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What does it take to stick around?: Molecular insights into biofilm formation by uropathogenic Escherichia coli

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What does it take to stick around?
Molecular insights into biofilm formation by uropathogenic *Escherichia coli*

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Existence in the biofilm state lends bacteria the opportunity to enjoy, at least for a finite amount of time, the benefits of a multicellular entity. The order of events leading to biofilm formation and disassembly has been the topic of interest for numerous studies, aiming to identify factors and mechanisms that underlie this dynamic developmental process. Of particular import is research leveraged at delineating biofilm formation by medically relevant microorganisms, as prevention or eradication of biofilm from medical devices and from within the host pose a serious challenge in the healthcare setting. Recent research describes how a transcriptional regulator modulates biofilm formation in uropathogenic *Escherichia coli* (UPEC) by affecting the expression of the type 1 adhesive organelles in response to extracellular signals.

The transition from a solitary to a multicellular lifestyle is a complex developmental process that is multi-faceted and dynamic in nature. It involves the orchestrated interplay of regulatory networks that translate extracellular signals to concerted gene expression patterns, thereby tailoring bacterial behavior in response to environmental changes. Initiation of biofilm formation requires the introduction of bacteria to a surface, a partly stochastic process that is driven by Brownian motion, gravitational forces and, where applicable, flagellar motility.[1,2] Upon intercepting the surface, adherence, mediated by extracellular adhesive appendages and adhesin proteins, becomes a property that is critical for successful biofilm development.

Uropathogenic *Escherichia coli* (UPEC) and other *E. coli* pathotypes rely heavily on type 1 pili,[2-7] which are multi-subunit adhesive organelles assembled by the chaperone usher pathway (CUP).[8] UPEC harbor numerous CUP pili systems, the differential expression of which is thought to facilitate colonization of different niches.[9-13] Type 1 pili mediate adherence largely via the FimH tip adhesin, which recognizes and binds mannosylated moieties on biotic and abiotic surfaces.[4,6,14-20] Within the host, FimH mediates UPEC binding to the bladder epithelium and is also required for proper formation of biofilm-like intracellular bacterial communities (IBCs) within bladder epithelial cells.[21]

Regulation of type 1 pili is complex, involving a number of cis- and trans-regulatory factors. The *fim* operon is under the control of a phase-variable promoter, *fimS*,[22] the orientation of which is primarily determined by the activity of FimB, FimE and other recombinases.[23,24] The expression and activity of each recombinase is in turn controlled by several transcriptional regulators.[24,25] Moreover, other regulatory proteins have been shown to influence *fim* transcription, including the nutrient-responsive Lrp, cAMP-CRP and the global regulator H-NS.[26-28] More recent studies have identified the QseC sensor kinase as another regulator that indirectly impacts expression of type 1 pili and interferes with UPEC biofilm formation.[12,29] It is thus apparent that an intricate network of regulatory components is in place to direct and fine-tune expression of type 1 pili in response to varying environmental conditions. Augmenting this complexity is a study by Vila et al.[30] published in this issue of *Virulence*, which describes an additional effector of type 1 pili expression and biofilm formation in UPEC.

Vila and colleagues initiated their studies by investigating the effects of increasing concentrations of salicylate on UPEC biofilm formation.[30] Salicylate is the active ingredient in aspirin and is widely used for its anti-inflammatory effects. It is also a critical intermediate in the biosynthetic pathway that leads to the synthesis of yersiniabactin, a bacterial siderophore that is prevalent among UPEC strains.[31-33] Previous investigations identified a bacterial transcriptional response to salicylate treatment, which leads to the upregulation of multiple drug resistance systems and induces appreciable phenotypic changes.[34] In the current study, Vila et al. observed an inverse relationship between biofilm formation and salicylate, such that sessility is no longer favored at high salicylate concentrations.[30] In an attempt to identify differentially expressed factors in the presence of

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high salicylate levels that may contribute to the observed reduction in biofilm, the authors performed proteome profiling using 2D-gel electrophoresis.[30] This approach revealed that a spot corresponding to FimA, the major pilin subunit of type 1 pili, was significantly reduced in the lysates of UPEC subjected to 1 mM of salicylate. Subsequent RT-PCR analysis verified a reduction in *fimA* transcript, indicating that the effect of high salicylate on type 1 pili occurs at the transcriptional level. Consistent with the observed *fim* downregulation, RT-PCR analysis also indicated a reduction in the transcript of *fimB*, the gene that encodes the recombinase responsible for switching the *fim* promoter, *fimS*, in the ON orientation.[23] Thus, combined, these data indicate that high salicylate concentrations lead to *fim* downregulation by altering the expression of FimB and resulting in switching *fimS* in the OFF orientation. Paradoxically, Vila et al. also report that at the time of their proteome analysis, assessment of *fimS* orientation in the corresponding cultures had not revealed significant phase-switch differences.[30] This apparent paradox is intriguing and could be pointing toward a transcriptional effect on *fim* expression that extends beyond the phase-switch. It is possible that upon downregulation of *fimB* in response to high salicylate, other recombinases present in this UPEC strain invert fimS ON, but *fim* operon transcription is impeded by the activity of a yet undefined transcriptional repressor or the inactivation of a transcriptional activator.

Interestingly, the authors also observed an increase in the expression of the MarA transcriptional regulator.[30] MarA has been previously shown to be upregulated in response to high levels of salicylate and is responsible for inducing the expression of multiple antibiotic resistance systems.[34,35] This implicates MarA induction with regulation of type 1 pili and biofilm formation. To further investigate this connection, Vila and colleagues generated a functional mutation in MarR, the transcriptional repressor of the *marRAB* operon, and investigated the effects of this mutation on the expression of *marA* and *fim*.[30] Indeed, in the isogenic *marR* mutant, *marA* was upregulated while *fimA* and *fimB* were downregulated, supporting a connection between MarA expression and reduced *fim* transcription.[30] Based on these results, the authors concluded that high levels of salicylate negatively impact UPEC biofilm formation by upregulating *marA*, which in turn downregulates type 1 pili expression, albeit via a mechanism that bears further scrutiny.

Collectively, this work points toward another pathway that is used by UPEC to monitor changes in the concentration of small molecules such as salicylate while in the biofilm state and mediate the appropriate cellular response. Notably, although high salicylate concentrations exert a detrimental effect on UPEC biofilm formation, as shown by this study, previous studies established that endogenously produced salicylate is a critical precursor for the biosynthesis of the yersiniabactin siderophore.[32,33] In UPEC, a metabolomic study by Henderson et al. demonstrated a prevalence of yersiniabactin in UPEC vs. coincident rectal isolates, indicating a role for this siderophore during pathogenesis.[31] Consistent with this hypothesis, yersiniabactin biosynthesis genes have been shown to be highly expressed in IBCs in a murine model of infection[36] (Hadjifrangiskou et al., unpublished). More recent studies have demonstrated that disruption of salicylate production by the yersiniabactin biosynthesis pathway in UPEC results in dramatic loss of UPEC pellicle biofilm, which is restored upon exogenous addition of micromolar concentrations of salicylate (Henderson and Hung et al., unpublished data). These findings underscore the significance of physiological concentrations of salicylate as a bacterial signaling molecule, the concentration of which plays a pivotal role on the fate of a UPEC biofilm. This emphasizes the delicate balance that needs to be struck between all participating components of the networks in place, which act as surveillance mechanisms, sampling the extracellular environment and modulating bacterial responses.

The study by Vila et al.[30] is the first to identify MarA as an effector of type 1 pili expression and places MarA in the arsenal of factors involved in resolving the bacterial dichotomy between motility and sessility. Upon induction of stress, downregulation of type 1 pili may be necessary for mobilization of UPEC and dispersal away from the biomass. It is thus possible that induction of *marA* coincides with upregulation of flagella. Previous studies identified a role for toxin-antitoxin systems in relaying stress signals and modulating the shift from the biofilm to the planktonic state.[37] The hierarchical network upstream of *marA* remains unclear. The membrane protein MppA has been previously identified as a potential membrane stress transducer that is found upstream of *marA*,[38] but more recent reports argue against such a relationship.[39] Further characterization of the role of MarA in UPEC biofilm formation and dispersal will provide new insights into the mechanism by which MarA gets induced and how exactly it exerts its regulatory function on type 1 pili and possibly other UPEC biofilm factors.

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