Illuminating Diatom Cell Biology with a Genetically Encodable Tag for Electron Microscopy and Subcellular Proteomics

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Project Goals

A proteomics-based approach to identify proteins that are nearby targets of interest can provide powerful insights into biological pathways. We have implemented APEX2 in *Phaeodactylum tricornutum* to facilitate cataloging of biological pathway-specific proteomes in this model diatom species. APEX2, a soybean-derived ascorbate peroxidase, is a genetically encodable tag for electron microscopy and subcellular proteomics. When fused to a protein of interest, it permits spatially resolved proteomic mapping by oxidizing biotin-phenol to short-lived phenoxyl radicals which can covalently react with proximal proteins. We envision APEX2 will allow us to dissect a range of cell biology questions in *Phaeodactylum tricornutum* and other transformable diatoms which will collectively inform our future strategies for exploiting the biotechnological potential of these biogeochemically and evolutionarily important microeukaryotes.

Abstract

Iron is crucial for organisms across the tree of life as it plays an important role in many key enzymes linked to photosynthesis, respiration and nitrogen fixation. Primary productivity in ~30% of the modern oceans is limited by iron availability. Our laboratory has identified phytotransferrins as a new group of high affinity ferric iron-binding proteins widespread among marine microeukaryotes. Phytotransferrin ISIP2a from *Phaeodactylum tricornutum* internalizes ferric iron via endocytosis, but the molecular details behind ion liberation, chemical speciation and subsequent intracellular allocation remain elusive. We are using APEX2 in *Phaeodactylum tricornutum* to identify additional vesicle-associated proteins involved in this endocytic process. After supplementing APEX2-positive cells with biotin-phenol and hydrogen peroxide, cells are lysed, biotinylated proteins recovered with streptavidin-coated beads and analyzed using mass spectrometry. We show successful heterologous expression of APEX2-tagged ISIP2a, provide evidence for retained APEX2 activity *in* vivo, and present preliminary proteomic data containing a range of endocytosis- and trafficking-related proteins.

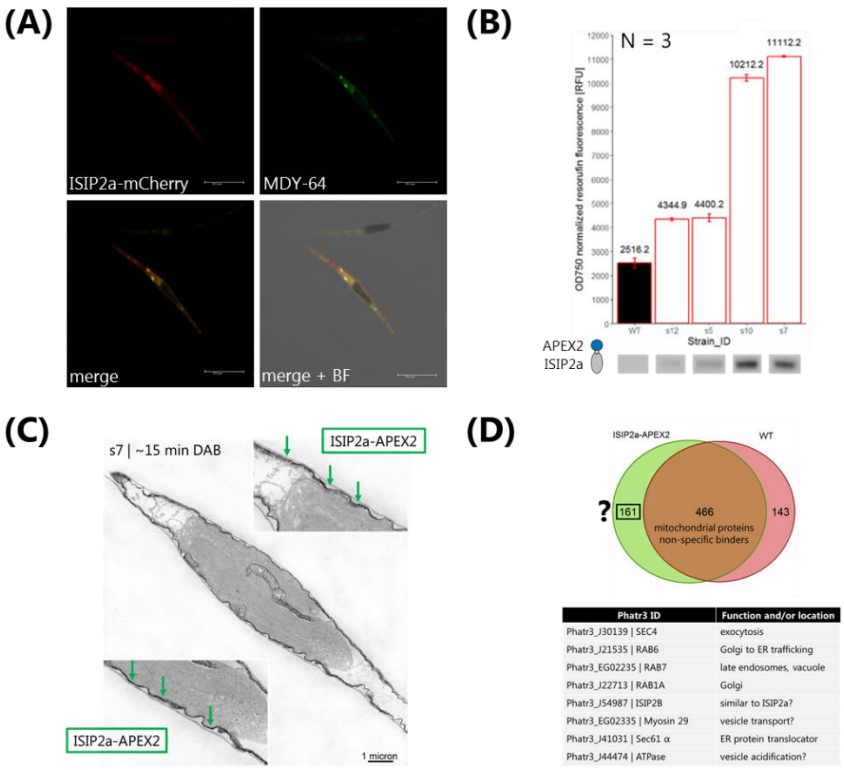

Figure 1 | ISIP2a and APEX2 in *Phaeodactylum tricornutum*. (A) ISIP2a-mCherry co-localizes with MDY-64 (membrane dye), further supporting ISIP2a internalization via endocytosis. *ΔISIP2a* genetic background. Scale bar is 10 µm. (B) A fluorescence-based assay data show retained *in vivo* APEX2 activity in 4 ISIP2a-APEX2 strains (empty bars). (C) APEX2 enables high resolution imaging of diatom proteins using electron microscopy. DAB (diaminobenzidine) is a contrast-generating substrate for APEX2. (D) Endocytosis-related proteins identified in a preliminary subcellular proteomics experiment using an ISIP2a-APEX2 strain.

References

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