Cell dispersal in biofilms: an extracellular DNA masks nature's strongest glue

KIRKPATRICK, Clare, VIOLLIER, Patrick

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MicroCommentary

Cell dispersal in biofilms: an extracellular DNA masks nature’s strongest glue

Clare L. Kirkpatrick and Patrick H. Viollier*
Department of Microbiology and Molecular Medicine, University of Geneva, 1 rue Michel-Servet, 1211 Geneva 4, Switzerland.

Summary

Growth in biofilms provides bacterial species with many advantages over growth in suspension, e.g. colonization of nutrient-rich areas. In the α-proteobacterium Caulobacter crescentus biofilm formation is facilitated through its asymmetric cell division, where one daughter cell becomes a motile flagellated swarmer cell able to colonize new surfaces while the other remains as a stalked cell attached to the substrate through the adhesive holdfast. The Caulobacter biofilm consists of stalked cells arranged either in a monolayer or in a multicellular ‘mushroom’ structure. In this issue of Molecular Microbiology, Berne et al. demonstrate that extracellular DNA (eDNA) from lysed cells prevents biofilm maturation. eDNA masks the adhesive properties of newly synthesized holdfast to enable the escape of swarmer cells from the biofilm. By contrast, holdfasts on previously attached stalked cells remain unaffected by eDNA. Surprisingly, the inhibitory effect was genus-specific, as only DNA from Caulobacter, but not from other genera, could interfere with biofilm maturation. This study reveals a new role for DNA in biofilms, as a regulatory rather than a structural component, and a novel mechanism to facilitate the escape of cells from biofilms. A compelling case is made for the existence of a new type of genus-specific ‘macromolecular language’.

A wide variety of bacteria grow into biofilms in an equally wide variety of environments. Biofilms can form on inorganic or organic surfaces, subject to the presence of the correct environmental signals (Goller and Romeo, 2008; Spormann, 2008; Karatan and Watnick, 2009). Due to its protective multicellular structure, a biofilm formed by bacterial pathogens often exhibits antibiotic resistance and contributes to bacterial persistence in the host (Hoiby et al., 2010). Commensal bacteria form biofilms in many different niches in the human host, in particular the skin, the oropharyngeal membranes and the intestine. Non-commensal bacteria such as Caulobacter crescentus use biofilms for the permanent colonization of suitable environmental niches. Caulobacter crescentus is an oligotrophic bacterium found in freshwater habitats, where nutrient supply is usually low. Caulobacter alternates between motile and sessile developmental states that arise from an asymmetric cell division (Skerker and Laub, 2004). One daughter cell, the stalked cell, remains attached to the substrate via a polysaccharide-based holdfast [also known as ‘nature’s strongest glue’ (Tsang et al., 2006)] at the tip of the polar stalk. The other sibling, a chemotactically active dispersal cell known as the swarmer cell, is equipped with a flagellum and pili at the old pole and cannot carry out chromosome replication. As the swarmer cell samples the environment for nutrients, occasional collisions with surfaces can lead to reversible attachment mediated by pili and/or the flagellum. In response to an unknown (but seemingly hard-wired) signal, the swarmer cell initiates a sequence of developmental events that result in a nascent stalked cell that replicates DNA and proceeds to division. These events include the synthesis of the holdfast at the flagellated pole, followed by the elaboration of the stalk and the concomitant disassembly of pili, the flagellum and the chemosensory apparatus. With the emergence of the holdfast, the initial transient attachment turns into an irreversible anchoring to the surface in a monolayer of cells that are permanently glued to the surface via the holdfast at the stalk tip (Bodenmiller et al., 2004; Levi and Jenal, 2006).

In environments that already contain sufficient nutrients to maintain a population, the swarmer cells will not significantly disperse but will collide and interact with the surface relatively near to their parent cells (Siegal-Gaskins and Crosson, 2008). Subsequently the swarmer-to-stalked cell

Accepted 14 June, 2010. *For correspondence. E-mail patrick.viollier@unige.ch; Tel. (+41) 22 379 41 75; Fax (+41) 22 379 55 02.

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transition leads to the formation of a biofilm, initially as a monolayer, followed by clonal growth of attached cells into multicellular ‘mushroom’ structures (Entcheva-Dimitrov and Spormann, 2004). Biofilms of various bacteria are encased in a protective extracellular matrix that may contain different types of macromolecules, including proteins, polysaccharides and DNA (Karatan and Watnick, 2009). As excessive growth of the biofilm might exhaust the nutrient supply, a mechanism for releasing cells from a biofilm to resume planktonic growth is advantageous. Biofilm dispersal is usually highly regulated and inducible by environmental cues (oxygen or nutrient depletion) or by intracellular or intercell signalling (c-di-GMP or quorum sensing) (Karatan and Watnick, 2009).

In this issue of Molecular Microbiology, Berne et al. (2010) investigate the role of extracellular DNA (eDNA) in Caulobacter biofilm dispersal. Contrary to biofilms from other bacterial lineages that incorporate DNA into the biofilm’s extracellular matrix and even require it as a structural component of the matrix (Whitchurch et al., 2002), Caulobacter eDNA inhibits surface colonization and biofilm maturation. The authors found that this effect was exerted by low molecular mass eDNA, as addition of purified intact genomic DNA from Caulobacter did not affect biofilm maturation. Moreover, progressive partial DNase I digestion of genomic DNA increased its inhibitory activity in inverse correlation to the molecular mass of the DNA fragments. Complete digestion with DNase I, but not with RNase or protease, resulted in loss of the inhibitory activity. eDNA concentration correlated positively with cell death and negatively with biofilm formation, arguing that death and lysis of cells within the biofilm is the source of the eDNA, rather than active secretion of DNA fragments from live cells. These findings raise the possibility that cell death in the biofilm follows a developmental programme, rather than occurring by chance. Caulobacter possesses several chromosomally encoded toxin–antitoxin (TA) systems, which could be candidates for such regulation. TA systems were originally identified on plasmids and are encoded as two-gene operons, where the toxin and antitoxin open reading frames are co-transcribed and often appear translationally coupled. However, the translation products commonly exhibit different stabilities, which provide the mechanistic basis of TA-mediated killing under specific conditions. Because the antitoxin is degraded more quickly than the toxin and the neutralization of the antitoxin by the toxin is stoichiometric rather than catalytic, an imbalance in steady-state levels towards the toxin will unleash the detrimental activity of the toxin and ultimately cause cell death. Such an imbalance can arise when cells lose the plasmid, ensuring that only plasmid-containing progeny remain viable. It might also occur when the rate of synthesis of TA proteins is altered. Interestingly, the messages of the Caulobacter TA operons are differentially induced by growth phase and under various cellular stresses, including oxidative stress and heat shock (Fiebig et al., 2010). As cells in biofilms experience a variety of stresses, these TA operons are strong candidates for density-regulated cell death, causing the release of eDNA and concomitant swarm cell dispersal from Caulobacter biofilms.

Berne et al. undertook several approaches to investigate how eDNA mediates swarm cell dispersal from biofilms. Fluorescence microscopy showed that the eDNA colocalized with the holdfast, which is required for permanent attachment of the stalked cells to the biofilm (Bodenmiller et al., 2004). The authors then employed an in vitro assay with holdfasts purified from a holdfast attachment (ΔhfaB) mutant. The ΔhfaB mutant synthesizes holdfasts that are not attached to the stalk and, hence, accumulate in the supernatant (Cole et al., 2003; Hardy et al., 2010). Binding studies with these purified holdfasts provided strong evidence that the holdfast is necessary and sufficient for DNA binding. Consistent with these in vitro data, a holdfast-deficient mutant could not bind DNA. Flow cell experiments demonstrated that although eDNA binds the holdfast of both swarm and stalked cells and prevents the attachment of differentiating swarm cells to the biofilm, it cannot dislodge cells pre-attached to the surface with their holdfasts. Therefore, the holdfast binding effect of eDNA is specifically inhibitory to the swarm cells (Fig. 1). Presumably, when a threshold level of cell death is reached in the biofilm, the concentration of eDNA from lysed cells becomes sufficient to bind the newly synthesized holdfasts, preventing further growth of the biofilm without dispersing the stalked cells from it. The eDNA thus directly regulates the homeostasis of the biofilm.

The specificity of the eDNA–holdfast interaction was investigated in Caulobacter biofilm growth experiments using spent media, eDNA purified from spent media or sonicated genomic DNA from other bacterial species. These experiments revealed that the form in which the eDNA was supplied was irrelevant, while the genus specificity was clear; only eDNA from Caulobacter prevented biofilm maturation. Even eDNA from bacteria with the same genomic GC content as Caulobacter crescentus (Rhodobacter capsulatus and Brevundimonas diminuta, 67% GC content) had no effect on biofilm growth. Some inhibition was detected in the more sensitive purified holdfast binding assay, but only with DNA from closely related genera, and the degree of inhibition was correlated with phylogenetic proximity rather than GC content. Therefore, the inhibitory effect on Caulobacter biofilms is not a general property of DNA, raising the question whether the sequence context is important for this activity. The authors used an in silico approach to search for enriched sequence motifs in the Caulobacter crescentus genome.
and then compare the frequency of these motifs in Caulobacter with their occurrence in the genomes of the other bacterial strains whose DNA was tested for Caulobacter biofilm inhibition. For a few of the identified motifs, the ordering of frequency of motif occurrence in each genome was correlated with the ordering based on efficiency of holdfast binding for that genome, but the absolute values of motif frequency and holdfast binding did not correlate with each other. Therefore, the mechanism of the observed species specificity of biofilm inhibition remains to be elucidated.

Whatever the mechanism, the species specificity of eDNA biofilm inhibition provides Caulobacter with an efficient modulator of biofilm density. Preventing the attachment of new swarmer cells while retaining the previously attached cells enables the population to take advantage of both the stability of the biofilm and the potential of other habitats, while enabling the dissemination of cells when conditions suddenly become unfavourable. Furthermore, this mechanism minimizes intraspecies competition during sedentary growth, while enabling the establishment of biofilms in the presence of non-kin competitors.

References


