Filming bacteria

Run your tongue against your teeth and you will feel a slippery, slimy coat. Anton van Leeuwenhoek scraped this film from his teeth and observed the free-floating bacteria in his primitive microscope. Scientists now appreciate that this slimy material, renamed biofilm, consists of microcolonies of bacteria organized into elaborate communities with the ability to cause chronic human diseases.

Formation of a biofilm is a complex developmental process that involves alteration of bacterial-cell physiology and formation of an extracellular matrix. It is a protected mode of growth that allows the bacteria to shield themselves from deleterious agents, such as bacteriophages, biocides or antimicrobials in the environment. For example, the biofilm of *Vibrio cholerae* is shed in the stool of cholera patients and survives to infect subsequent hosts. The ability of this biofilm to survive and retain infectivity is one reason why cholera occurs in epidemic form. Zhu and Mekalanos have previously shown that the virulence of *V. cholerae* is regulated by quorum sensing, the signaling mechanism by which bacteria monitor their cell population. In a recent report, Zhu and Mekalanos (*Dev. Cell* **5**, 647–656; 2003) suggest that the quorum-sensing pathway mediates dispersal of the bacteria from biofilms in order to permit colonization of new sites once a successful primary infection is established.

Severe enteric infection by *V. cholerae* requires cholera toxin and a pilus colonization factor. The expression of these two molecules is controlled by a series of signals originating from two membrane-localized complexes, ToxRS and TcpPH. The transcription of *tcpPH* is indirectly decreased by the activity of HapR, a quorum-sensing regulator. Zhu and Mekalanos show that mutation of *hapR* results in the formation of a thicker biofilm than in wild-type *V. cholerae*. Scanning electron microscopy of wild-type (left panel) and *hapR* mutant bacteria (middle panel) grown for 24 hours identifies

excess bright white irregularly shaped material (enlarged in the right panel) encasing the bacteria. The increased amount of biofilm in *hapR* mutants is due to the overexpression of the *vps* genes, which are required for exopolysaccharide synthesis and biofilm formation. Hence, HapR, in addition to its role in quorum sensing, also negatively regulates transcription of the *vps* genes and keeps the synthesis of biofilm in check.

How does the regulation of the amount of biofilm affect the survival of *V. cholerae* and its ability to infect the host? The bacteria must survive the acidic environment of the stomach before reaching its site of replication in the upper intestine. Therefore, inclusion of the bacterial cells in a biofilm might provide protection from this harsh environment. Zhu and Mekalanos show that the biofilm-associated *V. cholerae* are 1,000-fold more resistant to acidic pH than free-floating cells. Surprisingly, *hapR* biofilms have similar acid resistance to wild-type biofilms even though *hapR* biofilms are thicker. These and other data indicate that the physical structure of the biofilm does protect the bacteria, but the amount of film may be inconsequential to survival in acid.

However, the thicker biofilm may affect the infectivity of *V. cholerae*. The bacteria in the mutant biofilms show a ten-fold reduction in ability to colonize experimental animals, suggesting that the excess exopolysaccharide structure in the *hapR* mutants hinders colonization. Zhu and Mekalanos propose that the biofilm protects the bacteria as it passes through the stomach. Once in the intestine, however, the bacteria must detach from the biofilm and turn off quorum sensing in order to colonize the intestinal walls. Hence, the proper regulation of the biofilm is essential for both survival in a harsh environment and subsequent dispersal of the bacteria to infect the host.

Studies of other pathogenic bacterial biofilms show that antibiotic therapy can reverse the symptoms caused by the free-

floating bacterial cells, but generally fails to kill the biofilm. Furthermore, the protected microcolonies can also withstand the host immune response. These observations suggest that biofilm-mediated infections like cholera could be effectively targeted by developing a new class of antibacterial agents acting against different stages of biofilm maturation, perhaps by affecting specific genes that are activated (and deactivated) during exopolysaccharide matrix growth or quorum sensing. **Evelyn Jabri**

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