UNDER THE LENS

Filming flagella and pili in action

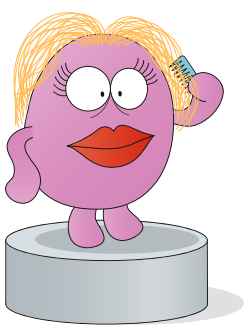
Valentine Lagage and Stephan Uphoff*

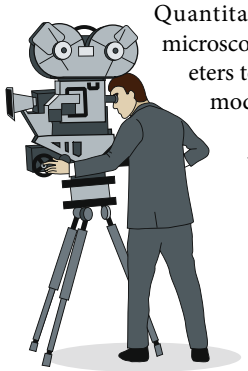
This month's Under the Lens discusses the utility of live-cell fluorescence labelling to record the dynamics of bacterial surface appendages.

Pili and flagella perform crucial actions on the bacterial cell surface such as motility, adhesion and the uptake and excretion of proteins and DNA. These structures can grow to many times the size of the cell, withstand and generate high forces and form and disassemble rapidly in response to environmental cues. Understanding the underlying molecular mechanisms of these features is the aim of intense ongoing research. Two recent studies demonstrate the value of microscopy in investigating cellular dynamics in bacteria.

Zhao et al.[1] examined the formation of flagella in *Escherichia coli*, which are used by these bacteria to swim towards nutrients, and in a separate study, Ellison et al.[2] observed the dynamics of type IV pili during the uptake of DNA by *Vibrio cholerae*. Both studies avoided using common fluorescent proteins because these bulky labels can interfere with protein export and functionality. Instead, the researchers took advantage of the ability to label specific components of the extracellular structures with small synthetic fluorophores. A tetracysteine tag, which can be labelled using a set of dyes called FlAsH (green) and ReAsH (red), was inserted into the flagellin protein. As these dyes only become fluorescent when bound to the tag, there is no need for extensive washing to remove any fluorescent background, allowing continuous labelling of the growing flagella. In a related approach, Ellison et al. generated a mutant of *V. cholerae* that encodes a cysteine residue in the major pilin subunit of the competence pili, which was subsequently used to conjugate a thiol-reactive maleimide fluorescent dye.

During flagellum growth, flagellin subunits traverse the flagellum tube and polymerize when they reach the end. Zhao et al. addressed what determines the flagellum growth rate by sequential labelling, first with ReAsH and then with FlAsH dyes. Using this approach the authors were able to visualize different stages of flagella assembly and found that the growth rate decreases as the flagellum elongates, consistent with the team's previous observation in *Vibrio alginolyticus*[3]. The authors also observed in real time that growth was not continuous but frequently paused. The dynamics of separate filaments on the same cell were correlated, indicating that their pauses had a common origin. The authors suspected that fluctuations in the supply of flagellin subunits could be the cause, and indeed found that over-expression of flagellin reduced the frequency of pauses. Quantitative data extracted from microscopy movies provided parameters to construct a mathematical model of flagella growth.





Credit: Philip Patenall/Springer Nature Limited

In contrast to flagella, type IV pili grow from the cell surface through the polymerization of subunits at the inner cell membrane. Seminal live microscopy experiments by Skerker and Berg[4] showed that bacteria are capable of building and retracting micrometer-long pili on a timescale of seconds. The retracted pili subunits appeared to be recycled into the inner membrane, which was confirmed in another study by Ellison et al[5]. The latest work by the same team related pili dynamics to the uptake of DNA from the environment, visualizing the entire sequence of events from DNA binding and transport to internalization[2]. Using fluorescently labelled DNA, they found that type IV pili of *V. cholerae* capture DNA at their ends. Retraction of pili generates sufficient force not only to bring DNA to the cell surface but also to bend and thread it through a pore in the outer membrane. A ratchet-like mechanism appears to initiate DNA uptake into the periplasm and drive directional transport. This study provides a basis for visualizing the entire process of horizontal gene transfer from DNA uptake to genetic transformation.

By examining living bacteria under the lens, microscopy complements structural, biochemical and genetic analyses to provide not only a more complete picture but also a gripping movie of biological action.

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Competing interests
The authors declare no competing interests.