Signal Transduction of Streptococci by Cyclic Dinucleotide Second Messengers

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Abstract

Since the discovery of cyclic dimeric guanosine 3',5'-monophosphate (c-di-GMP) in 1987, the role of cyclic dinucleotides in signal pathways has been extensively studied. Many receptors and effectors of cyclic dinucleotides have been identified which play important roles in cellular processes. Example of such effectors include cyclic dimeric adenosine 3',5'-monophosphate (c-di-AMP)-binding proteins and endoplasmic reticulum membrane adaptor. Accumulating evidence indicate that cyclic dinucleotides act as second messengers that not only regulate

the bacterial physiological processes but also affect host immune responses during infections. Streptococci species, which produce cyclic dinucleotides, are responsible for many human diseases. Numerous studies suggest that the cyclic dinucleotides are vital in signal transduction pathways as second messengers and influence the progression of infectious diseases. Here, we provide an overview of the molecular principles of cyclic dinucleotides synthesis and degradation and discuss recent progress on streptococcal signal transduction pathways by cyclic dinucleotide second messengers and their role in regulating host immune reaction. This review will provide a better understanding of the molecular mechanisms of streptococcal cyclic dinucleotide second messengers thereby revealing novel targets for preventing infections.

Introduction

In natural environments, bacteria are exposed to rapidly changing nutrient availability and physical conditions. To adapt to their environments, bacteria use efficient signal transduction systems to detect changes and to transduce the information (Galperin 2018). Therefore, signaling molecules are the most crucial part of these systems. Over the past few decades, nucleotide messengers, including linear and cyclic nucleotides have been shown to control important biological signaling cascades in prokaryotic cells (Gomelsky 2011, Kalia et al. 2013).

Linear nucleotides include guanosine 3',5'-bispyrophosphate (ppGpp) and guanosine 3'-diphosphate, 5'-triphosphate (pppGpp) (collectively known as (p)ppGpp) are important second messengers which are produced by bacteria in response to nutrient starvation, which reduces the growth rate of cells. (p)ppGpp

affects several vital biochemical processes in the bacteria including transcription, translation and DNA replication, thus regulating the virulence factors and biofilm formation (Kalia et al. 2013, Liu et al. 2015). Studies on *Streptococcus mutans* revealed the role of (p)ppGpp in stress tolerance, peptide signaling and competence development (Kaspar et al. 2016). Moreover, (p)ppGpp and its synthetases are involved in the pathogenesis of zoonotic *Streptococcus suis* (Zhu et al. 2016).

The cyclic mononucleotides, cyclic guanosine 3',5'-monophosphate (cAMP) and cyclic adenosine 3',5'-monophosphate (cGMP) are extensively used as second messengers in bacteria. cAMP is involved in transcription regulation by binding to cAMP-receptor protein (Crp), a transcription factor that regulates the transcription of a large number of promoters (Hengge et al. 2016). A study on type III CRISPR-Cas systems of *Streptococcus thermophilus* demonstrated that the cAMP synthesized by Cas10 binds the ribonuclease Csm6 thereby activating its nonspecific RNA degradation (Kazlauskiene et al. 2017). The role of cGMP in bacterial signaling become clear when it was detected in *Rhodospirillum centenum* and acted as a regulator of developmental process via cGMP-dependent transcription factor (Marden et al. 2011). Although numerous studies have investigated cAMP signaling in streptococci, the biology of cGMP in streptococci is not fully understood.

Recently, the cyclic dinucleotides (CDNs) including c-di-GMP, c-di-AMP and cyclic GMP-AMP (cGAMP) have emerged as key components in cellular signal transduction pathways (Drexler et al. 2017). They are also released or secreted by the bacteria and are recognized by the host innate immune system

(Danilchanka et al. 2013).

C-di-GMP was first described to mediate signals of allosteric activators of cellulose synthase in *Gluconacetobacter xylinus* (Ross et al. 1987). Since then, c-di-GMP and its metabolizing enzymes have been identified in many Gram-negative bacteria such as *Caulobacter crescentus, Pseudomonas aeruginosa, Vibrio cholera* and *Escherichia coli* (Kalia, Merey et al. 2013) and a few Gram-positive bacteria such as *Bacillus subtilis* and *Listeria monocytogenes* (Yan et al. 2010, Kalia, Merey et al. 2013). C-di-GMP mainly regulates the motility-to-sessility transition, and it is also associated with biofilm formation and virulence factor production (Commichau et al. 2018)

C-di-AMP was added to the list of nucleotide second messengers in 2009. Since then, it has been identified in several Gram-positive bacteria including *Bacillus subtilis* (Gándara and Alonso 2015), *Listeria monocytogenes* (Woodward et al. 2010), *Staphylococcus aureus* (Corrigan et al. 2011), and *Streptococcus* species such as *S. mutans* (Peng et al. 2016), *S. pneumoniae* (Bai et al. 2014), *S. pyogenes* (Kamegaya et al. 2011) *S. agalactiae* (the Group B *Streptococcus* [GBS]) (Andrade et al. 2016), and in a few Gram-negative bacteria including *Chlamydia trachomatis* (Barker et al. 2013) and *Borrelia burgdorferi* (Ye et al. 2014). The functions of c-di-AMP have been extensively studied in *streptococcus* such as biofilm formation, osmolyte transport, bacterial growth, and virulence factor expression (Corrigan and Grundling 2013, Commichau et al. 2018, Hall et al. 2018).

cGAMP is the most recently identified cyclic dinucleotide second messenger.

There are two types of cGAMP: 3',3'-cGAMP and 2'3'-cGAMP. 3',3'-cGAMP was first identified as a second messenger in *V. cholerae and* was required for chemotaxis and intestinal colonization (Davies et al. 2012). 2'3'-cGAMP was uncovered as a key factor that regulates innate immune signaling and antiviral response in eukaryotes (Krasteva et al. 2017).

The metabolism of cyclic dinucleotide second messengers

c-di-GMP

C-di-GMP is synthesized from two GTP molecules by diguanylate cyclases (DGCs) and is degraded into pGpG by phosphodiesterases (PDEs). DGC was first purified and tested in *G. xylinus* by Benziman and colleagues (Ross et al. 1987). Further biochemical and structural studies on DGCs provided the mechanistic understanding on c-di-GMP synthesis. In 1995, the gene for the pleiotropic protein D (PleD, one of the DGCs) was characterized and a novel C-terminal GGDEF (Gly-Gly-Asp-Glu-Phe) domain was identified (Hecht and Newton 1995). Further studies revealed the crystal structure of *C. crescent* PleD and showed that PleD is composed of two response regulator domains, receiver (REC) and GGDEF (Chan et al. 2004, Wassmann et al. 2007). The GGDEF (contains GGDEF and GGEEF, collectively called GGDEF) motif is conserved in most DGCs (Tal et al. 1998, Simm et al. 2004, De et al. 2009). Two Mg^{2+} or Mn^{2+} are required for the formation of phosphoester bond and the first two Gly residues of the GGDEF motif are responsible for GTP binding whereas the forth Glu residue is involved in metal ion coordination (Pei et al. 2001). The third Asp/Glu is responsible for catalysis and plays a role in metal coordination.

Hydrolysis of c-di-GMP is triggered by proteins carrying EAL (Glu-Ala-Leu) and

HD-GYP (His-Asp and Gly-Tyr-Pro) domains. EAL domain-containing PDE cleaves c-di-GMP into pGpG (5'-phosphoguanylul-guanosine) and further into two GMPs by oligoribonuclease (Orr et al. 2015). The activity of EAL-containing PDEs requires either Mg^{2+} or Mn^{2+} while its activity is strongly inhibited by Ca^{2+} and Zn^{2+} (Rao et al. 2008, Tchigvintsev et al. 2010). The activity of EAL domain-containing PDEs depends on the structure of a two-metal cation cluster which is required for the binding of divalent cations and c-di-GMP, and for catalysis (Salter et al. 2007). The EAL-containing PDEs that only have a single cation are catalytically inactive. Additional mutagenesis and structural studies classified the EAL domains into three categories: enzymatically active, potentially enzymatically active, and enzymatically inactive (Rao et al. 2009). HD-GYP domain containing proteins have resisted high-resolution crystallography, however, the crystal structure of a few HD-GYP containing PDEs has been reported (Kalia et al. 2013). One prototype of HD-GYP domain-containing protein is RpFg, which transforms c-di-GMP to GMP via 5'-pGpG as an intermediate (Nickels et al. 2011). Some hybrid proteins that contain both GGDEF and EAL or HD-GYP domains have also been reported. Although the majority of such proteins contain one intact active site, a few proteins have dual functions of DGC and PDE (Ferreira et al. 2008, Seshasayee et al. 2010).

c-di-AMP

C-di-AMP is synthesized by diadenylyl cyclase (DAC) enzymes from two molecules of ATP and is broken down to pApA by PDE enzymes. C-di-AMP is synthesized through the reaction of two molecules of ATP carried out by the nucleophilic attack at each 3'-OH group on the α-phosphate of the other ATP molecule, which leads to the ring closure accompanied by the release of two

molecules of pyrophosphate (Commichau et al. 2018). The enzymes responsible for producing c-di-AMP share a common catalytically active domain known as DAC domain. C-di-AMP has not been detected in an organism which does not encode an enzyme containing a DAC domain. In these enzymes, the DAC domains are often accompanied by other domains that regulate the enzymatic activity (Commichau et al. 2018). So far, five classes of cyclases with different regulatory domains have been identified: DisA (DNA integrity scanning protein A), CdaA, CdaS, CdaM, and CdaZ (Commichau et al. 2015, Kellenberger et al. 2015, Blötz et al. 2017, Commichau et al. 2018). Although most organisms possess one type of diadenylate cylase, some contain multiple enzymes such as *Clostridium* spp., which contains both CdaA and DisA. *B. subtilis* has three types of DACs, DisA, CdaA, and CdaS (Fahmi et al. 2017). The most common motifs that reside in DAC domain proteins are DGA (Asp-Gly-Ala) and RHR (Arg-His-Arg) which possess cyclase activity required for the synthesis of c-di-AMP (Gundlach et al. 2015, Rosenberg et al. 2015). Structural analysis of DACs has revealed that DisA, CdaS and CdaA have a conserved DAC domain of about 150 amino acids. In addition, a structure-based sequence alignment of these three proteins revealed sequence identities of 28% to 49% among their DAC domains (Huynh and Woodward 2016).

The c-di-AMP hydrolyzing enzyme PDE was first identified in *B. subtilis* and since then it has been found in a variety of bacteria including *S. aureus*, *L. monocytogenes*, and *Streptococcus* spp. (Rao et al. 2010, Huynh et al. 2015). PDEs degrade C-di-AMP into two molecules of AMP with an intermediate linear form of phosphoadenyl adenosine. Four classes of PDEs that specifically degrade c-di-AMP have been identified: GdpP, DhhP, PgpH and CdnP (cyclic

dinucleotide phosphodiesterase) (Huynh et al. 2016). The PDEs of PgpH and GdpP families cleave c-di-AMP to a linear dinucleotide pApA whereas Dhhp-type PDEs cleave pApA to the final product AMP. The cell wall-anchored PDE CdnP is responsible for degrading extracellular c-di-AMP to AMP (Commichau et al. 2018). The expression of PDEs varies in different bacterial species. For example, *L. monocytogenes* encodes GdpP and PgpH, whereas *Streptococcus* and *Staphylococcus* species express GdpP and Dhhp. The PDEs appear to be stimulated by both intracellular and extracellular stimuli but the specific mechanisms remain unknown. The GdpP-type PDE was first described in *L. lactis*, and later, gdpP orthologs were found in Firmicutes such as *E. aecalis, E. faecium*, *S. aureus, S. pneumoniae, S. mutans,* and *S. pyogenes* (Commichau et al. 2018). The GdpP-type PDEs contain two N-terminally located transmembrane helices which are accompanied by a PAS motif, a degenerated GGDEF motif, DHH and DHHA1 motifs (Huynh et al. 2016). DHH/DHHA1 domain family proteins are named due to the presence of a conserved Asp-His-His motif in their active sites which is essential for cleaving c-di-AMP to 5'-pApA (Rao et al. 2010, Corrigan et al. 2011). Dhhp also contains a DHH/DHHA1 domain which has been identified in several bacteria including *S. pneumoniae* (Cron et al. 2011)*, S. aureus* (Bowman et al. 2016)*, Mycobacterium spp*. (Tang et al. 2015), and *Borrelia burgdoferi* (Ye et al. 2014). The DhhP-type PDEs degrade c-di-AMP into AMP in a two-step process involving the formation of an intermediate 5'-pApA (Manikandan et al. 2014). PgpH-type PDEs are widespread among bacteria especially in Firmicutes. PgpH contains a histidine-aspartate (HD) domain in its C-terminus that is responsible for specific binding and degradation of c-di-AMP into 5'-pApA. In addition, PgpH possesses a 7TMR-HDEDD domain and seven transmembrane helices (Huynh et al. 2015). Cdnp is a recently discovered

extracellular cell-wall-anchored c-di-AMP hydrolase in GBS (Andrade et al. 2016), consisting of a 5'-nucleotidase domain and a metallophosphoesterase domain essential for enzyme activity.

cGAMP

3',3'-cGAMP and its synthase DncV were discovered in 2012 by Mekalano and colleagues while studying the contribution of VSP-1 (*Vibrio* seventh pandemic island-1) to the pathogenesis (Davies et al. 2012). DncV can also synthesize c-di-AMP and c-di-GMP in vitro, but cGAMP is the major product of DncV. Subsequently, three cGAMP-specific PDEs named V-cGAP1/2/3 in *V. cholerae* were reported, all of which contained the HD-GYP domains. Although all the three PDEs hydrolyze 3',3'-cGAMP to a linear 5'-pApG, only V-cGAP1 harbors 5'-nuleostidase activity which enables cleavage of 5'-pApG to 5'-ApG (Gao et al. 2015). In 2015, a study on GGDEF domain-containing enzymes isolated from *G. sulfurreducens* identified dubbed hybrid promiscuous (Hypr) GGDEF enzymes that produce either c-di-AMP or cGAMP, except c-di-GMP, depending on ATP-to-GTP ratios (Hallberg et al. 2016).

2',3'-cGAMP was first identified as a cyclic dinucleotide second messenger in mammals (Ablasser et al. 2013). One phosphodiester bond of 2',3'-cGAMP has a unique linkage between 2'-OH of GMP and 5'-phosphate of AMP, distinguishing the mammalian 2'3'-cGAMP from other bacterial cyclic dinucleotides (Zhang et al. 2013). 2'3'-GAMP is synthesized by a cytoplasmic nucleotidyl transferase cyclic GMP-AMP synthase (cGAS). The synthesis of cGAMP is a two-step reaction process with an intermediate product ppGpp(2'-5')A (Gao et al. 2013). Interestingly, the double stranded DNA in the cytoplasm can stimulate the

enzymatic activity of cGAS to produce cGAMP thereby inducing host immune reaction (Gao et al. 2013). In 2014, a glycoprotein named ENPP1 was reported on the plasma membrane and endoplasmic reticulum which can degrade 2',3'-cGAMP into AMP and GMP (Li et al. 2014).

The regulation of cyclic di-nucleotides in *Streptococci*

C-di-GMP can be sensed by RNAs, proteins, and is involved in many biological processes including switching between motile and sessile biofilm states, virulence, biofilm matrix formation and quorum sensing (Romling et al. 2005, Shanahan et al. 2012). C-di-GMP binding RNAs contain the GEMM (Genes for the Environment, Membranes and Motility) motif (Weinberg et al. 2007), and these RNAs are upstream of genes that encode diguanylate cyclases, phosphodiesterases and some other c-di-GMP-associated proteins. C-di-GMP binding proteins carry the GGDEF domains and the EAL domains containing the diguanylate cyclase and phosphodiesterase activity, respectively (Ramesh 2015). The discovery of c-di-GMP in *Streptococci* has not been reported, but the impact of extracellular c-di-GMP on *S. mutans* is not fully understood. It was shown that c-di-GMP reduced biofilm formation in *S. mutans* UA159 and inhibited the adherence of *S. mutans* to tooth surfaces suggesting that c-di-GMP may be used as a novel antimicrobial agent (Yan et al. 2010). Indeed, c-di-GMP can inhibit the occurrence and development of dental caries in rats (Yan et al.), but has no effect on the number of bacterial plagues. Moreover, C-di-GMP may indirectly affect *S. mutans* via other bacteria or mechanisms. However, further studies are needed to validate the protective effect of c-di-GMP on dental caries to boost the exploitation of its therapeutic effects.

Effectors that bind to c-di-AMP in *Streptococcus* include CabPA, CabPB, KtrA and TrkH potassium transporter subunits, OpuCA transporter subunit, as well as BusR transcriptional factor which are closely associated with bacterial biofilm formation and osmotic homeostasis (Peng et al. 2016, Devaux et al. 2018). In addition, c-di-AMP may influence *Streptococcus* cell phenotypes such as growth, fitness, and virulence. However, the signal transduction pathways downstream of c-di-AMP remain elusive. Apart from binding to protein adaptors, c-di-AMP can also bind some bacterial signal-sensing riboswitches such as *ydaO* riboswitch with high affinity and specificity. In this way, they regulate the cell wall metabolism, sporulation, and germination in eubacteria (Nelson et al. 2013).

The role of c-di-AMP in *S. mutans* has been extensively studied and the CabPA/VicR/GtfB signaling pathway regulated by c-di-AMP during *S. mutans* biofilm formation has been recently reported. C-di-AMP interacts with VicR through its binding protein (CabPA), and up-regulates *gtfB* expression. The *gtfB* gene encodes a glucosyltransferase which regulates the synthesis of water-insoluble glucans. Therefore, the effect of C-di-AMP on *gtfB* expression augmented EPS (extracellular polysaccharides) production and biofilm formation in *S. mutans*. Deletion of *pdeA* gene, which encodes PDE, increased c-di-AMP levels in *S. mutans* and upregulated the expression of *gtfB*. Moreover, CabPA, a c-di-AMP binding protein containing TrkA_C and TrkA_N domains with a molecular mass of ~25 kDa, can specifically interact with VicR, a response regulator of a two-component regulatory system in *S. mutans*. This provides the basic stimulus-response coupling mechanism allowing organisms to perceive and respond to changes in environmental conditions. It was demonstrated that increased c-di-AMP level in *S. mutans* due to *pdeA* deficiency promoted bacterial

colonization in Drosophila (Peng, Zhang et al. 2016). Besides, Cheng et al. (Cheng et al. 2016) showed that in-frame deletion of *cdaA* gene in *S. mutans* decreased c-di-AMP level and increased not only the production of EPS but also the sensitivity to hydrogen peroxide. The global analyses of gene expression revealed that genes with significantly altered expression were clustered in bacterial polysaccharide biosynthesis and oxidoreductase process. The deletion of *cdaA* which encodes CdaA in *S. mutans* resulted in a striking decrease in c-di-AMP levels and bacterial growth rate but increase in cell lysis. The *cdaA* deficiency altered the cell shape and size in *S. mutans* compared to the wild type bacteria. Furthermore, the Δ*cdaA* mutant displayed decreased competitiveness against *Streptococcus sanguinis* which increased the sensitivity of *S. mutans* Δ*cdaA* mutant to H_2O_2 produced by *S. sanguinis* and the production of EPS. A markedly enhanced glucosyltransferases (GTFs) activity was also observed in Δ*cdaA* mutant. Based on whole-genome microarrays, transcriptional changes in a set of functional genes were identified in the Δ*cdaA* mutant. The up-regulated genes included genes associated with cell envelope biogenesis, ribosomal structure and biogenesis, coenzyme metabolism, protein turnover, chaperones, post-translational modification, and translation. The down-regulated genes comprised of genes involved in carbohydrate transport and metabolism, cell secretion, and DNA replication, recombination and repair (Cheng et al. 2016). However, Peng et al. (Peng et al. 2016) reported opposite findings that DAC deficiency in *S. mutans* decreased the production of EPS and that the response of DAC mutant to oxidative stress is independent of gtfB. They observed that DAC deficiency significantly reduced the expression of gtfB, resulting in decreased biofilm formation in the mutant. This inhibited bacterial colonization by the mutant and reduced the cariogenicity of SmDAC deficiency on buccal and sulcal surfaces

of teeth. The authors also showed that the attenuated response to hydrogen peroxide stress in SmDAC deficiency contributes to low virulence. Moreover, Peng et al. provided corroborating evidence that *smDAC* mutant exhibits the typical pattern of Gtfs, but complementation of the mutant in a study by Cheng et al. failed to restore its EPS production ability compared to wild type. In contrast to the conclusion reached by Cheng et al. that EPS is required for bacterial resistance to hydrogen peroxide challenge, Peng et al. found that *SmDAC* mutant decreased GtfB and EPS levels and that deletion of *gtfB* did not affect the EPS levels of the *smDAC gtfB* double mutant (Cheng et al. 2016). These contradictory findings point to the complexity of the c-di- AMP signaling network in *S. mutans* and warrant further investigation.

In *S. pneumoniae*, deletion of *pde1* and *pde2* genes encoding PDE markedly increased c-di-AMP levels. Homeostasis of c-di-AMP is essential for pneumococcal growth and its associated pathogenesis which includes adherence to human epithelial cells, colonization and infection. Various mice models have shown that Pde1 and Pde2 are essential for bacterial biology and bacterial-induced diseases (Bai et al. 2013). In a subsequent study, CabP which directly interacts with a potassium transporter (SPD_0076) was identified in *S. pneumoniae*. C-di-AMP regulates potassium uptake by *S. pneumoniae* via its specific binding protein CabP which interacts with a potassium transporter. On the other hand, c-di-AMP reduces the interaction between CabP and SPD_0076 and impairs the ability of potassium uptake by CabP-SPD_0076 complex in *S. pneumonia* (Bai et al. 2014). It was found that high c-di-AMP levels enhanced the susceptibility of *S. pneumoniae* phosphodiesterase mutants to stress conditions such as heat shock, acidic environment, and osmotic stress. Thus, it is likely that

accumulation of c-di-AMP is toxic under specific incubation conditions and inhibits adaptation to the stresses in *S. pneumoniae*. Given that the deletion of c-di-AMP effector protein CabP reduced c-di-AMP levels in *S. pneumoniae*, it can be inferred that c-di-AMP and its binding protein mutually affect each other through a feedback loop and this relationship may is dependent on the pneumococcal potassium uptake process. In *S. pneumonia,* it was also demonstrated that mutations in *cdaA* reduced its enzymatic activity and the levels of c-di-AMP, leading to increased resistance to environmental stress in the Δ*pde1* Δ*pde2* mutant. Although CdaA plays a major role in restoring stress resistance, the effect of TrkH in reducing c-di-AMP levels in isolated suppressor mutant is minimal (Zarrella et al. 2018). The c-di-AMP/CabP/SPD_0076 pathway in *S. pneumoniae* regulates potassium uptake, but the mechanism of c-di-AMP in pneumococcal stress resistance is unknown.

In *S. pyogene* (Cho et al. 2013), c-di-AMP phosphodiesterase GdpP influences the maturation of SpeB, a major cysteine protease which can destroy human matrices such as fibronectin and vitronectin thus damaging the host tissues. The absence of GdpP decreases the ability to produce active SpeB. GdpP also influences the virulence of *S. pyogene* by decreasing the sensitivity to β-lactam antibiotic ampicillin in the GdpP null mutant. Re-introduction of GdpP enhanced SpeB processing in the ΔGdpP mutant, although the underlying mechanisms of this effect remain elusive. Moreover, the virulence of ΔGdpP declined in a murine model of soft tissue infection. All these results suggest that increased c-di-AMP levels resulting from GdpP deletion promote SpeB processing, pathogenicity of *S. pyogene* and its response to ampicillin. In S. pyogenes, SpyDAC was found to catalyze the in vivo biosynthesis of c-di-AMP from ATP.

C-di-AMP synthesis is essential for GBS in standard growth conditions but becomes dispensable afterwards due to the accumulation of other compensatory mutations, especially mutations in the genes encoding BusAB transporter responsible for osmolyte import in GBS. Previous experiments demonstrated that c-di-AMP binding transcriptional factor BusR is essential for osmotic homeostasis and that BusR negatively regulates *busAB* transcription by directly binding to the *busAB* promoter. Deletion of *gdpP* which encodes c-di-AMP phosphodiesterase increased c-di-AMP levels in GBS and led to hyperosmotic susceptibility by influencing the BusR transcriptional factor. C-di-AMP is not required for growth in a media without osmolyte which is attributed to the combination of dysregulated homeostasis of potassium and osmolyte uptake. However, high c-di-AMP levels avoids inhibitory effect in the presence of the osmolytes. Adaptation to the absence of c-di-AMP synthesis in Δ*dacA* mutants involves intertwined mutations including necessary mutations in the two *oppC* and *busB* genes as well as other compensatory mutations which alleviates BusB toxicity. In conclusion, c-di-AMP is a central regulator of osmotic homeostasis in GBS and acts by binding to KtrA and TrkH potassium transporter subunits, OpuCA osmolyte transporter subunit, as well as BusR transcriptional factor which represses the expression of BusAB osmolyte transporter. Furthermore, binding of full-length protein Eric containing chloride channel protein to c-di-AMP was not found in this study (Devaux et al. 2018). This intertwined network involves more than four kinds of c-di-AMP binding proteins responsible for potassium uptake or osmolyte import, and both of these processes are essential for osmotic homeostasis.

SSU98_1483 (ssDacA) is a diadenylate cyclase found in *S. suis serotype 2* (SS2)

(Du et al. 2015). GdpP is a c-di-AMP specific phosphodiesterase that degrades c-di-AMP and the *gdpP* mutant markedly increased the c-di-AMP levels in SS2. This suggests that c-di-AMP is a biological substrate of GdpP and that GdpP contributes to the homeostasis of c-di-AMP in SS2. Studies showed that the *gdpP* mutant strain exhibited lower growth rate than the wild type and complementation of *gdpP* mutant partly restored the growth rate. The deletion of *gdpP* also increased biofilm formation in SS2 and the mutant exhibited a significant reduction in hemolytic activity, invasion and adherence to HEp-2 cells compared to the parental strain which indicates that GdpP contributes to the virulence of SS2. GdpP or c-di-AMP influences the expression of virulence-related genes *cps2*, *sly, fpbs*, *mrp*, *ef* and *gdh.* These genes are significantly decreased in *gdpP* mutant based on qRT-PCR analysis. In murine infection models, the pathogenicity of *gdpP* mutant strain was attenuated compared with the wild-type strain, indicating that inactivation of GdpP weakens the virulence of SS2. Also, in vivo colonization experiments demonstrated that more wild-type strains were recovered from brain, spleen, lung and liver than the *gdpP* mutant strains after intraperitoneal inoculation with *gdp*P mutant or the wild type strain. This result reveals the significance of c-di-AMP homeostasis during SS2 infection. Deletion of *gdp*P weakened the growth and virulence but improved the biofilm formation ability of SS2 (Du et al. 2014).

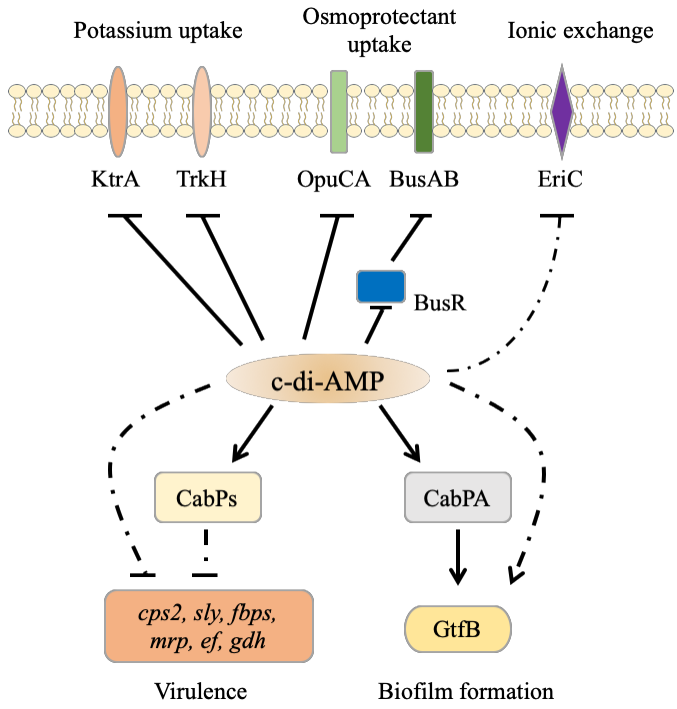

Figure 1. c-di-AMP signaling in *streptococcus*. The KtrA and TrkH potassium transporter subunits and OpuCA osmolyte transporter subunits are conserved c-di-AMP binding proteins. C-di-AMP can bind to BusR transcriptional factor, which is a repressor of osmolyte transporter BusAB. C-di-AMP might also regulate EriC, a chloride channel protein with 11 transmembrane domains. High level of c-di-AMP binds to CabPA, which regulates the expression of *gtfB* intermediated by histidine two-component system response regulator VicR. c-di-AMP also affect streptococcal virulence by influencing the putative expression of virulence-related genes *cps2*, *sly, fpbs*, *mrp*, *ef* and *gdh*.

A recent study showed that (Severin et al. 2018) cGAMP can activate a phospholipase Vibrio (CapV) which degrades phospholipids in El Tor biotype of *Vibrio cholerae* cell membrane. However, the presence of this phospholipase in *Streptococcus* has not been reported given that it has recently been identified as

a second messenger in bacteria.

The interaction between host cells and *Streptococcus* are regulated by CDNs

With the development of new techniques, novel host cell receptors sensing c-di-AMP or c-di-GMP have been reported. C-di-AMP and c-di-GMP trigger type I interferons via STING including MITA and MPYS which further activate TBK1 kinase and IRF3 transcription factor (Burdette et al. 2011, Chen et al. 2016, Ruiz-Moreno et al. 2018). Besides, helicase DEAD box polypeptide 41 (DDX41) was also found to be one of a pattern recognition receptor (PRR) which senses c-di-AMP or c-di-GMP, and interacts and co-localizes with the STING adaptor (Parvatiyar et al. 2012).The oxidoreductase RECON is another CDN host receptor which antagonizes STING activation and downregulates NF-kB activation. And CDN inhibition or gene knockout of RECON leads to increasing NF-kB activation and a decline in bacterial survival (McFarland et al. 2017). Recently, ERAdP that acts as a direct sensor of c-di-AMP in *L. monocytogenes* infection has been reported. C-di-AMP binding to a C-terminal domain of ERAdP results in dimerization of ERAdP leading to the activation of the kinase TAK1 which activates NF-κB to induce the production of cytokines in host innate immune cells (Moretti et al. 2017).

c-di-GMP is a unique bacterial intracellular second messenger which promotes protective innate and adaptive immunity against various invasive bacterial infections including, but not limited to *S. pneumoniae and S. pyogenes* (Ogunniyi et al. 2008, Yan et al. 2009). Intranasal pretreatment of mice with c-di-GMP led to a significant decrease in pneumococcal bacterial load in lungs and blood after

serotypes 2 and 3 challenge, and also reduced lung titers after serotype 4 challenge. Coincidentally, intraperitoneal co-administration of c-di-GMP significantly increased antigen-specific antibody titers as well as the survival of mice. It was also observed that c-di-GMP has no significant direct short-term influence on *S. pneumoniae* growth either *in vitro* or *in vivo* (Ogunniyi et al. 2008). Intranasal c-di-GMP administration induces pulmonary dendritic cell recruitment and proinflammatory cytokine/chemokine responses in mice. Similarly, co-administration of c-di-GMP with pneumococcal surface adhesion A (PsaA) led to a strong antigen-specific serum immunoglobulin G (IgG) and secretory IgA antibody response at multiple mucosal surfaces. Importantly, the immunized mice showed a significantly reduced nasopharyngeal *S. pneumoniae* colonization (Yan et al. 2009). Moreover, c-di-GMP not only induced a higher Ab production including IgG1 and IgA but also conferred a better protection against *S. pneumoniae* infection than the mammalian 2'3'-cGAMP. However, c-di-GMP did not cause strong inflammatory responses or lung injury but enhanced type II (IFNγ) and III IFN (IFN λ) production and generated of T_H1, T_H2, and T_H17 responses through the production of polarizing cytokines including T_H1 polarizing cytokines IL-12p70 and IFNγ, T_H2 polarizing cytokines IL-5 and IL-13, and T_H17 polarizing cytokines IL-23, IL-6 and TGF-β1 by activating pinocytosis-efficient-DCs. C-di-GMP also enhanced Ag uptake dependent on MPYS (STING, MITA) expression in $CD11C^+$ cells (Blaauboer et al. 2015).

In addition, c-di-AMP influences the interaction between host cells and *Streptococcus* by signaling through factors that regulate this process. In GBS, IFN-β production is mainly regulated through cGAS-STING-dependent recognition of bacterial DNA. GBS expresses an ectonucleotidase CdnP which

hydrolyzes extracellular bacterial c-di-AMP and reduces its levels. Inactivation of CdnP leads to c-di-AMP accumulation outside the bacteria and an increase in IFN-β levels *in vivo* which promotes GBS killing by the host and reduces GBS virulence. This process is mainly due to enhanced DNA sensing by cGAS and STING-dependent sensing of c-di-AMP. Therefore, GBS expresses CdnP at its surface to promote its virulence (Andrade et al. 2016). Furthermore, a degraded RNA sensor in monocytes and macrophages of human Toll-Like Receptor 8 (TLR8) and IFN regulatory factor 5 (IRF5) form a signaling pathway that regulates GBS sensing and cytokine activation in host cells (Ehrnstrom et al. 2017).

Studies have shown that cGAMP is an important node in host innate immune responses to bacterial DNAs and is a strong activator of STING which induces interferon-β production through activation of IRF3 in mammalian cells transfected with either the DNA or infected with DNA virus (Wu et al. 2013). It was reported that cGAMP activates B cells through STING pathway when cytoplasmic DNA is sensed *in vivo.* Human B cells can be activated by cGAMP *in vitro* thus increasing IgM production (Chen et al. 2016). cGAS senses and binds to cytosolic DNA and activates the synthesis of cGAMP and subsequent production of type I interferon (Cai et al. 2014). During the defense against *Neisseria gonorrhoeae* (GC), both cGAMP and TLR4, known as a host pattern recognition receptor, contribute to IFN-β activation in the immune cells. TLRs and cGAS functions synergistically when host innate immunity is confronted with GC invasion (Andrade et al. 2016).

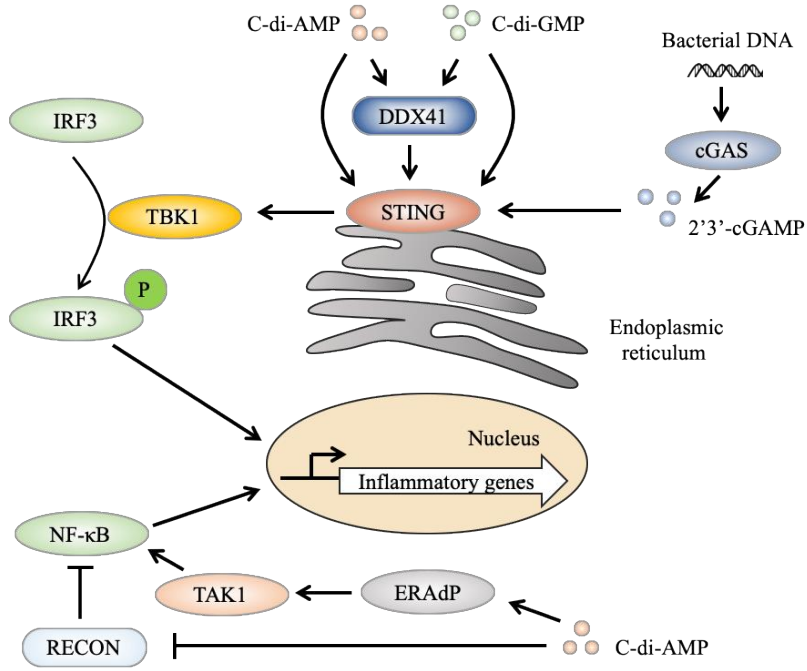

Fig 2. Induction of host immune responses by CDNs. Bacterial c-di-AMP and c-di-GMP bind to eukaryotic helicase DDX41, which in turn interacts and activates the co-receptor STING. Some evidence suggest that these two dinucleotides interact directly with STING. Activated STING can then activate the kinase TBK1, leading to phosphorylation of transcription factor IRF3 triggering its translocating to the nucleus, further promoting the expression of IFNβ and activation of type Ⅰ interferon response. In addition, bacterial double-strand DNA may be sensed by eukaryotic cGAMP synthase cGAS. High levels of 2'3'-cGAMP interacts and activates STING, leading to activation of type Ⅰ interferon response through TBK1/IRF3 pathway. The oxidoreductase RECON is another cyclic dinucleotide host receptor which prevents the activation of transcription factor NF-κB. Recently, it was found that the ER membrane adaptor ERAdP acts as a direct sensor for c-di-AMP, which causes dimerization of ERAdP. This in turn interacts and activates the kinase TAK1.Activated TAK1 initiates activation and translocation of NF-κB, leading to the production of cytokines.

Conclusions and prospect

In this review, the regulatory networks of c-di-GMP, c-di-AMP, and cGAMP cyclic nucleotide second messengers are summarized. Cyclic nucleotide second messengers play important roles in both prokaryotes and eukaryotes. They mediate bacterial growth, fitness, virulence, and regulate innate immune responses in host cells via intricate signaling networks. Cyclic nucleotide signaling molecules are rapidly internalized by cellular diffusion and act in highly specific manner forming a multilayered signaling scaffold (Jenal et al. 2017). However, our understanding on the role of cyclic dinucleotide signaling is far from being complete. In *Streptococcus*, a diverse group of more than 100 recognized species which cause invasive diseases (Andam et al. 2015), it is important to explore the specific mechanisms of signal transduction based on nucleotide signaling molecules to develop effective strategies for controlling infections caused by these pathogens. It is possible that there are other unidentified cyclic dinucleotides which may be discovered in future research. In addition, future studies on cyclic dinucleotide should focus on identifying more cyclic di-nucleotides receptor and effector proteins to fill the gaps in our understanding of the cyclic di-nucleotide messengers-based network. *yadO* riboswitch is the only identified c-di-AMP binding RNA and majority of bacteria that produce c-di-AMP do not contain this riboswitch (Fahmi et al. 2017). Thus, identification of potential c-di-AMP binding RNA should also be a focus of further research. Moreover, the specific environmental input signals that influence cyclic di-nucleotide second messenger pathways remain to be identified.

It has been proposed that c-di-GMP can be administered as an effective broad spectrum immunomodulator and vaccine adjuvant to prevent infectious diseases.

This also implies that c-di-GMP has the potential to be a mucosal vaccine adjuvant. Moreover, a combination of LCP based peptide vaccine with c-di-AMP targeted against *S. pyogenes* infection reduced antigen dose and maintained vaccine efficacy providing a strong evidence that c-di-GMP can be used as a vaccine adjuvant (Schulze et al. 2017). In a study on mice model, it was found that c-di-GMP exhibited better mucosal adjuvant response than cGAMP.

Compared to c-di-GMP, the c-di-AMP signaling network appears to be far simpler since most organisms contain only a single DAC enzyme. However, in view of the various phenotypes associated with the levels of c-di-AMP, it is possible that there are many c-di-AMP effectors. This might be the case for other second messenger systems which function through many effectors and regulate many physiological processes. cGAMP has been identified in bacteria and mammals, however, the presence of cGAMP in bacteria is limited to an only a few species. Given that the enzymes responsible for cGAMP synthesis also synthesize c-di-GMP or c-di-AMP, we postulate that cGAMP signaling network may be interrelated with other cyclic di-nucleotide signal networks. Moreover, there are no reports on cGAMP producing *Streptococcus* or cGAMP signal pathway related physiological processes in *Streptococcus* and warrant further investigation.

Given the indispensable roles of cyclic dinucleotide second messengers in bacteria and in host cells, there have been efforts to interfere with cyclic dinucleotide signaling (Opoku-Temeng et al. 2016). However, only a handful of inhibitors of cyclic dinucleotide signaling have been identified. We believe that with the development of high-throughput screening methods, other small molecule regulators of cyclic signal pathways will be identified. Considering the

importance of cyclic dinucleotide in bacteria cellular process and host immune reactions, the development of novel cyclic nucleotide inhibitors may have a tremendous impact on clinical practice.

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References

Ablasser, A., et al. (2013). cGAS produces a 2 ‘-5 ‘-linked cyclic dinucleotide second messenger that activates STING. Nature 498(7454): 380-384. doi: 10.1038/nature12306

Andam, C. P. and, W. P. (2015). Mechanisms of genome evolution of Streptococcus. Infect Genet Evol 33: 334-342. doi: 10.1016/j.meegid.2014.11.007

Andrade, W. A., et al. (2016). Type I Interferon Induction by Neisseria gonorrhoeae: Dual Requirement of Cyclic GMP-AMP Synthase and Toll-like Receptor 4. Cell Rep 15(11): 2438-2448. doi: 10.1016/j.celrep.2016.05.030

Andrade, Warrison A., et al. (2016). Group B Streptococcus Degrades Cyclic-di-AMP to Modulate STING-Dependent Type I Interferon Production. Cell Host Microbe 20(1): 49-59. doi: 10.1016/j.chom.2016.06.003

Bai, Y., et al. (2013). Two DHH subfamily 1 proteins in Streptococcus

pneumoniae possess cyclic di-AMP phosphodiesterase activity and affect bacterial growth and virulence. J Bacteriol 195(22): 5123-5132. doi: 10.1128/JB.00769-13

Bai, Y., et al. (2014). Cyclic Di-AMP Impairs Potassium Uptake Mediated by a Cyclic Di-AMP Binding Protein in Streptococcus pneumoniae. J Bacteriol 196(3): 614. doi: 10.1128/JB.01041-13

Barker, J. R., et al. (2013). STING-dependent recognition of cyclic di-AMP mediates type I interferon responses during Chlamydia trachomatis infection. MBio 4(3): e00018-00013. doi: 10.1128/mBio.00018-13

Blaauboer, S. M., et al. (2015). The mucosal adjuvant cyclic di-GMP enhances antigen uptake and selectively activates pinocytosis-efficient cells in vivo. Elife 4: e06670. doi: 10.7554/eLife.06670

Blötz, C., et al. (2017). Identification of the components involved in cyclic di-AMP signaling in Mycoplasma pneumoniae. Front Microbiol 8: 1328. doi: 10.3389/fmicb.2017.01328

Bowman, L., et al. (2016). New insights into the cyclic di-adenosine monophosphate (c-di-AMP) degradation pathway and the requirement of the cyclic-dinucleotide for acid stress resistance in Staphylococcus aureus. J Biol Chem 291(53): 26970-26986. doi: 10.1074/jbc.M116.747709

Burdette, D. L., et al. (2011). STING is a direct innate immune sensor of cyclic di-GMP. Nature 478(7370): 515-518. doi: 10.1038/nature10429

Cai, X., et al. (2014). The cGAS-cGAMP-STING pathway of cytosolic DNA sensing and signaling. Mol Cell 54(2): 289-296. doi: 10.1016/j.molcel.2014.03.040

Chan, C., et al. (2004). Structural basis of activity and allosteric control of diguanylate cyclase. Proc Natl Acad Sci U S A 101(49): 17084. doi:

10.1073/pnas.0406134101

Chen, Q., et al. (2016). Regulation and function of the cGAS-STING pathway of cytosolic DNA sensing. Nat Immunol 17(10): 1142-1149. doi: 10.1038/ni.3558

Cheng, X., et al. (2016). Regulation of oxidative response and extracellular polysaccharide synthesis by a diadenylate cyclase in Streptococcus mutans. Environ Microbiol 18(3): 904-922. doi: 10.1111/1462-2920.13123

Cho, K. H. and S. O. Kang (2013). Streptococcus pyogenes c-di-AMP Phosphodiesterase, GdpP, Influences SpeB Processing and Virulence. PLoS One 8(7): e69425. doi: 10.1371/journal.pone.0069425

Commichau, F. M., et al. (2015). A jack of all trades: the multiple roles of the unique essential second messenger cyclic di-AMP. Mol Microbiol 97(2): 189-204. doi: 10.1111/mmi.13026

Commichau, F. M., et al. (2018). A Delicate Connection: c-di-AMP Affects Cell Integrity by Controlling Osmolyte Transport. Trends Microbiol 26(3): 175-185. doi: 10.1016/j.tim.2017.09.003

Commichau, F. M., et al. (2018). Making and breaking of an essential poison: the cyclases and phosphodiesterases that produce and degrade the essential second messenger cyclic di-AMP in bacteria. J Bacteriol 201(1). pii: e00462-18. doi: 10.1128/JB.00462-18

Corrigan, R. M., et al. (2011). c-di-AMP is a new second messenger in Staphylococcus aureus with a role in controlling cell size and envelope stress. PLoS Pathog 7(9): e1002217. doi: 10.1371/journal.ppat.1002217

Corrigan, R. M. and A. Grundling (2013). Cyclic di-AMP: another second messenger enters the fray. Nat Rev Microbiol 11(8): 513-524. doi: 10.1038/nrmicro3069

Cron, L., et al. (2011). Two DHH subfamily 1 proteins contribute to

pneumococcal virulence and confer protection against pneumococcal disease. Infect Immun 79(9):3697-710. doi: 10.1128/IAI.01383-10

Danilchanka, O. and J. J. Mekalanos (2013). Cyclic dinucleotides and the innate immune response. Cell 154(5): 962-970. doi: 10.1016/j.cell.2013.08.014

Davies, B. W., et al. (2012). Coordinated regulation of accessory genetic elements produces cyclic di-nucleotides for V. cholerae virulence. Cell 149(2): 358-370. doi: 10.1016/j.cell.2012.01.053

Davies, Bryan W., et al. (2012). Coordinated Regulation of Accessory Genetic Elements Produces Cyclic Di-Nucleotides for V. cholerae Virulence. Cell 149(2): 358-370. doi: 10.1016/j.cell.2012.01.053

De, N., et al. (2009). Determinants for the activation and autoinhibition of the diguanylate cyclase response regulator WspR. J Mol Biol 393(3): 619-633. doi: 10.1016/j.jmb.2009.08.030

Devaux, L., et al. (2018). Cyclic di-AMP regulation of osmotic homeostasis is essential in Group B Streptococcus. PLoS Genet 14(4): e1007342. doi: 10.1371/journal.pgen.1007342

Drexler, D. J., et al. (2017). Structural and Biophysical Analysis of the Soluble DHH/DHHA1-Type Phosphodiesterase TM1595 from Thermotoga maritima. Structure 25(12): 1887-1897 e1884. doi: 10.1016/j.str.2017.10.001

Du, B., et al. (2014). Functional analysis of c-di-AMP phosphodiesterase, GdpP, in Streptococcus suis serotype 2. Microbiol Res 169(9-10): 749-758. doi: 10.1016/j.micres.2014.01.002

Du, B. and J. H. Sun (2015). Diadenylate cyclase evaluation of ssDacA (SSU98_1483) in Streptococcus suis serotype 2. Genet Mol Res 14(2): 6917-6924. doi: 10.4238/2015.June.18.34

Ehrnstrom, B., et al. (2017). Toll-Like Receptor 8 Is a Major Sensor of Group B

Streptococcus But Not Escherichia coli in Human Primary Monocytes and Macrophages. Front Immunol 8: 1243. doi: 10.3389/fimmu.2017.01243

Fahmi, T., et al. (2017). c-di-AMP: An Essential Molecule in the Signaling Pathways that Regulate the Viability and Virulence of Gram-Positive Bacteria. Genes (Basel) 8(8). doi: 10.3390/genes8080197

Ferreira, R. B., et al. (2008). Vibrio parahaemolyticus ScrC modulates cyclic dimeric GMP regulation of gene expression relevant to growth on surfaces. J Bacteriol 190(3): 851-860. doi: 10.1128/JB.01462-07

Galperin, M. Y. (2018). What bacteria want. Environ Microbiol. doi: 10.1111/1462-2920.14398

Gándara, C. and J. C. Alonso (2015). DisA and c-di-AMP act at the intersection between DNA-damage response and stress homeostasis in exponentially growing Bacillus subtilis cells. DNA Repair 27: 1-8. doi: 10.1016/j.dnarep.2014.12.007

Gao, J., et al. (2015). Identification and characterization of phosphodiesterases that specifically degrade 3′3′-cyclic GMP-AMP. Cell Research 25: 539. doi: 10.1038/cr.2015.40

Gao, P., et al. (2013). Cyclic [G(2′,5′)pA(3′,5′)p] Is the Metazoan Second Messenger Produced by DNA-Activated Cyclic GMP-AMP Synthase. Cell 153(5): 1094-1107. doi: 10.1016/j.cell.2013.04.046

Gomelsky, M. (2011). cAMP, c-di-GMP, c-di-AMP and now cGMP: bacteria use them all! Mol Microbiol 79(3): 562-565. doi: 10.1111/j.1365-2958.2010.07514.x

Gundlach, J., et al. (2015). An essential poison: synthesis and degradation of cyclic di-AMP in Bacillus subtilis. J Bacteriol 197(20):3265-74. doi: 10.1128/JB.00564-15

Hall, C. L. and V. T. Lee (2018). Cyclic-di-GMP regulation of virulence in

bacterial pathogens. Wiley Interdiscip Rev RNA 9(1). doi: 10.1002/wrna.1454

Hallberg, Z. F., et al. (2016). Hybrid promiscuous (Hypr) GGDEF enzymes produce cyclic AMP-GMP (3', 3'-cGAMP). Proc Natl Acad Sci U S A 113(7): 1790-1795. doi: 10.1073/pnas.1515287113

Hecht, G. B. and A. Newton (1995). Identification of a novel response regulator required for the swarmer-to-stalked-cell transition in Caulobacter crescentus. J Bacteriol 177(21): 6223-6229. doi: 10.1128/jb.177.21.6223-6229.1995

Hengge, R., et al. (2016). Bacterial Signal Transduction by Cyclic Di-GMP and Other Nucleotide Second Messengers. J Bacteriol 198(1): 15-26. doi: 10.1128/jb.00331-15

Huynh, T. N., et al. (2015). An HD-domain phosphodiesterase mediates cooperative hydrolysis of c-di-AMP to affect bacterial growth and virulence. Proc Natl Acad Sci U S A 112(7): E747-E756. doi: 10.1073/pnas.1416485112

Huynh, T. N. and J. J. Woodward (2016). Too much of a good thing: regulated depletion of c-di-AMP in the bacterial cytoplasm. Curr Opin Microbiol 30: 22-29. doi: 10.1016/j.mib.2015.12.007

Jenal, U., et al. (2017). Cyclic di-GMP: second messenger extraordinaire. Nat Rev Microbiol 15(5): 271-284. doi: 10.1038/nrmicro.2016.190

Kalia, D., et al. (2013). Nucleotide, c-di-GMP, c-di-AMP, cGMP, cAMP, (p)ppGpp signaling in bacteria and implications in pathogenesis. Chem Soc Rev 42(1): 305-341. doi: 10.1039/c2cs35206k

Kamegaya, T., et al. (2011). Identification of a Streptococcus pyogenes SF370 gene involved in production of c-di-AMP. Nagoya J Med Sci 73(1-2): 49-57. doi: 10.18999/nagjms.73.1-2.49

Kaspar, J., et al. (2016). An Essential Role for (p)ppGpp in the Integration of Stress Tolerance, Peptide Signaling, and Competence Development in

Streptococcus mutans. Front Microbiol 7:1162. doi: 10.3389/fmicb.2016.01162

Kazlauskiene, M., et al. (2017). A cyclic oligonucleotide signaling pathway in type III CRISPR-Cas systems. Science 357(6351): 605-609. doi: 10.1126/science.aao0100

Kellenberger, C. A., et al. (2015). RNA-based fluorescent biosensors for live cell imaging of second messenger cyclic di-AMP. J Am Chem Soc 137(20): 6432-6435. doi: 10.1021/jacs.5b00275

Krasteva, P. V. and H. Sondermann (2017). Versatile modes of cellular regulation via cyclic dinucleotides. Nat Chem Biol 13(4): 350-359. doi: 10.1038/nchembio.2337

Li, L., et al. (2014). Hydrolysis of 2′3′-cGAMP by ENPP1 and design of nonhydrolyzable analogs. Nat Chem Biol 10: 1043. doi: 10.1038/nchembio.1661

Liu, K., et al. (2015). Diversity in (p)ppGpp metabolism and effectors. Curr Opin Microbiol 24: 72-79. doi: 10.1016/j.mib.2015.01.012

Manikandan, K., et al. (2014). Two-step synthesis and hydrolysis of cyclic di-AMP in Mycobacterium tuberculosis. PLoS One 9(1): e86096. doi: 10.1371/journal.pone.0086096

Marden, J. N., et al. (2011). Cyclic GMP controls Rhodospirillum centenum cyst development. Mol Microbiol 79(3): 600-615. doi: 10.1111/j.1365-2958.2010.07513.x

McFarland, A. P., et al. (2017). Sensing of Bacterial Cyclic Dinucleotides by the Oxidoreductase RECON Promotes NF-kappaB Activation and Shapes a Proinflammatory Antibacterial State. Immunity 46(3): 433-445. doi: 10.1016/j.immuni.2017.02.014

Moretti, J., et al. (2017). STING Senses Microbial Viability to Orchestrate Stress-Mediated Autophagy of the Endoplasmic Reticulum. Cell 171(4): 809-823

e813. doi: 10.1016/j.cell.2017.09.034

Nelson, J. W., et al. (2013). Riboswitches in eubacteria sense the second messenger c-di-AMP. Nat Chem Biol 9(12): 834-839. doi: 10.1038/nchembio.1363

Nickels, B. E. and S. L. Dove (2011). NanoRNAs: a class of small RNAs that can prime transcription initiation in bacteria. J Mol Biol 412(5): 772-781. doi: 10.1016/j.jmb.2011.06.015

Ogunniyi, A. D., et al. (2008). c-di-GMP is an effective immunomodulator and vaccine adjuvant against pneumococcal infection. Vaccine 26(36): 4676-4685. doi: 10.1016/j.vaccine.2008.06.099

Opoku-Temeng, C., et al. (2016). Cyclic dinucleotide (c-di-GMP, c-di-AMP, and cGAMP) signalings have come of age to be inhibited by small molecules. Chem Commun (Camb) 52(60): 9327-9342. doi: 10.1039/c6cc03439j

Orr, M. W., et al. (2015). Oligoribonuclease is the primary degradative enzyme for pGpG in Pseudomonas aeruginosa that is required for cyclic-di-GMP turnover. Proc Natl Acad Sci U S A 112(36):E5048-57. doi: 10.1073/pnas.1507245112

Parvatiyar, K., et al. (2012). The helicase DDX41 recognizes the bacterial secondary messengers cyclic di-GMP and cyclic di-AMP to activate a type I interferon immune response. Nat Immunol 13(12): 1155-1161. doi: 10.1038/ni.2460

Pei, J. and N. V. Grishin (2001). GGDEF domain is homologous to adenylyl cyclase. Proteins. 2001 Feb 1;42(2):210-6. doi: 10.1002/1097-0134(20010201)42:2<210::AID-PROT80>3.0.CO;2-8

Peng, X., et al. (2016). Effects of diadenylate cyclase deficiency on synthesis of extracellular polysaccharide matrix of Streptococcus mutans revisit. Environ

Microbiol 18(11): 3612-3619. doi: 10.1111/1462-2920.13440

Peng, X., et al. (2016). Cyclic di-AMP mediates biofilm formation. Mol Microbiol 99(5): 945-959. doi: 10.1111/mmi.13277

Ramesh, A. (2015). Second messenger - Sensing riboswitches in bacteria. Semin Cell Dev Biol 47-48: 3-8. doi: 10.1016/j.semcdb.2015.10.019

Rao, F., et al. (2009). The functional role of a conserved loop in EAL domain-based cyclic di-GMP-specific phosphodiesterase. J Bacteriol 191(15): 4722-4731. doi: 10.1128/JB.00327-09

Rao, F., et al. (2010). YybT is a signaling protein that contains a cyclic dinucleotide phosphodiesterase domain and a GGDEF domain with ATPase activity. J Biol Chem 1;285(1):473-82. doi: 10.1074/jbc.M109.040238

Rao, F., et al. (2008). Catalytic mechanism of cyclic di-GMP-specific phosphodiesterase: a study of the EAL domain-containing RocR from Pseudomonas aeruginosa. J Bacteriol 190(10): 3622-3631. doi: 10.1128/JB.00165-08

Romling, U., et al. (2005). C-di-GMP: the dawning of a novel bacterial signalling system. Mol Microbiol 57(3): 629-639. doi: 10.1111/j.1365-2958.2005.04697.x

Rosenberg, J., et al. (2015). Structural and biochemical analysis of the essential diadenylate cyclase CdaA from Listeria monocytogenes. J Bacteriol 190(10):3622-31. doi: 10.1128/JB.00165-08

Ross, P., et al. (1987). Regulation of cellulose synthesis in Acetobacter xylinum by cyclic diguanylic acid. Nature 325: 279. doi: 10.1038/325279a0

Ruiz-Moreno, J. S., et al. (2018). The cGAS/STING Pathway Detects Streptococcus pneumoniae but Appears Dispensable for Antipneumococcal Defense in Mice and Humans. Infect Immun 86(3). doi: 10.1128/IAI.00849-17

Salter, E. A. and A. Wierzbicki (2007). The mechanism of cyclic nucleotide

hydrolysis in the phosphodiesterase catalytic site. J Phys Chem B 111(17): 4547-4552. doi: 10.1021/jp066582+

Schulze, K., et al. (2017). Bivalent mucosal peptide vaccines administered using the LCP carrier system stimulate protective immune responses against Streptococcus pyogenes infection. Nanomedicine 13(8): 2463-2474. doi: 10.1016/j.nano.2017.08.015

Seshasayee, A. S., et al. (2010). Comparative genomics of cyclic-di-GMP signalling in bacteria: post-translational regulation and catalytic activity. Nucleic Acids Res 38(18):5970-81. doi: 10.1093/nar/gkq382

Severin, G. B., et al. (2018). Direct activation of a phospholipase by cyclic GMP-AMP in El Tor Vibrio cholerae. Proc Natl Acad Sci U S A 115(26): E6048-E6055. doi: 10.1073/pnas.1801233115

Shanahan, C. A. and S. A. Strobel (2012). The bacterial second messenger c-di-GMP: probing interactions with protein and RNA binding partners using cyclic dinucleotide analogs. Org Biomol Chem 10(46): 9113-9129. doi: 10.1039/c2ob26724a

Simm, R., et al. (2004). GGDEF and EAL domains inversely regulate cyclic di-GMP levels and transition from sessility to motility. Mol Microbiol 53(4): 1123-1134. doi: 10.1111/j.1365-2958.2004.04206.x

Tal, R., et al. (1998). Three cdg operons control cellular turnover of cyclic di-GMP in Acetobacter xylinum: genetic organization and occurrence of conserved domains in isoenzymes. J Bacteriol 180(17): 4416-4425. https://jb.asm.org/content/180/17/4416.short

Tang, Q., et al. (2015). Functional analysis of a c-di-AMP-specific phosphodiesterase MsPDE from Mycobacterium smegmatis. Int J Biol Sci 11(7):813-24. doi: 10.7150/ijbs.11797

Tchigvintsev, A., et al. (2010). Structural insight into the mechanism of c-di-GMP hydrolysis by EAL domain phosphodiesterases. J Mol Biol 402(3):524-38. doi: 10.1016/j.jmb.2010.07.050

Wassmann, P., et al. (2007). Structure of BeF3−-modified response regulator PleD: implications for diguanylate cyclase activation, catalysis, and feedback inhibition. Structure 15(8): 915-927. doi: 10.1016/j.str.2007.06.016

Weinberg, Z., et al. (2007). Identification of 22 candidate structured RNAs in bacteria using the CMfinder comparative genomics pipeline. Nucleic Acids Res 35(14): 4809-4819. doi: 10.1093/nar/gkm487

Woodward, J. J., et al. (2010). c-di-AMP secreted by intracellular Listeria monocytogenes activates a host type I interferon response. Science 328(5986): 1703-1705. doi: 10.1126/science.1189801

Wu, J., et al. (2013). Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. Science 339(6121): 826-830. doi: 10.1126/science.1229963

Yan, H., et al. (2009). 3',5'-Cyclic diguanylic acid elicits mucosal immunity against bacterial infection. Biochem Biophys Res Commun 387(3): 581-584. doi: 10.1016/j.bbrc.2009.07.061

Yan, W., et al. (2010). The effect of c-di-GMP (3'-5'-cyclic diguanylic acid) on the biofilm formation and adherence of Streptococcus mutans. Microbiol Res 165(2): 87-96. doi: 10.1016/j.micres.2008.10.001

Yan, W., et al. (2012). [Exogenous 3', 5'-cyclic diguanylic acid prevents caries formation in rats]. Nan Fang Yi Ke Da Xue Xue Bao 32(5):639-42.

Ye, M., et al. (2014). DhhP, a c-di-AMP phosphodiesterase of Borrelia burgdorferi, is essential for cell growth and virulence. Infect Immun 82(5):1840-9. doi: 10.1128/IAI.00030-14

Zarrella, T. M., et al. (2018). Stress Suppressor Screening Leads to Detection of Regulation of Cyclic di-AMP Homeostasis by a Trk Family Effector Protein in Streptococcus pneumoniae. J Bacteriol 200(12). doi: 10.1128/JB.00045-18

Zhang, X., et al. (2013). Cyclic GMP-AMP Containing Mixed Phosphodiester Linkages Is An Endogenous High-Affinity Ligand for STING. Molecular Cell 51(2): 226-235. doi: 10.1016/j.molcel.2013.05.022

Zhu, J., et al. (2016). (p)ppGpp synthetases regulate the pathogenesis of zoonotic Streptococcus suis. Microbiol Res 191: 1-11. doi: 10.1016/j.micres.2016.05.007