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Editorial

The symbolic power of nucleotide second messengers – or how prokaryotes link sensing and responding to their outside world

Editorial

Nucleotide second messengers are key components of molecular information processing pathways and networks that allow bacteria and archaea to navigate and adapt to an ever changing external world. Being produced and/or degraded by often membrane-associated enzymes that can sense and react to environmental or cellular changes, the intracellular second messenger molecules are in turn sensed by cellular effectors—usually proteins or protein domains, sometimes riboswitches—that trigger directly associated target systems to produce specific molecular reactions. Thus, second messengers are informational molecules, which stand as intracellular molecular symbols or signs for something else, namely, some potentially life-threatening condition, and which inform the executive machinery of the cell that an appropriate adaptive response is urgently needed. It is probably not accidental that two major classes of intracellular molecules with symbolic functions are both derivatives of nucleotides although there is a clear functional division of labour between them. The nucleotide polymers DNA and RNA function in the storage and effectuation of genetic information that has to be maintained over long times. By constrast, nucleotide second messengers are tiny RNAs, which consist only of one or two, often cyclic nucleotides, that have to transiently represent environmental or cellular states in real time. This requires high dynamics of second messenger production and degradation by specific enzymes whose expression and activity has to be tightly controlled by sensory input. It is likely that second messenger signaling is the evolutionary oldest form of molecular information processing, rudimentary forms of which may have already evolved in the early RNA world. The current picture of second messenger signaling in Prokaryotes, however, is of amazing complexity as outlined by Hengge et al. in the introductory review of this special issue, which also serves as a state-of-the-art report from a recent international symposium on the topic.

Overall, two general principles governing second messenger signaling are *representation* and *specificity*—in fact, representation requires specificity in order to be unequivocal. Historically, it was a fortunate coincidence that the first second messenger system that molecular microbiology came across was cAMP/CRP signaling in *Escherichia coli*, which embodies these principles in the most simple form. Here, just one enzyme (adenylate cyclase) synthesizes cAMP from ATP in response to and as an intracellular proxy for glucose starvation—in fact, when the enzyme senses the glucose transport system to run out of substrate. The freely diffusible cAMP then allosterically triggers the effector/target component, the transcription factor CRP, to directly bind specific DNA operator sites and change the expression of alternative carbon source scavenging genes (Green et al. 2014). However, as described by Krol et al. in this special issue, signaling by cAMP/CRP can play different physiological roles in other bacterial species, some CRP-like proteins bind alternative second messengers such as cGMP or c-di-GMP or other ligands, and certain alpha-proteobacteria possess multiple CRP-like proteins as well as multiple adenylate/guanylate cyclases. As shown by Mantovani et al., some cyanobacteria use cAMP as a signal for high availability of bicarbonate, i.e. the substrate for photosynthesis-driven carbon fixation by RuBisCO. Here, cAMP allosterically controls the PII-like signaling protein SbtB, which by direct interaction can attenuate the activity of the bicarbonate transporter SbtA.

Also the 'alarmone' (p)ppGpp was initially discovered and studied extensively in the *E. coli* model, where its cellular concentration inversely correlates with the growth rate, i.e. (p)ppGpp is an intracellular indicator of nutrient limitation and other growth-restrictive conditions. By binding to RNA polymerase directly, it triggers the 'stringent' response and—at higher cellular levels—the RpoS (σ^S)-mediated stationary phase response (Hauryliuk et al. 2015). However, new (p)ppGpp-binding effectors were found even in *E. coli* as shown here by Brückner et al. for a decisive enzyme in LPS biosynthesis. In Gram-positive bacteria, (p)ppGpp was discovered to serve a plethora of effector/target systems. Its physiological effects include modulating the heat shock response and thermotolerance as outlined by Driller et al. as well as antibiotic tolerance, general stress tolerance, persistance and survival as described by Salzer et al. in this special issue. Despite this apparent diversity in output reactions, (p)ppGpp, which usually is produced by only one or a couple of enzymes in a given species, can be seen as the second messenger that generally balances growth against survival in response to nutrient availability and other stress impact (Anderson et al. 2021).

Then came c-di-GMP. It was discovered in the late 1980ies as an initially inconspicous molecule that could enhance the activity of cellulose synthase in a weirdly cellulose-overproducing bacterium nowadays called *Komagataeibacter xylinus*. Yet, researchers began to really take notice of c-di-GMP only some fifteen years later, when it became apparent that enzymes that can make or break it—diguanylate cyclases (DGC) and specific phosphodiesterases (PDE)—are not only ubiquitously encoded in bacterial genomes, but that many species have entire batteries of these enzymes featuring highly diverse sensory input domains. Further research over the recent two decades has shown that c-di-GMP effector components and output reactions are often equally diverse even in single species (Hengge 2009, Jenal et al. 2017). So, what

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kind of conditions does this signaling molecule actually stand for? What about specificity of signaling via this second messenger, if it is produced by multiple enzymes—present and active at the same time—and if it acts on multiple and highly different target processes? Could it be that tiny non-compartimentalized bacterial cells somehow manage to operate several signaling pathways in parallel without noisy cross-talk, even though they all rely on the same *diffusible* second messenger (Hengge 2009)? Research performed in recent years has demonstrated this to be indeed the case. In their review, Junkermeier and Hengge summarize the underlying principles of such highly specific local c-di-GMP signaling, in which c-di-GMP producing and degrading enzymes team up with specific effector-target components in larger protein complexes while an abundant and highly active phosphodiesterase sweeps away the 'spill over' of c-di-GMP in the cytoplasm, and they define the criteria to experimentally detect local signaling. Scherhag et al. present a novel proteomic method to identify proteins present in complexes with membrane-associated c-di-GMP-related enzymes. Spatial polarity of c-di-GMP signaling by positioning single enzymes or such complexes at only one cell pole can generate asymmetric cell division and heterogeneity of phenotypic behaviour within bacterial populations as described for several bacterial species in the review by Kreiling and Thormann. Starting from the observation that certain cyanobacteria possess c-di-GMP-related enzymes with photoreceptor domains, Enomoto et al. summarize current knowledge on c-di-GMP signaling in light-controlled behaviour in these bacteria, which includes motility and phototaxis as well as cell-cell aggregation.

The last twenty years of research have shown the unparalleled diversity of signal input and of the molecular mechanisms of output reactions in c-di-GMP signaling. Nevertheless, the emerging unifying physiological role of c-di-GMP-mediated control seems adhesion and multicellular behaviour—no matter, whether it regulates the production of various fimbriae or highly diverse extracellular matrix components in biofilms, asymmetric cell division where a mother cell remains attached to a surface or biofilm-associated virulence determinants. Furthermore, c-di-GMP-driven multicellularity as a globally protective and maintenance energy-saving strategy is tightly intertwined with the (p)ppGpp-controlled growth-versus-survival balance (Hengge 2020).

c-di-AMP is a more recently discovered signaling dinucleotide, which is prominently present in Gram-positive bacteria, where it is synthesized by one to three diadenylate cyclases (DAC), and in certain archaea. It usually binds to different effector/target systems whose physiological function is associated with ion (mainly K^+), osmotic and cell wall homeostasis and the associated links into central metabolism, as outlined here for the model bacterium *Bacillus subtilis* by Jörg Stülke. Major targets are for instance K^+ and osmolyte transport systems, which are c-di-AMP-controlled via various effector proteins or domains as well as riboswitches. Variations of these themes in the Gram-positive intracellular pathogen *Listeria monocytogenes*, including a link to (p)ppGpp signaling, are described by Schwedt et al. in this special issue. That c-di-AMP is essential for the bacteria that make it but is absent in humans, also provides the basis for DACs potentially serving as antimicrobial drug targets. Such an approach is described by an article by Neumann et al. in this issue. As inhabitants of soil with its huge changes in osmotic conditions due to intermittent rainfall and drought, various *Streptomyces* also make use of c-di-AMP as an internal osmosignaling molecule as reported by Bhowmick et al. here. In the cyanobacterium *Synechocistis*, c-di-AMP is not only involved in osmoregulation, but also in the diurnal control of photosynthesis-related metabolic processes. The review by Mantovani et al. also summarizes how c-di-AMP accumulates upon illumination, which allows it to bind and activate the PII-like signaling protein SbtB for interacting with the glycogen branching enzyme (GlgB), which stimulates the accumulation of glycogen as a carbon storage compound required to later make it through the night. By competitively binding AMP, cAMP (as already mentioned above) and c-di-AMP, SbtB thus integrates signals reflecting the energy state, the carbon supply and the diurnal cycle. To complete the current c-di-AMP picture, van der Does et al. report that c-di-AMP and its physiological function in controlling osmotic adaptation have even be found in archaea, along with several other nucleotide second messengers of yet uncharacterized functions, but with the notable exception of the guanine-based nucleotides c-di-GMP and (p)ppGpp.

Furthermore, some old and new pecularities in second messenger signaling are covered by this special issue. One of these is Ap_4A, a linear dinucleotide produced as a side reaction by aminoacyl-tRNA synthetases that was observed already decades ago. As described by Zegarra et al., Ap_4A is currently emerging as a potential general stress signaling molecule with putative roles in modulating the heat shock response and mRNA metabolism in bacteria and possibly also in stress response pathways in eukaryotes up to humans. As covered in the review by Hengge et al., a most recent discovery in the second messenger field has been the role of many highly specific (oligo)nucleotide signaling molecules in various phage resistance systems, which essentially trigger cellular suicide mechanisms that result in abortive phage infection and thereby in protection of the remaining bacterial population.

As mentioned initially, signaling by second messengers acting as symbolic or semiotic molecules is likely to represent a quite ancient form of signal transduction that has now reached enormous complexity through billions of years of evolution. In addition, it may have given rise to more modern versions of molecular information processing and regulation. Thus, many bacterial species contain proteins with enzymatically inactive or 'degenerate' GGDEF or EAL domains, which—instead of making or degrading c-di-GMP, respectively—evolved to engage in direct and specific regulatory protein-protein interactions (Hengge 2009). After all, also two-component systems use a nucleotide (ATP) for signal propagation, but instead of converting it into a diffusible second messenger, its (γ–)phosphate moiety is used to modify and thereby link the activities of the sensor kinases—that feature similar sensor domains as second messenger-generating enzymes—with the cognate response regulators as effector/target components. In the course of an evolutionary trajectory from simple nucleotide-based enzymatic products assuming symbolic functions via the emergence of parallel signaling by locally acting second messengers, these signal transducing systems have finally attained unprecedented signaling specificity by relying entirely on complex protein-protein recognition.

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