule depends on the appropriate biogenesis which is a complex process aided by different biogenesis factors. Experimental studies provide that GTPase Obg interacts with other biogenesis factors for the initial processing of the pre-50S ribosomal subunit in *E. coli*. In this regard, the coordination of ObgE is extremely vital for the maturation of the core part of the large ribosomal subunit. Incorporation of the late assembly proteins (bL36 and uL16) and proper folding of the 23S rRNA helix H89 is controlled by ObgE which is

regarded as the rate-determining step for the maturation of the 50S subunits (Nikolay et al., 2021). Conversely, depletion of Obg from the genetic background causes significant alternation in the ribosome profile and accumulation of the pre-50S ribosomal subunit in *E. coli* (Sato et al., 2005). Mutation of ObgE affects pre-16Sr-RNA processing, ribosomal protein levels, thereby considerably reducing 70S ribosome levels. This evidence implicates that the ObgE is an important factor for ribosome biogenesis in different bacteria. On another note, Obg-like protein is important for the functioning of the human mitochondrial ribosome which is required for oxidative phosphorylation. Human Obg-like protein GTPBP5 specifically interacts with large mitoribosomal unit and several other assembly factors that function in ribosomal maturation (Cipullo et al., 2021). Mutation in GTPBP5 leads to severe impairment in oxidative phosphorylation associated with decreased mitochondrial translation which is a potential cause of different metabolic diseases in humans. In a nutshell, Obg-like proteins play an important role in the assembly and fitness of ribosomes thereby controlling the protein translation in different system.

1.9 | Alleviation of antibiotic stress

Obg is an extremely important protein regulating different functions of bacteria. This conserved GTPase is known to be associated with persister formation in different bacteria, therefore directly or indirectly plays a role in antibiotic resistance. In clinically important organism, *Pseudomonas aeruginosa* (associated with cystic fibrosis) the expression of full length Obg transcript is enhanced significantly whereas different other genes including those are involved in exerting virulence property has been prematurely terminated in presence of macrolide azithromycin (Konikkat et al., 2021). As Obg has structural interaction with alarmone ppGpp that controls the gene expression under stringent condition by relieving the pause of RNA polymerase across the length of gene, helps in the up-regulation of obg transcript. Another very interesting aspect is seen in *Acinetobacter baumannii*. The mutant form of GTPase Obg triggers the synthesis of ppGpp from pppGpp, thereby inhibiting the fatty acid biosynthesis required during the formation of outer membrane vesicles (OMV). These OMV maintains the membrane lipid asymmetry (MLA) in *A. baumannii*, which is crucial for membrane integrity, lipid homeostasis and antibiotic sensitivity. This is another example where the antibiotic resistance property of clinically important bacteria is influenced by a multifunctional GTPase (Powers et al., 2020). As stated earlier, ppGpp mediated transcriptional activation HokB results in decrease of membrane potential, respiration slow down and inducting dormancy (Verstraeten et al., 2015). It also protects bacteria from antibiotic stress. Thus, Obg plays its role as conserved regulator of antibiotic tolerance in various bacteria.

1.10 | Obg and cancer

Obg was also found to have some functional role to play in cancer therapy. OLA1 or Obg-like ATPase 1 which is a P-loop GTPase, belongs to the TRAFAC class of the Obg family. OLA1 can perform both the functions of GTPase and ATPase (Liu et al., 2020). One of the recently conducted studies showed that OLA1 is capable of forming a complex with BRCA1 (breast cancer 1) and BARD1 (BRCA1-associated RING domain) protein by interacting with γ- tubulin, resulting in assigning of RACK1 (receptors for activated C kinase 1) for centrosome regulation (Liu et al., 2020; Matsuzawa et al., 2014; Yoshino et al., 2018; Yoshino et al., 2019). It was also revealed that OLA1 increases the anti-apoptotic ability and demonstrates its regulatory role to promote resistance to chemotherapy in breast cancer. Further investigation suggested that chemotherapy of breast cancer patients can be improved by developing OLA1 as a potential target (Liu et al., 2020). Developmentally regulated GTPase family-1 and 2 belong to Obg class that are primarily responsible for maintaining protein translation in eukaryotes. These DRG1 and DRG2 have been implicated in cell growth and proliferation of different cancer cell lines and loss of gene function leads to the loss of growth of lung cancer cell (Lu et al., 2016). Probably they have been associated with the cell cycling process of eukaryotes and engaged in the stimulation of important cell cycle regulators such as C-Myc, Ras etc.

2 | DISCUSSION

In this review, we have discussed the structural features of the conserved GTPase ObgE and how that is correlated with the function of the protein. Principally, the Obg protein family belongs to the TRAFAC (translation factor) class of P-loop GTPases that is conserved from bacteria to eukaryotes. This GTPase was first discovered in *Bacillus subtilis* associated with the sporulation property of the bacteria. Later it appeared to be important for other bacterial species. Analysis of sequences of the different Obg classes of proteins shows certain modifications across different members. Although almost all of the Obg class of proteins consist of a basic structure; N-terminal

glycine-rich sequences that form the characteristic fold found among all Obg-like proteins, a central conserve nucleotide-binding domain, and a non-conserved C-terminal domain participates in various biological functions in bacteria. Depending upon the requirement, this GTPase modifies itself gradually to attain its present structure. The Obg homologs are widely present in eukaryotic organelles and participate in GTP binding and hydrolysis function. However, in a few special cases, such as the rice homolog OsYchF and the human homolog hOLA1 (human Obg-like ATPase 1) was found to bind and hydrolyze ATP more efficiently than GTP (Bang et al., 2009; Hirano et al., 2006; Scott et al., 2000). The sequence comparison of the nucleotide-binding domain of Obg protein will help us to understand the basis of discrepancy of biochemical properties within members from various domains of life as well as the course of evolution in which the protein gets diverged from its ancient ancestor.

The conserved Ras-like GTP binding domain of Obg-like proteins remains in between N- terminal Obg fold and intrinsically disordered C-terminal domain described in the previous analysis by W. Versees group. The central Ras-like GTPase domain of ObgE (150-340 residues) takes characteristics conformation upon binding with guanosine nucleotides. In the GDP bound form of ObgE, major interaction takes place through different switch regions (switch 1 and switch 2) and partially from the conformation of the N-terminal Obg fold. Particularly two helices of switch 2, α1 and α2 play a vital role in this context. The intermediate switch loop remains in "open" conformation where they do not interact with the nucleotide and closely resembles the conformation of these loops in the nucleotide-free form of the *B. subtilis* Obg crystal structure (Buglino et al., 2002). Due to partial unwinding of the helix, switch II adopted a different conformation in the crystal structure of Obg in *T. thermophilus* (Kukimoto-Niino et al., 2004). The nucleotide-binding site of the G domain present in Obg crystal interacts with the C-terminal domain of the adjacent molecule in *T. thermophilus*. Surprisingly, the N-terminal domain is rotated drastically by almost 180 degrees around the G domain axis. Therefore, there is a structural rearrangement of different domains of Obg structure as the central G domain interacts with guanosine nucleotide. It is noticeable that there is not much change in conformation within the G domain of the GTPase whereas major changes occur on the other part of Obg particularly in the C-terminal domain due to the presence of certain amino acids that are not conserved across different species. Similarly, the conserved glycine rich N-terminal domain that interacts with switch motifs during the interaction with GTP is pivotal in this context. Similar interfaces are observed in other large G proteins (EF-Tu, EF-G, SRP GTP) where GTP binding and hydrolysis trigger the intra-molecular domain rearrangement. The structure-based functional analysis is therefore useful for the interpretation of the genetic and biological experiments aimed at elucidating Obg-mediated pathways in the cell. The similarity in domain architecture in the P-loop region possibly indicates the same thing can happen in other bacteria as well as they can produce stringent responses during the stressful condition.

One of the most promising phenomena is the interaction between large subunit proteins of ribosome and Obg which stabilizes the ribosome structure and ensures fitness for the translation process. Amino-terminal domain of Obg interacts with large subunit protein L13 of *B. subtilis* via a cognate sequence almost identical in both binding partners (Scott et al., 2000). Bioinformatic modeling has validated the important H-bond interactions between the Obg fold and the L13 (Lee et al., 2009). The same thing has been reported in the case of *E. coli*, where L13 protein that has been known as early assembly protein of ribosome structure, interacts with ObgE to stabilize 50S subunit structure. Apart from this major interaction, Obg has also been shown to have interaction with other small and large subunit proteins S4, S7, L2, L4 and L17 are primarily known for their interaction with ribosomal RNA (Sato et al., 2005).

Concomitantly, Obg helps in maintaining the folding process of ribosomal RNA GTP-bound form of Obg binds to 16S and 23S rRNA but the detailed mechanism of this interaction is not understood due to lack of structural analysis. Being activated by GTP binding, Obg may either differentially maintain the structural integrity of ribosomal RNA and proteins that are directly involved in the construction of ribosome structure or stabilize the structure of rRNAs and/or rRNA-ribosomal protein complexes in the ribosome maturation process. Again, the N- terminal domain of Obg plays a crucial role in the interaction with rRNA helices of 50S subunits that includes helix 89, helix 90, helix 91, helix 93, and the A-loop. Mutation of the different residues in the NTD-Obg can lead to 50S maturation defect, 23S r RNA processing, and reduced binding of several 50S proteins that have been discussed earlier (Feng et al., 2014). This GTPase may have a further role in maintaining ribosomal RNA structure as it is shown to have interaction with RNA helicase CsdA which is an integral part of mRNA stability. Similarly, a co-localization study has indicated the association with chaperone protein DnaK that is required during the molecular assembly of 50S subunit. Detailed molecular analysis is required to explore the underlying mechanism of ribosome biogenesis.

In vitro analysis shows that the Obg balances the production of alarmone ppGpp, a central regulator of gene expression and translational attenuation under stress condition. In *Vibrio cholerae*, Obg-like GTPase CgtA shown to have interacted with ppGpp hydrolase SpoT (Raskin et al., 2007). SpoT acts as hydrolase of ppGpp thereby reducing its concentration in cellular environment. Similar interaction between ObgE and SpoT has been documented in *E.coli*. Although the detail mechanism of interaction is not clear yet but this is an indication of Obg mediated regulation of SpoT activity in different bacteria. As the presence of ppGpp depends on the activity of the SpoT therefore Obg-like GTPases also have an effect on stringent response. The connection between ObgE and stress has been well documented in pathogen *Legionella pneumophila*, where its expression is elevated during intracellular survival (Rankin & Isberg, et al., 2002). Actually, biochemical and structural analysis indicated that bacterial and human homolog of Obg GTPase directly interacts with ppGpp via its active site (Buglino et al., 2002). Crystal structure analysis of *B. subtilis* Obg protein reveals that ppGpp is bound to the G1 and G5 region of the GTPase domain although no exogenous ppGpp has been added during the preparation. ppGpp binds to the second monomer of Obg at almost similar site where GDP binds to it. Unfortunately, no direct evidence has been obtained about the direct contact between Obg and ppGpp. The presence of ppGpp in the active site of Obg may be due to the artifact of the starvation and therefore may be closely linked to the stress responsive pathway of the bacterial system.

Obg is a small p-loop GTPase of diverse functions from bacteria to humans. The idiosyncrasy of the functional diversity lies on the structural aspect of the protein. The unique structural feature of the conserved GTPase makes it an interesting candidate for potential drug targeting. Obg can be targeted for the delivery of the drug in such cases where there is no protection against infection by administering vaccine or the scope of conventional drug or antibiotic treatment is limited (Bonventre et al., 2016). In such cases where there is no protection by administering drug or vaccine, inhibition of Obg function might be important issue in terms of controlling the growth and virulence of different organisms inside the host condition. The simple reason behind this approach is Obg being such a common biological target which is essential for the physiology of the microorganism and well conserved across various species. After having an idea of the structure of different chemical compounds from bioinformatic analysis that can fit into the binding site of GTP, we can develop novel inhibitory compounds or enhance the efficacy of the existing one by chemical modification that can effectively bind to the GTP binding pocket of the protein can perturb GTP binding and hydrolysis function. This is how the concept of repurposing of drug comes into play. As GTP binding leads to conformational stabilization of the protein necessary for different biological function, the activity of the GTPase can be manipulated. This idea can be implemented to other biological targets and can emerge as important therapeutics in the future. Therefore, the overall idea of the review is to highlight the structure based functional analysis of this conserved GTPase that can open up the scope of translational research in future.

AUTHOR CONTRIBUTION

AB designed the manuscript. AB, AC, SH, PK, DS wrote the manuscript. AB and KS proofread the manuscript.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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