Reply to "Precedence for the Structural Role of Flagella in Biofilms"

Regine Hengge

Institute of Biology, Microbiology, Freie Universität Berlin, Berlin, Germany

In the Serra et al. paper (1), we addressed the question of spatial physiological heterogeneity in *Escherichia coli* macrocolonies that were grown for up to 7 days on agar plates. These are architectonically structured biofilms that develop at a wet medium/air interface. In essence, we observed a two-layer architecture (with a transition zone of changing physiology in between). The bottom layer, which is close to the nutrient-providing agar and extends to the rims of the colonies, exhibits the visual hallmarks of a vegetative growth zone with rod-shaped dividing cells that produce flagella, which get entangled by rotation and thereby generate a filamentous mesh that contributes to cohesion of the colony and overall macrostructure. In contrast, the surface layer consists of ovoid starving cells that are surrounded by a thick network of σ^S-controlled amyloid *curli* fibers that are essential to produce complex macrocolony structures.

One of the outcomes of our study is the proposal of a novel architectural role of flagella in macrocolony biofilms that is not related to motility. In the Discussion, we related our findings to earlier observations that flagella in general are involved in biofilm formation (2, 3). However, we refrain from extending our proposal to submerged biofilms in flow cell devices for several reasons. Not only does the latter type of model biofilm have a "mushroom"-like architecture different from macrocolonies, with nutrients being provided by the surrounding liquid medium, but flagella have been shown by several groups to be required or dispensable for biofilm formation depending on the media and exact conditions used (4–7). The study on temporal gene expression in *E. coli* K-12 biofilms (8) mentioned in the letter by Dr. Wood showed that flagellum genes are expressed also in mature flow cell biofilms and interpreted this in the context of "the previously proposed hypothesis that motility is important for initial attachment as well as movement along the surface in *E. coli*" (2). More-recent studies have shown unexpected, but motility-related, roles of flagella also in mature flow cell biofilms of *Pseudomonas* and *Bacillus* (9, 10). In conclusion, not only should caution be applied in comparing macrocolonies and flow cell-grown biofilms, but elucidating the potential role(s) of flagella in submerged mature biofilms of *E. coli* clearly requires further studies.

The second issue raised by Thomas Wood in his letter refers to renaming *E. coli* genes, which—while not being pertinent to the Serra et al. paper—may be an issue of general interest for the *E. coli* community. When the *E. coli* genome sequence was annotated, protein-encoding genes of unknown functions were given "y" designations. As a community, we in principle have several options to deal with y genes when functions become apparent. (i) With y designations being unequivocal, we could stop renaming genes altogether. While y designations are admittedly mnemonically suboptimal, this would at least avoid further proliferation of gene names and confusing cases where two different genes have been given the same name (11). (ii) We could rename genes only in cases that fulfill a "gold standard" criterion, i.e., when the direct molecular function of the gene product as well as its physiological role have been fully demonstrated *in vitro* and *in vivo*. (iii) We could continue to rename genes based just on mutant phenotypes, i.e., essentially continue with the practice of the pregenomics era. In doing so, we risk using inappropriate names because a phenotype may be affected very indirectly and (in particular for more globally acting regulators) may reflect only a minor effect of a more global function. On the other hand, an increasing number of gene designations, possibly also due to re-renaming when molecular functions are finally demonstrated, may not pose a problem as long as all the names eventually make their way into EcoCyc.

Personally, I prefer the second option or not renaming y genes anymore. The *ymgB*/*ariR* case is actually a good illustration of the problems arising with the third option. The *E. coli* genome sequence suggested *ymgB* to be one of the genes of an obvious operon (*ygcZ-ymgABC*) which encodes four small proteins (of 78 to 90 amino acids only). In 2007, Thomas Wood and coworkers (12) reported that mutations in *ymgA*, *ymgB*, and *ymgC* affect biofilm formation. In addition, a mutation in *ymgB* (but not in the other genes) also results in increased sensitivity to acid that is no longer modulated by indole. While no molecular mechanism was provided to explain these phenotypes, the crystal structure of YmgB was reported and was found to bear structural similarity to Hha (a small regulatory protein that interacts with the nucleoid-associated protein H-NS but does not show any functional overlap with *ymgB*). On the basis of these data, YmgB was renamed AriR (regulator of acid resistance influenced by indole).

In 2009, a paper published by my group (13) showed that the promoter region of the *ygcZ-ymgABC* operon is a direct target of a MerR-like transcription factor (YcgE), which is equally directly antagonized by a blue-light-sensing EAL domain protein (YcgF). Moreover, we could demonstrate genetically that YmgB (and to a lesser extent also YmgA) activates the Rcs phosphorelay system and thereby indirectly (i) stimulates colanic acid production and the expression of *bdm* and *ybgS* (known target genes of the RcsB response regulator) and (ii) inhibits the expression of CsgD and therefore *curli* genes. The finding that the Ymg system stimulates Rcs activity can finally also explain the indirect contribution of YmgB to acid resistance, since RcsB, which can form various heterodimers, is now known to also team up with the GadE regulator to control the expression of acid resistance genes (14). Now that research begins to focus on the direct molecular functions of the Ymg proteins, a gene name dilemma is becoming apparent. How should YmgA or the other operon gene products be named? Is it really appropriate to call YmgB an "acid resistance regulator"? In

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Address correspondence to rhenggea@zedat.fu-berlin.de.

my opinion, final names, although they are likely to reflect some regulatory role in the Rcs system, should be given to these genes only when the direct molecular functions of their gene products have been shown unequivocally.

With respect to citations, it should be noted that in the Tschowri et al. paper of 2009, which demonstrated Ymg proteins to act upstream of the Rcs system, the previous work by Lee et al. (12) as well as by Domka et al. (8) was appropriately cited. The 2012 paper by Tschowri et al. (15) to which Thomas Wood refers in his letter does not deal with the YmgB protein, but it demonstrates the close functional and probably evolutionary relationship between the blue-light-sensing YcgF/YcgE system and the YciR/MlrA system, which controls the expression of the curli regulator CsgD. Based on the direct interaction of YcgF and YcgE and the blue-light-modulated YcgF-dependent release of YcgE from promoter DNA demonstrated with purified proteins *in vitro*, it was finally also proposed to rename *ycgF* and *ycgE*, respectively, *bluF* and *bluR*.

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