Spatial Proteomics in Diatoms: Towards a Systems View of Biosilificaiton and Beyond

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Abstract

Diatoms are unicellular photosynthetic algae with intricately ornamented species-specific silica cell walls. The complete understanding of the molecular mechanism guiding the morphogenesis of this hybrid biomaterial remains elusive. To elucidate unknown, but speculated, proteins required for diatom cell wall biosynthesis, we aim to apply proximity-based proteome mapping to the model diatom species *Thalassiosira pseudonana* used in the engineered ascorbate peroxidase APEX2. In addition to advancing our understanding of the long-standing biological problem of diatom silica evolution, these efforts will provide a platform to start cataloging proteomes of other partially understood biological processes in diatoms.

Diatoms

Importance

• Biomonitoring tool for aquatic environments.
• Produce about 20% of the Earth’s oxygen.
• Crucial for C, N and Si biogeochemical cycles.

Model organisms

• Evolution (secondary endosymbiosis).
• Photosynthesis (pyrenoendogy).
• Diatom-bacteria interactions and signaling.
• Silica (SiO₂) biomineralization.

Biosilicification in Diatoms

Figure 1 | Morphological diversity of diatoms. (A) It is estimated there are 100,000 diatom species each encasing itself in a unique glassy shell (Kröger, 2007). (B) Diatom star made by Klaus Kemp, a specialist in diatom microslides and diatom arrangements.

Figure 2 | Biosilicification in diatoms. (A) Diatom cell wall is synthesized inside an acidic compartment called the silica deposition vesicle (SV). (B) It is hypothesized that the interaction of silafins with long-chain polyamines (LCPA) inside the SDV leads to the formation of an organic matrix, the structure of which is controlled by the silafins (Kröger, 2007; Kröger & Poulsen, 2008).

Spatial Proteomics in *Thalassiosira pseudonana*

*Thalassiosira pseudonana*

• Marine centric diatom.
• 14th sequenced diatom species (~11,800 genes).

Overview of the Approach

Figure 6 | Spatial proteomics in *Thalassiosira pseudonana*. (A) *T. pseudonana* strain expressing a TpSil3p-APEX2 protein. (B) Synchronized *T. pseudonana* strain is supplemented with biotin-phenol and (C) hydrogen peroxide to initiate biotin labeling, (red, biotin). (D) Cell lysate is enriched for biotinylated proteins followed by their identification with mass spectrometry. Some expected hits: silafin-modifying enzymes, SDV-sorted receptors and proteins involved in vesicle transport and interactions with cytoskeleton, Si(OH)₂⁻, interacting proteins, and lys1 oxidases.

Results

Conjugation of *T. pseudonana*

Figure 7 | Conjugation of *T. pseudonana*. (A) Genetically modified *T. pseudonana* cells appear on agar plates supplemented with 50 or 100 ng/ml nourseothricin after ~10 days. (B) Transgenic cells from colonies growing in the presence of antibiotic strain type control (left) and isolated colonies containing conjugated cells (right) and on DNA purified from cells (right) yields the expected 0.8 kb product. Side arrows: 0.85 kb (black), 0.65 kb (red).

Endogenous Protein Biotinylation in *T. pseudonana*

Figure 9 | Endogenous biotinylation state in *T. pseudonana*. (A) SDS-PAGE gel stained with Coomassie Fluor Orange (CFO). (B) Streptavidin blot with HRP-conjugated streptavidin. Details: MQ, MQ water; D, A. lysis buffers; O2, D7, A7: *T. pseudonana* lysates using three different lysis protocols; B: 0.4 μg biotinylated BSA (~46.5 kDa); Ec, *E. coli* (N85a) lysate; Sc: S. cerevisiae (BY4743) lysate. *E. coli* contains 1 trimeric biotinylated protein complex, *S. cerevisiae* 5 biotinylated proteins whereas at least 4 proteins in *T. pseudonana* that are predicted to contain biotin are yet to be characterized.

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Ongoing Work

• Western blot and Amplex UltraRed assay to confirm TpSil3p-APEX2 expression and activity.
• Designing and building alternative diatom artificial chromosomes for spatial proteomics.
• BirA-, HRP-, and 3xFLAG-tagged TpSil3p
• Streptavidin pull-down and identification of endogenous biotinylated proteins in *T. pseudonana*.

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