MINIREVIEW PROLOGUE

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 291, NO. 24, pp. 12527–12528, June 10, 2016

Introduction to Biofilms Thematic Minireview Series*

Published, JBC Papers in Press, April 21, 2016, DOI 10.1074/jbc.R116.734103

Norma M. Allewell[1]

From the Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, Maryland 20742

The biofilms that many bacteria and fungi produce enable them to form communities, adhere tightly to surfaces, evade host immunity, and resist antibiotics. Pathogenic microorganisms that form biofilms are very difficult to eradicate and thus are a frequent source of life-threatening, hospital-acquired infections. This series of five minireviews from the Journal of Biological Chemistry provides a broad overview of our current understanding of biofilms and the challenges that remain. The structure, biosynthesis, and biological function of the biofilms produced by pathogenic fungi are the subject of the first article, by Sheppard and Howell. Gunn, Bakaletz, and Wozniak focus on the biochemistry and structure of bacterial biofilms, how these structures enable bacteria to evade host immunity, and current and developing strategies for overcoming this resistance. The third and fourth articles present two of the best understood cell signaling pathways involved in biofilm formation. Valentini and Filloux focus on cyclic di-GMP, while Kavanaugh and Horswill discuss the quorum-sensing (*agr*) system and the relationship between quorum sensing and biofilm formation. Mechanisms of antibiotic resistance, particularly the role of efflux pumps and the development of persister cells, are the topics of the final article by Van Acker and Coenye. The advances described in this series guarantee that ongoing interdisciplinary and international efforts will lead to new insights into the basic biology of biofilm formation, as well as new strategies for therapeutic interventions.

Many bacterial and fungal cells have the ability to form biofilms that enable them to exist as communities and to adhere tightly to surfaces. Biofilm formation requires a switch from a motile to a sessile life style, as well as generation of an extracellular matrix. The biofilms of pathogenic microorganisms are a major medical issue, because they are difficult to eradicate and thus contribute to many chronic infections. Well known bacterial examples include *Staphylococcus aureus*, which causes diseases that range from simple skin infections to sepsis, endocarditis, and osteomyelitis, and *Pseudomonas aeruginosa*, often associated with respiratory tract infections in cystic fibrosis. The yeast *Candida albicans* is an example of a fungus with biofilm-forming abilities that enable it to colonize mucosal surfaces and contaminate the surfaces of surgical equipment.

Understanding how biofilms form, respond to environmental cues, and contribute to disease is a complex, multi-faceted challenge. This thematic minireview series focus on five major aspects: the structure, biosynthesis, and function of both bacterial and fungal biofilms; the roles of biofilms in acute and chronic disease; signaling by cyclic di-GMP; the relationship between quorum sensing and biofilm formation; and the role of efflux pumps and persister cells in promoting antibiotic tolerance and resistance.

Sheppard and Howell (1) discuss the structure, biosynthesis, and function of biofilms formed by the pathogenic fungi, *Candida albicans* and *Aspergillus fumigatus*, and compare them with their better known bacterial counterparts. Despite the lack of sequence homology among the fungal and bacterial biosynthetic enzymes, Sheppard and Howell report striking functional similarities in the enzymes and the glycans they produce in biofilm formation, drug resistance, and immune evasion. Potential therapeutic strategies with either specific enzyme inhibitors or enzymes that degrade components of the biofilm show promise for the treatment of these biofilm-associated fungal infections.

The biochemistry of the extracellular material of bacterial biofilms, how this structure enables pathogenic bacteria to avoid host immunity, and existing and developing strategies to overcome this evasion are the subjects of the minireview by Gunn, Bakaletz, and Wozniak (2). Three Gram-negative bacteria (*Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Salmonella enterica*) are used as examples. The extracellular material in the biofilms of these organisms has four major components, exopolysaccharides, extracellular DNA, several types of proteins, and outer membrane vesicles, all of which contribute to promote resistance to host immunity. The physical structure of the biofilm impedes penetration and engulfment by host cells, the DNA and polysaccharides bind and sequester antimicrobial molecules produced by host cells, and the vesicles are believed to traffic materials. The bacterial cells also respond by producing factors that limit the oxidative and non-oxidative capabilities of phagocytic cells and by producing additional extracellular material. Developing novel therapeutic strategies for treating both acute infections and chronic diseases associated with these pathogens requires sophisticated animal models and effective methods for disrupting biofilms both *in vivo* and *in vitro*. The review concludes with an assessment of existing animal models and the use of matrix-degrading enzymes, immunotherapeutics, and small molecule inhibitors to disrupt biofilms.

The transition between motile and sessile lifestyles during biofilm formation involves metabolic, physiological, and phenotypic changes that are coordinated by intracellular signaling pathways that respond to environmental cues. The second mes-

* The author declares that she has no conflicts of interest with the contents of this article.

[1] To whom correspondence should be addressed. E-mail: allewell@umd.edu.

senger cyclic di-GMP (c-di-GMP)[2] acts as the transducing signal for many of these pathways and is the subject of the minireview by Valentini and Filloux (3). Cellular levels of c-di-GMP depend on the relative activities of two families of enzymes, diguanylate cyclases (DGCs) and phosphodiesterases (PDEs). Although each family has a characteristic structural motif, individual members have additional domains that may segregate their activity temporally or spatially, thus enabling different members of each family to respond to different signals and regulate different cellular processes. Valentini and Filloux describe biofilm formation as a developmental process in which cells use c-di-GMP as a checkpoint for transitions between distinct phases, using *P. aeruginosa* as a model organism. Emerging opportunities and challenges include new imaging methods for detecting heterogeneous c-di-GMP levels in individual cells, investigating cross-talk between c-AMP- and c-di-GMP-regulated pathways, and understanding the role of c-di-GMP in regulating antimicrobial resistance.

Kavanaugh and Horswill (4) focus on a second central regulator of biofilm formation and infection, the quorum-sensing system (or *agr* system) of *Staphylococcus*. The quorum-sensing system is turned on at high cell densities and controls the production of exo-toxins and exo-enzymes required for infection and biofilm production and dispersal. The signaling pathway consists of an auto-inducing peptide (AIP), a histidine kinase that is activated by AIP, and the response regulator, a protein that, when phosphorylated by the kinase, binds to four promoters on the chromosome. One of these promoters (P2) is the promoter for the operon for the signaling pathway, which is therefore auto-inducible. The second (P3) controls the production of RNAIII, which up-regulates both exo-toxin and exo-enzyme production via protein-RNA interactions.

Despite these advances in understanding quorum sensing at the molecular level, Kavanaugh and Horswill emphasize that very little is known about how quorum sensing functions in the human environment. The *agr* system appears to operate differently in standard culture conditions and in the human environment, and the conditions required for colonization in humans may be different from the conditions required for infection. Host factors such as serum components, reactive oxygen species, and low pH are likely to play an important role.

Biofilms frequently increase the ability of bacteria to tolerate antibiotics used in treating infectious disease. However, the mechanisms that confirm this resistance are not well understood. The minireview by Van Acker and Coenye (5) focuses on two mechanisms that have been shown to confer protection. At the molecular level, efflux pumps work to maintain low intracellular concentrations of the antibiotic by removing it from the cell. At the cellular level, a subpopulation of cells called persister cells has a phenotype that enables them to survive exposure to high levels of antibiotics and to begin growing again when the antibiotic is removed.

The most thoroughly studied efflux pumps are members of the RND (resistance-nodulation-division) superfamily, whose members characteristically have inner and membrane proteins linked by a periplasmic membrane fusion protein. Several studies in *Escherichia coli*, *S. enterica*, *Klebsiella pneumoniae*, and *P. aeruginosa* have demonstrated linkages between expression and inhibition of pumps and biofilm formation and dispersal, and a transcriptional factor that may link expression of pump genes and biofilm formation has been identified. However, other studies have failed to demonstrate this linkage, indicating that additional factors may also be involved.

The mechanisms that give rise to persister cells are not well understood. A key question, still under debate, is whether persister cells are simply dormant cells with inactive antibiotic targets, or cells with a different metabolism that is actively maintained. Because several factors appear to be involved in the persister phenotype: the oxidative stress, stringent and SOS responses, toxin-antitoxin modules, and levels of metabolic activity, Van Acker and Coenye suggest that the dormant and active models may not be irreconcilable.

During the past decade, substantial progress has been made in recognizing the importance of biofilms in chronic infections, as well as understanding the biochemical and cellular processes that lead to biofilm formation *in vitro*, although many questions remain. Much less is known about how biofilm-forming pathogens interact with their hosts *in vivo*, and about the mechanisms that enable biofilms to resist the action of antibiotics and both innate and acquired immune effectors *in vitro* and *in vivo*. Advances on both fronts are essential for preventing and treating infections. Progress will require technologies that will enable sophisticated analyses of the biochemistry and cell biology of biofilm-forming microorganisms *in vivo*, as well ongoing development of animal models that faithfully mimic chronic infections. In addition, there is a need for innovative methods for disrupting biofilms that do not require antibiotics. Hospital-acquired infections, frequently involving biofilms, are a leading cause of death and thus a major problem not only for individuals but also for health care facilities. The ongoing international and interdisciplinary efforts to understand both the basic biology and pathogenesis of biofilms give confidence that there will be substantial progress in developing new avenues for therapeutic interventions in the coming years.

[2] The abbreviation used is: c-di-GMP, cyclic di-GMP.

References

1. Sheppard, D. C., and Howell, P. L. (2016) Biofilm exopolysaccharides of pathogenic fungi: lessons from bacteria. *J. Biol. Chem.* **291,** 12529–12537
2. Gunn, J. S., Bakaletz, L. O., and Wozniak, D. J. (2016) What's on the outside matters: the role of the extracellular polymeric substance of Gram-negative biofilms in evading host immunity and as a target for therapeutic intervention. *J. Biol. Chem.* **291,** 12538–12546
3. Valentini, M., and Filloux, A. (2016) Biofilms and cyclic di-GMP (c-di-GMP) signaling: lessons from *Pseudomonas aeruginosa* and other bacteria. *J. Biol. Chem.* **291,** 12547–12555
4. Kavanaugh, J. S., and Horswill, A. R. (2016) Impact of environmental cues on staphylococcal quorum sensing and biofilm development. *J. Biol. Chem.* **291,** 12556–12564
5. Van Acker, H., and Coenye, T. (2016) The role of efflux and physiological adaptation in biofilm tolerance and resistance. *J. Biol. Chem.* **291,** 12565–12572