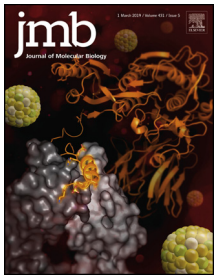
Conference Report



Recent Advances and Current Trends in Nucleotide Second Messenger Signaling in Bacteria

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Abstract

The "International Symposium on Nucleotide Second Messenger Signaling in Bacteria" (September 30–October 3, 2018, Berlin), which was organized within the framework of DFG Priority Programme 1879 (www.spp1879.de), brought together 125 participants from 20 countries to discuss recent progress and future trends in this field. Even 50 years after its discovery, (p)ppGpp is venturing into exciting new fields, especially in gram-positive bacteria. After triggering the current renaissance in bacterial second messenger research, c-di-GMP is becoming ever more global with abounding new molecular mechanisms of action and physiological functions. The more recently discovered c-di-AMP is rapidly catching up and has now been found even in archaea, with its function in osmotic homeostasis being conserved across kingdom boundaries. Small modules associated with mobile genetic elements, which make and react to numerous novel mixed cyclic dinucleotides, seem to roam around rather freely in the bacterial world. Finally, many novel and old nucleotide molecules are still lurking around in search of a function. Across many talks it became apparent that (p)ppGpp, c-di-GMP and GTP/ATP can share and compete for binding sites (e.g., the Walker A motif in GTP/ATPases) with intriguing regulatory consequences, thus contributing to the emergent trend of systemwide networks that interconnect diverse signaling nucleotides. Overall, this inspiring conference made it clear that second messenger signaling is currently one of the most dynamic and exciting areas in microbial molecular biology and physiology, with major impacts ranging from microbial systems biology and ecology to infection biology.

Introduction

Nucleotide second messengers are key components of signal transduction networks that link sensory input with the regulatory output responses in all living cells. While research with bacteria has provided early textbook examples such as cyclic 3′–5′ adenosine phosphate (cAMP) and guanosine-(penta)tetra-phosphate ((p)ppGpp), which in gram-negative bacteria control genome-wide gene expression [1,2], the astonishing diversity, mechanistic complexity and pervasive roles of bacterial second messenger signaling have become apparent only recently.

This dramatic progress has been triggered by the discovery of bis-(3′,5′)-cyclic diguanosine monophosphate (c-di-GMP) as a ubiquitous "life-style"

signaling molecule that promotes adhesion and biofilm formation while antagonizing motility of single planktonic bacteria. Moreover, c-di-GMP controls bacterial cell cycle progression, development and virulence [3–5]. Research on c-di-GMP has also sparked intense interest in additional nucleotide-based second messengers. This led to the more recent identification of bis-(3′,5′)-cyclic diadenosine monophosphate (c-di-AMP), which turned out to be a key player in cell wall homeostasis and osmoregulation in gram-positive bacteria [6,7], and the mixed purine dinucleotide (3′,3′)-cyclic AMP–GMP (c-AMP–GMP or cGAMP) that was first discovered in the context of *Vibrio cholerae* virulence [8,9]. A structurally closely related nucleotide, (2′,3′)-cyclic GMP–AMP ("non-canonical" c-GAMP or 2′,3′-cGAMP), is produced by cGAMP synthase (cGAS) in mammalian cells in response to cytosolic dsDNA and is involved in the mammalian innate immune response [10,11]. Intracellular pathogenic bacteria actually cross-talk into this response via their own cyclic dinucleotides [12,13].

Knowledge on the molecular signaling mechanisms involving these cyclic dinucleotides is currently accumulating at a steep rate [4,14–18] and—despite its discovery about 50 years ago [19]—also (p)ppGpp is back on the agenda with many new targets found beyond its classical interaction with RNA polymerase [20–22]. In order to further promote and organize this field of research, the priority program "Nucleotide Second Messenger Signaling in Bacteria" was established in 2016 in Germany (SPP 1879, funded by the Deutsche Forschungsgemeinschaft; www.spp1879.de). Besides funding research, this includes the possibility to organize dedicated conferences to allow the exchange of the newest findings and views. This review reports on the first *DFG-SPP1879 International Symposium on Nucleotide Second Messenger Signaling in Bacteria* organized by Regine Hengge and Mihaela Pruteanu (Humboldt-Universität zu Berlin) in Berlin (Germany) from 29th September to 3rd October 2018, with this meeting building upon an international symposium of similar format that also took place in Berlin in 2015 [23]. The meeting covered the entire range from signaling input via sensory domains as well as the structure and function of enzymes that make and break nucleotide second messengers to the molecular and physiological functions controlled by these signaling molecules in diverse bacteria, both in the environment and in their interactions with hosts.[†]

(p)ppGpp—Still Magic!

Stress-dependent synthesis of (p)ppGpp was first observed by Cashel and Gallant in the late 1960's as a "magic spot" appearing on autoradiograms of extracts of amino acid-starved wild-type "stringent" *Escherichia coli* cells. This spot was not observed in "relaxed" *E. coli* strains, which—due to mutations in "*rel*" genes—had lost starvation-induced inhibition of rRNA synthesis, suggesting a direct association between increased intracellular levels of this nucleotide and the stringent response [19].

This cellular stress response system is highly conserved in bacteria [2,24] and is also present in eukaryotic organisms, for example, in the chloroplasts of plants [25]. Research in many laboratories established that (p)ppGpp synthesis and hydrolysis are catalyzed by RelA/SpoT homologs (Rel), which usually combine a hydrolase domain (RSH) followed by a synthetase domain with additional regulatory domains; that is, in general, these enzymes are conditionally bifunctional and can synthesize and hydrolyze (p)ppGpp. Rel enzyme activity can be modulated by association with uncharged tRNAs and the ribosome, thereby allowing the sensing of amino acid starvation. In addition to these large multidomain RSH enzymes, shorter monofunctional small alarmone synthetases (SAS) or small alarmone hydrolases containing only the synthetase or hydrolase domains, respectively, are present in many bacteria [2,24]. Synthesis and hydrolysis of (p)ppGpp allow cells to activate or inhibit different cellular pathways by modulating various enzyme activities (Fig. 1), which translates into a direct or indirect control of replication, transcription or translation, in response not only to amino acid starvation but also to various other signals or stresses [14,20,21,27–29]. Interestingly, the ability to recognize different starvation and stress signals and the resulting response pattern of the stringent response system appear to be quite adaptable, as observed for microorganisms evolved to occupy distinct niches or environments. The stringent response, with its ability to inhibit translation and growth, was also implicated in persister formation and development of antibiotic tolerance [30,31] as well as survival of pathogens during infection [32].

It comes therefore as no surprise that even 50 years after its discovery this fascinating system continues to be actively investigated in many laboratories around the world. This general interest in stringent response was also reflected by the scientific program of the symposium, where the first session—"A classic revisited—(p)ppGpp)"—was devoted to the stringent response with its diverse facets. The session was opened by Jade Wang (University of Wisconsin at Madison) whose laboratory had previously demonstrated that high (p)ppGpp levels interfere directly with cellular GTP synthesis in *Bacillus subtilis* cells, resulting in many of the known stringent response phenotypes [33–35]. She could demonstrate that stringent response and (p)ppGpp signaling are intricately involved in persister cell formation of *B. subtilis*. In particular, her group's experiments could unravel and dissect the specific roles of the RSH (RelA) and two SAS (YwaC and YjbM) in regulated and spontanous persister formation and demonstrate

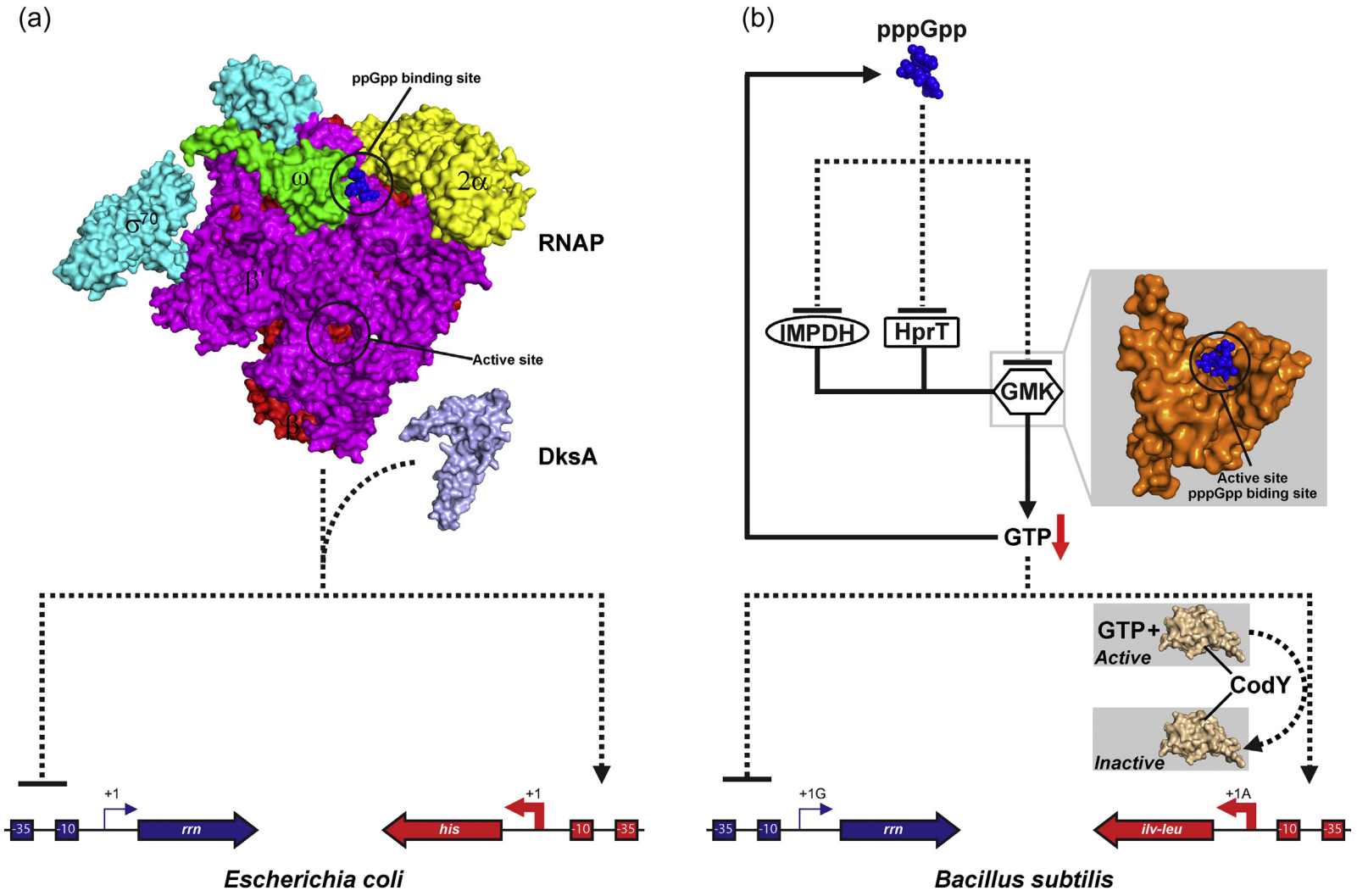

Fig. 1. Divergent mechanisms of transcription initiation by (p)ppGpp in gram-negative *E. coli* and gram-positive *B. subtilis*. (a) In *E. coli*, the tripartite interaction between RNA polymerase (RNAP), (p)ppGpp and DksA controls transcription initiation, with (p)ppGpp binding to an interface between the β′ and ω subunits of RNAP and DksA binding to the secondary channel [26]. While ribosomal genes (*rrn*) are negatively affected, amino acid biosynthesis genes are usually positively regulated by (p)ppGpp/DksA [24]. (b) In *B. subtilis*, (p)ppGpp controls GTP levels by directly inhibiting IMP dehydrogenase (IMPDH), guanylate kinase (GMK) and hypoxanthine–guanine phosphoribosyltransferase (HprT) and by passively consuming GTP during its own synthesis. Lower GTP levels have a direct negative impact on transcription initiation of ribosomal promoters (which initiate with a GTP), but activate transcription at amino acid biosynthesis genes, in part through inactivating CodY (whose C-terminal domain is shown here only). Solid lines indicate biosynthetic pathways, and dotted lines indicate regulatory effects. This figure was previously published [21] and is used here with permission.

the importance of the control of GTP concentration by (p)ppGpp [36] in persistence.

Dipankar Chatterji (Indian Institute of Science, Bangalore), whose laboratory works on nucleotide second messengers in *Mycobacterium smegmatis* [37,38], presented data on RelZ (previously MS_RHII-RSD), a new variant of a SAS fused to a RNase HII domain [39]. Active hexameric RelZ is involved in the removal of stalled R-loops, that is, the triple-stranded nucleic acid structure consisting of a nascent RNA strand hybridizing to the complementary DNA strand, which is associated with the displacement of the non-template strand [40]. Since RelZ, like other alarmone synthetases, can synthesize pGpp from GMP and ATP and this activity appears to be controlled by RNA and may become more important during slow growth or in stationary phase where GMP levels are enhanced, he further emphasized that also pGpp may have an important and comparable role to (p)ppGpp as a second messenger.

Kürşad Turgay (Leibniz Universität, Hannover) presented experiments demonstrating a fast increase of cellular (p)ppGpp levels with a strong and pleiotropic impact on survival and the control of transcription and translation during heat stress. These experiments suggest that *B. subtilis* cells respond to heat-mediated protein unfolding and aggregation by decreasing translation via the (p)ppGpp-mediated stringent response to concurrently reduce the protein load for the cellular protein quality control system.

Using pull-down assays, Rebecca Corrigan (University of Sheffield) found that (p)ppGpp-binding proteins in *Staphylococcus aureus* include the GTPases Era, RsgA, RbgA and HflX, which are all involved in ribosome assembly, with (p)ppGpp binding impacting their function and interactome. Thereby, she could address the important question by which mechanisms stringent response can affect translation [41]. Interestingly, (p)ppGpp does not only directly compete with GTP for the same binding site in these GTPases, but they could also observe a direct interaction between Era and CshA (a RNA helicase) with Rel (RSH), thereby influencing cellular (p)ppGpp levels [42].

Régis Hallez (University of Namur) reported experiments revealing the molecular basis of a direct connection between the nitrogen-sensing PTS system and how nitrogen starvation results in the induction of the stringent response in *Caulobacter crescentus* [43]. They could demonstrate that the hydrolase activity of the RSH SpoT is inhibited by the phosphorylated $EIIA^{Ntr}$ of the PTS^{Ntr} system by directly competing with the regulatory ACT domain of the RSH SpoT, thus participating in cellular (p)ppGpp accumulation [44].

In the last talk in this session, Viktoriya Shyp (from the group of Urs Jenal at the Biozentrum, University of Basel) introduced *C. crescentus* SmbA, a protein that possesses overlapping binding sites for c-di-GMP and ppGpp, which are competing for interaction with this site. SmbA could thus be a factor that integrates nutritional clues with the inverse regulation of cell cycle progression by these two global second messengers.

In his keynote talk, Mike Cashel (NIH, Bethesda) gave a broad personal and historical perspective on the discovery and development of research on the stringent response over 50 years after the first observation and characterization of the (p)ppGpp second messenger [19]. While reviewing research progress over the decades, he acknowledged and explained the involvement of many colleagues and co-workers. He highlighted recent progress on identifying the regulatory mechanisms of growth rate inhibition acting via DNA replication, synthesis and transcription, the differential effects of ppGpp *versus* pppGpp and the potential of (p)ppApp to antagonize (p)ppGpp at RNA polymerase in *E. coli* [45–48]. Interestingly, different regulatory effects where observed when *E. coli* was exposed to nutrient limitations. In particular, the specific response was dependent on how limitation was achieved, the chemical nature of the limiting nutrient or on the inefficient utilization of excess nutrients. In each case, (p)ppGpp seems to play strikingly different roles in growth adaptation [46,49,50]. Most recently, the influence of the stringent response during diauxic shifts and the connection between cellular acetyl phosphate and (p)ppGpp levels was investigated [51,52]. This excellent overview, introducing the nuts and bolts of this research field as well as the personal and serendipitous aspects of research, was an inspiring closure and transition to the next sessions on other bacterial nucleotide second messengers, which more recently came into the focus of research.

Sensory Input into c-di-GMP Signaling

c-di-GMP is synthesized from GTP by diguanylate cyclases (DGCs) characterized by the GGDEF domain (the GGDEF sequence motif represents the conserved active site or A-site). Most, but not all DGCs also contain a secondary binding site for c-di-GMP (I-site), which allosterically inhibits c-di-GMP synthesis once certain cellular levels have been reached. Degradation of c-di-GMP is mediated by specific phosphodiesterases (PDEs), which can feature either EAL or HD-GYP domains [53]. Often GGDEF and EAL domains also occur together in single "composite" proteins, with one of the two domains providing enzymatic activity and the other, usually degenerate domain playing a regulatory role. Most bacteria possess multiple DGCs and PDEs. A majority of these DGCs and PDEs, many of which are membrane-associated, harbor diverse N-terminal sensory input domains that control their activities in response to primary intra- or extracellular signals [54]. As DGCs are active in the dimeric or oligomeric state, signal input via their N-terminal domains usually controls dimerization [55].

Common sensory input domains of DGCs and PDEs are PAS domains which can use FAD or heme as cofactors thereby allowing the perception of oxygen, redox changes or light, but which can also serve as dimerisation domains [56–59]. Also, a BLUF domain has been found to control the activity of an EAL domain in response to light [60]. DgcZ (YdeH) activity in *E. coli* is inhibited by zinc binding to its N-terminal CZB domain [61]. Transmembrane signaling can originate at periplasmic domains as for instance the redox-sensitive periplasmic CSS domains, which are found exclusively at the N-termini of a subclass of EAL domain PDEs [62]. Another periplasmic sensory domain is the CHASE8 (cyclases/histidine kinases associated sensory) domain of DgcN (YfiN), which is inhibited by a directly interacting periplasmic protein, YfiR, that also serves as a redox sensor [63]. However, many putative sensory domains still remain uncharacterized, including, for example, various GAPES (*ga*mma-proteobacterial *p*eriplasmic *s*ensory) domains or the membrane-intrinsic MASE1 and MASE2 domains [64,65]. Moreover, only few small molecules potentially binding to sensory input domains have been identified so far.

Pseudomonas aeruginosa produces phenazines, which serve as antibiotics but also act as extracellular freely diffusible electron acceptors. Phenazines allow *P. aeruginosa* to build higher macrocolonies despite the steep vertical oxygen gradients that form in this type of biofilm. By contrast, mutants unable to synthesize phenazines produce more exopolysaccharide matrix, which results in flatter biofilms that buckle into wrinkled colonies, thereby increasing their oxygen-exposed surface area [66–68]. Lars Dietrich (Columbia University, New York) showed that the underlying regulatory mechanism involves RmcA (PA0575), a c-di-GMP-degrading PDE consisting of a transmembrane domain and four PAS domain followed by a GGDEF domain, which is degenerate in important amino acids and therefore enzymatically inactive (GGDEF^{deg}), and an EAL domain [69] (Fig. 2). RmcA activity was found to be stimulated by phenazines *in vivo*, with *rmcA* mutants showing strong colony wrinkling. RmcA also binds phenazines *in vitro*, possibly via one of the PAS domains. In addition, he reported on the microscopic observation that *P. aeruginosa* macrocolony biofilms form “clonal striations that align with the z-axis” in the hypoxic zones. This seems to be a rather general c-di-GMP-controlled phenomenon since similar growth patterns termed “vertical pillars” have been observed in *E. coli* macrocolonies [70]. By approaching the *P. aeruginosa* protein RmcA/PA0575 from a biochemical angle, Serena Rinaldo (Sapienza University of Rome) showed that its PDE activity is allosterically controlled by GTP, through the enzymatically inactive GGDEF domain [71]. *In vivo*, the protein is activated by exogenous arginine through its periplasmic Venus Flytrap (VFT) domain [72]. VFT domains represent a widespread strategy for nutrient recognition that is also found in histidine kinases of the BvgS family [73] or for instance in L-glutamate (umami)-sensing domains in human taste receptors [74].

The nitric oxide (NO)-driven dispersal of *P. aeruginosa* biofilms depends on NbdA, another complex c-di-GMP-degrading PDE in this bacterium, which features an N-terminal MHYT domain followed by a combination of a GGDEF^{deg} and an EAL domain [75–77]. Nicole Frankenberg-Dinkel (Universität Kaiserslautern) showed that the expression of NbdA is under the control of the alternative sigma factor RpoS. However, unlike previously postulated [75,76], NO-induced activation of the PDE activity seems to be indirect, since NbdA recombinantly expressed in *E. coli* was active—complementing a lack of the major *E. coli* PDE PdeH—even in the absence of NO.

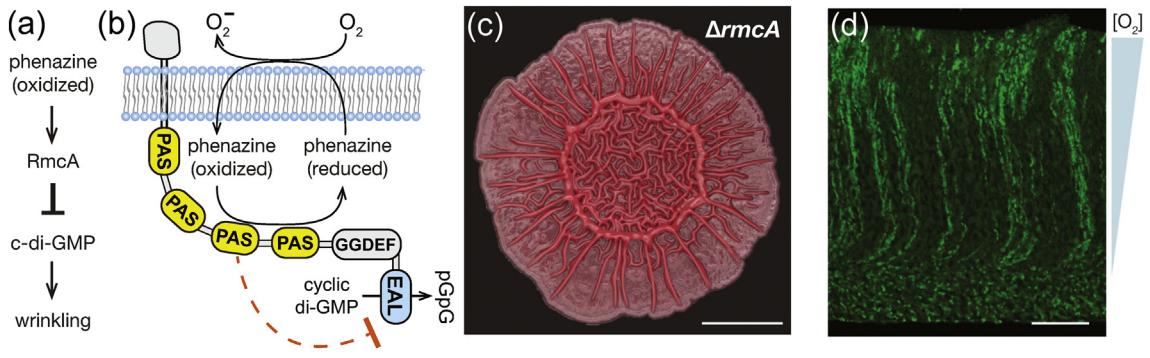

Fig. 2. Redox regulation of *P. aeruginosa* colony biofilm formation. (a) Pathway linking phenazines to 3D structure formation (wrinkling) in macrocolonies. (b) Domain architecture of the PDE RmcA [69]. Phenazines interfere with the activity of the c-di-GMP-degrading EAL domain of RmcA. (c) Macrocolony biofilm of a Δ*rmcA* strain of *P. aeruginosa*. Scale is 5 mm. (d) Vertical macrocolony thin section with 2% YFP-labeled cells showing clonal populations of cells arranged along the oxygen gradient. Scale is 20 μm. The figure was kindly provided by Lars Dietrich.

High Specificity Local c-di-GMP Signaling

Most bacterial species possess multiple enzymes that make or break c-di-GMP, often with surprisingly distinct and specific output functions. Such high target specificity has led to theoretical concepts of "local" c-di-GMP signaling involving direct interactions between specific DGC/PDE pairs and c-di-GMP-binding effector/target systems [78]. This has been studied in detail in the complex c-di-GMP signaling network that controls the production of extracellular matrix polymers in *E. coli*, that is, amyloid curli fibers and phosphoethanolamine-modified cellulose (pEtN cellulose) [79–82]. As shown by Regine Hengge (Humboldt Universität zu Berlin), mutations that eliminate locally acting DGCs and PDEs (DgcE, PdeR, DgcM, DgcC) in this regulatory network in *E. coli* (Fig. 3) drastically affect matrix production but do not significantly change the global cellular c-di-GMP pool that was found to remain surprisingly low even under conditions of matrix production. She proposed a novel model of local c-di-GMP signaling, in which a single strongly expressed master PDE—PdeH in *E. coli*—dynamically eradicates global effects of several active DGCs by strongly draining the global c-di-GMP pool, which both enables and restricts these DGCs to serve as local c-di-GMP sources that activate nearby specific effector/target systems within multi-protein complexes [91]. This principle was further illustrated by DgcC (YaiC; AgfA in *Salmonella*), which has long been known to be specifically required for cellulose biosynthesis [92,93] and which has now been found by the Hengge group to interact directly with distinct partner proteins within the large c-di-GMP-controlled cellulose synthesis, secretion and modification machinery [82,94,95]. A mathematical reaction–diffusion model—presented by Nadeshda Malysheva (from the group of Max von Kleist, Freie Universität Berlin) on one of the two prize-winning posters—confirmed that co-localization of a c-di-GMP source, sink and effector/target strongly increases the signaling probability through a localized, yet open source of c-di-GMP such as DgcC to an adjacent effector/target system such as the BcsA component of cellulose synthase. The model also shows that very short input signals (i.e., noise) get filtered out, while only a few free c-di-GMP molecules in the localized environment are sufficient to accurately transduce incoming signals that stimulate c-di-GMP production.

Bacterial biofilms exhibit pronounced antibiotic tolerance [96,97]. While this property is usually believed to be multifactorial, Karin Sauer (Binghamton University) pointed out that antimicrobial tolerance of biofilms of *P. aeruginosa* specifically depends on the two-component hybrid sensor kinase SagS, which promotes biofilm formation and antibiotic tolerance via distinct pathways [98]. Only the antibiotic tolerance pathway requires the c-di-GMP-binding MerR-like transcription factor BrlR, which activates the expression of distinct multidrug efflux pumps already early during biofilm formation [99–101]. As the missing link between SagS and BrlR, recent work in her group identified the DGC SicA (PA3177), which is specifically dedicated to induce antibiotic tolerance via BrlR without affecting biofilm formation in general, thus probably representing yet another case of local c-di-GMP signaling.

In general, bacterial swimming motility is negatively affected by c-di-GMP. As presented by Kai Thormann (Universität Gießen), *Shewanella putrefaciens* which features a polar flagellum supported by several lateral flagella, represents a particular intriguing variation on this theme [102,103]. Among its >50 proteins contributing to c-di-GMP control, a single one, PdeB, specifically controls swimming behavior. PdeB is a PDE consisting of a transmembrane domain, a HAMP domain, an enzymatically inactive yet c-di-GMP-binding GGDEF domain and an active EAL domain. PdeB is recruited to the flagellated pole by direct interaction—via its GGDEF domain—with the polar marker protein HubP [104]. Surprisingly, however,

Fig. 3. Roles of cAMP, (p)ppGpp and c-di-GMP in the biofilm matrix control network in *E. coli* K-12. While cAMP/CRP is required to express flagellar genes [83], (p)ppGpp and c-di-GMP both contribute to inversely coordinating the flagellar and the biofilm matrix transcription factor cascades (indicated by light blue and light red boxes I to III, respectively). Increasing cellular (p)ppGpp levels downregulate expression of the flagellar master regulator FlhDC [84], activate *rpoS* transcription [85,86] and promote the binding of σ^S to RNAP core enzyme [87]. c-di-GMP affects flagella function and biofilm matrix production via two distinct regulatory modules. In signaling module A, c-di-GMP antagonistically controlled by PdeH and DgcE inhibits flagellar rotation via YcgR [88–90] and affects the interactions of PdeR, DgcM and the transcription factor MlrA to activate expression of CsgD, which is required for the production of both curli fibers and pEtN cellulose [79–81]. By contrast, signaling module B specifically controls pEtN cellulose biosynthesis. Here, CsgD-controlled DgcC, which directly interacts with the cellulose synthase complex (BcsAB–BcsEFG), generates the local c-di-GMP required to activate cellulose synthesis by BcsA and—via the transmembrane BcsEF pathway—to activate PEtN modification of cellulose by BcsG. The regulatory network is arranged as to also roughly reflect the spatial order of physiological differentiation from the bottom (left side) to the top (right side) of a macrocolony biofilm, which follows nutrient and oxygen gradients and controls the extracellular matrix architecture as visualized with the matrix dye thioflavin S in a vertical section of a macrocolony (shown at the bottom of the figure; scale is 10 μm). At the right side, morphotypes of macrocolony biofilms of wild-type *E. coli* K-12 strain AR3110 and knockout mutants lacking the indicated DGCs and PDEs are shown (grown on agar plates containing the matrix dye Congo red; scale is 5 mm). The figure was provided by Regine Hengge. The macrocolony section and the macrocolony morphotype images were published previously and are used here with permission [70,91].

PdeB is not involved in polar flagellum assembly, rotation or chemotaxis, but its polar localization in the complex with HubP rather affects the production of the lateral flagella and their control of rotation by the c-di-GMP-binding protein YcgR. In addition to its role in recruiting multiprotein complexes involved in

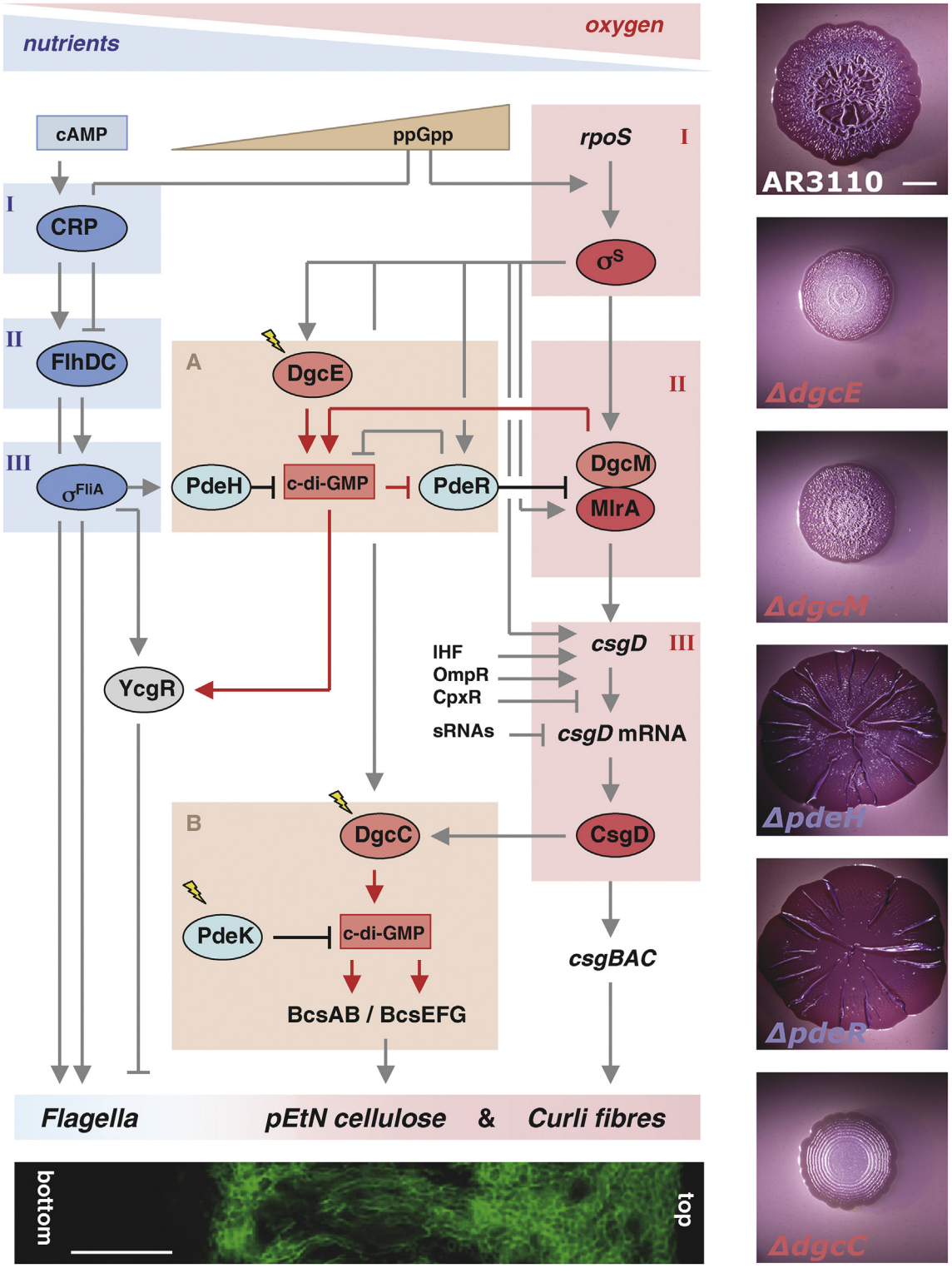

Fig. 3 (*legend on previous page*)

chromosome segregation and motility to the appropriate cell pole, HubP seems to emerge as a local coordinator of highly specific c-di-GMP signaling in *S. putrefaciens*.

Taken together, a number of criteria for local c-di-GMP signaling emerge from these and other recent studies. These are (i) high target specificity of distinct DGCs and/or PDEs, (ii) direct interactions between these enzymes and c-di-GMP-binding effector/target systems, with the complexes formed being sometimes localized to specific cellular positions, (iii) no effects on cellular c-di-GMP levels of knockout mutations in genes for DGCs and/or PDEs that have clear target-specific phenotypes, and, in some cases, (iv) severalfold lower cellular c-di-GMP levels than the K_d's of c-di-GMP-binding effectors, even under conditions where the respective pathway is active [91].

Diversity of c-di-GMP-Binding Effectors and Their Targets

C-di-GMP-mediated signal transduction pathways involve a wide variety of c-di-GMP binding proteins, which interact with diverse downstream targets to orchestrate equally diverse bacterial responses [105,106]. Thus, a session of the symposium concentrated on c-di-GMP-binding effectors and their targets. Among the best-characterized and most widespread c-di-GMP binding proteins are the PilZ domain proteins, which bind c-di-GMP with K_d's in the (sub)micromolar range [107–110], but novel c-di-GMP-binding domains continue to be discovered such as for instance the MshEN domain [111]. Michael Galperin (NCBI-NIH) discussed the evolution of c-di-GMP signaling and presented examples of c-di-GMP binding proteins that emerged from widespread protein families through acquisition of diverse c-di-GMP-binding motifs. As in some cases binding of c-di-GMP seems to require just a flexible loop with an Arg residue, he expects additional c-di-GMP receptors with narrow phylogenetic distribution in distinct bacterial taxa to be identified in the future. On the other hand, certain c-di-GMP receptors may lose the ability to bind c-di-GMP but retain their regulatory properties.

Juan Sanjuan (CSIC-Granada) reported on c-di-GMP binding to the C-terminal domain of BgsA (C-BgsA). BgsA is the glycosyltransferase involved in the production of a linear mixed-linkage β-glucan, an exopolysaccharide important for *Sinorhizobium meliloti* colonization of its plant host, alfalfa. BgsA shares significant sequence and structural similarities with bacterial cellulose synthase BcsA, except for the c-di-GMP binding C-terminal domains. Whereas in cellulose synthases, c-di-GMP binding to the C-terminal PilZ domains seems to release a "gating loop" that inhibits glycosyltransferase activity [109], the data suggest a true activation mechanism of BgsA enzyme activity by c-di-GMP binding to C-BgsA [112].

Lotte Søgaard-Anderson (Max Planck Institute for Terrestrial Microbiology) presented the work of her research group on how c-di-GMP regulates growth and development in *Myxococcus xanthus* [113]. To search for novel c-di-GMP binding effectors in *M. xanthus*, they applied a capture compound mass spectrometry approach. CdbA, a ribbon–helix–helix DNA-binding protein, and CdbB were identified as c-di-GMP binding proteins. Structural analyses of the essential CdbA protein together with functional studies suggest that CdbA together with a PilZ domain protein might be involved in regulating chromosome organization and cell division.

Tom Landgraf (from the group of Harald Schwalbe, Goethe-Universität Frankfurt) shed light in his talk on a post-transcriptional mechanism in *Clostridium difficile* that uses a c-di-GMP riboswitch (Cd1) to control flagellar protein expression [114]. He applied a single-nucleotide extension strategy in combination with 2D NMR analysis and presented new data on an aptamer domain minimal motif. Structural and kinetic features of the Cd1-riboswitch upon c-di-GMP binding were presented that provided a deeper understanding of this riboswitch.

Holger Sondermann (Cornell University) presented new data on the structure and function of the *P. aeruginosa* oligoribonuclease or "nano-RNase" Orn. An *orn* mutant is characterized by accumulation of the c-di-GMP cleavage product pGpG, which leads to a hyperbiofilm phenotype due to overproduction of the pel polysaccharide since pGpG feedback inhibits c-di-GMP-specific EAL domain PDEs [115,116]. Based on the crystal structure of the Orn dimer, he demonstrated how the enzyme is optimized for dinucleotides with a 5′-phosphate, explaining its strict substrate length preference for dinucleotides. Interestingly, an Orn homolog also exists in eukaryotic mitochondria, with its depletion causing severe phenotypes [117]. Crystal structures of the human homolog show that substrate recognition is conserved in eukaryotes.

Biofilms and Beyond—The Physiological Versatility of c-di-GMP Signaling

Since the discovery of c-di-GMP in 1987 [118], the list of bacterial phenotypes controlled by this cyclic dinucleotide has steadily increased. Nevertheless, the role of c-di-GMP is often reduced to that of a "regulator of bacterial lifestyle transitions." At the meeting, new c-di-GMP functions and mechanisms have been presented.

An exciting and to date largely unexplored regulatory process is c-di-GMP-controlled alteration of ribosome functions by post-translational modification of ribosomal proteins as presented by Jake Malone (University of East Anglia/John Innes Centre, Norwich). In their studies on c-di-GMP signaling in the plant growth-

promoting rhizobacterium *Pseudomonas fluorescens*, Malone and co-workers discovered c-di-GMP binding by the RimK protein, which modifies the ribosomal protein RpsF by the addition of glutamate residues (Fig. 4). Using a global proteomics approach, they showed that this RpsF modification results in profound proteome alterations affecting *P. fluorescens* motility, virulence and plant colonization [119]. In addition to binding c-di-GMP, RimK interacts with the PDE RimA and the newly identified poly-glutamate protease, RimB. These interactions are proposed to fine-tune the degree of ribosomal glutamylation for optimal proteomic adaptation to specific environmental conditions [120].

As shown by Anke Becker (Phillips-Universität Marburg), c-di-GMP also controls plant colonization by the rhizobacterium *S. meliloti*, which can exist either as a free-living organism or in symbiosis with a leguminous plant host. The exopolysaccharides succinoglycan and galactoglucan contribute to *S. meliloti* biofilm formation as well as sliding on surfaces, and promote establishment of productive symbiosis. Work in the Becker group has demonstrated that the *uxs1-Smb20463* gene cluster is involved in the production of yet another putative EPS. Expression of this gene cluster is directly controlled by an AraC-like transcription factor CuxR whose dimerization and DNA binding is promoted by c-di-GMP binding. As shown by structural analysis, binding of c-di-GMP to CuxR is highly reminiscent of that of PilZ domains despite the absence of any sequence similarities between the two proteins [121]. However, in *S. meliloti*, c-di-GMP signaling reaches far beyond EPS-associated functions and is also linked to quorum sensing, peptidoglycan biogenesis and cell division by yet unknown mechanisms [122].

Two talks focused on functions of c-di-GMP in the human pathogen *V. cholerae*. Fitnat Yildiz (University of California, Santa Cruz) showed new data on the mechanism how c-di-GMP controls the dynamic behavior of the mannose-sensitive hemagglutination (MSHA) pilus, which is vital for surface colonization and biofilm formation in *V. cholerae* [123,124]. Using an elegant way to visualize MSHA pili with thiol-reactive fluorescent dyes (Fig. 5), they showed for the first time that these cellular appendages can extend and retract and thus are dynamic structures. c-di-GMP is a key factor controlling these dynamics through binding to the MSHA extension ATPase, MshE, which has provided the prototype of the novel c-di-GMP-binding MshEN domain [111,125].

Chris Waters (Michigan State University, East Lansing) presented his current model of the multi-layered regulation of motility in *V. cholerae*. This cascade involves the transcription factor FlrA, which—if not bound by c-di-GMP—induces biosynthesis of flagellar genes at low c-di-GMP concentrations [126] and the c-di-GMP-responsive Vc2 riboswitch, which in its unbound state allows maximal expression of the TfoY protein to stimulate disperse motility. He further showed that the c-di-GMP-binding regulators VpsR and VpsT, which have been shown to induce biofilm formation in *V. cholerae*, also regulate DNA repair and oxidative stress responses and a type II secretion system [127,128]. Thus, c-di-GMP-controlled stress-response functions may contribute to stress tolerance of *V. cholerae* biofilms.

In the predatory bacterium *Bdellovibrio bacteriovorus*, invasion into the prey periplasm and other lifecycle processes are under c-di-GMP control [129]. Andy Lovering (University of Birmingham) talked about structural work on the GGDEF diguanylate cyclase DgcB of *B. bacteriovorus*, which is a GGDEF enzyme that specifically contributes to initiation of predation since a Δ*dgcB* mutant shows an invasion defect [130]. In their collaborative studies, he and Liz Sockett (University of Nottingham) addressed the question which stimulus drives DgcB activity and found that phosphorylation of a threonine in the flexible N-terminal tail of DgcB stimulates c-di-GMP production. Moreover, a cAMP-binding hydrolase interacts with DgcB suggesting a link between cAMP and c-di-GMP signaling in *Bdellovibrio*.

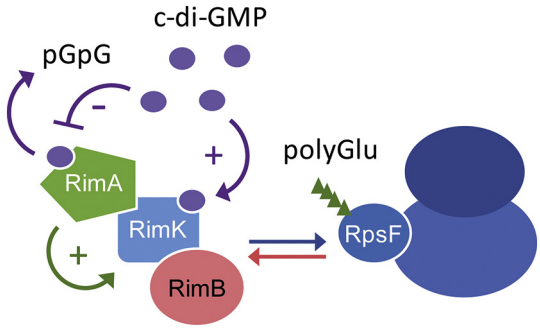

Fig. 4. Role of c-di-GMP in ribosomal modification. In *P. fluorescens*, the c-di-GMP-binding RimK protein controls the RpsF-dependent poly-glutamylation of ribosomal proteins. RimA is a directly associated c-di-GMP-degrading PDE, and RimB is an accessory protein of not yet fully clarified function [119,120]. The figure was kindly provided by Jake Malone.

c-di-AMP—A Key Factor in Osmotic Homeostasis of Gram-Positive Bacteria

Among bacterial second messengers, cyclic di-AMP has recently attracted much attention. This cyclic dinucleotide has been discovered only 10 years ago in the course of the structural analysis of the DNA integrity scanning protein DisA [131]. c-di-AMP is a second messenger that under most conditions is essential for many bacteria that produce it, as observed for the model bacterium *B. subtilis*, the pathogens

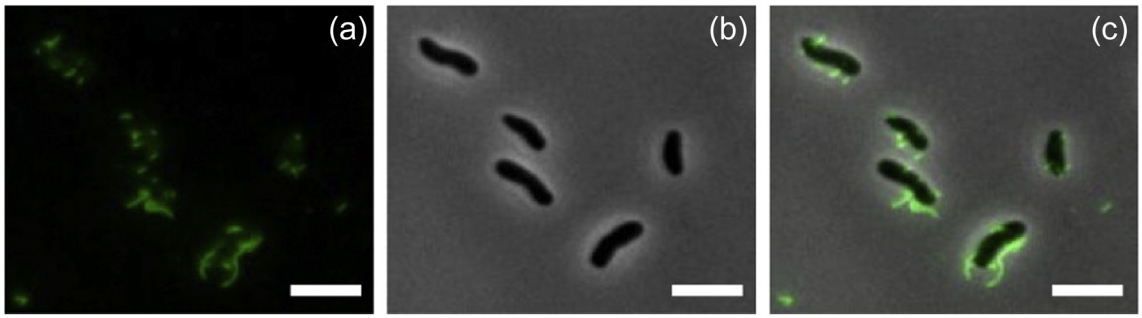

Fig. 5. Visualization of the c-di-GMP-controlled *V. cholerae* type IVa MSHA pili by thiol-reactive fluorescent dye labeling. (a) Within the major pilin subunit MshA [123], a surface-exposed threonine residue was mutated to a cysteine (T70C) in *V. cholerae* strain A1552, to allow for labeling with thiol-reactive fluorescent dyes. Here, MSHA pili were specifically labeled with Alexa Fluor 488 C5 maleimide dye (Thermo Scientific). (b) Phase contrast image of labeled cells, imaged underneath a 1% agarose pad. Imaging was performed on a Zeiss Axiovert 200 phase contrast microscope, with a 63× Plan-Apochromat1.4NA Ph3 oil objective and 1.6× auxiliary magnification, and images were processed with Image J. (c) Overlaying of fluorescent and phase contrast images demonstrates the distribution and localization of labeled pili along the cell body. Scale bar for all images is 2 μm. The figure was kindly provided by Kyle A. Floyd and Fitnat Yildiz.

S. aureus, *Listeria monocytogenes*, and *Streptococcus pneumoniae*, as well as the minimal genome bacterium *Mycoplasma pneumoniae* [6,7,132]. By contrast, too high accumulation of c-di-AMP can become toxic—this second messenger has therefore been called an "essential poison" [133]. So far, c-di-AMP is the only signaling nucleotide found to regulate by binding to two different classes of target molecules, proteins and RNA, within a single biological pathway: in *B. subtilis* c-di-AMP binding controls the activity of a potassium transporter as well as the riboswitch that modulates its expression [134,135]. In the past few years, c-di-AMP targets have been identified in several gram-positive bacteria, and these studies have revealed that c-di-AMP has major functions in the control of potassium and osmotic homeostasis as well as in the regulation of the citric acid cycle, the central hub in bacterial energy metabolism [134,136–139]. Several recent studies on c-di-AMP essentiality in *L. monocytogenes*, *B. subtilis* and *S. aureus* have demonstrated that in fact c-di-AMP is dispensable for bacteria under specific conditions, and that strains lacking the second messenger rapidly acquire suppressor mutations that affect potassium homeostasis and osmoprotection [140–142].

At the conference, news on the synthesis and degradation of c-di-AMP were presented as well as novel insights into the function of this second messenger. Angelika Gründling (Imperial College London) reported on novel findings concerning the control of c-di-AMP synthesis by the diadenylate cyclase DacA in *S. aureus*. Given the conserved organization of the *dacA* gene in an operon with the *glmM* gene encoding the phosphoglucosamine mutase and the observed interaction between the GlmM and DacA proteins in *B. subtilis* and *Lactococcus lactis* [133,143], the possible control of DacA activity by GlmM has been studied in *S. aureus*. Upon interaction, GlmM completely inhibits c-di-AMP synthesis by DacA, while GlmM's own activity is not affected by this interaction. The structural analysis of DacA revealed the formation of inactive dimers, as also reported for the *L. monocytogenes* enzyme [144]. However, DacA is capable of forming larger oligomers that exhibit enzymatic activity. Structural analyses revealed that GlmM directly blocks the active site of DacA, thus explaining the inhibition of enzyme activity.

Importantly, the field of c-di-AMP signaling research has recently been extended to streptomycetes. Degradation of c-di-AMP was the topic of the presentation of Natalia Tschowri (Humboldt Universität zu Berlin), whose laboratory studies c-di-AMP signaling in *Streptomyces venezuelae*. In these bacteria, which belong to the actinobacteria, this second messenger is not essential. While a DisA-type diadenylate cyclase is present, none of the known classes of c-di-AMP-degrading PDEs [16] are encoded in the genome of *S. venezuelae* and related bacteria. Using a combination of bioinformatic and experimental analyses, Natalia Tschowri and her team could identify four promising candidate proteins, one of which is widely conserved in actinobacteria and is capable of degrading c-di-AMP. c-di-AMP is required for growth of *S. venezuelae* at increased salt concentrations, thus supporting the idea of a general role of the second messenger in osmotic homeostasis. In addition, overproduction of c-di-AMP interferes with spore maturation indicating that c-di-AMP is involved in the control of development in *S. venezuelae*.

Sonja-Verena Albers (University of Freiburg) demonstrated c-di-AMP synthesis in the halophilic euryarchaeon *Haloferax volcanii* and thus extended the analysis of c-di-AMP signaling even beyond the domain of bacteria. In euryarchaeota, a novel type of diadenylate cyclases, called DacZ, is present. These enzymes consist of an enzymatically active domain fused to an uncharacterized C-terminal pyruvate

kinase-like domain. Interestingly, as in many bacteria, c-di-AMP is both essential and—at high concentrations—toxic for *H. volcanii*, suggesting a role in a homeostatic process. The conserved arrangement of the archaeal *dacZ* gene with a gene encoding a mechanosensitive channel points to a role of c-di-AMP in osmoregulation related to that described in gram-positive bacteria [145]. Indeed, a reduced c-di-AMP level in *H. volcanii* resulted in an increase of cell volume at low salt concentrations, which might result from an increased influx of water.

Two talks presented new insights into downstream signaling by c-di-AMP. Adnan Syed (from the group of Rich Losick at Harvard University) discussed the role of c-di-AMP in extracellular DNA release by *S. aureus* during biofilm formation. A *S. aureus* mutant that accumulates c-di-AMP due to the absence of the only c-di-AMP degrading PDE is impaired in biofilm formation and in the release of extracellular DNA [146]. Interestingly, a mutant lacking the transcription factor XdrA also showed reduced eDNA secretion and elevated levels of c-di-AMP, with overexpression of the PDE GdpP suppressing this effect. Obviously, this study has discovered a new regulatory link between c-di-AMP levels and bacterial activities at the community level.

Jörg Stülke (University of Göttingen) discussed the role of c-di-AMP in maintaining the cellular homeostasis of potassium and glutamate, the two most abundant ions in every living cell (Fig. 6). While c-di-AMP is essential for *B. subtilis* on complex medium, a strain lacking the second messenger is viable at low potassium concentrations and in the absence of externally added glutamate [147]. The presence of either ion provokes the acquisition of suppressor mutants that affect potassium and/or glutamate homeostasis. Moreover, both ions affect the intracellular concentration of c-di-AMP. Interestingly, the conserved PII-like c-di-AMP binding protein DarA [153] seems to be required for efficient glutamate synthesis under conditions of severe potassium limitation. Both potassium and glutamate, a precursor for the osmoprotectant proline, play key roles in osmoprotection, supporting the implication of c-di-AMP in osmotic homeostasis [145].

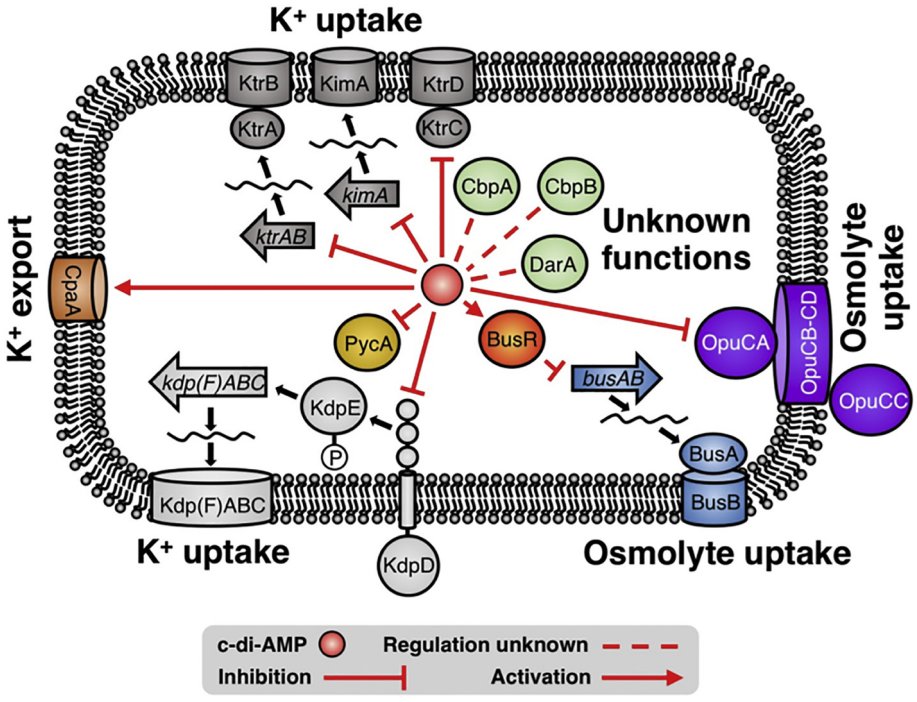

Fig. 6. Overview on the cellular functions of c-di-AMP. The expression of genes encoding potassium transporters is controlled by c-di-AMP, in *B. subtilis* via a c-di-AMP-responsive riboswitch, and in *S. aureus* via the KdpD sensor kinase [135,147,148]. c-di-AMP directly binds to the KtrC subunit of the KtrCD potassium uptake systems of *S. aureus* [134]. Moreover, c-di-AMP stimulates the activity of the *S. aureus* potassium exporter CpaA [149]. The transport activity of the *S. aureus* and *L. monocytogenes* Opu osmolyte uptake system is inhibited by c-di-AMP [150,151]. In lactic acid bacteria, transcriptional repression of the *busAB* genes encoding an osmoprotectant transporter is mediated by BusR, for which c-di-AMP acts as a co-factor for DNA binding [138,152]. The pyruvate carboxylase PycA of *L. monocytogenes* is inhibited by c-di-AMP [136]. The functions of the target proteins CbpA and CbpB from *L. monocytogenes* and DarA, which is conserved in several gram-positive bacteria, have not yet been unraveled [134,136,153]. This figure was previously published [16] and is used here with permission. Artwork was by Fabian Commichau.

Recently Discovered Cyclic Nucleotides with Mixed Base Composition

The final session of the symposium focused on novel nucleotide second messengers and host interactions. The mixed cyclic dinucleotide c-AMP–GMP (also termed cAG or 3′,3′-cGAMP) was first discovered as the product of the enzyme DncV in the El Tor strain of *V. cholerae* and affects *V. cholerae* motility and intestinal colonization in mammalian hosts [8]. The *hy*brid *pr*omiscuous (Hypr) sub-class of GGDEF enzymes can use both ATP and GTP as substrates and is thus capable of producing three bacterial cyclic dinucleotides, that it, 3′,3′-cGAMP as well as c-di-GMP and c-di-AMP [154]. Ming Hammond (University of Utah, Salt Lake City) reported on the role of Hypr GGDEF enzymes in 3′,3′-cGAMP signaling in *Geobacter* (Fig. 7), which uses corresponding riboswitches to regulate genes associated with extracellular electron transfer, an activity that involves bacterial colonization on surfaces [9,155]. Using gene knockouts and riboswitch reporters, her collaborators showed that 3′,3′-cGAMP and c-di-GMP control distinct phenotypes in *Geobacter sulfurreducens*. Combining structural analysis of the Hypr GGDEF domain from *G. metallireducens* GacA (GSU1658, the founding member of Hypr GGDEF proteins) with kinetic modeling, she provided evidence for how Hypr variants of the homodimeric GGDEF enzyme class promote production of 3′,3′-cGAMP over c-di-GMP and the structural basis for substrate binding, cooperative effects, and catalysis.

Chris Waters (Michigan State University, East Lansing) presented the discovery of CapV, a phospholipase in *V. cholerae* that is the first protein receptor for 3′,3′-cGAMP in bacteria [156]. As shown by Geoffrey Severin (from the Waters group) on a poster that won one of the two poster prizes, binding of 3′,3′-cGAMP to CapV, which is encoded by a gene right next to *dncV* on the VSP-1 genomic island, induces membrane remodeling and the generation of free fatty acids although the function of this system is not yet known.

Cyclic dinucleotides also play central roles in host immunity and bacterial pathogenesis. 2′,3′-Cyclic AMP–GMP (“noncanonical “cGAMP or 2′,3′-cGAMP) is produced by cGAS in mammalian cells in response

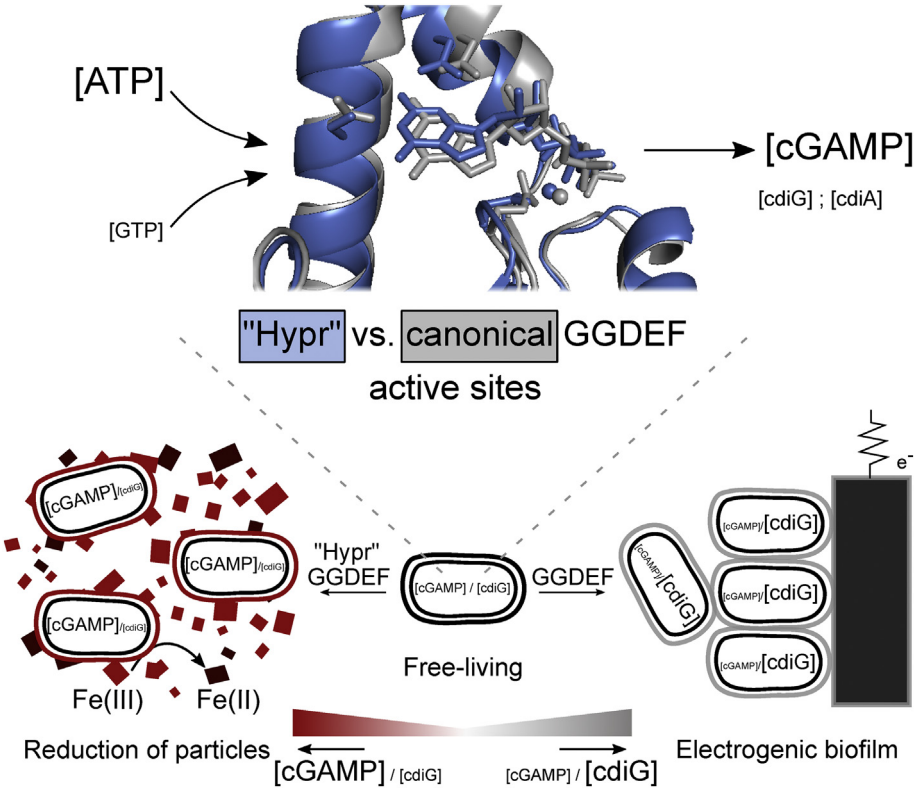

Fig. 7. Roles of 3′,3′-cGAMP and c-di-GMP in *G. sulfurreducens*. The Hypr GGDEF dinucleotide cyclase GacA (previously termed GSU1658 or DncG) produces 3′,3′-cGAMP in *G. sulfurreducens* [154] to control a specific surface-dependent lifestyle that is different from biofilm formation, demonstrating divergence from the canonical cyclic di-GMP signaling paradigm. The figure was kindly provided by Ming Hammond.

to cytosolic dsDNA and is involved in the mammalian innate immune response by binding to the cyclic dinucleotide receptor STING (*st*imulator of *I*F*N g*enes) [157]. However, the mammalian 2′,3′-cGAMP binds to STING with a much greater affinity than the bacterial 3′,3′-cGAMP. The group of Joshua Woodward (University of Washington, Seattle) recently identified a new cytosolic pattern recognition receptor, the host oxidoreductase RECON (reductase controlling NF-κB), that shows high affinity binding of the bacterial nucleotides c-di-AMP and 3′,3′-cGAMP, but not of the mammalian 2′,3′-GAMP [158]. During infection with the intracellular bacterium *L. monocytogenes*, which secretes c-di-AMP into the cytosol of infected host cells [13], c-di-AMP binding attenuates RECON's enzyme activity and its negative control of the NF-kB pathway, thereby leading to increased NF-κB activation and reduced bacterial survival. Furthermore, Joshua Woodward also showed that during infection of RECON-deficient hepatocytes, which exhibit hyperinflammatory responses, *L. monocytogenes* exhibits significantly enhanced cell-to-cell spread, with the bacterial cells displaying longer actin tails and increased speed. This behavior is triggered by NF-κB-dependent iNOS expression and NO production [159]. Thus, bacterial c-di-AMP—via RECON—leads to an enhanced NF-kB response, which on the one hand is detrimental to the bacteria, but at the same time also triggers their evasion by enhancing intercellular spreading (Fig. 8).

Philip Kranzusch (Harvard Medical School) reported on their recent discovery of new cGAS/DncV-like nucleotidyltransferase enzymes (DC-NTases) in bacteria that synthesize diverse cyclic nucleotides. Despite very low sequence similarity (<10% identity), mammalian cGAS and bacterial DncV are remarkably similar in structure and show a mechanistically conserved sequential NTase activity, which differs from the mechanism of classical GGDEF domain diguanylate cyclases [160]. Working in collaboration with John Mekalanos' group (Harvard Medical School, Boston), they experimentally characterized the enzymatic products of 66 recombinantly produced purified CD-NTases, and defined diverse mixed cyclic dinucleotides and alternative nucleotide signals. Based on a structural biology approach, the rules dictating how distinct nucleotide products are formed by different CD-NTases could be defined. Bioinformatic genome analyses building upon previous research [161] revealed that DC-NTases comprise an extremely diverse family of >5500 representatives that cluster into eight specific clades. The CD-NTase-encoding *dnc* genes occur on mobile genetic elements in nearly every bacterial phylum, usually in direct combination with genes that seem to encode effector proteins that bind the cognate nucleotides, as already mentioned above for *dncV* and *capV* in *V. cholerae* [156]. The physiological consequence for the bacteria and/or their host is currently unknown.

Conclusions and Perspectives

In general, structures and molecular mechanisms of the various families of enyzmes that make and break nucleotide messengers in bacteria are now well characterized but may still bring surprises (Sondermann, Chatterji, Hammond). However, a still widely open field is the diverse stimuli and potential accessory components that provide for signal input via the usual N-terminal sensory domains of these signal transducing enzymes (Dietrich, Rinaldo, Frankenberg-Dinkel, Lovering). Research highlighted at the meeting has also shown that the list of molecular mechanisms and cellular functions in bacteria that are controlled by nucleotide second messengers is continuously expanding. Recently, a new signal input and many new (p)ppGpp-controlled targets have been found, mostly in gram-positive bacteria (Wang, Turgay, Hallez, Cashel). The diversity of c-di-GMP-binding effectors has already become the topic of dedicated reviews [105,106,162], and more recently discovered effectors were presented at the conference (Malone, SanJuan, Søgaard-Andersen, Yildiz, Becker), but given the structural malleability of c-di-GMP and a minimal binding requirement of just one arginine in a flexible loop of an effector or receptor protein (Galperin), we can safely predict that even more c-di-GMP-binding domains will be discovered in the future.

In addition, numerous intersections between different second messenger signaling pathways within larger cellular regulatory networks are currently emerging. Particularly intriguing are observations that the two guanine nucleotides c-di-GMP and (p)ppGpp can compete with GTP or ATP binding at Walker A motifs in for instance ribosome-modifying GTPases (Corrigan) or in ATPase domains of two-component histidine kinases [163] and thereby can interfere with the corresponding signaling pathways. However, also at other effector binding sites, c-di-GMP and (p)ppGpp can directly compete with each other, which can result in an antagonistic control of a regulatory output (Shyp and Jenal). On the other hand, bacteria with many diguanylate cyclases and PDEs can manage to operate several c-di-GMP signaling pathways with distinct inputs and outputs in parallel by using an abundant and highly active master PDE that drains the global cellular c-di-GMP pool to such a low level that different effector/target systems become dependent on distinct co-localized DGCs for locally satisfying the needs of their respective c-di-GMP-binding sites (Hengge).

Also new physiological functions under the control of ubiquitously occurring nucleotides c-di-GMP or (p)ppGpp continue to be discovered. Besides generally regulating single-cell motility and multicellular adhesion in an antagonistic manner as well as the cell cycle, development and virulence in certain bacterial species, c-di-GMP can also affect chromosome segregation

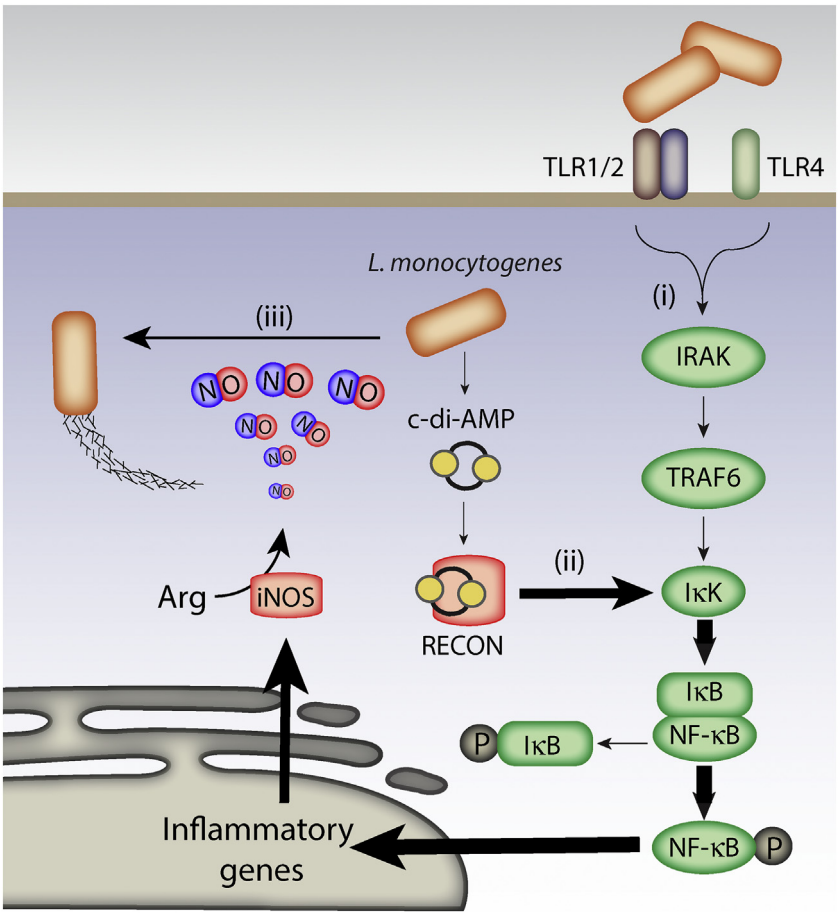

Fig. 8. RECON sensing of c-di-AMP promotes the spread of *L. monocytogenes*. (i) TLR activation by bacteria initiates NF-kB dependent inflammation. (ii) Upon cytosolic invasion, c-di-AMP secreted by *L. monocytogenes* is bound by RECON, resulting in augmented inflammatory gene expression [13,158]. (iii) Elevated expression of iNOS, results in enhanced NO production, which promotes *L. monocytogenes* actin-based motility and cell-to-cell spread [159]. The figure was kindly provided by Joshua Woodward.

(Søgaard-Anderson) and multi-drug efflux pump-mediated antibiotic tolerance in biofilms (Sauer). By contrast, the function of c-di-AMP signaling seems to be somewhat more narrowly focused on homeostasis of potassium and glutamate in the context of osmoregulation (Gründling, Stülke) but now also in development (Tschowri). Strikingly, this function is conserved even in archaea (Albers), which suggests that c-di-AMP may have found its role already quite early in evolution. At the other end of the spectrum, a novel type of structurally flexible dinucleotide cyclase (Dnc), which is associated with mobile genetic elements, has allowed the evolution of a diversity of new mixed cyclic dinucleotides and even larger cyclic nucleotides that seem to target specific genetically linked effector proteins (Kranzusch, Waters). Moreover, bacteria can use various cyclic dinucleotides to manipulate host cell signaling (Woodward).

Overall, this highly inspiring conference has made it amply clear that nucleotide second messenger signaling in bacteria is a highly dynamic field of research that will continue to provide us with exciting discoveries. These will have both fundamental and applied implications in molecular and environmental microbiology as well as in infection biology and biotechnology. The DFG Priority Programme 1879 will continue to accompany, focus and showcase this development with a next *International Symposium on Bacterial Nucleotide Second Messenger Signaling* that is currently being planned for 2021.

Acknowledgments

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ppGpp

†This report focusses on the 31 oral presentations given during the conference, with only a few of the 77 poster presentations mentioned. We try to provide the relevant literature wherever possible (published until December 31, 2018). Unreferenced statements refer to unpublished results presented at the conference, with their inclusion here being authorized by the respective presenters.

Abbreviations used:
cAMP, cyclic 3′-5′ adenosine phosphate; (p)ppGpp, guanosine (pentagon)tetra phosphate; c-di-GMP, bis-(3′,5′)-cyclic diguanosine monophosphate; c-di-AMP, bis-(3′,5′)-cyclic diadenosine monophosphate; cGAS, cGAMP synthase; c-AMP–GMP or cGAMP, (3′,3′)-cyclic-AMP–GMP; SAS, small alarmone synthetase; DGC, diguanylate cyclase; PDE, phosphodiesterase; NO, nitric oxide; pEtN cellulose, phosphoethanolamine-modified cellulose; MSHA, mannose-sensitive hemagglutination.

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