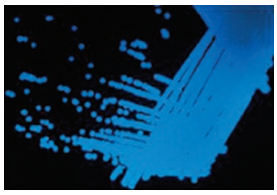
Sense and insensibility



Quorum sensing is a fascinating process that bacteria use to communicate with other bacterial cells. Small molecules called autoinducers are secreted and detected by adjacent bacterial cells, and—when the local concentration of bacteria reaches a certain threshold—the accumulation and binding of the autoinducers to their receptors elicit a number of cellular responses, including bioluminescence and siderophore production. LuxN is the receptor for AI-1, a potent autoinducer from Gram-negative bacterium *Vibrio harveyi*, and the three-dimensional structure of this membrane protein is not known. Bassler and colleagues screened a library of approximately 30,000 LuxN point mutants to identify inactivating mutations, which would confer a dark phenotype even in the presence of AI-1. This screen revealed that the AI-1 binding site is composed of amino acids from four transmembrane helices and two loops on the periplasmic side of the bacterial inner membrane. The authors then screened a library of 35,000 synthetic small molecules to discover non-natural compounds that could antagonize the AI-1–LuxN interaction, finding several structurally distinct antagonists, including C450-0730, which seem to directly compete with the AI-1 binding site on LuxN. In addition to locating the AI-1 binding site on LuxN, the authors unexpectedly found several activating LuxN mutants, which were more sensitive to the autoinducer than the wild-type protein. Although the exact molecular mechanism underlying this observation has yet to be elucidated, the authors propose that these amino acid changes may actually alter the equilibrium between the kinase ('on') and phosphatase ('off') states of LuxN, enabling the receptor to be turned on in the presence of lower concentrations of AI-1. (*Cell* **134**, 461–473, 2008) *JMF*

Cascade defense

Many prokaryotes contain regions of small palindromic repeats similar to the DNA of invaders such as phage and thought to act as a defense system against subsequent infection. These so-called CRISPR regions are often associated with *cas* (CRISPR associated) genes, which have been shown to be essential for host defense. Data from van der Oost and colleagues now test the role of the *cas* genes that are found in the *Escherichia coli* K12 strain but are missing from the BL21 (DE3) strain. By tagging five genes known as *casA–E*, the authors define a complex, called Cascade, some members of which are necessary and sufficient for generation of ~60-nt RNAs representing CRISPR sequences. In addition, Cascade isolation can pull down CRISPR RNAs. Deleting *casE* in particular completely eliminated the 60-nt RNAs, and the gene seems to be essential both for recapitulating pre-CRISPR RNA processing in the *E. coli* BL21 (DE3) strain and for *in vitro* endonucleolytic cleavage of RNA by the purified complex. On the basis of the published structure of a *Thermus thermophilus* CasE-related protein, the authors generated CasE mutated at His20, which is incapable of CRISPR processing. To begin to test the role of these activities in host defense, *E. coli* was supplied with a sequence expected to provide resistance to phage λ infection, an outcome indeed observed, but only if the Cascade complex genes were also present. Together, these results suggest that the Cascade complex has a key role in processing and is associated with CRISPR RNAs, providing a backdrop for further analysis of the mechanism by which this prokaryotic self-defense system operates. (*Science* **321**, 960–964, 2008) *SL*

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Running rings around termination

In bacteria, replication is arrested by means of a protein, Tus, acting at a specific, polar sequence, *Ter*. Two models have been proposed to account for the termination process. In one, the replicative helicase, DnaB in *Escherichia coli*, physically interacts with a Tus bound at *Ter*. In the other model, the unwinding produced by the helicase allows flipping out of a conserved C in the *Ter* sequence, which is captured by Tus; no interaction with DnaB would be required. A recent study by Bastia and colleagues has probed whether direct interaction of the hexameric ring helicase DnaB with Tus, and duplex unwinding near the putative flipped base, are required for termination. The authors devised a substrate that loaded DnaB but caused the helicase hexamer to slide over, rather than unwind, the neighboring duplex region containing the *Ter* sequence. With this substrate, they first determined that sliding of the helicase could be blocked by Tus-*Ter* in the blocking orientation. By adding intrastrand cross-links in the vicinity of *Ter*, they then showed that the helicase does not need to form a bubble to get termination; this suggests that flipping out of the C is not essential. A mutant Tus protein that is defective in interaction with DnaB also showed an effect on termination efficiency. These data support the idea that the physical interaction of Tus-DnaB, rather than any opening up of *Ter* and remodeling of Tus to bind an extrahelical C, initiates replication termination. This result suggests that RNA polymerase, which halts transcription when it encounters a properly oriented *Ter* site, may have a similar interaction domain and that other ring helicases that are insensitive to *Ter* do not. (*Proc. Natl. Acad. Sci. USA*, published online 15 August 2008, doi: 10.1073/pnas.0805898105) *AKE*

Sensing metabolites

Two-component systems have a central role in the ability of bacteria to respond to environmental changes. They are composed of two elements: a transmembrane sensor kinase and a cytoplasmic response regulator. The former contains a modular periplasmic domain that recognizes specific ligands and a conserved cytoplasmic kinase that undergoes autophosphorylation upon ligand binding. The phosphate is then transferred to the cytoplasmic response regulator. To gain insight into ligand sensing by two-component systems, Cheung and Hendrickson solved the crystal structures of periplasmic domains from two sensor kinases: DcuS from *Escherichia coli* and DctB from *Vibrio cholerae*. Both domains recognize C_4-dicarboxylates, but the DctB domain has 270 amino acids compared to only 140 amino acids in the DcuS domain. The DctB structure was shown to contain two subdomains of a similar fold, each of which is also structurally similar to DcuS. All are similar to the sensor domains from citrate sensor CitA and magnesium sensor PhoQ, despite low sequence similarity, leading the authors to define the PDC (for PhoQ-DcuS-CitA) fold. The DcuS domain was crystallized with its ligand malate, whereas the DctB sensor domain structure showed a succinate molecule, presumably from the *E. coli* cytosol, bound to the distal subdomain via interactions similar to those in the DcuS–malate complex. Protein loops surround the ligand pockets from DcuS and DctB, suggesting that ligand-dependent conformational changes are likely to occur. This work provides an initial step in understanding how these periplasmic ligands cause activation of the cytoplasmic kinase domain. (*J. Biol. Chem.*, published online 12 August 2008, doi: 10.1074/jbc.M805253200) *IC*