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Regulation of prokaryotic gene expression by eukaryotic-like enzymes

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Summary

A growing body of evidence indicates that serine/threonine kinases (STK) and phosphatases (STP) regulate gene expression in prokaryotic organisms. As prokaryotic STKs and STPs are not DNA binding proteins, regulation of gene expression is accomplished through post-translational modification of their targets. These include two-component response regulators, DNA binding proteins and proteins that mediate transcription and translation. This review summarizes our current understanding of how STKs and STPs mediate gene expression in prokaryotes. Further studies to identify environmental signals that trigger the signaling cascade and elucidation of mechanisms that regulate cross-talk between eukaryotic-like signaling enzymes, two-component systems, and components of the transcriptional and translational machinery will facilitate a greater understanding of prokaryotic gene regulation.

Introduction

Coordinated gene expression is critical for all living organisms to adapt to their environment. In prokaryotes, timely expression of factors important for environmental adaptation was thought to be primarily achieved by the action of two-component systems (TCSs) consisting of sensor histidine kinases (HKs) and cognate DNA binding response regulators (RRs) (for reviews see [1–3]). However, recent studies have shown that prokaryotes encode signaling enzymes commonly found in eukaryotes and include serine/threonine kinases (STKs) and phosphatases (STPs) (for recent reviews see [4,5]). Analysis of mutants that lack these signaling enzymes has facilitated our understanding of how serine/threonine kinases and phosphatases contribute to regulation of gene expression in prokaryotes. STK and STP mediated gene expression is important for cellular processes such as growth, virulence, antibiotic resistance and secondary metabolite production. Although they are not DNA binding proteins, STK and STPs mediate prokaryotic gene expression through post-translational modification of a variety of targets, including two-component response regulators or critical components of prokaryotic transcriptional and translational machinery. This review summarizes how reversible phosphorylation by STKs and STPs facilitate prokaryotic gene expression.

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Interaction with response regulators and transcription factors

Cross-talk between eukaryotic-like serine/threonine kinases (STKs) and phosphatases (STPs) with two-component systems (TCSs) is known to occur in a number of prokaryotes [6–13]. Post-translational modifications of TCSs by STKs affect virulence, iron transport, antibiotic production and antibiotic resistance [6–13]. In some prokaryotes, it is thought that the membrane-associated STK senses external signals and regulates the DNA-binding activity of response regulators (RR) (see Figure 1A). This mechanism provides an additional level of control of two-component mediated gene expression and increases the versatility of the organism for environmental adaptation. Below, we have included examples of how STK mediated post-translational modification of response regulators affects gene expression.

Streptococcus agalactiae (also known as Group B Streptococcus or GBS) is an important human pathogen. The TCS known as CovR/CovS (Cov = control of virulence) regulates the expression of over 100 GBS genes [14–16]. Thirty genes comprise the core regulon [16,17], including CovR/S repression of an important GBS toxin known as β-hemolysin/cytolysin (β-H/C) [14,15]. Aspartate phosphorylation of CovR by its sensor kinase (CovS) enhances CovR binding to the β-H/C promoter P*cylE* [6,14,15]. Conversely, Stk1 phosphorylation of CovR at a threonine residue (T65) decreases promoter DNA binding and alleviates repression of β-H/C [6]. Thus, Stk1 phosphorylation of CovR allows GBS to fine tune the expression of an important virulence factor and possibly facilitates the transition of GBS from commensal to invasive niches.

The TCS CovR/S was originally identified in the human pathogen *Streptococcus pyogenes* (also known as Group A Streptococcus or GAS) [18,19]. In GAS, CovR/S regulates the expression of a number of virulence genes [20,21] and Bugrysheva *et al* described that the *S. pyogenes* STK (SP-STK) regulates the expression of genes that are linked to and are independent of CovR/S [22]. Agarwal *et al* recently showed that SP-STK phosphorylates GAS CovR at threonine residues [23], similar to GBS. SP-STK and SP-STP mutants exhibit changes in capsule and hemolysin expression [23,24], presumably due to a link with the CovR/S system. Additionally, SP-STK was also shown to phosphorylate WalR of the WalRK TCS [23] which may explain the decrease in expression of a cell division protein *cdhA* in GAS mutants lacking SP-STK [24,25]. Although the role of SP-STK phosphorylation on WalR and CovR promoter binding and gene regulation remains to be elucidated [23], these observations provide evidence for the interaction between TCS and eukaryotic-like signaling enzymes in bacterial pathogens.

In the human pathogen *Streptococcus pneumoniae,* RitR (Rit = repressor of iron transport) is a stand alone transcriptional regulator that negatively regulates expression of an iron uptake ATP-binding cassette (ABC) transporter known as Piu. RitR is homologous to CovR of GBS (45% homology, see [26]), is important for virulence of *S. pneumoniae* and is reversibly phosphorylated by the kinase StkP and the phosphatase PhpP [7]. STK phosphorylation was localized to the DNA-binding domain of RitR suggesting that phosphorylation might regulate binding to the *piu* promoter (P*piu*, [26]), though this was not examined. Surprisingly, both PhpP and StkP positively regulate Piu as mutations in either decreased *piu* expression [7]. The exact mechanism of how StkP and PhpP regulate RitR function and Piu expression is unclear [7]. As the complex consisting of RitR and PhpP bound to P*piu* was dissolved upon addition of StkP, Ulijaz *et al* hypothesize that phosphorylation of RitR by StkP blocks the formation of a ternary complex between PhpP, RitR and P*piu* [7]. How S/T phosphorylation regulates RitR mediated gene expression and iron acquisition of *S. pneumoniae* remains to be clarified.

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Similar to CovR and RitR, EmbR is a transcriptional regulator belonging to the OmpR-like family and is important for cell wall biosynthesis of the human pathogen *Mycobacterium tuberculosis* (MTB, [8,10]). EmbR regulates expression of three arabinosyltransferases (*embC*, *A*, and *B*) that are important for modulation of the mycobacterial cell wall, MTB virulence and ethambutol resistance [8–10]). EmbR contains a DNA binding domain, transcriptional activator domain and a forkhead-associated (FHA) phosphoprotein recognition domain [8,10]. Mutations in three residues of the FHA domain (Arg312, Ser326 and Asn348) abrogated phosphorylation of EmbR by the STK PknH [8]. While threonine phosphorylation inhibited CovR DNA binding [6], phosphorylation of EmbR by the kinase PknH enhanced its ability to bind to promoter DNA [10]. PknH expression increased during macrophage infection and the kinase promotes virulence of MTB through its ability to control expression of the *embCAB* genes [10]. Interestingly, EmbR was also described to be phosphorylated by other mycobacterial serine/threonine kinases known as PknA and PknB and is also dephosphorylated by the phosphatase Mstp [9]. Collectively, these observations indicate that regulation of virulence through post-translational modification of EmbR is important for regulation of gene expression in MTB infections.

AfsR of *Streptomyces coelicolor* is a homolog of EmbR of MTB [8]. AfsR is a global regulatory protein that controls the expression of two pigmented antibiotics namely, actinorhodin and undecylprodigiosin [27]. AfsR was found to be phosphorylated at serine and threonine residues by the kinase AfsK [11]. Loss of AfsK resulted in a significant decrease in actinorhodin production suggesting that phosphorylation of AfsR is necessary for its function [11]. Paradoxically, other STKs are predicted to regulate AfsR function as AfsR was still found to be phosphorylated in an AfsK mutant strain [11]. Whether phosphorylation of AfsR occurs at different amino acid residues in the absence of AfsK and the consequent effect on gene expression requires additional study. Collectively, these studies indicate that multiple STKs control the expression of secondary metabolite production in *S. coelicolor* through phosphorylation of the global regulator AfsR.

A global transcriptional regulator important for virulence of *Staphylococcus aureus* is the Staphylococcus accessory regulator, SarA (for a recent review see [28]). SarA is known to be involved in the regulation of over 100 *S. aureus* genes including positive regulation of the *agr* locus and negative regulation of itself [28–30]. SarA was recently suggested to be phosphorylated at threonine residues by the STK (Stk1) that is conserved in all *S. aureus* strains [12]. Phosphorylation of SarA by Stk1 led to a four fold increase in binding to P2*agr* and a four fold decrease in binding to P1*sar* [12]. The *in vivo* relevance of Stk1 regulation of SarA is unclear because the observations of Didier *et al* imply that the absence of Stk1 should lead to decreased *sarA* transcription and decreased expression of the Agr locus. However, microarray analysis of a *pknB* (*stk1*) mutant in *S. aureus* 8325 indicated an increase in expression of both *agrA* and *agrC* [31]. Also, expression of Agr genes in a *stk1* mutant of *S. aureus* Newman was similar to the WT [32]. In addition, there was no change in *sarA* expression in the *stk1* mutants from either 8325 or Newman strains [31,32]. Didier *et al* also suggest that SarA is phosphorylated by a second serine threonine kinase Stk2 (found only in certain *S. aureus* strains such as N315) at serine residues (SA0077, [12]). However, further studies are essential to understand the downstream implications of SarA phosphorylation on regulation of gene expression and *S. aureus* virulence.

In addition to SarA, *S. aureus* Stk1 has also been described to phosphorylate another global transcriptional regulator, MgrA [13,33,34]. MgrA regulates expression of the multi-drug efflux pump, NorA [35,36]. Phosphorylation by PknB prevents MgrA binding to the *norA* promoter, thus increasing *norA* transcription and resistance to certain fluoroquinolone antibiotics [13]. The link between PknB and MgrA regulation was discovered because of conflicting results on whether MgrA is a positive or negative regulator of *norA* expression

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[13]. Truong-Bolduc *et al* discovered that MgrA is a positive regulator of *norA* in *rsbU*- strains and a negative regulator of *norA* in *rsbU*+ strains [13]. *rsbU* is required for activation of the sigB regulon which controls the expression of multiple genes in response to stress [37]. Transcription of *pknB* is similar in both *rsbU*+ and *rsbU*− strains [13]. Whether RsbU and/or SigB expression affects PknB regulation of MgrA is not known. Regardless, MgrA mediated regulation of *S. aureus* resistance to certain fluoroquinolone antibiotics is controlled through PknB phosphorylation.

One of the first STKs characterized was from *M. xanthus*, a gram-negative soil bacterium that exhibits vegetative growth and forms fruiting bodies in response to environmental stress [38,39]. Pkn8 and Pkn14 are two serine/threonine kinases of *M. xanthus* that regulate MrpC, an essential transcription factor necessary for activating *fruA* expression during fruiting body development [40]. The membrane kinase Pkn8 phosphorylates the cytoplasmic kinase Pkn14 which in turn phosphorylates MrpC [40]. MrpC binds and activates its own promoter and phosphorylation by Pkn14 prevents promoter DNA binding [41]. Pkn8/Pkn14-mediated phosphorylation of MrpC represses *mrpC* expression during vegetative growth and allows for timely expression of *fruA* and fruiting body development [40]. Thus, the membrane bound STK Pkn8 enables survival of *M. xanthus* by sensing an external signal and initiating a phosphorylation cascade that regulates fruiting body development in response to environmental stress.

Interaction with transcription/translation components

In this section, we provide examples of the role of STKs and STPs in regulation of prokaryotic transcriptional and translational machinery. Similar to the role of histones in eukaryotes, histone-like proteins have been described to regulate DNA transcription and replication in bacteria [42–46]. In *E. coli*, the DNA-binding histone-like protein HU regulates transcription of approximately 8% of the genome [47]. It is thought that binding of histone-like proteins can introduce structural changes to the DNA which facilitate or prevent binding of other regulatory proteins to DNA. Recent studies have indicated that histone-like proteins are phosphorylated by STKs such as Stk1 of *S. aureus* [32] and SP-STK of *S. pyogenes* [24] (see Figure 1B). While the role of phosphorylation on the DNA binding ability of histone-like proteins in *S. aureus* and *S. pneumoniae* is not known, the serine/threonine kinase Prk2 of *M. xanthus* was described to phosphorylate HUα and HUβ of *E. coli* and phosphorylation of HUα at threonine 59 (T59) prevented DNA binding [48]. HUα and HUβ are highly conserved in bacteria and form a heterodimer for regulation of gene expression [49,50]. These observations indicate that post-translational modification of histone-like proteins by STKs may affect DNA binding or introduce structural changes to DNA that either directly regulates transcription or affects the binding of regulators that mediate transcription.

In addition to phosphorylating histone-like proteins to control transcription, prokaryotic STK and STP enzymes have also been found to reversibly phosphorylate RNA and DNA polymerases directly (see Figure 1C). Some transcriptional activators interact with the C-terminal domain of the alpha subunit of RNA polymerase (RpoA) in order to function properly [51,52]. RpoA was identified as a substrate of *S. pneumoniae* StkP [52], suggesting that StkP may regulate gene expression by controlling the interaction of certain transcription factors, like RitR [7], with RpoA. In *L. monocytogenes*, the catalytic domain of the STK PrkA was shown to interact with the alpha subunit of DNA polymerase III PolC, the DNA-directed RNA polymerase subunits alpha and beta (RpoA and RpoB) and the recombination protein RecA [53]. The interaction of these proteins implies a role for PrkA in transcriptional regulation of *L. monocytogenes*. Understanding the *in vivo* relevance (*e.g.*

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changes in gene expression) due to of S/T phosphorylation of transcription machinery will further establish the relevance of these *in vitro* observations.

A number of prokaryotic STKs and STPs have been found to phosphorylate elongation factors. In prokaryotes, elongation factors play a critical role in protein biosynthesis. Given that transcription and translation is coupled in prokaryotes, these observations provide an additional mechanism by which STKs and STPs regulate prokaryotic gene expression. Translation elongation is initiated by three elongation factors: EF-Tu, EF-Ts and EF-G [54,55]. EF-Tu delivers aminoacyl-tRNAs to the ribosome and associates with GTP [54,55]. EF-Ts acts as a guanine nucleotide exchange factor (GEF) on EF-Tu and EF-G is an additional essential GTPase involved in mRNA and tRNA translocation [54,55]. Phosphorylation of EF-Tu has been found to prevent binding to aminoacyl-tRNAs and thus inhibits translation elongation [54]. In eukaryotes, the functional homologs of EF-Tu, EF-Ts and EF-G (eEF1A, eEF1B and eEF2 respectively) are known to be regulated via phosphorylation in order to maintain the appropriate rate of protein synthesis under a variety of conditions including starvation and growth stimulation [54,56–59]. Therefore, mechanisms that regulate prokaryotic translational elongation may also serve to control the rate of protein synthesis in response to the environment.

Pereira *et al* hypothesized that post-translational modification of elongation factors allows bacteria to direct protein translation to specific mRNAs required for growth phase transition or in response to different growth environments sensed by the kinase [4]. In the spore-forming soil bacterium *B. subtilis*, EF-G and EF-Tu are reversibly phosphorylated by PrpC (STP) and PrkC (STK), which regulates bacterial germination from dormant spores [60–63]. Thus, exit from dormancy may be controlled through specific expression of germination genes via phosphorylation of elongation factors. Similarly, EF-Tu was found to be a substrate of PknB in MTB [64]. Sajid *et al* found that phosphorylation of EF-Tu by PknB reduced its interaction with GTP, increased resistance to EF-Tu specific antibiotics, and caused an overall decrease in protein synthesis which can promote dormancy in MTB [64]. EF-Tu is also a substrate for Stp in *L. monocytogenes* [54], and PrkA (STK) was found to interact with both EF-Tu and EF-G [53]. Archambaud *et al* suggested that phosphorylation of EF-Tu in *L. monocytogenes* allows the bacterium to adapt to the stressful environments encountered in the host by regulating the expression of specific genes [54]. Recently, EF-Tu and ATP-dependent RNA and DNA helicases were found to be phosphorylated by Stk1 in GBS Stp1 mutants [65]. As increased phosphorylation of GBS proteins were observed in the absence of Stp1, the authors predict that Stp1 regulates Stk1 phosphorylation of its targets for appropriate regulation of gene expression and GBS autolysis [65]. Taken together, STK-directed phosphorylation of prokaryotic factors could either lead to a specific change in protein expression and/or function. These changes enable the organism to respond to environment signals for regulation of gene expression and survival.

Conclusion

Regulation of gene expression by eukaryotic-like serine/threonine kinases and phosphatases appears to be a conserved function in prokaryotes. Although some STK and STP mediated gene regulation can be explained through the post-translational modifications of TCSs, DNA binding proteins, transcription and translation machinery (summarized in Table 1 and Figure 1), further studies will be essential for a complete understanding of the signaling mechanisms. In *S. mutans* approximately 4% of the genome was shown to be regulated by the STK PknB, but no targets have been identified to date [66]. In addition, microarray analysis of kinase mutants in *Staphylococcus aureus* [31,32], *Streptococcus pyogenes* [22], *Streptococcus pneumoniae* [67] and GBS (L. Rajagopal *et al*, unpublished) revealed that a large number of genes are regulated by STKs in these organisms, which may not be limited

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to the mechanisms described to date. Therefore, our understanding of prokaryotic STP and STK signaling is still in its infancy.

It is noteworthy that signals that activate prokaryotic STK and STP enzymes have not been extensively described. In *B. subtilis*, unlinked peptidoglycan was shown to be an activating ligand for PrkC and mediates exit from dormancy [61]. More recently, a link between peptidoglycan biosynthesis and/or remodeling has been described for StkP of *S. pneumoniae* (see Sham *et al* review this issue). Therefore, understanding the activating signals for STK in prokaryotes will provide further insight into signaling mechanisms that mediate prokaryotic gene expression.

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Highlights

- Serine/threonine kinases and phosphatases regulate prokaryotic gene expression
- Signaling mediates growth, virulence and antibiotic resistance
- Targets include DNA-binding regulators, transcriptional and translational machinery

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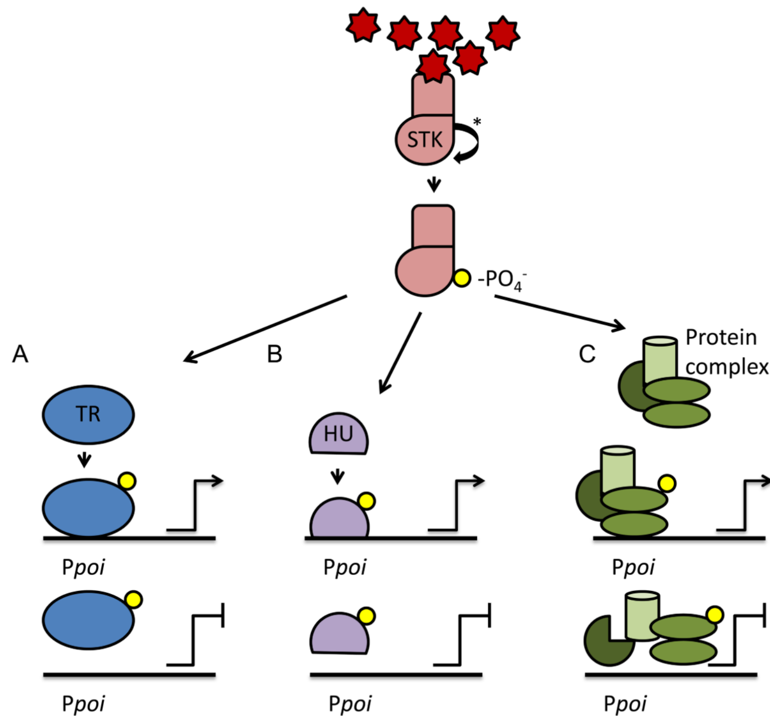

Figure 1.
Schematic representation of known mechanisms of STK-mediated regulation of gene expression. Prokaryotic serine/threonine kinases (STK) are membrane bound protein kinases that autophosphorylate in response to an activating environmental signal. This phosphate group can then be transferred to different targets within the bacterial cell to modulate gene expression. Known targets include A) transcriptional regulators (TR) which include DNA-binding response regulators (RR) of two-component systems (TCS), B) DNA-binding histone-like proteins (HU), and C) proteins of transcriptional or translational machinery. Phosphorylation of these targets has been shown to induce or repress gene expression allowing the bacteria to adapt appropriately to their changing external environment.

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Table 1

Targets of eukaryotic-like enzymes that mediate gene expression in prokaryotes

Strain	Targets
Streptococcus agalactiae/ Group B Streptococcus	DNA-binding Response regulator, CovR
	Elongation factor Tu, EF-Tu
	ATP-dependent RNA helicase
	ATP-dependent DNA helicase
Streptococcus pyogenes/ Group A Streptococcus	DNA-binding histone-like protein, SP-HLP
	DNA-binding Response regulator, CovR
	DNA-binding Response regulator, WalR
Streptococcus pneumoniae	DNA-binding Response regulator, RitR
	DNA-directed RNA polymerase subunitα, RpoA
Mycobacterium tuberculosis	DNA-binding Response regulator, EmbR
	Elongation factor Tu, EF-Tu
Streptomyces coelicolor	DNA-binding Response regulator, AfsR
Staphylococcus aureus	DNA-binding histone-like protein, HU
	Global transcriptional regulator, MgrA
	Global transcriptional regulator, SarA
Myxococcus xanthus	Transcription factor, MrpC
	DNA-binding histone-like protein, Huα
	DNA-binding histone-like protein, Huβ
Listeria monocytogenes	DNA Polymerase III PolC, α subunit
	DNA-directed RNA polymerase subunit α, RpoA
	DNA-directed RNA polymerase subunit β, RpoB
	Recombinant protein, RecA
	Elongation factor Tu, EF-Tu
	Elongation factor G, EF-G

Strain	Targets
Bacillus subtilis	Elongation factor G, EF-G
	Elongation factor Tu, EF-Tu

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