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Sustained sensing as an emerging principle in second messenger signaling systems

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Bacteria utilize a diverse set of nucleotide second messengers to regulate cellular responses by binding macromolecular receptors (RNAs and proteins). Recent studies on cyclic di-GMP (c-di-GMP) have shown that this signaling molecule binds multiple receptors to regulate different steps in the same biological process. We propose this property of the same molecule regulating multiple steps in the same process is biologically meaningful and have termed this phenomenon 'sustained sensing'. Here, we discuss the recent findings that support the concept of sustained sensing of c-di-GMP levels and provide additional examples that support the utilization of sustained sensing by other second messengers. Sustained sensing may be widespread in bacteria and provides an additional level of complexity in prokaryotic signal transduction networks.

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Introduction

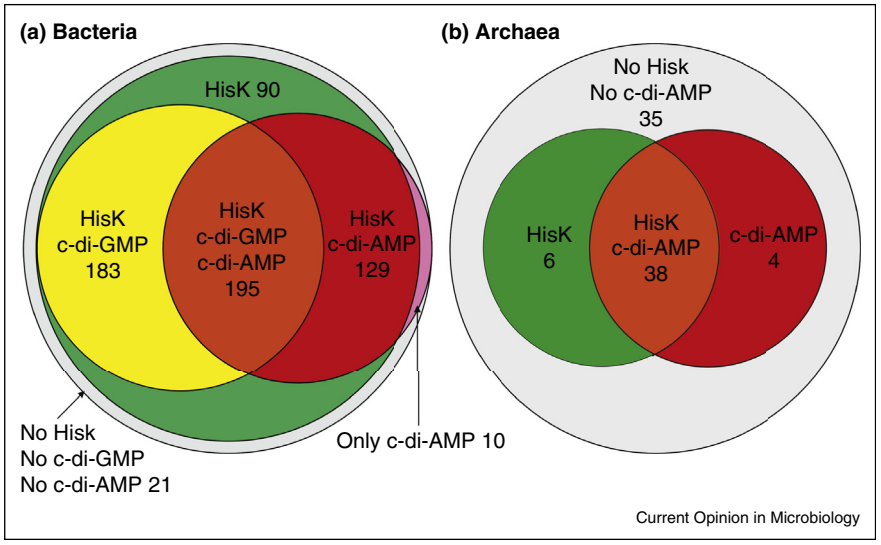
Bacteria utilize signal transduction systems to enact suitable responses to changing environments in order to optimize growth and survival. This is typically a three step process in which the signal is detected, cellular response is triggered, and then the response is terminated to return the system to its initial state. Some of the most widely utilized signal transduction mechanisms in bacteria are the second messenger signaling systems (reviewed in [1]). These signaling systems are found in the majority of bacterial and archaeal species that have completely sequenced genomes (Figure 1). Recently, c-di-GMP, cyclic di-AMP (c-di-AMP) and cyclic-AMP-GMP (cAGMP) have been characterized as new second messengers in bacteria that regulate transcription initiation, post-initiation regulation of the mRNA, and allosteric regulation of translated proteins (Figure 2). Here, we discuss the recent observations that c-di-GMP second messenger signaling may regulate the same phenotype at multiple regulatory steps. We introduce the term 'sustained sensing' to describe this phenomenon. We describe several examples of sustained sensing, the mechanism and utility of sustained sensing, and discuss whether sustained sensing could be a general principle of secondary signaling molecules. If sustained sensing proves to be conserved, it represents another layer of complexity in second messenger signaling.

Discovery of c-di-GMP, its receptors and their implications for second messenger signaling systems

The cyclic dinucleotide second messenger c-di-GMP was first described in 1987 by the Benziman lab as an allosteric activator of cellulose synthase in *Acetobacter xylinum* (since renamed *Komagataeibacter xylinus*) [2]. Since then, c-di-GMP has been shown to be a widely utilized signaling molecule in diverse bacterial species, generally regulating major lifestyle transitions such as the switch from motile, free-swimming planktonic cells to sessile, biofilm-dwelling forms (see [3] for a comprehensive review of c-di-GMP signaling). C-di-GMP regulates these processes by binding to receptors that include transcription factors [4••,5,6,7,8], riboswitches [9•,10•], and other proteins such as those containing a PilZ domain [11,12] and reviewed in [3,13,14]. For many years, the number of c-di-GMP receptors identified in any given genome was far below the number of c-di-GMP synthetases and hydrolases encoded in the same genome. However, just in the past several years, a variety of new c-di-GMP receptors have been characterized [15••,16,17] that partly resolved that conundrum. Furthermore, recent studies show an emerging pattern in which c-di-GMP appears to regulate the same cellular processes at multiple levels. We have proposed to refer to this phenomenon as 'sustained sensing' [15••].

Two examples of sustained sensing that have been described for c-di-GMP are regulation of the synthesis of the Pel polysaccharide in *Pseudomonas aeruginosa* and the mannose-sensitive hemagglutinin (MSHA) pilus *in Vibrio cholerae* (Table 1). In the first example, c-di-GMP regulates *P. aeruginosa* Pel polysaccharide by interacting with

Figure 1



(a) Co-occurrence of two-component systems, c-di-GMP-mediated (yellow) and/or c-di-AMP-mediated (red) regulation in 628 bacterial genera, covered by the recent version of the COG database [62]. There are 90 genera with HisK only (green), 183 with HisK and c-di-GMP (yellow), 195 with HisK and c-di-AMP (red), and 129 with all three signaling systems (orange). There are 10 genera that have only c-di-AMP (pink) and 21 genera that lack all three signaling systems (gray). **(b)** Co-occurrence of two-component systems and c-di-AMP-mediated regulation in 83 archaeal genera, covered by the recent version of the COG database. There are 6 genera with HisK only (green), 38 with both HisK and c-di-AMP (orange), 4 with c-di-AMP only (red). There are 35 genera that lack HisK and c-di-AMP signaling systems (gray). Archaea do not encode genes with COGs related to c-di-GMP signaling.

two protein receptors. The first receptor is FleQ, a transcription factor that enhances transcription of the *pel* operon [4••,17]. The second receptor is PelD, which allows Pel polysaccharide synthesis upon binding to c-di-GMP [18••]. In the second example, c-di-GMP regulates the MSHA pilus in *V. cholerae* also at two levels, the transcriptional level and the level of secretion. Elevated levels of c-di-GMP increase transcription of the *msh* operon that encodes proteins responsible for the assembly and function of the MSHA pilus [19•]. The second c-di-GMP-dependent regulatory step is its binding to secretory ATPase MshE in order to allow export of the MshA pilus to the cell surface [15••,20••].

In addition to regulating transcription factors and protein complexes, c-di-GMP regulates RNA through direct binding to at least two types of riboswitches [9•,10•]. In many cases, these riboswitches are upstream of operons known to be regulated by c-di-GMP, such as those encoding flagella, which are negatively regulated by c-di-GMP binding, and type IV pili, which are positively regulated by c-di-GMP binding in *C. difficile* [10•,21]. Interestingly, the PilB1 (CD3512) protein encoded in the operon downstream of the c-di-GMP-responsive riboswitch in *C. difficile* is an MshE homolog (36.3% identity) that also contains the R9 and Q32 residues that are important for c-di-GMP binding in MshE [15••]. If PilB1 indeed binds c-di-GMP in a manner similar to MshE, type IV pili biogenesis in *C. difficile* would serve as another example of c-di-GMP sustained sensing. In contrast to *C. difficile*, *Clostridium perfringens* has a similar *pil* operon with an apparent c-di-GMP binding MshE-like ATPase (CPF_2570) that, however, is not preceded by a c-di-GMP riboswitch. Therefore, in *C. perfringens*, c-di-GMP appears to regulate formation of type IV pili solely at the level of secretion, indicating that sustained sensing is not involved in regulation of this system. This comparison suggests that recombination of these regulatory elements may aid organisms to adapt their regulatory systems for their unique niches.

Mechanism of sustained sensing

Sustained sensing occurs when two c-di-GMP receptors are involved in regulating the same process. If the receptors for mRNA regulation and post-translational allosteric regulation have different relative affinities, it could result in different regulatory mechanisms playing the key role under certain conditions. There are three theoretically possible scenarios: first, the affinity (the inverse of dissociation constant (K_d)) of receptor 1 is lower than receptor 2 ($K_{d1} > K_{d2}$); second, the affinity of receptor 1 is higher than receptor 2 ($K_{d1} < K_{d2}$); and third, the affinity for both receptors are the same ($K_{d1} = K_{d2}$) (Figure 3). The different combinations of relative affinities of the two regulated steps permit additional possibilities of c-di-GMP regulation beyond a simple on and off switch.