Probing the initial steps of bacterial biofilm formation: dynamic and molecular principles of surface-based cell motility and mechanosensation

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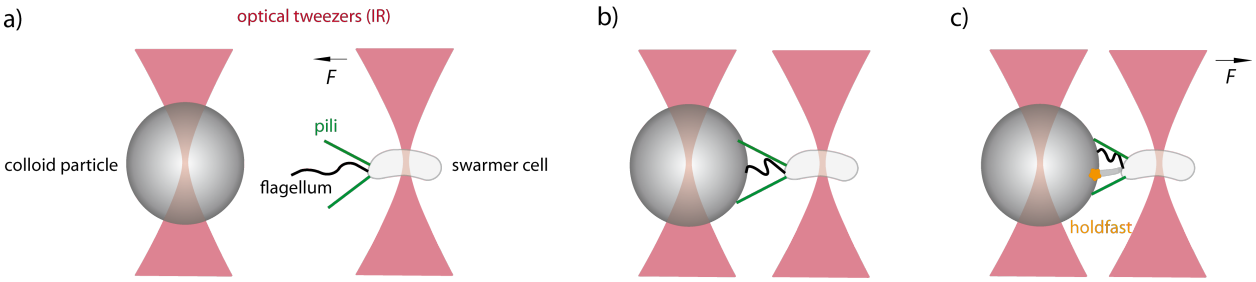
Background

The gram-negative bacterium *Caulobacter crescentus*, which is widely distributed in lakes and rivers, is an important model organism for studying the regulation of the cell cycle, asymmetric cell division, and cellular differentiation.[1-3] An obligate asymmetric division produces two daughter cells with distinct morphologies, behavior, and replicative potential: a motile swarmer cell and a sessile stalked cell. The swarmer cell is equipped with a single flagellum and a chemosensory apparatus, which direct movement in chemical gradients. The stalked cell strongly adheres to surfaces via an exopolysaccharide adhesin, the holdfast, which is located at the end of a cell protrusion, the stalk. During the normal *Caulobacter* life cycle, swarmer cells differentiate into stalked cells after a defined period of motility. This developmental transition coincides with the initiation of chromosome replication and cell division, processes that take place only in the sessile cell type. Thus, *Caulobacter* cells continuously oscillate between two different developmental and reproductive stages, offering an exemplary model system to dissect the molecular and cellular basis for the motile-sessile switch and adaptation to surface-based growth. This transition bears behavioral changes that are highly relevant for growth and persistence of many environmental and pathogenic bacteria. E.g. surface colonization and biofilm formation are key features of chronic infections of numerous human pathogens.[4] Just how exactly this behavioral change is staged and adjusted to environmental cues is still largely unknown. Likewise, how bacterial cells specifically recognize and respond to mechanical signals is poorly understood.



Scheme 1. Using a double trap optical tweezers setup, a trapped swarmer cell will be approached to a surface (trapped colloid particle with modifiable surface). The approaching (a) and attachment (b) of the cell to the surface can be imaged and the acting forces (c) can be measured in parallel.

Work plan

Caulobacter swarmer cells are able to adhere to surfaces through their polar pili followed by irreversibly anchoring through the formation of an adhesive holdfast exopolysaccharide (EPS) at the same cell pole. While this motile-sessile transition occurs within 10-20 minutes in liquid, the swarmer cell is able to adhere to surface within seconds when challenged with surface. While the molecular and cellular mechanisms involved in surface recognition (mechanosensation) and rapid attachment are widely unknown the process can easily be monitored by using a fluorescently labeled lectin that specifically binds to the emerging holdfast EPS. Preliminary studies have indicated that pili and the rotary flagellum play a critical role in this process. Moreover, rapid surface attachment depends on the controlled production of the second messenger cyclic di-GMP. Such studies have led to a model where mechanical sensing occurs by pili-mediated obstruction of the

rotary nanomotor in close proximity of the surface. Motor obstruction is then sensed through some unknown mechanism and transferred to the cell interior to increase c-di-GMP levels and activate the holdfast synthesis machinery. To probe this model and to analyze the mechanism of mechanosensation in more detail, we propose to use a microfluidic-based optical tweezers setup[5]. This will allow to challenge swarmer cells with surface in a highly controlled manner and to study the resulting cellular and molecular events of attaching cells. In scheme 1 the experimental setup is illustrated.

A trap of optical double tweezers will be used to directly catch swarmer cells in order to approach and contact the cells to the surface of a colloidal particle, which is hold by a second optical trap. For imaging the cell motility and cell adsorption mechanism as well as for analyzing the applied and acting forces, we use fast imaging by cameras with frame rates of 400 Hz (fluorescence) and >1000 Hz (bright field). Photo toxicity resulting from the optical setup will be minimized by using IR laser beams and by adding oxygen scavengers into the buffer solution. In such a setup bacteria are able to survive for several tens of minutes in the strong light field of the optical traps[6]. The colloid particle in the second trap emulates the adsorbing surface. Owing to the large variety of commercially available colloidal particles, different surfaces, such as PEG-coated, positively or negatively charged particles, surface geometries/radii and surface roughness can be probed in a straightforward manner. For a controlled exchange of solutions, generation of concentration gradients and calibration of the optical tweezers, the setup will be combined with a microfluidic device.

Initial experiments will optimize optical trapping of swarmer cells and the subsequent surface challenge and attachment to the colloidal particle. High-speed bright field and fluorescence imaging will be used to observe the attachment process. Formation of the EPS holdfast adhesin and binding of fluorescent lectin will be used as readout. Using this experimental system we will then analyze the impact of flagellar rotation, pili and holdfast biogenesis on the attachment process. Defined *Caulobacter* mutants lacking specific components of the motor, pili biogenesis and function, as well as c-di-GMP dependent signal transduction will be analyzed with respect to a specific influence on the adsorption process. Moreover, due to the combined fast imaging and optical tweezers setup, the generated and acting forces can be measured in parallel. After cell attachment, the traction forces of the pili and the holding forces of the holdfast can be measured by moving the two traps apart from each other. Additionally, the impact of different surface properties can be probed by replacing the colloid particles in the second trap with particles of differing surface coatings. Finally, this experimental system allows monitoring the initiation and progression of the cell cycle program in response to challenging cells with surface. For this, we will combine the optical trap with fluorescence microscopy to temporally and spatially resolve specific cellular processes upon surface exposure.

The experiments described here will provide an elegant entry into the analysis of cellular mechanosensation in an experimentally tractable system. Moreover, these studies will shed light on the molecular and cellular processes involved in the very first steps of bacterial surface colonization and biofilm formation. A better understanding of these processes on a molecular level will provide novel opportunities for specific interference with biofilms of bacterial pathogens and with chronic infections, their most prominent clinical manifestation in the human host.

We seek a highly motivated and well-trained individual with a specific interest in biophysics of cellular behavior. Chemists, nanoscientists, biologists, or physicists with a strong interest in interdisciplinary research and a multidisciplinary educational background are encouraged to apply.

References

1. P. D. Curtis, Y. V. Brun, *Microbiol Mol Biol Rev* **74**, 13 (2010).

2. T. Schirmer, U. Jenal, *Nature Rev Microbiology* **7**, 724 (2009).

3. A. Böhm et al., *Cell* **141**, 107 (2010).

4. S. Furukawa, S. L. Kuchma, G. A. O'Toole, *J Bacteriol* **188**, 1211 (2006).

5. M. Engstler et al., *NNFM* **119**, 43 (2012); E. Stellamanns, *PhD-Thesis*, University of Göttingen (2011).

6. M. Koch, A. Rohrbach, *Nature Photonics* **6**, 680 (2012).