Nitrate sensing and uptake in diatoms: from molecular evolution to functional characterisation

Anna Santin

Thesis submitted for the degree of
Doctor of Philosophy (PhD)
in Life, Health and Chemical Sciences

October 2022

Abstract

Diatoms constitute one of the most important phytoplankton groups in the ocean, thanks to their adaptative capacity to face environmental variations, among which nutrient availability. To cope with fluctuations, diatoms own sophisticated mechanisms, still largely unknown, to sense and transport nutrients from the external environment and to reallocate them inside the cell.

This PhD thesis provides the first characterisation of the Nitrate/Peptide Transporter Family (NPFs) in diatoms. NPFs are putative low-affinity nitrate transporters, known in other organisms to be active at high nitrate concentrations, rarely found in the ocean. Beside nitrate, NPFs have been shown to recognise a remarkably broad range of diverse substrates in organisms where they were characterised, ranging from di- and tripeptides in bacteria, to a wide variety of different molecules in plants, such as phytohormones. However, for diatom NPFs exploration is still at its infancy.

Using a multilevel approach which integrated omics, phylogenetic, structural and expression analyses, we revealed an evolutionary divergence into two distinct branches, with a different predicted subcellular localisation suggesting functional diversification.

In order to understand the function of diatom NPFs, and to explain the apparent contradiction of the presence of low-affinity nitrate transporters in an environment in which nitrate levels are never very high, we used reverse genetics approaches. We generated overexpressing strains and CRISPR/Cas9 loss of function mutants in the model species *Phaeodactylum tricornutum*.

Functional characterisation of the mutants suggested that the two different *P. tricornutum* NPFs could be respectively required for the regulation of intracellular nitrogen fluxes, especially nitrate reallocation from the vacuole, and for internal pH regulation and ion transport across chloroplast membranes. So, diatom NPFs evolved to regulate intracellular nutrient and ion transport, rather than uptake them from the external environment, adding new pieces to the complex puzzle of diatom physiology which contributes to their ecological success.