



The Diverse Functional Roles of Elongation Factor Tu (EF-Tu) in Microbial Pathogenesis

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Elongation factor thermal unstable Tu (EF-Tu) is a G protein that catalyzes the binding of aminoacyl-tRNA to the A-site of the ribosome inside living cells. Structural and biochemical studies have described the complex interactions needed to effect canonical function. However, EF-Tu has evolved the capacity to execute diverse functions on the extracellular surface of both eukaryote and prokaryote cells. EF-Tu can traffic to, and is retained on, cell surfaces where can interact with membrane receptors and with extracellular matrix on the surface of plant and animal cells. Our structural studies indicate that short linear motifs (SLiMs) in surface exposed, non-conserved regions of the molecule may play a key role in the moonlighting functions ascribed to this ancient, highly abundant protein. Here we explore the diverse moonlighting functions relating to pathogenesis of EF-Tu in bacteria and examine putative SLiMs on surface-exposed regions of the molecule.

Keywords: EF-Tu, moonlighting, bacteria, adhesion, chaperone activities

INTRODUCTION

Elongation Factor Thermo Unstable (EF-Tu) is one the most abundant proteins found in bacteria, comprising up to 6% of the total protein expressed in *Escherichia coli* (Furano, 1975) and as high as 10% of the total protein expressed in the genome reduced pathogen *Mycoplasma pneumoniae* (Dallo et al., 2002). The primary, canonical function of EF-Tu is to transport aminoacylated tRNAs to the ribosome (Sprinzl, 1994). Ef-Tu has been a therapeutic target for antibiotics (elfamycins) since the 1970s (Wolf et al., 1974; Prezioso et al., 2017). However, current issues with elfamycins' poor pharmacokinetics and solubility has prevented their commercialization as therapeutic agents (Prezioso et al., 2017).

Diverse functions have been ascribed to EF-Tu many of which include important virulence traits in Gram positive and Gram-negative pathogenic bacteria. To effect alternate virulence-associated functions, including adhesion to host extracellular matrix components, EF-Tu must gain access and be retained on the extracellular surface. This poses a challenge as signal secretion motifs are absent in this highly structured protein, and motifs required for binding diverse host cell surface receptor and matrix molecules must evolve without jeopardizing structural constraints needed to execute canonical function as a G protein. Here we refer to secondary functions as “moonlighting” functions. The concept of protein moonlighting is well established in eukaryotes (Jeffery, 1999; Huberts and van der Klei, 2010; Petit et al., 2014; Min et al., 2016; Yoon et al., 2018), and is rapidly gaining traction in prokaryotes (Henderson and Martin, 2011, 2013;

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Wang et al., 2013b; Kainulainen and Korhonen, 2014; Jeffery, 2018; Ebner and Götz, 2019) indicating that it is an ancient and evolutionally conserved phenomenon. Although EF-Tu executes various functions in eukaryotes, a review of the moonlighting roles of EF-Tu in bacteria is lacking. Therefore, this review has a focus to discuss the ever-expanding moonlighting roles of EF-Tu in prokaryotes, and how these roles relate to pathogenesis.

STRUCTURE AND FUNCTION OF EF-Tu

Structural Analysis of EF-Tu

Elongation factors (Table 1) in bacteria (e.g., EF-Tu also known as EF1A) and in eukaryotes (e.g., the eukaryotic Elongation Factor 1 Complex [eEF1A]) all have the same primary and critical function to shuttle aminoacylated tRNAs to the ribosome during protein translation. A codon-anticodon system ensures that the correct amino acid is added to the growing protein chain, a process that consumes guanosine triphosphate (GTP) prior to releasing the elongation factor from the aminoacyl tRNA. However, bacteria and eukaryotes differ in the mechanism by which they recharge the elongation factor/guanosine diphosphate (GDP) complex. This recharging function is executed by the Elongation Factor Thermo stable (EF-Ts) in prokaryotes and by eukaryotic Elongation Factor 1B (eEF1B) in eukaryotes (Cacan et al., 2013) (Figure 1).

EF-Tu is comprised of three domains known as domains i, ii and iii which have evolved a high degree of molecular flexibility. To perform its canonical function, EF-Tu must form a functional binding pocket for an aminoacyl-tRNA, and to achieve this, domain i must become aligned more closely to domains ii and iii (i.e., they must move by around 90°) (Kjeldgaard et al., 1993). The extent of intramolecular movement needed to accommodate the aminoacyl-tRNA is about one third of the protein's total diameter, indicating how significant this conformational change is (Sprinzl, 1994). Once the incoming aminoacyl-tRNA has docked with the mRNA, GTPase activity induces a reverse conformational change enabling the release of EF-Tu from the ribosome (Polekhina et al., 1996). The structural and functional constraints needed to execute these critical molecular interactions ensure that key domains within EF-Tu evolve slowly compared to molecules that perform their functions on the cell surface, where they face constant challenge from the host's immunological defenses and undergo diversifying selection. As such, EF-Tu is considered to be an ancient molecule that is comprised of domains that are highly conserved in phylogenetically diverse prokaryotes (Filer and Furano, 1980). This sequence conservation extends to EF-Tu homologs in eukaryotes, which have also evolved a similar overall protein synthesis pathway (Ejiri, 2002).

Genetic Evolution of EF-Tu

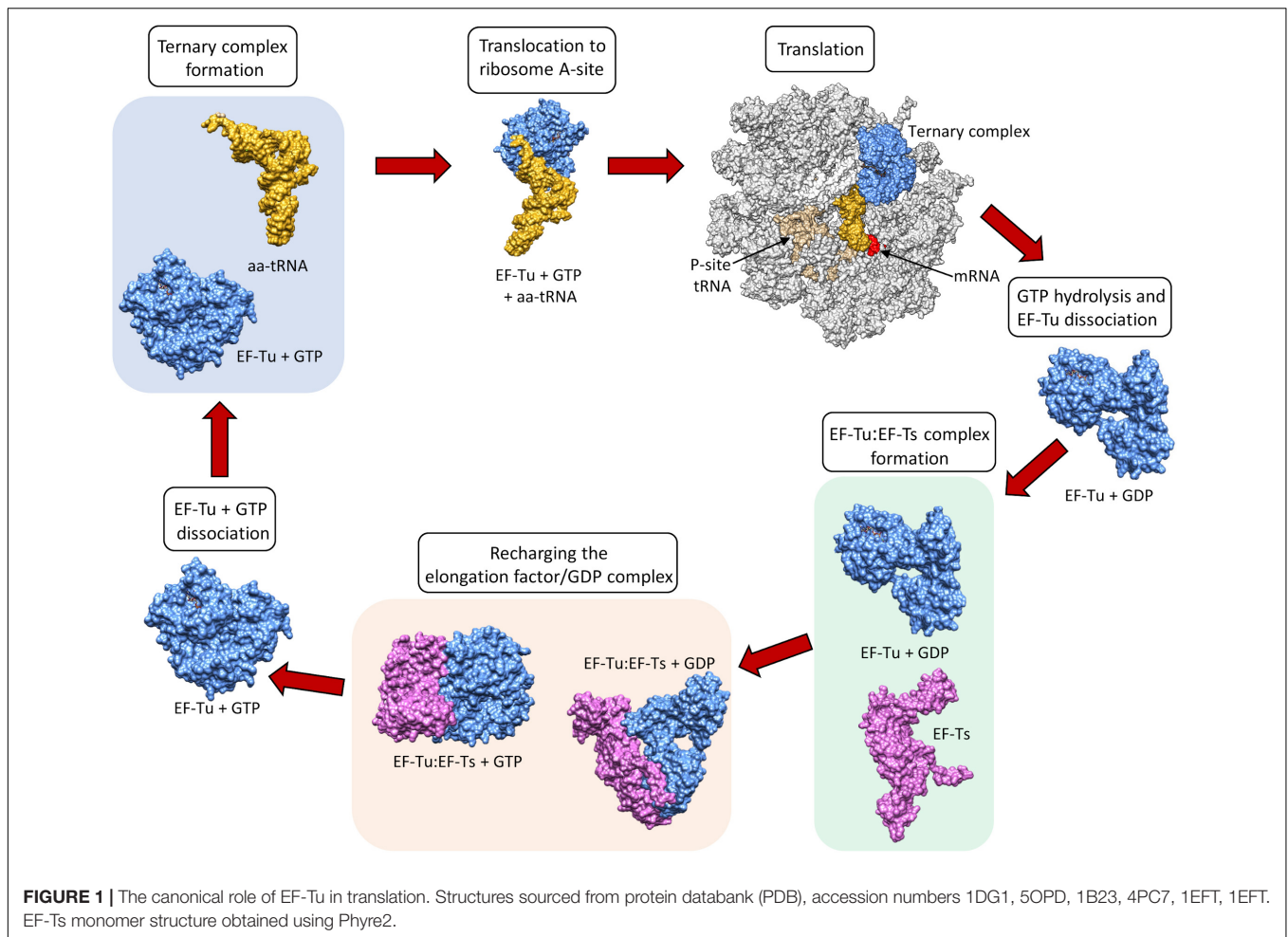
In bacteria, EF-Tu is encoded by the *tuf* gene. *tuf* has a highly conserved genomic location and amino acid sequence, and has been used in the construction of phylogenetic trees for species discrimination (Iwabe et al., 1989; Baldauf et al., 1996; Mignard and Flandrois, 2007; Shin et al., 2009; Li et al., 2012; Caamano-Antelo et al., 2015). Amongst different bacterial species the EF-Tu

sequences have less than 30% sequence divergence (Lathe and Bork, 2001). Low G + C Gram positive bacteria carry only a single copy of *tuf* (Ke et al., 2000). In contrast, many enteric bacteria have two copies (*tufA* and *tufB*) while three *tuf*-like genes have been identified in *Streptomyces ramocissimus* (Filer and Furano, 1981; Vijgenboom et al., 1994). In species with two copies of the gene, the two genes differ by less than 1.4%, based on nucleotide comparison (Lathe and Bork, 2001). In some bacteria with two copies of *tuf*, deletion of one copy of the gene is not lethal to the bacterium (Hughes, 1990; Zuurmond et al., 1999). It has been postulated that a second copy of this gene (which is mainly conserved in Gram negative bacteria) evolved before the branching of eubacteria (Lathe and Bork, 2001). The cause for the intermittent presence of the second copy of *tuf* within eubacteria has been debated. It has been proposed that the second copy arose by lateral gene transfer, at least within Enterococci (Ke et al., 2000), whilst others argue that lateral gene transfer is unlikely in translation factors and attribute the discontinuous observation of a second *tuf* gene to the theory that it had been randomly lost in some lineages (Lathe and Bork, 2001).

Eukaryotes have two isoforms of EF-Tu known as eEF1A1 and eEF1A2 (Table 1), with each sharing 96% amino acid similarity (Abbas et al., 2015). Both isoforms are also highly expressed representing 1–11% of the total protein expressed (Slobin, 1980; Abbas et al., 2015). Some cells express just one of the eEF1A isoforms, while both are expressed after muscle trauma and in some tumor cell types (Bosutti et al., 2007; Abbas et al., 2015). The number of genes encoding eEF1A varies widely within eukaryotes, from ten in maize to four in rice (Ejiri, 2002).

MOONLIGHTING PROTEINS IN BACTERIA

There is now overwhelming evidence that proteins with canonical functions in the bacterial cytosol also perform important tasks on the bacterial cell surface (Sanchez et al., 2008; Kainulainen and Korhonen, 2014; Ebner and Götz, 2019). EF-Tu features prominently in many of these studies. Many moonlighting proteins are ancient, highly expressed enzymes, or are proteins that perform essential roles in glycolysis, respiration and respond to stress (Henderson and Martin, 2011). There is evidence that only a subset of cytosolic proteins can traffic onto the cell because other highly expressed cytosolic proteins are not observed on the cell surface or in extracellular secretions (Vanden Bergh et al., 2013). Mass spectrometry studies have been instrumental in revealing the identities of surface accessible proteins that are not predicted to reside on the cell surface that have canonical functions in the bacterial cytosol (Jeffery, 2005; Robinson et al., 2013; Jarocki et al., 2015; Tacchi et al., 2016; Wang and Jeffery, 2016; Widjaja et al., 2017). The presence of surface-associated moonlighting proteins has been confirmed using fluorescence and electron microscopy (Bergmann et al., 2001; Candela et al., 2010; Yamaguchi et al., 2010; Robinson et al., 2013; Grundel et al., 2015; Jarocki et al., 2015). It is notable that purified, soluble moonlighting protein fails to



associate with the surface when exogenously incubated with bacterial cells (Saad et al., 2009) suggesting that posttranslational modification(s) that occur in the host bacteria and/or passage through the cell membrane may be important events in a protein's ability to moonlight on the cell surface. Another unusual feature of protein moonlighting is that not all strains belonging to the same species present moonlighting proteins on their cell surface. For example, only a subset of pathogenic *E. coli* express surface GAPDH which binds host molecules (Egea et al., 2007). Finally, it is now known that moonlighting proteins are processed on the surface of bacterial pathogens. Processing is expected to increase protein disorder and alter function compared to the full length proteoform (Tacchi et al., 2016). Here we present key studies that describe the salient features that define the diverse moonlighting functions of EF-Tu related to pathogenesis (Figure 2 and Table 2).

EF-Tu Is Exposed on the Surface of Bacteria

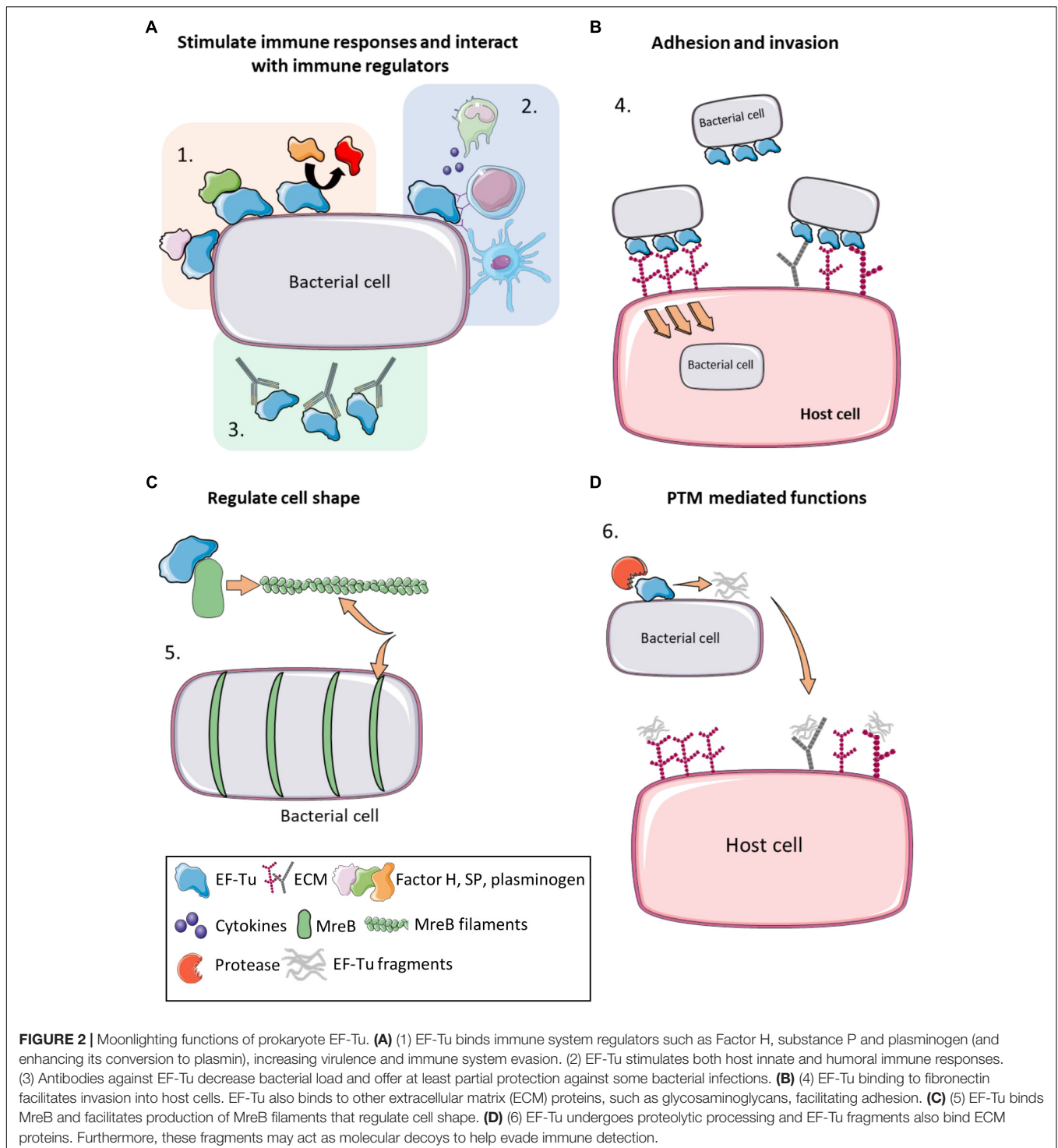
EF-Tu was first described as having a moonlighting function on the cell surface of *M. pneumoniae* (Dallo et al., 2002). As EF-Tu has now been found on the

TABLE 1 | Elongation Factors in eukaryotes and their equivalent title in prokaryotes.

Eukaryotic protein	Prokaryotic equivalent
eEF1/EF-1	EF1/EF-T
eEF1A/(e)EF-1 α	EF1A/EF-Tu
eEF1A1/eEF1 α 1 eEF1A2/eEF1 α 2	2 isoforms
eEF1B/(e)EF-1 β	
eEF1B β γ δ (animals)	3 subunits
eEF1B α β γ (yeast)	2 subunits
eEF1B β γ δ (plants)	3 subunits
eEF2/EF-2	EF2/EF-G
eEF3/EF-3	N/A

Both the new nomenclature and the old are shown below in the format of current/old. Although new nomenclature has been established for over a decade, many articles still use the old nomenclature. Indeed, the old nomenclature is more widely used in the prokaryotic literature and that is why EF1A is referred to as EF-Tu in this review. Adapted from Ejiri (2002) and Sasikumar et al. (2012).

surface of a wide range of prokaryotes (Table 3), the potential mechanisms behind its extracellular locale shall be summarized here.



Typically, newly synthesized proteins destined for the cell surface possess either signal peptides or signal motifs that are recognized by transport machinery and translocated through the cytoplasmic membrane (Green and Mecsas, 2016). However, conventionally cytosolic proteins lacking signal sequences, like EF-Tu, have also been identified extracellularly. These proteins, termed non-classically secreted proteins (Wang et al., 2016),

can be differentiated from other cytosolic proteins by assessing properties such as amino acid composition and structurally disordered regions (Bendtsen et al., 2005). Indeed, EF-Tu was one protein used to construct the feature-based non-classically secreted protein prediction software SecretomeP (Bendtsen et al., 2005). However, the actual mechanisms behind non-classical protein secretion remain a topic for debate. While there are

TABLE 2 | List of moonlighting functions published for EF-Tu in prokaryotes.

Species	Moonlighting function	Year	References
<i>Acidovorax avenae</i>	Rice plants recognize the central amino acids (175-225aa) of EF-Tu as a PAMP	2014	Furukawa et al., 2014
<i>Acinetobacter baumannii</i>	Binds fibronectin	2012	Dallo et al., 2012
	Binds plasminogen	2015	Koenigs et al., 2015
<i>Actinobacillus seminis</i>	Binds fibrinogen and fibronectin	2018	Montes-García et al., 2018
<i>Bacillus anthracis</i>	Binds plasminogen to evade C3b-dependent innate immunity	2011	Chung et al., 2011
<i>Bacillus cereus</i>	Target for Substance P (SP)	2013	Mijouin et al., 2013
		2019	N'Diaye et al., 2019
<i>Bacillus subtilis</i>	Binds calcium ions	2009	Dominguez et al., 2009
	Role in cell shape maintenance, colocalizes and modulates MreB filament formation	2010	Defeu Soufo et al., 2010,
		2015	2015
<i>Escherichia coli</i>	Cleaved in response to phage infection inducing phage exclusion induction	1994	Bingham et al., 2000
		1998	Georgiou et al., 1998
	<i>Arabidopsis thaliana</i> recognizes the first 18 aa of EF-Tu as a PAMP	2000	Yu and Snyder, 1994
		2004	Kunze et al., 2004
	Interacts and modulates MreB filament formation	2005	Butland et al., 2005
	Interacts with DsbA	2014	Premkumar et al., 2014
<i>Francisella tularensis</i>	Interacts with THP-1 nucleolin	2008	Barel et al., 2008
<i>Gallibacterium anatis</i>	Forms filaments, binds fibronectin and fibrinogen	2017	López-Ochoa et al., 2017
<i>Helicobacter pylori</i>	Adheres to THP-1 cells, novel potential adhesion factor	2016	Chiu et al., 2016
<i>Klebsiella pneumonia</i>	Virulence factor for Leukopenia caused by <i>Klebsiella pneumonia</i>	2014	Liu et al., 2014
<i>Lactobacillus johnsonii</i>	Attachment to human intestinal cells and mucins, and participates in host immunomodulation (IL-8 production)	2004	Granato et al., 2004
<i>Lactobacillus delbrueckii</i>	Binds mucin	2013	Dhanani and Bagchi, 2013
<i>Lactobacillus paraplantarum</i>	Modulates biofilm formation	2017	Liu et al., 2017
<i>Lactobacillus plantarum</i>	Adheres to Caco-2 cells	2008	Ramiah et al., 2008
	Binds mucin	2011	Dhanani et al., 2011
	Binds actin	2013	Dhanani and Bagchi, 2013
		2018	Peng et al., 2018
<i>Leptospira interrogans serovar Copenhageni</i>	Binds Factor H and plasminogen (and other ECM)	2013	Wolff et al., 2013
<i>Listeria monocytogenes</i>	Binds plasminogen	2004	Schaumburg et al., 2004
	Induces dendritic cell maturation	2016	Mirzaei et al., 2016
<i>Mycobacterium avium</i> subsp. <i>Paratuberculosis</i>	Binds fibronectin	2014	Viale et al., 2014
<i>Mycoplasma fermentans</i>	Interacts with the intracytoplasmic domain of CD21 (EBV/C3d receptor)	2005	Balbo et al., 2005
<i>Mycoplasma hyopneumoniae</i>	Fragments bind heparin and fibronectin	2016	Tacchi et al., 2016
	Binds A594 cells, fetuin, actin, heparin, and plasminogen	2017	Widjaja et al., 2017
	Binds fibronectin	2018	Yu et al., 2018
<i>Mycoplasma pneumoniae</i>	Binds fibronectin Binds A594 cells, fetuin, actin, heparin, and plasminogen	2002	Dallo et al., 2002
		2008	Balasubramanian et al., 2008
		2017	Widjaja et al., 2017
<i>Pseudomonas aeruginosa</i>	Binds Factor H and plasminogen	2007	Kunert et al., 2007
	Trimethylation of the lysine allowing binding to platelet-activating receptor	2013	Barbier et al., 2013
	Part of the TVISS	2015	Whitney et al., 2015
<i>Staphylococcus aureus</i>	Target for Substance P (SP)	2016	N'Diaye et al., 2016
<i>Staphylococcus epidermidis</i>	Target for Substance P (SP)	2016	N'Diaye et al., 2016
<i>Streptococcus gordonii</i>	Binds saliva mucin MUC7	2009	Kesimer et al., 2009
<i>Streptococcus pneumoniae</i>	Binds Factor H, FHL-1, CFHR1 and plasminogen	2014	Mohan et al., 2014

Table is arranged based on function, showing different species have evolved the same EF-Tu moonlighting functions. The majority of these moonlighting functions have only been described in the last decade.

translocation systems that do not require signal peptides, such as the Holin–Antiholin system, ABC transporters, and a type seven secretion system in Gram-positive bacteria (Götz et al., 2015),

these only account for a small portion of non-classically secreted proteins (Wang et al., 2016). Therefore, the presence of cytosolic proteins in extracellular locations is often linked with cell lysis.

TABLE 3 | Literature reporting the identification of bacterial EF-Tu in non-cytoplasmic locations.

Species	Surface exposed [†]	References	
		Secretome	Immunoproteome
<i>Actinobacillus seminis</i>	Montes-García et al., 2018		
<i>Arskubacterium ikkense</i>		Lylloff et al., 2016	
<i>Bacillus anthracis</i>		Kim et al., 2014	
<i>Bacillus cereus</i>		Clair et al., 2013; Laouami et al., 2014; Voros et al., 2014; Madeira et al., 2015; Omer et al., 2015;	
<i>Bacteroides fragilis</i>	Wilson et al., 2015	Wilson et al., 2015	
<i>Borrelia burgdorferi</i>			Carrasco et al., 2015
<i>Brucella abortus</i>		Jain et al., 2014	
<i>Burkholderia pseudomallei</i>		Burtnick et al., 2014 Schwarz et al., 2014	
<i>Caulobacter crescentus</i>	Cao and Bazemore-Walker, 2014		
<i>Cellulomonas fimi</i>		Wakarchuk et al., 2016	
<i>Cellulomonas flavigena</i>		Wakarchuk et al., 2016	
<i>Desulfotomaculum reducens</i>	Dalla Vecchia et al., 2014		
<i>Enterococcus faecalis</i>	Sinnige et al., 2015	Arntzen et al., 2015	
<i>Escherichia coli</i>		Boysen et al., 2015	Kudva et al., 2015
<i>Gallibacterium anatis</i>	López-Ochoa et al., 2017		
<i>Haemophilus influenzae</i>	Thofte et al., 2018		
<i>Helicobacter pylori</i>		Chiu et al., 2016 Snider et al., 2016	
<i>Klebsiella pneumoniae</i>			Liu et al., 2014
<i>Lactobacillus rhamnosus</i>	Espino et al., 2015		Espino et al., 2015
<i>Leptospira biflexa</i>	Wolff et al., 2013; Stewart et al., 2015		
<i>Leptospira borgpetersenii</i>	Wolff et al., 2013		
<i>Leptospira interrogans</i>	Wolff et al., 2013	Eshghi et al., 2015	
<i>Leptospira kirschneri</i>	Wolff et al., 2013		
<i>Leptospira noguchii</i>	Wolff et al., 2013		
<i>Leptospira santarosai</i>	Wolff et al., 2013		
<i>Listeria monocytogenes</i>	Tiong et al., 2015	Rychli et al., 2016	
<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i>		Viale et al., 2014	Viale et al., 2014
<i>Mycobacterium tuberculosis</i>		Chande et al., 2015	
<i>Mycoplasma hyopneumoniae</i>	Tacchi et al., 2016; Yu et al., 2018		
<i>Mycoplasma mycoides</i> subsp. <i>capri</i>			Churchward et al., 2015
<i>Mycoplasma pneumoniae</i>	Widjaja et al., 2017		
<i>Neisseria meningitidis</i>		Newcombe et al., 2014	Newcombe et al., 2014
<i>Propionibacterium freudenreichii</i>	Le Marechal et al., 2015		
<i>Pseudomonas aeruginosa</i>	Kunert et al., 2007	Reales-Calderon et al., 2015	
<i>Pseudomonas syringae</i>		Schumacher et al., 2014	
<i>Roseobacter pomeroyi</i>		Christie-Oleza et al., 2015a	
<i>Staphylococcus aureus</i>		Peton et al., 2014; Liew et al., 2015; Mishra and Horswill, 2017	Kloppot et al., 2015
<i>Staphylococcus carnosus</i>		Nega et al., 2015	
<i>Staphylococcus epidermidis</i>		Siljamaki et al., 2014	
<i>Streptococcus gordonii</i>		Maddi et al., 2014	
<i>Streptococcus agalactiae</i>	Yang et al., 2018		
<i>Streptococcus pneumoniae</i>	Mohan et al., 2014	Pribyl et al., 2014	

(Continued)

TABLE 3 | Continued

Species	Surface exposed [†]	References	
		Secretome	Immunoproteome
<i>Streptococcus thermophilus</i>	Pribyl et al., 2014; Jimenez-Munguia et al., 2015		
<i>Streptomyces scabiei</i>	Lecomte et al., 2014		
<i>Synechococcus</i> sp.		Komeil et al., 2014;	
<i>Tistlia consotensis</i>		Padilla-Reynaud et al., 2015	
<i>Vibrio cholerae</i>		Christie-Oleza et al., 2015b	
<i>Vibrio parahaemolyticus</i>		Rubiano-Labrador et al., 2015	
<i>Xanthomonas citri</i> subsp. <i>citri</i>		Altindis et al., 2015	
<i>Xylella fastidiosa</i>		He et al., 2015	
		Ferreira et al., 2016	
		Nascimento et al., 2016	

Articles searched between 2014–2018 identified EF-Tu present on the surface or in secretions of many bacterial species. Antibodies to this protein were also identified in a number of studies. This table includes data from accessible **Supplementary Files**. Key search terms used were, but not limited to: exoproteome, secretome, surfacome, surfaceome, immunoproteome, and secretomic. Table excludes any data that was only bioinformatically determined. [†]Includes surfacome, outer membrane, specific protein analysis under any condition.

There is evidence supporting cytosolic protein excretion through cell lysis, and there is evidence supporting excretion through alternative secretion pathways (reviewed in Wang et al., 2013a). In addition to specific secretion pathways, cytosolic protein excretion can also occur through compromised membrane integrity, translational and osmotic stress, the protein's biochemical and structural properties, and via membrane vesicles (MVs) (Outer Membrane Vesicles [OMVs] in Gram-negative bacteria) (reviewed in Ebner and Götz, 2019).

Multiple mechanism may contribute to the surface location of EF-Tu; however, there are substantial reports of MVs carrying EF-Tu. Bacterial MVs are nanoparticles produced through processes such as membrane blebbing and endolysin-triggered cell death and contain various membrane and cytosolic proteins, as well as lipopolysaccharides, peptidoglycan and DNA (Turnbull et al., 2016; Toyofuku et al., 2019). MVs are involved in diverse biological processes, including virulence, biofilm development, quorum sensing, horizontal gene transfer, and exportation of cellular components (Toyofuku et al., 2019). EF-Tu is present within MVs derived from Gram-positive bacteria including *Listeria monocytogenes* (Coelho et al., 2019), *Mycobacterium bovis*, *Mycobacterium smegmatis*, *Mycobacterium tuberculosis* (Prados-Rosales et al., 2011), *Staphylococcus aureus* (Lee et al., 2009; Wang et al., 2018), *Streptococcus agalactiae* (Surve et al., 2016), *Streptococcus pneumoniae* (Olaya-Abril et al., 2014), and *Streptococcus pyogenes* (Resch et al., 2016); Gram-negative bacteria including *Acinetobacter baumannii* (Kwon et al., 2009), *Bacteroides fragilis* (Zakharzhevskaya et al., 2017), *Cronobacter sakazakii* (Alzahrani et al., 2015), *Escherichia coli* (Lee et al., 2007), *Francisella novicida* (Pierson et al., 2011), *Haemophilus influenzae* (Sharpe et al., 2011), *Klebsiella pneumoniae* (Lee et al., 2012), *Neisseria gonorrhoeae* (Pérez-Cruz et al., 2015), *Neisseria meningitidis* (Vipond et al., 2006), and *Pseudomonas aeruginosa* (Choi et al., 2011); and in six *Mycoplasma* species (Gaurivaud et al., 2018). Indeed, EF-Tu was reported as one of the most abundant protein in some of these studies (Lee et al., 2009;

Pérez-Cruz et al., 2015; Gaurivaud et al., 2018). Interestingly, several MVs that contain EF-Tu have been reported to increase virulence (Surve et al., 2016), modulate immune responses (Prados-Rosales et al., 2011; Sharpe et al., 2011; Alzahrani et al., 2015), and offer protection to infection via immunization (Vipond et al., 2006; Pierson et al., 2011; Olaya-Abril et al., 2014). As the number of MV-encapsulated proteins varied dramatically within this subset of studies, ranging from 8 in *S. agalactiae* to 416 in *F. novicida*, it is not possible to determine the exact role EF-Tu plays in these processes in most instances. However, in the case of *S. pneumoniae* MVs, which were shown to have high immunogenic capacity and induce protective responses in mice, EF-Tu was one of 15/161 MV proteins that were immunogenic and one of two proteins from which antibodies were generated against in immunized mice (Olaya-Abril et al., 2014).

EF-Tu Stimulates a Humoral Immune Response and Interacts With Host Immune Regulators

Antibodies against EF-Tu have also been detected in a range of natural infections, including those caused by *Mycoplasma hyopneumoniae* (Pinto et al., 2007), *Chlamydia trachomatis* (Sanchez-Campillo et al., 1999) and *K. pneumoniae* (Liu et al., 2014). Recombinant EF-Tu (rEF-Tu) from *Mycoplasma ovipneumoniae* induces an immune response in mice, increasing levels of IgG, TNF- α , IFN- γ , IL-12(p70), IL-4, IL-5, and IL-6. Sera from mice immunized with rEF-Tu also reduced *M. ovipneumoniae* growth (Jiang et al., 2016). In *Mycoplasma fermentans*, EF-Tu interacts specifically with the C-terminal 34 amino acids of CD21 in human B lymphoma cells (Balbo et al., 2005). CD21 receptors on the B cells enable the complement system to influence B-cell activation and maturation. The implication is that *Mycoplasma* species are involved in malignancies (reviewed in Vande Voorde et al., 2014)

and the interaction between EF-Tu and CD21 may be significant in this regard. EF-Tu from *L. monocytogenes* is the main activator of host dendritic cells (DC) (Mirzaei et al., 2016), antigen presenting cells (APCs) that play a key role in host immune modulation. Activation of DCs is achieved when *L. monocytogenes* interacts with the pattern recognition receptors (PRRs) on the surface of DCs (Mirzaei et al., 2016). These PRRs recognize bacterial proteins known as PAMPS (pathogen-associated molecular pattern). In *L. monocytogenes* EF-Tu was identified as a potent immuno-stimulatory effector in this process indicating that EF-Tu is a candidate for DC maturation-based therapies (Mirzaei et al., 2016).

Recombinant EF-Tu (rEF-Tu) has recently shown promising results as a vaccine candidate against bacterial pathogens. Immunization with rEF-Tu elicited both Th1 and Th2-type responses against *Streptococcus suis* and anti-rEF-Tu sera reduced viable load detection in porcine blood (Feng et al., 2018). Mice immunized with rEF-Tu demonstrated significant protection against lethal challenges with *S. pneumoniae* and increased cytokine, IgG1 and IgG2a, and CD4⁺ T-cell production (Nagai et al., 2019). rEF-Tu mediated protection against *S. pneumoniae* has also been demonstrated in fish (Yang et al., 2018), and partial protection against *H. influenzae* was achieved in mice (Thofte et al., 2018).

Besides stimulating an immune response in mammals (Figure 2A), EF-Tu is also a recognized PAMP in plants (Kunze et al., 2004; Furukawa et al., 2014). EF-Tu is secreted by via unknown mechanisms in soil dwelling, plant-pathogenic bacteria and is recognized by membrane-associated PRRs found on the extracellular surface of root epithelial cells in different plant species. The interaction between PRRs and PAMPS identifies the bacteria as an infectious threat, triggering a signal transduction cascade, that elicits an innate immune response (Zipfel, 2008) that includes the production of reactive-oxygen species and programmed cell death (Zipfel, 2008).

Both monocots and dicots use EF-Tu to notify their immune system of an infection. There is however, another level of sophistication to this interaction (Furukawa et al., 2014). Different plant species are known to have evolved recognition mechanisms in their respective PRRs that interact with different regions in the EF-Tu molecule. Rice PRRs recognize the amino acids (aa) 175-225 of EF-Tu, termed EFa50, from the plant pathogenic bacteria *Acidovorax citrulli* (formerly *Acidovorax avenae*) (Furukawa et al., 2014) whereas *Arabidopsis thaliana* recognizes the first 18 aa of EF-Tu from *E. coli* (termed elf18) (Kunze et al., 2004). Although elf18 does not illicit a response to this pathogen in rice plants, engineering the Arabidopsis PRR for elf18 into the rice plant enabled rice to recognize elf18 and respond by increasing resistance to bacterial attack (Lu et al., 2015). This proof-of-concept experiment demonstrated that PRRs can be engineered into the genomes of different crop species and may be beneficial to the farming and food production industry. Transfer of PRRs has already been demonstrated on food crops such as tomatoes and wheat (Lacombe et al., 2010; Schoonbeek et al., 2015). Earlier structural studies of EF-Tu suggested that the first 12 amino acids of EF-Tu are exposed on the surface and the dodecapeptide can act as a competitive

inhibitor of the elf18 elicitor (Kunze et al., 2004). The first 12aa of EF-Tu can also suppress the apoptotic response in plant cells allowing bacterial pathogens sufficient time and nutrient resources to colonize and replicate within plant cells (Igarashi et al., 2013). elf18 and EFa50 do not seem to have similar properties, but more recent structural studies have shown that they do appear to be (at least partially) surface exposed on the EF-Tu molecule (Furukawa et al., 2014). These data indicate that these two PAMPS can interact with PRRs on plant cell surfaces.

EF-Tu has also been shown to bind selectively to neuropeptide hormone substance P (SP) (Mijouin et al., 2013; N'Diaye et al., 2016). SP belongs to the tachykinin family of neuropeptides released by nerve and inflammatory cells (Datar et al., 2004) and is linked to many inflammatory diseases because it binds to the neurokinin 1 receptor (NK-1R) which stimulates pro-inflammatory responses (O'Connor et al., 2004). The inability to trigger NK-1R decreases bacterial clearance and increases death rates in mouse models of infection (Verdrengh and Tarkowski, 2008). SP and/or NK-1R have been linked to disease caused by infectious agents (Douglas et al., 2001; Schwartz et al., 2013), autoimmune disorders (Mantyh et al., 1988), psychological disturbances (Fehder et al., 1997; Herpfer and Lieb, 2005; McLean, 2005; Ebner and Singewald, 2006; Carpenter et al., 2008), cancer (Esteban et al., 2006), atopic dermatitis (Toyoda et al., 2002), and cell proliferation (Goode et al., 2003). EF-Tu from *S. aureus*, *S. epidermidis*, and *B. cereus* has been shown to bind SP, with an associated increase in virulence and biofilm formation (Mijouin et al., 2013; N'Diaye et al., 2016).

rEF-Tu derived from *Lactobacillus johnsonii* triggers a pro-inflammatory response in HT29 cells and increased IL-8 secretion in the presence of CD14 (Granato et al., 2004). IL-8 is known to increase levels of calcium ions within the cell in which it is expressed (Tuschil et al., 1992; Schorr et al., 1999). In *Bacillus subtilis* EF-Tu is a calcium binding protein (Dominguez et al., 2009). However, the link between calcium and EF-Tu in prokaryotes remains tenuous and further studies are needed to investigate the voracity of this association and its implication in the inflammatory response.

EF-Tu Has a Role in Adherence to Host Molecules

Infection

Adhesion to host cells and molecules is fundamental to pathogenesis in many bacterial species as it facilitates colonization, invasion, and host immune subversion (Stones and Krachler, 2016). In many instances, moonlighting functions for EF-Tu are associated with a role in adherence to a range of host molecules and host cells (Figure 2B). This does not appear to be a trend in specific phylogenetic groups of prokaryotes, as it extends through a range of bacteria. EF-Tu resides on the surface of *Francisella tularensis* where it binds to the RGG domain of nucleolin on the surface of the human monocytic cell line THP-1 (Barel et al., 2008). The HB-19 pseudopeptide irreversibly binds to the RGG domain in the C-terminus of nucleolin (Nisole et al., 1999, 2002) and effectively blocks attachment of *Francisella tularensis* (Barel et al., 2008). Consistent with

these experiments, EF-Tu and a 32 kDa cleavage fragment of EF-Tu were recovered during affinity chromatography pull-down experiments using nucleolin as bait (Barel et al., 2008). It is notable that cleavage fragments of EF-Tu have been previously described in the cytoplasm and membrane fraction of *L. monocytogenes* (Archambaud et al., 2005) and more recently on the extracellular surfaces of *S. aureus*, *Mycoplasma hyopneumoniae* and *M. pneumoniae* (Widjaja et al., 2015, 2017). Despite this finding, data describing the cleavage products in *L. monocytogenes* has not been reported but is an interesting observation nonetheless that warrants further investigation.

Fibronectin (Fn) is a key component of the extracellular matrix. It is a glycoprotein that binds to integrins embedded in eukaryote cell membranes and provides support and anchors cells to substrata. Many bacterial pathogens and commensals express adhesins that bind Fn and these interactions can trigger cytoskeletal rearrangements that promote host cell invasion (Massey et al., 2001; Deutscher et al., 2010; Seymour et al., 2010, 2012; Henderson et al., 2011; Bogema et al., 2012; Raymond et al., 2015, 2018). Interestingly, Fn also plays a role as a signaling molecule so, binding Fn may serve other functions for the bacteria, compounding their infectivity (Sandig et al., 2009). Indeed, many bacteria have a repertoire of dedicated, secreted adhesins that target Fn (Henderson et al., 2011).

EF-Tu localizes to both the outer membrane (OM) and outer membrane vesicles (OMV) of *A. baumannii* and binds to DsbA (Premkumar et al., 2014), a protein important in protein folding and maturation (Heras et al., 2009). In its external location in *A. baumannii* EF-Tu directly binds Fn (Dallo et al., 2012). The genome-reduced, human pathogen, *M. pneumoniae* also displays EF-Tu on its cell surface and plays an important role in interactions with Fn (Dallo et al., 2002; Widjaja et al., 2017). Anti-EF-Tu antibodies are able to prevent the binding of *M. pneumoniae* to immobilized Fn demonstrating the specificity of this interaction (Dallo et al., 2002). Binding of EF-Tu is confined to the C-terminal region of EF-Tu, with two Fn-binding regions being identified at amino acid positions 192-292 and 314-394 (Balasubramanian et al., 2008). The specificity of the Fn binding domain between amino acids 314-394 was confirmed when peptides spanning this region blocked the binding capacity of EF-Tu to Fn by 62% (Balasubramanian et al., 2009). Notably, EF-Tu from *Mycoplasma genitalium*, which shares 96% identity with EF-Tu from *M. pneumoniae*, does not bind Fn (Balasubramanian et al., 2009). Experimental comparison between the two sequences identified the residues S343, P345 and T357 to be key in the interaction with Fn (Balasubramanian et al., 2009). However, the Fn-binding *A. baumannii* EF-Tu does not possess these key binding residues identified in *M. pneumoniae* (see **Supplementary File**), suggesting an alternative Fn-binding mechanism. More recently, we have shown that EF-Tu from *M. pneumoniae* is a multifunctional, adhesive moonlighting protein that can bind fetuin, heparin, actin, as well as to plasminogen, vitronectin, lactoferrin, laminin, and fibrinogen (Widjaja et al., 2017).

The exogenous addition of soluble Fn is known to promote the ability of *Mycobacterium avium* subsp. *paratuberculosis* to attach and invade two epithelial cell lines (Secott et al., 2002)

but the identity of bacterial cell surface receptor(s) for Fn were not known. EF-Tu is a surface exposed cell wall protein in *Mycobacterium avium* subspecies *paratuberculosis* (MAP) (Viale et al., 2014). With the importance of Fn to MAP adhesion and invasion (Secott et al., 2002), and as EF-Tu is known to bind Fn in *M. pneumoniae* (Dallo et al., 2002; Balasubramanian et al., 2008) and *A. baumannii* (Dallo et al., 2012), it was investigated for its role as a Fn-binding protein. Knowledge of the Fn-binding regions of EF-Tu from *M. pneumoniae* was used to map putative Fn-binding regions in EF-Tu from *A. baumannii* (Dallo et al., 2012). Although the two previously identified Fn-binding regions (Balasubramanian et al., 2008) only showed 73 and 69% identity respectively in the EF-Tu homolog from MAP, ELISA assays showed that MAP EF-Tu binds Fn in a dose-dependent manner (Viale et al., 2014).

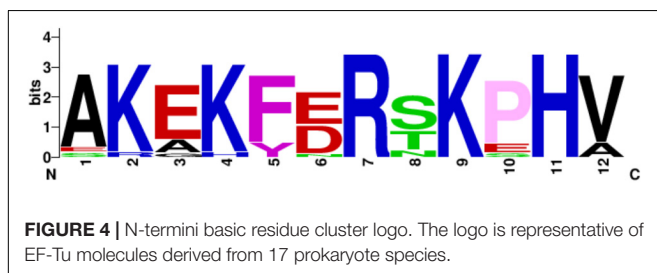
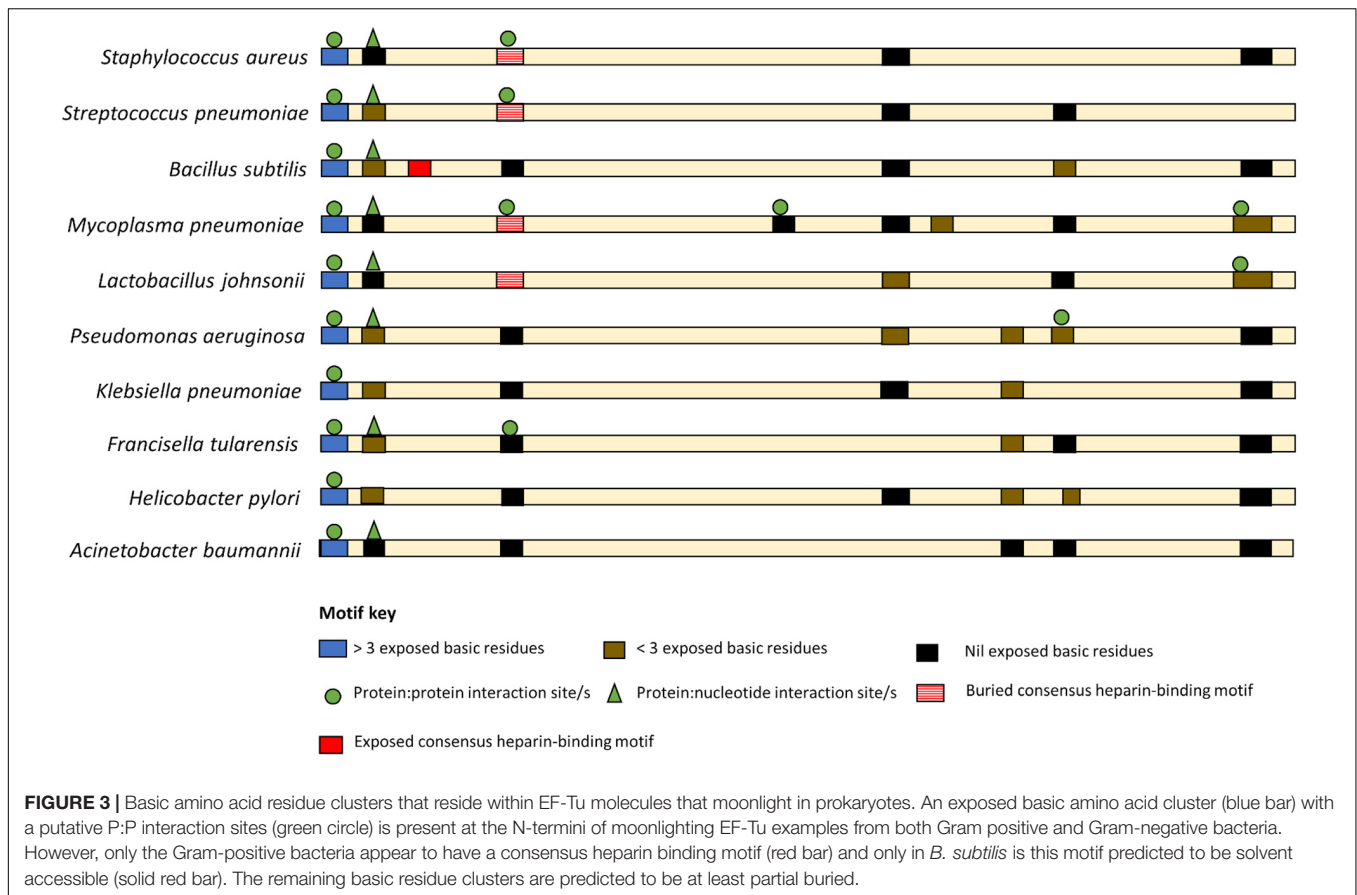
EF-Tu binds to human intestinal cells and mucins in a pH-dependent manner in the probiotic bacterium, *Lactobacillus johnsonii* suggesting a role for EF-Tu in gut colonization (Granato et al., 2004). At pH 5, EF-Tu derived from *L. johnsonii* was able to bind to the human colorectal adenocarcinoma cell line HT29 cells, undifferentiated CACO-2 cells and mucins isolated from HT29-MTX cells (Granato et al., 2004). The ability of EF-Tu to bind mucins extends to other anatomical locations, including the saliva mucin, MUC7 (Kesimer et al., 2009). EF-Tu was identified as one of six proteins to bind this mucin in *Streptococcus gordonii*, a major oral colonizer (Kesimer et al., 2009).

When *H. pylori* is co-cultured with THP-1 cells, expression of EF-Tu is upregulated and secreted and shown to localize to the surface of the human monocytic cell line THP-1 implicating it in host adhesion (Chiu et al., 2016).

While there are currently no universal moonlighting motifs (Babady et al., 2007), basic residues, both singularly and in clusters, are known to be binding anchors for anionic glycosaminoglycans (Jayaraman et al., 2000), are key in plasminogen (Jarocki et al., 2015), DNA (Ruyechan and Olson, 1992) and actin-binding (Peng et al., 2018), and have been linked with biofilm formation (Shanks et al., 2005; Green et al., 2013). Such basic residue clusters are found throughout the sequences of EF-Tus with reported moonlighting functions (**Figure 3** and **Supplementary File**).

A basic residue cluster is located at the N-termini of EF-Tu molecules with known moonlighting functions (**Figure 3** and **Table 2**). This short linear motif (SLiM) has at least three surface exposed basic residues, resides within a region of protein disorder, and possesses predicted protein:protein interaction sites. The arginine and lysine residues in positions 7 and 9 are unconserved in some bacterial species, thus may have arisen from advantageous point mutations (**Figure 4**).

Interestingly, the moonlighting EF-Tu from *Mycoplasma fermentans* has a unique, highly surface exposed, N-terminal extension that is 39 amino acids in length and possess an additional basic residue cluster at ³¹nKmKgKy³⁸ (see **Supplementary File**). Whether *M. fermentans* EF-Tu harbors moonlighting adhesive capabilities is currently unknown and may warrant future investigation.



Apart from the N-termini, the remaining basic residue clusters observed in EF-Tu molecules that moonlight are at least partially buried, which may impede their ability to bind host molecules. However selective cell surface proteolysis, described in detail in Section “Post-translational Modifications of EF-Tu,” can overcome some of the structural impediments.

EF-Tu Binds Innate Immune Effectors

Many bacterial pathogens of medical (Sanderson-Smith et al., 2012) and veterinary (Raymond and Djordjevic, 2015) significance have the ability to bind and activate plasminogen, a process that relies on interactions between basic amino acid residues on surface-accessible bacterial adhesins and kringle domains in plasminogen (Figure 2A). EF-Tu is also utilized by bacteria to dampen the host immune response.

The ability of EF-Tu to bind to host complement factors and plasminogen has been demonstrated in *Pseudomonas aeruginosa* (Kunert et al., 2007), *Leptospira* sp. (Wolff et al., 2013), *Streptococcus pneumoniae* (Mohan et al., 2014) *M. pneumoniae* (plasminogen only) (Widjaja et al., 2017) and *Acinetobacter baumannii* (plasminogen only) (Koenigs et al., 2015). The complement system is part of the innate immune system which non-specifically acts to clear the body of infection by lysing bacteria (Lambris et al., 2008). Complement factors bound on the bacterial surface remain active, and plasminogen can be converted to plasmin in its bound state (Kunert et al., 2007; Wolff et al., 2013; Mohan et al., 2014; Raymond and Djordjevic, 2015). This allows the bacteria to regulate and utilize these components for their own gain. By recruiting FH, FH1 and plasminogen the bacteria are able to inactivate C3b via a cleavage event mediated by FH, FH1 and plasmin (Lambris et al., 2008). The C3b complement factor is an important opsonization trigger that binds and labels bacteria ready for opsonization by the host immune system (Lambris et al., 2008). By degrading C3b, the bacteria are able to overcome this aspect of the innate immune system (Lambris et al., 2008). Additionally, the recruitment of plasmin to the bacterial cell surface plays an important role in degrading ECM and facilitating tissue invasion (Lahteenmaki et al., 2000; Bhattacharya et al., 2012). By binding complement factors such as Factor-H, FHL-1 and CFHR1 the bacterium

is able to suppress this process and evade the host innate immune response.

Bacterial infections can also lead to a reduction in white blood cell (WBC) counts in infected hosts leading to leukopenia. Isolates of *K. pneumoniae* from patients with leukopenia express higher levels of EF-Tu compared with *K. pneumoniae* isolates from patients with leucocytosis (Liu et al., 2014) suggesting that EF-Tu may be a pathogenicity factor in *K. pneumoniae*-based leukopenia (Liu et al., 2014). Interestingly, EF-Tu is upregulated in *Mycobacterium* species that have been phagocytosed by macrophages. The purpose of this upregulation has yet to be determined, but may further support the idea of a role for EF-Tu in bacterial evasion of the host immune system (Monahan et al., 2001).

Not only do bacteria have to defend themselves against the host immune response, but often they must defend themselves against each other. Due to the competitive environment in which bacteria live, they have developed toxins that target other bacterial cells. The Type VI secretion system (T6SS) can deliver toxin molecules into the cytosol of competing bacteria inhabiting the same niche (Pukatzki et al., 2006; Coulthurst, 2013; Cianfanelli et al., 2016). For the T6SS effector molecule in *P. aeruginosa* to enact its toxic effect, it must interact with EF-Tu prior to delivery into the recipient's cytoplasm (Whitney et al., 2015). The effector molecule and EF-Tu directly bind to each other in members of the *Pseudomonas* genus (Whitney et al., 2015). These observations suggest that EF-Tu has a wide variety of moonlighting functions relating to pathogenesis in different prokaryote species.

Cell Shape

Some bacteria can change cell shape as a protective strategy against the immune system. By changing shape, bacteria can become less easily engulfed by phagocytes, and can enhance biofilm formation thereby increasing persistence in the host (van Teeseling et al., 2017). Moreover, major actin-like cytoskeletal proteins, such as MreB, have a role in virulence. For example, in *P. aeruginosa* MreB regulates the type IV pili assemblage (Cowles and Gitai, 2010); in *H. pylori* MreB regulates the secretion of virulence factors (Waidner et al., 2009); and in *Salmonella enterica* serovar Typhimurium a disruption in the *mreB* gene led to downregulation of genes involved in pathogenicity (Bulmer et al., 2012; Doble et al., 2012).

The hypothesis that EF-Tu may interact with the cytoskeleton in prokaryotes is not a new idea, with the concept being introduced as early as the 1970s when EF-Tu was shown to form filaments (Beck, 1979). More recently, the formation of amyloid-like filaments by *Gallibacterium anatis* EF-Tu has been linked to biofilm formation (López-Ochoa et al., 2017). EF-Tu also interacts with MreB in *E. coli* (Butland et al., 2005). MreB forms helical filaments beneath the cell membrane and is essential for regulating cell shape (Jones et al., 2001). As it is well known that eukaryotic EF-Tu (eEF1A) interacts with actin, and influences cell shape, it is conceivable that this moonlighting function also occurs in prokaryotes. In *B. subtilis* and *E. coli* (Defeu Soufo et al., 2010, 2015), EF-Tu modulates the formation of MreB filaments by binding MreB in a ratio of 1:1 (Defeu Soufo et al., 2015)

(Figure 2C). One hypothesis suggests a link between EF-Tu cell concentration and cytoskeletal function. By reducing the expression of EF-Tu in the cell, cell shape can be modulated from the typical rod-like appearance to an abnormal cell shape (Defeu Soufo et al., 2010). Alteration of cell shape is due to disruption of the process that places MreB in helical structures beneath the membrane (Defeu Soufo et al., 2010). Further studies investigated whether EF-Tu's role in the translation mechanism was directly related to the population of EF-Tu molecules that interact with MreB, or whether separate populations of EF-Tu are generated for these alternate roles. Treatment of bacterial cells with kirromycin, which inhibits the release of EF-Tu from the ribosome, failed to interfere with EF-Tu localization in the cytoskeleton or its interactions with MreB filaments (Defeu Soufo et al., 2010). As previous studies have only revealed an interaction between *tufB* with *mreB* (not *tufA*) (Butland et al., 2005), it is tantalizing to consider whether only one of the *tuf* genes is responsible for the alternate function of cytoskeletal integrity. This might suggest that evolution of two *tuf* genes is useful in the delegation of EF-Tu moonlighting roles.

Post-translational Modifications of EF-Tu

Proteolytic processing is an irreversible post-translational modification (PTM) that can result in a loss, gain or change of function in a protein, as well as in degradation (Turk, 2006). Processing may be a mechanism to unlock moonlighting functions that are inherent in the newly created cleavage fragments via a mechanism similar to ectodomain shedding (Raymond et al., 2013; Tacchi et al., 2016) (Figure 2D). Furthermore, cleavage fragments may serve as competitive inhibitors to host immune cells. Host cytokines, chemokines, enzymes, antimicrobial peptides and growth factors all bind ECM components, including heparin and fibronectin, to control immune responses such as leukocyte emigration through tissue (Gill et al., 2010). By binding to the same ligands as host effector molecules (Kaneider et al., 2007; Krachler and Orth, 2013), bacterial proteins and their cleavage fragments may dampen an immune response. Additionally, cleavage may result in the loss of antigenic epitopes in surface proteins thereby circumventing host immune detection.

Cell surface EF-Tu is proteolytically processed in *M. hypopneumoniae* (Tacchi et al., 2016; Berry et al., 2017; Widjaja et al., 2017). Cleavage fragments of EF-Tu are retained on the cell surface and recovered during affinity chromatography using different host molecules as bait (Tacchi et al., 2016). The cleavage of EF-Tu has recently been demonstrated to be more widespread than previously appreciated with cleavage sites now also mapped in the *M. pneumoniae* and *S. aureus* (Scherl et al., 2005; Plikat et al., 2007; Widjaja et al., 2017). Processing generates fragments that are predicted to be more structural disordered and exposes regions of EF-Tu that are normally inaccessible to the aqueous environment. In particular we have shown that novel SLiMs enriched in positive charges are exposed allowing them to bind to a range of host molecules (Widjaja et al., 2017). Processing has so far been described in bacteria that belong to the low G + C Firmicutes. Many protein:protein and protein:nucleic acid interactions require correctly spaced

positive charges derived from lysine, arginine and histidine side chains in short regions of peptide sequence. Amino acids with positively charged side chain residues are encoded by A:T rich triplet codons and members of the low G + C Firmicutes are well suited to employ this as a strategy to expand their functional proteome. Single amino acid substitutions caused by single nucleotide polymorphisms (SNPs) have previously been described as pathogenicity-enhancing (Weissman et al., 2003). Specifically, SNPs in *E. coli* and *S. typhimurium* adhesin genes have led to distinctive pathogenicity-enhanced phenotypes (Sokurenko et al., 1998; Pouttu et al., 1999; Boddicker et al., 2002). SNPs can provide bacteria a selective advantage, leading to niche expansion and ultimately, novel species (Weissman et al., 2003). We propose that the accumulation of positively charged residues via SNPs in SLiMs facilitates binding interactions with diverse host molecules (Widjaja et al., 2017). Processing presents a mechanism to release fragments, each with the potential to expose a different repertoire of SLiMs to the aqueous environment compared with the parent molecule, and generate protein multifunctionality (Widjaja et al., 2017).

Bacterial EF-Tus are also the target for reversible PTMs such as phosphorylation, methylation and acetylation. Phosphorylation of EF-Tu has been identified in *E. coli* (Lippmann et al., 1993), *Thermus thermophilus* (Lippmann et al., 1993), *B. subtilis* (Levine et al., 2006), *Corynebacterium glutamicum* (Bendt et al., 2003), *Streptomyces collinus* (Mikulik and Zhulanova, 1995), *Thiobacillus ferrooxidans* (Seeger et al., 1996), *S. pneumoniae* (Sun et al., 2010), *M. genitalium* (Su et al., 2007), and *M. pneumoniae* (Su et al., 2007). While phosphorylation of EF-Tu lowers its binding affinity to GTP, subsequently reducing protein synthesis, the PTM also inhibits binding by the antibiotic kirromycin, a specific inhibitor of EF-Tu (Archambaud et al., 2005; Sajid et al., 2011). Furthermore, the associated decreased bacterial growth facilitated by EF-Tu phosphorylation has been implicated as an acclimation measure to stress conditions during infection (Archambaud et al., 2005). Similarly, Van Noort et al. (1986) demonstrated that EF-Tu methylation in *E. coli* lowers GTP hydrolysis and suggest a more accurate translation process as a result.

Lysine acetylation and lysine glutarylation has been described in EF-Tu from *Mycobacterium tuberculosis* (Xie et al., 2015, 2016). These modifications can affect protein-protein and protein:nucleic acid interactions and it may be important to the pathogenicity of *M. tuberculosis*, although this is yet to be determined. Evidence for the role of PTMs in EF-Tu moonlighting functions have been described in *P. aeruginosa*, where the trimethylation of the lysine at residue 5, allows

EF-Tu to structurally mimic phosphorylcholine (Barbier et al., 2013). This modification means that EF-Tu can specifically bind a platelet-activating receptor, resulting in successful bacterial adhesion (Barbier et al., 2013).

CONCLUDING REMARKS

EF-Tu has evolved to be a multifunctional protein in a wide variety of pathogenic bacteria. While moonlighting functions vary among microbial species there is a common theme for roles in adherence and in immune regulation. The understanding of how this essential and highly expressed protein evolved moonlighting functions is an active area of research and is likely that more diverse and important roles are yet to be discovered.

AUTHOR CONTRIBUTIONS

KH and SD conceived and co-wrote the first draft with input from all authors for the final draft. VJ created the figures and contributed significantly to the revised manuscript. All authors contributed to revision, read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2019.02351/full#supplementary-material>

FILE S1 | Amino acid sequence analysis of EF-Tus with moonlighting functions.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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