Think globally, act locally: How bacteria integrate local decisions with their global cellular programme

Urs Jenal

Biozentrum, University of Basel, Basel, Switzerland. Correspondence to: urs.jenal@unibas.ch

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It has long been speculated that the bacterial second messenger c-di-GMP, a key molecular switch of bacterial community behaviour and persistence, can trigger specific cellular processes both on a global level as well as through some local action that prevents crosstalk originating from rapid diffusion of the signalling molecule. In this issue, Lindenberg *et al* (2013) report on a mechanism that not only helps to explain how spatial sequestration of c-di-GMP signalling components could be organized within bacterial cells, but also demonstrates how global and local command levels are interconnected.

One of the fundamental changes in behaviour of bacterial cells, the switch from motile single cells to surface grown sessile communities called biofilms, is regulated by the second messenger c-di-GMP. C-di-GMP is widespread in the bacterial world influencing different cellular processes on the transcriptional, translational and post-translational level (Hengge, 2009). Synthesis and degradation of c-di-GMP are catalysed by GGDEF domain diguanylate cyclases (DGCs) and by EAL or HD-GYP domain phosphodiesterases (PDEs), respectively (Schirmer and Jenal, 2009). Differential cellular concentrations of c-di-GMP resulting from the regulated activities of DGCs and PDEs are converted into specific readouts by a range of effector proteins and RNAs. Most bacteria encode more than one member of the GGDEF and EAL/HD-GYP domain families and many organisms contain a multitude of these components. This observation raised the question if multiple DGCs and PDEs contribute to a common cellular pool and thus converge into co-regulating related cellular processes or if there is functional sequestration that allows the cell to activate distinct processes in parallel without significant crosstalk. Such functional sequestration was predicted in several bacteria by genetic studies demonstrating that individual DGCs or PDEs target distinct c-di-GMP-dependent processes.

In general, two different regulatory scenarios can explain c-di-GMP signalling specificity. Serial activation of DGCs could lead to a graded response regulating a series of downstream effectors in a defined order, which is dictated primarily by the allosteric inhibition constants of the DGCs and by the binding affinities of the effector molecules (Christen *et al*, 2006; Pultz *et al*, 2012). Although still

serving a common c-di-GMP pool, in such a setting DGCs would have kinetic specificity for a given readout. As an alternative, it was proposed that signalling specificity involves some local organization of DGCs and/or PDEs generating separate pools of c-di-GMP. Evidence in favour of spatial sequestration comes from examples demonstrating that DGCs and PDEs can directly interact with each other and that they can localize to specific subcellular sites (Abel *et al*, 2011; Tuckerman *et al*, 2011). Despite of rapid diffusion of

Figure 1 Model for the regulation of the motile-sessile switch by hierarchical modules of c-di-GMP-metabolizing enzymes in *E. coli*. In the motile single-cell state, the global cellular programme set by module I is dominated by the PDE YhjH, which licences cell motility and keeps module II inactive. Under these conditions, *csgD* expression is OFF as a result of the inhibitory action of YciR on YdaM and MlrA (left). Upon induction of the DGC YegE by the stationary phase sigma factor sigma S, module I induces a global increase of c-di-GMP levels (right). High levels of c-di-GMP activate YcgR to interfere with cell motility and, through the inactivation of YciR, stimulate the transcription of *csgD* and curli formation.

c-di-GMP, spatial sequestration of DGCs and PDEs in microcompartments of larger complexes might couple specific signalling input directly with downstream targets. In this issue, Lindenberg *et al* (2013) propose a surprising new mechanism for local action of c-di-GMP that resembles this signalling architecture, but avoids the need for spatial gradients of c-di-GMP.

In *E. coli*, the motile-sessile switch involves the inverse transcriptional control by the stationary sigma factor sigma S and by the flagellar master regulator FlhDC (Pesavento *et al*, 2008). The flagellar regulon includes the structural components of the rotary nanomotor and the PDE YhjH, which acts as a gatekeeper for motility (Boehm *et al*, 2010). Sigma S, in turn, mediates the expression of several components involved in c-di-GMP metabolism including the DGCs YegE and YdaM, as well as the PDE YciR (Weber *et al*, 2006). Genetic studies indicated functional sequestration of these components since *yegE*, but not *ydaM*, mutants were able to suppress the motility defect of cells lacking YhjH (Pesavento *et al*, 2008). In contrast, all four c-di-GMP components (YegE, YhjH, YdaM and YciR) are involved in mediating the expression of *CsgD*, a central biofilm regulator that activates the genes for cellulose matrix and curli fibres (Figure 1). Transcription of *csgD* requires RNAP containing sigma S and MlrA, a transcription factor of the MerR family. Moreover, genome-wide transcription studies had indicated that YdaM, YciR and MlrA act specifically and exclusively to control *csgD* transcription. This called for a mechanism explaining how YdaM and YciR could act so specifically on one cellular process, while YegE and YhjH, although involved in the regulation of the very same process, are more pleiotropic.

In their study, Lindenberg *et al* (2013) first used genetic epistasis experiments to dissect the interactions of the upstream components of *csgD* expression control. These experiments led the authors to conclude that YegE/YhjH (module I) and YdaM/YciR (module II) operate at different levels and that module I controls *csgD* expression through module II. A key observation was that mutants lacking YciR were 'blind' to the input of YegE and YhjH, but were still strongly affected by the DGC YdaM. Likewise, while the YegE could be substituted by a heterologous DGC, this was not possible for YdaM, indicating that YdaM plays a more specific role in *csgD* expression. Epistasis thus defined a cascade of two c-di-GMP signalling modules directing *csgD* transcription with the PDE YciR that acts as connector between the two modules (Figure 1). Finally, epistasis also positioned MlrA downstream of module II indicating that YdaM/YciR specifically control the activity of this transcription factor.

These genetic experiments were in line with the idea that YdaM and YciR act on *csgD* within an enclosed, 'locally acting' signalling compartment. In agreement with such a notion, protein-protein interaction studies demonstrated direct interactions between YdaM, YciR and MlrA. Robust interactions were observed between the catalytic domains of YdaM and YciR, respectively, and the C-terminal domain of MlrA. As the C terminus of other MerR-like regulators functions as ligand-binding domain, MlrA may indeed be the direct target of these two enzymes. Interaction studies with individual subdomains and truncated variants further indicated that the N-terminal PAS domains of YdaM and YciR are not only involved in the pairwise interaction of the two

enzymes, but also modulate their interaction with MlrA. Apparently, the three proteins form a tight and possibly dynamic complex with various domain contacts.

In a next step, the authors targeted specific substructures of the module II complex. While strains lacking YciR showed hyper-activation of curli expression, YciR active site mutants not only lost PDE activity but also showed constitutive inhibition of *csgD*. This argued that the EAL domain of YciR, although being a *bona fide* PDE, also serves to sense c-di-GMP levels from module I and to transmit this information to the downstream components. The signalling logic would predict that YciR is an antagonist of its partner YdaM and that c-di-GMP binding in the active site of YciR would alleviate YdaM activity. In support of this idea, Lindenberg *et al* (2013) showed that YdaM DGC activity was effectively inhibited by stoichiometric amounts of YciR *in vitro*.

Finally, the authors investigated the role of the DGC YdaM for *csgD* expression control. Similar to YciR, strains expressing a catalytic mutant of YdaM showed a very different phenotype from mutants lacking YdaM altogether. While *csgD* expression was completely abolished in a *ydaM*-null mutant, transcription of *csgD* was reduced only by 50% in the presence of a catalytically inactive YdaM. This argued that YdaM-derived c-di-GMP, although contributing to *csgD* control, is not essential for MlrA activation. Rather, as the authors hypothesize, YdaM may activate MlrA by direct interaction. But then why would c-di-GMP originating from YdaM be important for *csgD* control at all? On the basis of some preliminary evidence, the authors argue that YdaM-derived c-di-GMP feeds back into the general, module I-generated pool, which by antagonizing YciR contributes to MlrA activation and *csgD* transcription (Figure 1). If so, module II (YdaM/YciR) would not only constitute a regulatory switch to activate MlrA in response to c-di-GMP generated by the upstream module I, but would at the same time constitute a positive-feedback loop for the higher-level global c-di-GMP system and by that would contribute to a rapid and robust behavioural switch upon entry of cells into stationary phase.

The case is now out for further scrutiny. Questions that require future attention concern the mechanistic details of this elaborate switch. Also, it will be interesting to test to which extent the catalytic activity of YciR can be uncoupled from its trigger function. If binding of c-di-GMP to YciR but not its cleavage is important for the trigger, one would expect that an EAL domain mutant lacking the conserved catalytic base could still function as trigger. The study by Lindenberg *et al* shows that YciR is an enzyme and a trigger. But is the catalytic activity of YciR important for *csgD* control or is this protein primarily acting as a sensor and trigger? The idea that c-di-GMP is both sensed and degraded at the same time seems counterintuitive, as is the idea that YciR would be active as PDE in a state of high cellular c-di-GMP that drives the cells into biofilms.

The work by Lindenberg *et al* (2013) thus exposes members of the DGC and PDE enzyme families as bifunctional proteins that, in addition to their catalytic functions, can control gene expression in response to substrate availability. Proteins coupling metabolite availability to gene expression control are common in bacteria and were recently termed trigger enzymes

(Commichau and Stülke, 2008). The finding that c-di-GMP signalling takes advantage of trigger enzymes is exciting, as it provides a novel entry point into the mechanistic and cellular analysis of this complex regulatory network in bacteria. It will be interesting to investigate which other DGCs and PDEs are trigger enzymes and how such components can be distinguished from conventional DGCs and PDEs. Furthermore, it is conceivable that c-di-GMP signalling employs triggers beyond gene expression control, that is, to

regulate specific processes post-translationally. And finally, it is possible that similar 'local' signalling modules exist that instead of using a trigger mechanism are controlled by the confined production of c-di-GMP and the coupled activation of a c-di-GMP effector output component.

Conflict of interest

The author declares that he has no conflict of interest.

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