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MicroCommentary

Biofilm dispersal: deciding when it is better to travel

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Summary

Bacteria live predominantly in biofilms, and the internal signal cyclic diguanylate (c-di-GMP) is a universal signal that governs the formation and the dispersal of these communities. *Pseudomonas aeruginosa* is one of the most important reference systems for studying bacterial biofilms and contains numerous diguanylate cyclases (DGCs) for synthesizing c-di-GMP and phosphodiesterases (PDEs) for degrading c-di-GMP. However, few studies have discerned how cells in biofilms respond to their environment to regulate c-di-GMP concentrations through this sophisticated network of enzymes. Basu Roy and Sauer (2014) provide insights on how cells disperse in response to an increase in nutrient levels. Their results show that the inner membrane protein NicD is a DGC that controls dispersal by sensing nutrient levels: when glutamate concentrations are increased, NicD is dephosphorylated, which increases c-di-GMP levels and leads to phosphorylation and processing of dispersal regulator BdlA. Processing of BdlA leads to activation of PDE DipA, which results in a net reduction of c-di-GMP and biofilm dispersal. These results suggest biofilm dispersal relies on surprisingly dynamic c-di-GMP concentrations as a result of a sophisticated interaction between DGCs and PDEs.

Most bacteria are starving and living in communities known as biofilms (Donlan and Costerton, 2002), as opposed to living as a free-floating form. These biofilms protect bacteria from stress. Hence, it is within biofilms that the myriad functions of these organisms occur including both beneficial (e.g. global recycling of nutrients and bioremediation) and deleterious ones (e.g. chronic infections) (Wood *et al.*, 2011). Cells form biofilms in response to stress (Zhang *et al.*, 2007); i.e. in response to environmental signals, and biofilm formation is elegantly regulated (Kostakioti *et al.*, 2013). The cells of biofilms are cemented to each other via polysaccharides, proteins, and DNA (Kostakioti *et al.*, 2013); however, biofilms are dynamic, and cells may leave the biofilm for several reasons including the search for a better environment when conditions are unfavourable and to spread when conditions are favourable. This detachment or dispersal requires that the cement that holds the cells together be degraded (Kaplan, 2010) and is also elegantly regulated and involves sensory circuits (Karatan and Watnick, 2009).

In general, increasing concentrations of the second messenger c-di-GMP lead to enhanced biofilm formation. In *Pseudomonas aeruginosa*, for example, increasing c-di-GMP by activating DGC TpbB through phosphorylation, increases mature biofilm formation by 150-fold as a result of 28-fold greater polysaccharide production (Ueda and Wood, 2009).

A key regulator for biofilm dispersal in *P. aeruginosa* is the cytoplasmic regulator BdlA (biofilm dispersal locus A) (Morgan *et al.*, 2006). BdlA was identified by recognizing that bacteria sense gradients in nutrients during dispersal; hence, BdlA was found by searching the genome for chemotaxis-related proteins that have Per-Arnt-Sint (PAS) domains, which are used by bacteria to respond to their environment (Morgan *et al.*, 2006). Along with its two N-terminal PAS domains [one of which binds haem (Petrova and Sauer, 2012a)], BdlA also contains a transduction/methyl-accepting chemotaxis protein domain (TarH/MCP) and is most similar to the *Escherichia coli* chemotaxis protein Aer (30% identity). BdlA is a general biofilm dispersal regulator since it controls dispersal related to several compounds including increases in glutamate, silver, mercury, arsenate, and succinate (Morgan *et al.*, 2006) and nitric oxide (Barraud *et al.*, 2009). Note the deletion of *bdlA* reduces biofilm formation by about sevenfold and increases c-di-GMP about sixfold (Morgan *et al.*, 2006). Additionally, BdlA plays a role in pathogenicity in that cells which lack *bdlA* are more virulent in chronic infections and less virulent in acute infections (Li *et al.*, 2014).

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Critically, BdlA functions as a dispersal regulator after post-transcriptional regulation, and the Sauer group used their discovery of the importance of phosphorylation in biofilm formation in *P. aeruginosa* (Petrova and Sauer, 2009) and the knowledge that dispersal is reduced when phosphate transfer is reduced by inhibiting phosphatases (Sauer *et al*., 2004) to guide them in determining that BdlA is phosphorylated only in biofilms at tyrosine-238 and then cleaved to release a 120 aa N-terminal fragment PASa and the remainder of the protein, PASb-TarH-BdlA (Petrova and Sauer, 2012b). BdlA cleavage appears to be guided by chaperone ClpD that activates protease ClpP (Petrova and Sauer, 2012b), and the protein fragments likely oligomerize via the PAS domains to make BdlA functional (Petrova and Sauer, 2012b) as well as likely oligomerize with PDE DipA (Petrova and Sauer, 2012a). Hence, the extent of BdlA post-transcriptional processing is remarkable and reminiscent of the processing of the eukaryotic signal insulin (Petrova and Sauer, 2012b).

A breakthrough in understanding how cells sense their environment was made when the Sauer group linked the activity of DGC GcbA (PA4833) to this processing of BdlA and biofilm dispersal (Petrova and Sauer, 2012b). This suggested that the paradigm that increasing c-di-GMP levels increases biofilm formation (Povolotsky and Hengge, 2012) is perhaps too simplistic and that more sophisticated models of c-di-GMP regulation are required. Upon reflection, this makes sense since c-di-GMP levels have to be low to increase motility so cells can reach the surface to initiate biofilm formation, then c-di-GMP levels should increase to stimulate biofilm maturation, then c-di-GMP concentrations should be become low again for dispersal. Hence, low c-di-GMP concentrations actually promote initial biofilm formation as has been shown for inactivation of DGCs in *E. coli* which increase early biofilm formation considerably; for example, inactivation of DGCs YeaI, YedQ, and YfiN increase initial biofilm formation by 30-fold, 12-fold, and 18-fold respectively (Sanchez-Torres *et al*., 2011). Therefore, there is precedence for low levels of c-di-GMP increasing biofilm formation, which is contrary to the current accepted paradigm, which makes room for the new idea here that a DGC has to be stimulated to initiate biofilm dispersal via BdlA, with subsequent activation of PDEs.

Basu Roy and Sauer (2014) build on their initial discovery for the importance of DGCs for biofilm dispersal (Petrova and Sauer, 2012b) by identifying through a bioinformatics search for carbohydrate-binding proteins the periplasmic protein that recognizes the change in the carbohydrate concentration that causes dispersal, DGC NicD (nutrient-induced cyclase D, formerly PA4929) (Fig. 1). NicD is clearly shown to be an active DGC, and NicD is required for a specific dispersal response in that it is required to disperse with glutamate and succinate but not with nitric oxide nor with ammonium chloride. The putative periplasmic, carbohydrate-binding region of NicD along with its GGDEF region are required for dispersal with glutamate, and c-di-GMP levels increase when NicD is produced in *P. aeruginosa* and glutamate is added. Phosphorylation of NicD seems to decrease upon glutamate addition (so the dephosphorylated form of NicD is active for producing c-di-GMP) but surprisingly phosphorylation increases upon NO and ammonium chloride addition. BdlA is phosphorylated only in the presence of NicD, and pull-down experiments show NicD associates with BdlA and PDE DipA. Also, BdlA phosphorylation was inversely related to NicD phosphorylation, and BdlA did not increase swarming in a *dipA* knockout. Critically, the *dipA* and *bdlA* mutants do not disperse in the presence of glutamate. Hence, the importance of the study lies in (i) the identification of a membrane protein responsible for a specific dispersal response (DGC NicD) as a result of responding to an outside cue (a seminal achievement), (ii) the realization that c-di-GMP concentrations are dynamic during biofilm dispersal in that they first increase (through DGC NicD upon carbohydrate sensing) and then decrease (through PDE DipA), and (iii) the likelihood that eukaryotic specific signal transduction systems that require protein processing may be evolved from bacterial systems such NicD–BdlA–DipA.

Although much has been determined for biofilm dispersal through BdlA, some areas for future research include determining what enzyme is responsible for phosphorylating DGC NicD, where is it phosphorylated, how does the dephosphorylation increase its activity, and how is the phosphate transferred to BdlA (direct or indirect transfer) (Fig. 1). Also, how are these post-transcriptional modifications regulated by nutrient changes. Furthermore, given the number of DGCs in *P. aeruginosa*, are there roles for other membrane-associated DGCs in regulating BdlA that would result in additional specific biofilm dispersal responses that utilize BdlA as a common means to activate PDEs (since BdlA is important for not only sensing glutamate but for also dispersal with succinate, silver, mercury, arsenate, and nitric oxide)? Also, determining the structure of the tentatively oligomerized two PAS fragments of BdlA and the structure of these fragments oligomerized with PDE DipA would shed light on how oligomerization stimulates PDE activity. It would also be interesting to determine what are the likely redox-dependent signals that interact with BdlA via is haem group and whether these interactions impact its phosphorylation and role as a biofilm dispersal regulator. Since BdlA is conserved in numerous pathogens including *Pseudomonas syringae*, *Yersinia* sp., *Helicobacter* sp., and *Pantoea* sp. (Petrova and Sauer, 2012b), it is clear that the authors have chosen to decipher an important biofilm dispersal system that is applicable to many

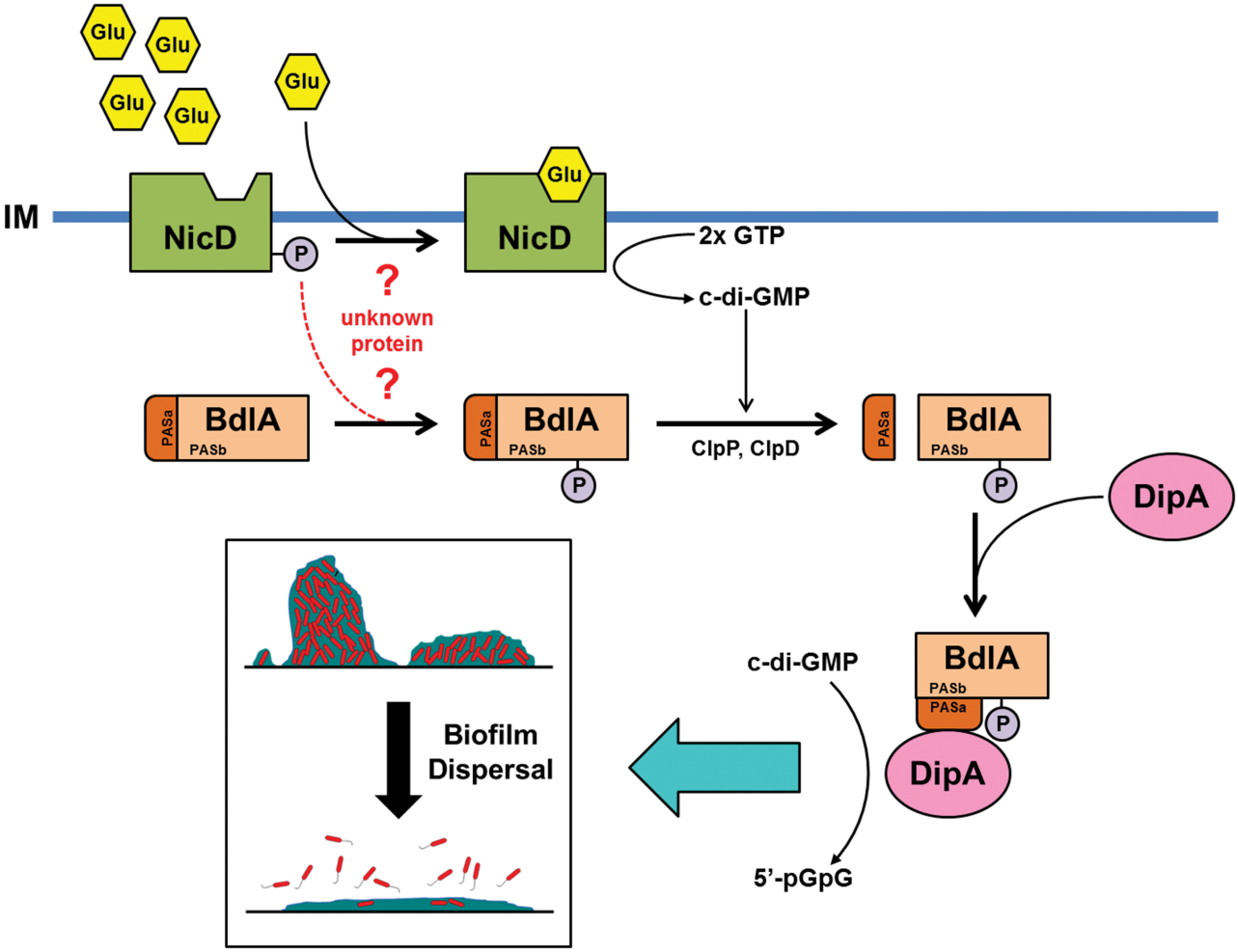

Fig. 1. Mechanism for glutamate-triggered biofilm dispersal in *P. aeruginosa* identified by Basu Roy and Sauer (2014). Glutamate addition results in dephosphorylation of NicD which activates it as a DGC. The increase in c-di-GMP results in phosphorylation of BdlA which leads to cleavage of the N-terminal PASa region of BdlA. The processed form of BdlA with its remaining PAS group (PASb) and its cleaved PASa region likely interact with the PAS region of DipA to result in activation of DipA as a phosphodiesterase. The ultimate reduction in c-di-GMP results in biofilm dispersal. The abbreviation Glu is for glutamate, IM is the inner membrane, NicD is the membrane-bound diguanylate cyclase, P indicates phosphorylation, GTP is guanosine triphosphate, c-di-GMP is cyclic diguanylate, BdlA is the biofilm dispersal regulator that contains two PAS domains (PASa and PASb), DipA is a cytosolic phosphatase, 5′-pGpG is 5′-phosphoguanylyl-guanosine, and ? indicates unknown aspects of the mechanism.

bacteria related to human health; hence, the BdlA network is an excellent candidate for drug development to inhibit the dispersal of pathogens.

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