228. Isotopic and click chemistry-based approaches for tracking activity in single cells and newly synthesized proteins in uncultured microorganisms catalyzing anaerobic methane oxidation

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Project Goals: Quantifying the activity of uncultured microbial cells within the environment has been a long standing challenge, but represents a key element in understandinging the function, dynamics and resiliency of microbial communities and consortia. Here we describe two complementary methodologies for measuring cellular activity (protein synthesis) by environmental microorganisms using 15N stable isotope probing (SIP) and bioorthogonal noncanonical amino acid tagging, or BONCAT, a click-chemistry method which labels newly synthesized proteins after assimilation of a methionine analog (Hatzenpichler et al., 2014). These methods were coupled with fluorescence and ion imaging (nanoSIMS) techniques for determination of the activity of individual cells within structured microbial consortia mediating the anaerobic oxidation of methane (AOM). At the level of the proteome, 15N-SIP and BONCAT are being coupled with environmental proteomics, allowing for the identification of newly synthesized microbial proteins in environmental samples incubated under different geochemical conditions.

Combinations of BONCAT and 15N-SIP being used to develop a more comprehensive understanding of the potential mechanisms underpinning the anaerobic oxidation of methane (AOM)- a globally important sink for methane. In methane-saturated anoxic sediments, uncultured methane-oxidizing 'ANME' archaea are frequently observed in structured consortia with sulfate-reducing deltaproteobacteria. This physical association has been historically interpreted as evidence for syntrophically-mediated AOM coupled with sulfate-reduction, but the specific mechanism(s) has remained elusive. Through activity-based tracking of methane-oxidizing archaea and associated bacteria, we are gaining new insights into the interspecies interactions occurring within these consortia and the specific pathways used for electron/ metabolite exchange during AOM. Protein- SIP analyses revealed information on the ecophysiology of slow growing methane- oxidizing consortia and specific proteins that are actively synthesized during AOM. Several ortholog proteins believed to be involved in the "reverse methanogenesis" and sulfate reduction pathways were detected, with roughly 1/3 of the total detected protein pool (704 proteins) showing evidence of 15N enrichment. A direct comparison of patterns in 15N labeling of proteins with the newly synthesized protein fraction detected by BONCAT is underway. FISH-nanoSIMS quantification of 15N assimilation by individual archaea and sulfate-reducing bacteria in AOM consortia revealed spatial patterns in activity that are inconsistent with conventional syntrophy based on molecular diffusion and instead appear to support an alternative interspecies electron transfer mechanism, likely facilitated by extracellular multiheme cytochromes. BONCAT experiments were used to track de novo protein synthesis in AOM consortia from sulfidic sediments and CH4 bioreactors over time, revealing enhanced protein synthesis by ANME-SRB consortia in the presence of CH4. The application of fluorescence-based cell sorting of individual, translationally active and FISH-stained consortia from sediments for the generation of targeted ANME consortia metagenomes is in progress, and data from this genome sequencing effort will be valuable for further refinement of our proteomics reference database and will facilitate comparative

genomics studies of the distinct methane-oxidizing archaeal lineages.

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