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GUEST COMMENTARY

Cyclic Dimeric GMP Signaling and Regulation of Surface-Associated Developmental Programs▽

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Cyclic dimeric GMP (c-di-GMP) has emerged as a ubiquitous second messenger in bacteria that controls the transition from the free-living, motile lifestyle to the biofilm lifestyle (5, 12, 19). Production and degradation of c-di-GMP are controlled by proteins containing the GGDEF and EAL or HD-GYP domains, respectively. The GGDEF domain harbors intrinsic diguanylate cyclase (DGC) activity, and the EAL or HD-GYP domain has c-di-GMP phosphodiesterase (PDE) activity (4, 21–23). c-di-GMP was initially identified in *Gluconacetobacter xylinus* as an allosteric activator of cellulose synthase (20). Recent studies have shown that c-di-GMP modulates cell differentiation, biofilm formation, motility, and virulence in a diverse group of microorganisms (5, 12, 19). Our understanding of the biological processes and target molecules controlled by c-di-GMP signaling is increasing. However, at present, we have a limited understanding of the mechanisms through which c-di-GMP affects biofilm formation, mechanisms by which the activity of GGDEF/EAL proteins are regulated, and how multiple GGDEF and EAL domain proteins contribute to cellular c-di-GMP levels. In this issue, a new study by Ferreira et al. (8) is beginning to shed light on these questions in *Vibrio parahaemolyticus*, a facultative human pathogen responsible for the most common *Vibrio*-associated, seafood-borne gastroenteritis.

STICKING OR SWARMING

Biofilms are surface-attached microbial communities composed of microorganisms and the extracellular polymers they produce. Biofilm formation is thought to be a developmental process that begins when a bacterium attaches to a surface. After the initial attachment, colonization of a surface is mediated by movement and growth of attached bacteria and production of biofilm matrix components (exopolysaccharides, proteins, nucleic acids, membrane vesicles), leading to the development of mature biofilm structures that are characterized by pillars and channels (18).

Many bacterial processes are involved in biofilm formation. One such process is flagellum-mediated motility, which facilitates attachment to surfaces and colonization of surfaces. *V. parahaemolyticus* possesses two flagellar systems: a polar flagellum and peritrichously arranged lateral flagella (Laf) (16). When grown in liquid environments, *V. parahaemolyticus* is polarly flagellated. However, when on a surface or in a medium of high viscosity, the organism produces lateral flagella, which enable the bacterium to move over and colonize surfaces in a process called swarming motility. When grown on a surface, *V. parahaemolyticus* is also capable of producing a capsular polysaccharide that enables biofilm formation (6, 7, 10). Thus, biofilm formation in *V. parahaemolyticus* involves not only a switch from a motile planktonic state to a surface-attached state but also a switch between the surface-associated motile (swarming) and surface-associated sessile (biofilm) states. It appears that c-di-GMP signaling is involved in the latter process in *V. parahaemolyticus*.

Ferreira et al. (8) and two earlier studies (1, 13) have shown that an increase in cellular c-di-GMP levels prevents swarming motility and promotes biofilm formation. These studies determined that the *scrABC* and *scrG* loci (*s*warming and *c*apsular polysaccharide gene *r*egulation), which were originally identified by their ability to induce *laf* gene expression when grown in liquid medium (a condition that normally represses *laf* gene expression), inversely regulate swarming motility and biofilm formation in *V. parahaemolyticus*. In the *scrABC* operon, *scrA* is predicted to encode a pyridoxal-phosphate-dependent enzyme, *scrB* encodes an extracellular solute-binding protein, and *scrC* encodes a protein that harbors both GGDEF and EAL domains on the same polypeptide and is predicted to be a bifunctional protein with DGC and PDE activities (1, 8). ScrG harbors a PAS domain and a tandemly arranged GGDEF-EAL output module. ScrG lacks the conserved residues in the GGDEF domain that are critical for DGC activity. The ScrG EAL domain is also not conserved (ESL); however, ScrG functions as a PDE (13). Null mutants of *scrABC* and of *scrG* exhibit decreased *laf* and increased *cps* gene expression. Consequently, these mutants have reduced swarming motility and enhanced biofilm formation. Epistasis analysis indicates that ScrG and ScrABC act in the same regulatory circuitry and that *scrG* and *scrABC* double mutants show a cumulative effect at the level of *laf* and *cps* gene expression. It is noteworthy that neither *scrG* nor *scrC* mutants exhibit a defect in polar flagellum production and swimming motility, indicating that these specific proteins are not involved in swimming-versus-swarming decision making (1, 13). Although we now know many of the molecular players, we still do not understand the molecular mechanism(s) through which c-di-GMP controls the transcription of the *laf* and *cps* genes.

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Recent studies have revealed that c-di-GMP signaling also inversely regulates swarming motility and biofilm formation in *Pseudomonas aeruginosa*. Swarming motility in *P. aeruginosa* is mediated by the polar flagellum and rhamnolipid surfactants (3). Biofilm formation requires the production of glucose- and/or mannose-rich exopolysaccharides, produced by *pel* and *psl* loci, respectively (9, 11, 15). It was shown that SadC, a membrane-localized diguanylate cyclase, inversely regulates biofilm formation and swarming motility in *P. aeruginosa* (17). The *sadC* mutant is defective in transitioning from reversible to irreversible surface attachment and has increased swarming motility and decreased biofilm formation. Another component of c-di-GMP signaling regulating surface-associated behaviors in *P. aeruginosa* is BifA, a membrane-localized protein with PDE activity (14). The *bifA* mutant has a hyperbiofilm phenotype and an increased capacity to produce the Pel polysaccharide and is defective for swarming motility. One of the main effector proteins controlling inverse regulation of swarming motility and biofilm formation is SadB, a cytoplasmically localized protein capable of modulating flagellar reversal in a viscosity-dependent manner (2). SadB mutants exhibit increased swarming motility and a decrease in biofilm formation. Transcription of *pel* genes is not altered in SadB mutants, indicating that its effect on Pel exopolysaccharide production is at the posttranslational level (2). The effects of SadB on biofilm formation and swarming are mediated by modulation of flagellar rotation only in high-viscosity environments by the components of the chemotaxis IV cluster system (2). Epistasis analysis has revealed that SadC, BifA, and SadB are components of a c-di-GMP signaling network that controls surface-associated motile and sessile behaviors, via a chemotaxis-like regulatory system, in *P. aeruginosa* (14, 17).

MODULATION OF ACTIVITY OF GGDEF-EAL PROTEINS

One of the least understood aspects of c-di-GMP signaling is how the activity of proteins containing conserved GGDEF-EAL modules is regulated. The study by Ferreira et al. also contributes to our understanding of the enzymology of bifunctional proteins. Ferreira et al. showed that overexpression of *scrABC* induces the expression of *laf* genes and represses the expression of *cps* genes (8). Interestingly, overexpression of *scrC* in the absence of *scrAB* does not induce the expression of *laf* genes but induces *cps* gene expression (8). This finding suggests that ScrC activity is modulated by ScrAB. Through genetic analysis, coupled with c-di-GMP quantification, the authors show that ScrC is a bifunctional enzyme. ScrC can act as a DGC and is capable of producing c-di-GMP. However, when produced together with ScrA and ScrB, ScrC functions as a PDE and degrades c-di-GMP. It is proposed by Ferreira et al. that the activity of ScrC could be modulated by environmental factors; however, ScrC does not possess sensory modules. The authors propose that ScrB, which is localized to the periplasm, can function as a "sensory module." According to their model, in response to an environmental signal, ScrB interacts with the periplasmic domain of ScrC and modulates the activity of cytoplasmically localized GGDEF-EAL output domains.

Another elegant example of a bifunctional c-di-GMP signaling protein is bacteriophytochrome BphG1 from *Rhodobacter sphaeroides* (24). Bacteriophytochromes function as red/far red light receptors that often regulate pigment production and motility; however, the role of BphG1 remains unknown. BphG1 harbors a PAS-GAF-PHY photosensory module and a tandemly arranged GGDEF-EAL output module. It has been shown that BphG1 can function either as a light-dependent DGC or as a light-independent PDE (24). The switch between the opposite enzymatic activities is predicted to be mediated by cleavage of the C-terminal EAL domain. It is proposed that a strong dimerization between EAL domains of BphG1 prevents "productive dimerization" of the GGDEF domains critical for DGC activity, which "locks" BphG1 in the PDE mode. Upon cleavage of the EAL domain, the GGDEF domain regains flexibility and can undergo conformational changes, which are activated by red light and lead to expression of DGC activity.

Understanding how bacteria sense and adapt to life on surfaces is fundamental to our understanding of the molecular mechanisms of biofilm formation and dissolution. It is clear that c-di-GMP signaling is an integral part of biofilm development dynamics. c-di-GMP not only regulates the transition from the motile planktonic to the sessile life style in a diverse group of microorganisms but also regulates the transition from a surface-attached motile to a sessile life style. A major question in c-di-GMP signaling is how c-di-GMP production turns into a specific cellular response. It is becoming clear that c-di-GMP can exert its effect at both the transcriptional and posttranslational levels. Determining the molecular mechanisms through which c-di-GMP affects biofilm development dynamics remains a challenging but exciting area of investigation.

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