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Some bacteria have the remarkable ability to switch from a free-swimming, planktonic phase to coexistence in a complex biofilm. In this issue of *NSMB*, the structures of the ligand-bound c-di-GMP riboswitch give insight into some of the molecular processes linked to lifestyle changes but also suggest a potential avenue for applications that begs exploration.

From flocking common grackles, birds that in their hundreds shake the branches of autumnal oak trees and then eat the dislodged acorns, to dolphins that act together to trap hapless fish and then feast, the advantages of cooperative lifestyles are clear. Such examples can be found across kingdoms, from symbiotic lichen to the more sinister bacterial biofilms that form aquatic reservoirs of *Vibrio cholerae*, the causative agent of cholera. *V. cholerae* can cause an acute diarrheal disease that is a leading cause of morbidity and mortality worldwide. Lethal as this organism can be, it is hard not to be fascinated by its ability to move between its different niches, from estuary waters to the human small intestine, an ability requiring orchestrated changes in gene expression that affect diverse pathways, including the flagellar and exopolysaccharide biosynthesis systems.

V. cholerae exists naturally in both salt- and freshwater environments, and its persistence in such reservoirs provides a pool for the initiation of outbreaks as the bacteria leave these havens and reach their human hosts via contaminated food or water. Cholera outbreaks can take a heavy toll and be quite persistent, particularly if sanitary conditions are poor and the population is malnourished. Indeed, a recent outbreak in Zimbabwe that began in 2008 had, by July 2009, resulted in 98,592 reported cases. In addition, these outbreaks can expand into worldwide pandemics.

In the aquatic environment, *V. cholerae* can be found as a free-living, motile form or in a sedentary mode, for example, forming biofilms on chitin, an abundant polysaccharide that is the main component of the arthropod exoskeleton. The switch between these lifestyles is dependent upon a variety of factors and involves inputs from various pathways, including the quorum-sensing system—a logical input given that biofilm formation would require some sense of population density. The pathways have been linked to a number of nucleotide-based second messengers, a key one being cyclic di-guanosine monophosphate (c-di-GMP). The regulation of this second messenger is surprisingly complex. The enzymes that synthesize and degrade c-di-GMP are widespread amongst bacterial species, and multiple versions are often found in a single species, indicating potential complexity in the spatial and temporal regulation of c-di-GMP synthesis and turnover. In addition, the systems for sensing c-di-GMP are diverse and involve multiple protein domains, including the PilZ domain and FleQ, PelD and I-type c-di-GMP–binding effectors.

More recently, a conserved riboswitch, originally called the GEMM riboswitch (RNA element occurring in "genes for the environment, membranes and motility"), has been uncovered. Riboswitches reside in the 5′ regions of particular genes and carry two domains, a ligand binding aptamer domain and an effector region, called the expression platform, that modulates gene expression in response to ligand binding. On pages 1212 and 1218 of this issue, Ferré-D'Amaré and colleagues and Strobel and colleagues present the structures of a ligand-bound c-di-GMP riboswitch from upstream of the *tfoX* gene in *V. cholerae*. In addition, Ferré-D'Amaré and colleagues present small-angle X-ray scattering (SAXS) models of the free and ligand-bound forms. Both crystal structures indicate the basis of ligand-binding specificity, and indeed Strobel and colleagues, through rational mutagenesis, alter the recognition potential of the switch to one that better sees c-di-AMP, a molecule whose function is currently enigmatic. Furthermore, according to the Strobel paper, this riboswitch has a remarkably tight binding affinity, 10 pM (compared to <50 nm-1 μM for the c-di-GMP-binding PilZ protein domains), with a ligand binding half life estimated at 1 month, such that "ligand binding is effectively irreversible on the biological timescale." Determining the implications of the insights gained in these papers for the pathogenesis and lifestyle shifts of *V. cholerae*, and indeed other pathogens, will require further work.

Although the therapeutic potential of these studies against pathogenesis or virulence pathways is rather unclear—though c-di-GMP–responsive genes would be a bacteria-specific target—they highlight how daunting the tasks of understanding and targeting bacterial pathways are. Indeed, much remains to be understood about the basic molecular biology of the pathogens that have such devastating effects on their hosts. At present, countries across the globe are gritting their teeth and have expended, or are preparing to expend, resources to deal with the spread of H1N1 influenza, also known as swine flu. What is clear, as we are biologically and financially accosted by such epidemics, is that we need to further fund basic research—especially given that it continues to surprise us and open potential new therapeutic avenues—as well as research that then capitalizes on this knowledge and explores which targets are viable for therapeutic purposes. In just the last couple of years, the unexpected existence of the c-di-GMP–responsive riboswitches in pathogenic bacteria, and now the structural basis for the ligand selectivity of these switches, have been revealed. An important next step will be to understand whether targeting these riboswitches through structure-based drug design may prove therapeutic. This would ensure that the full potential of this type of research is reached and further elevate the findings in such fields beyond fascinating intellectual insights. Such symbiosis between further basic research and meaningful follow-up is a win-win situation. ■