Visualizing microbial interactions and CRISPR-Cas interference using FISH applied to environmental archaeal biofilms

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Background incl. aims
Identifying microbial virus-host interactions is essential to understand viral dynamics in ecosystems and their impact on nutrient cycling. In environmental samples, their identification relies mostly on bioinformatic approaches, i.e., detecting matches between spacers encoded in clustered regularly interspaced short palindromic repeats (CRISPR) systems and their corresponding protospacers present in viral genomes. To complement and confirm these predictions, we established sophisticated microscopy techniques. First, by employing virus-targeted direct-gene fluorescence in situ hybridization (virusFISH), we demonstrated the lytic life cycle of an in silico predicted virus to infect Candidatus Altiarchaeum hamiconexum, an uncultivated free-living, biofilm-forming archaeon, via fluorescence microscopy. Secondly, we characterized the morphological changes of altiarchaeal virocells, i.e. virus-infected prokaryotic cells, using correlative fluorescence and scanning electron microscopy. This effort culminated in a protocol to link a viral genome to the ultrastructure of its virus-like particle and to co-localize these with their host. However, the CRISPR-Cas immune response in single environmental virocells remains unexplored due to current technical constraints.

Methods
As a next step in expanding our microscopy toolkit for the study of virus-host interactions in environmental samples, we utilize various FISH-based methods to visualize the presence and activity of CRISPR-Cas systems within a biofilm of Ca. Altiarchaeum hamiconexum. In the first step, we developed HRP-probes to target the host crRNA of 11 abundant spacer that target a host-specific lytic virus. As the CRISPR spacer diversity in altiarchaeal biofilm is complex, the CARD-FISH based amplification method enhances the signal intensity of low-abundant crRNA in environmental samples.

Furthermore, we developed a distinct set of probes to traget the mRNA of the cas3 protein. Amplification of the signal was achieved by employing 50 different oligonucleotide probes, each fluorescently labeled with so called FLAP tails, targeting the same mRNA segment. The established protocol can also be combined with virusFISH, allowing a nuanced perspective on the dynamic interplay between the CRISPR system and actively infecting viruses in a natural ecosystem.

Results
Our findings uncover the spatial expression of specific CRISPR spacer sets targeting one virus population within an environmental archaeal biofilm, with a confluence of the most abundant spacers targeting the same virus covering nearly the entire biofilm. Additionally, we are able to detect mRNA of the Cas3 protein within our environmental sample. Our two independent fluorescence microscopy methods enabled us detecting active CRISPR-Cas systems in environmental samples along with the targeted viral genomes.

Conclusions
Spacers targeting the same virus within a biofilm can be heterogeneously expressed across the individual cells, resulting in an active defense of the population. Nevertheless, active infections are observed in these biofilms, suggesting an ongoing competition between the host defense system and the virus. In sum, our results and newly developed techniques open novel avenues to understand the intricate relationships between viruses and their microbial hosts.

Keywords:

Microbiology, virology, FlSH, CRISPR, archaea

Reference:

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