Signal Synthesis for a Rapid Response

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Control of signal production, crucial to cell-cell communication, is usually at the level of transcription for the density-dependent bacterial signaling. Cheng et al. (2010) provide a structural basis for a novel mechanism that directly regulates the signal synthase.

Although unicellular, bacteria can coordinate individuals within the community for collective behaviors using small signaling molecules. The levels of synthesized signaling molecules reflect the bacterial population density within a given community (Dunny and Winans, 1999). Many bacteria selectively turn on certain genes only when their population rises above a threshold density, a process known as quorum sensing (QS). This process offers an effective way to orchestrate microbial activities, conserving energy and often providing advantages during the infection of host organisms. QS-regulated activities among different bacteria include virulence gene expression, antibiotic production, biofilm formation, bioluminescence, plasmid transfer, and surface motility.

One major class of QS-signal molecules used by Gram-negative bacteria are acylated homoserine lactones (AHLs) (Fuqua and Greenberg, 2002). An AHL-mediated QS system was first discovered in *Vibrio fischeri*, where it controls bioluminescence. The key players in the canonical AHL-based QS circuit are the LuxI-type synthases, which synthesize AHLs, and the LuxR-type transcription factors, whose DNA-binding functions are AHL responsive. Expression of genes encoding the LuxI-type synthases is often under transcriptional control of their cognate LuxR-type proteins. As a result of this positive feedback, AHL concentrations increase drastically once the bacteria reach a quorum, desensitizing the system to subsequent fluctuations in AHL levels. A second AHL-mediated QS system in *V. fischeri* consists of AinS (AHL synthase, unrelated to LuxI) and AinR (AHL sensor) (Ng and Bassler, 2009), and employs two-component signaling to regulate gene expression. AinS also exhibits positive feedback on regulator gene transcription.

α, β-unsaturated fatty acids are an emerging class of signals, termed diffusible signal factors (DSF). First identified in the plant pathogen *Xanthomonas campestris* (Barber et al., 1997), DSF-mediated biological processes are largely associated with pathogenicity in several important pathogens (Boon et al., 2008). The *X. campestris* DSF, *cis*-11-methyl-2-dodecenoic acid (Wang et al., 2004), is synthesized by RpfF, unrelated to LuxI-type proteins. The DSF-sensing mechanism involves RpfC, a two-component histidine kinase (HK) that contains two additional domains compared with typical HK proteins. The RpfC REC domain resembles the receiver domains of response regulators. Following phopshorylation from the HK domain, the REC domain passes the phosphoryl group to a histidine phosphotransfer (HPT) domain, which in turn relays the phosphoryl group to the cognate response regulator RpfG. RpfC controls two independent pathways, one via the HPT domain and the other through the REC domain (He et al., 2006). While the entire phosphorelay (sensor kinase → REC → HPT) is essential to activate virulence gene expression, only the REC domain is required to repress DSF biosynthesis. Strikingly, the downstream target of RpfC in DSF biosynthesis is DSF synthase RpfF via direct protein-protein interaction, and phosphorylation of RpfC presumably releases RpfF. Transcription of *rpfF* is not regulated by DSF; thus, unlike the transcriptional positive feedback on AHL synthases, autoregulation of DSF production is post-translational.

In this issue of *Structure*, Cheng et al. (2010) report structural and mutational studies of the interaction between RpfF and RpfC. They solved crystal structures of RpfF alone and RpfF in complex with the RpfC REC domain and found that RpfF adopts the same enoyl-CoA hydratase/isomerase fold in both forms. This enzyme superfamily contains two Glu residues for catalytic function and a nearby hydrophobic patch for substrate binding, as shown by crystallographic studies (Benning et al., 2000). A structurally equivalent assembly is found in RpfF, and point mutations of either Glu residues or residues lining the hydrophobic patch abolished DSF production, implicating that it is the substrate binding site for DSF biosynthesis. However, structural modeling with acyl-CoA as the DSF precursor shows that both the dimethyl and the CoA moieties exhibit steric clashes with helices α9 and α10 in RpfF structure, suggesting that the structure represents an inactive state. Conformational changes of both helices are required to accommodate the substrate; interestingly, both helices adopt a different conformation in substrate-bound enoyl-CoA hydratases. Within the RpfF-REC complex, the structural change in α10 is forestalled due to extensive intermolecular interaction; therefore, RpfF is locked in the inactive form. Mutational studies on the interacting residues support the functional importance of this interface in inhibiting DSF synthesis. Taken together, the structural and mutational analyses establish a basis for a novel mechanism in regulating DSF production via direct protein-protein interactions. Compared with transcriptional regulation, control at the protein level offers a rapid response to dissociate RpfF for DSF amplification that leads to the timely expression of virulence factors.

This work represents an important step in dissecting the complex DSF-mediated signaling mechanism. Many questions remain. A precursor of DSF, likely to be a lipid, has not been identified. Such information is important in understanding the

enzymatic mechanism of RpfF and the diversity of DSF-like signals that may correlate with bacterial physiology. Structural information on the substrate-acceptance state of RpfF will reveal the determinants to unlock RpfF from RpfC inhibition. Given that the phosphorylatable REC Asp512 is not located in the complex interface, phosphorylation-induced dissociation of REC-RpfF presumably involves domain rearrangement and sequestering the RpfF binding interface; however, this model remains to be confirmed. Finally, mechanistic understandings of DSF perception and transduction may provide insights into why RpfC has evolved into such a different mechanism to efficiently regulate two biological pathways. DSF-mediated activities are frequently related to bacterial virulence expression, and in light of the rise of antibiotic resistance, information on DSF-signaling may afford a rational approach to treat bacterial infections.

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