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Article Addendum

Cell signaling in marine diatoms

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Marine photosynthetic microorganisms (phytoplankton) are the basis of marine foodwebs and are responsible for nearly 50% of the global annual carbon-based primary production.[1] Phytoplankton can grow rapidly and form massive blooms that can be regulated by environmental factors such as nutrients and light availability and biotic interaction with grazers and viruses.[2,3] Their crucial role in drawing down atmospheric CO_2 and their potential use for future biofuel production[4] raises the critical need for better understanding of fundamental features of their biology.[5] Although traditionally phytoplankton were considered passive drifters with the currents (from Greek- "Planktos"), our recent reports demonstrate how cells employ a complex mechanism to sense changes in environmental cues and activate chemical-based defense strategies.

Diatoms are a highly diverse and abundant group of phytoplankton in the aquatic environment. As photosynthetic secondary endosymbionts, they are responsible for about 25% of global primary productivity,[6] and play a central role in the biogeochemical cycling of important nutrients such as carbon, nitrogen and silica.

Their evolutionary and ecological success in contemporary oceans suggests they utilize sophisticated mechanisms to monitor and adapt appropriately to changes in environmental stress conditions.[7] Only recently we have began to elucidate how chemical signals (e.g., infochemicals) derived from biotic interactions have the potential to shape complex community structures in aquatic systems.[8,9]

When diatom populations are subjected to grazing or nutrient stress, cells can rapidly induce the biosynthesis of diatom-derived unsaturated aldehydes (DD).[10,11] These oxylipins, products of the fatty-acid oxidation pathway, can be derived both from plasma membrane-localized phospholipid and chloroplast membrane-localized glycolipid precursors.[12,13] DD molecules have been implicated in diatom's chemical defense against competing species[14] and as birth control of copepods, their principal grazers. Thus, these aldehydes may lead to compromised transfer of energy through the marine food web. They can also posses teratogenic and pro-apoptotic activities in human carcinoma cells.[10]

We recently demonstrated how diatoms can accurately sense a potent DD (2E,4*E/Z*-decadienal) and employ it as a signaling molecule to control diatom population sizes.[15] This aldehyde triggered a dose-dependent calcium transient that has derived from intracellular store,[15] in contrast to the external origin of cytosolic calcium induced in response to abiotic stress.[7] Subsequently, Ca^{2+} increase led to nitric oxide (NO) generation by a calcium-dependent NO synthase-like activity,[15] resulting in cell death in diatoms. Interestingly, cells pretreated with a sublethal dose of this aldehyde prior to application of lethal dose had far better survival and growth rates than cells treated with only a single high dose. These results suggest that signaling levels of DD may immunize cells, stimulating resistance to lethal DD concentrations. Perception of the immunized signal modulated calcium and NO signatures.

Furthermore, healthy diatom cells were able to utilize NO-based system to monitor stressed bystanders cells. Therefore, monitoring of this infochemical concentrations thereby provides a sophisticated stress surveillance system whereby subthreshold levels could serve as an early-warning protective mechanism, and lethal doses would initiate the cascade responsible for cell death and bloom termination (Fig. 1).[15] Utilization of the gaseous NO molecule for regulating cell fate in marine phytoplankton provides a novel context for understanding cell signaling and diatom cell-cell communication during algal bloom succession.

NO is widely used as a signaling molecule in vasodilation, neurotransmission and immune system responses in animals and in defense and hormonal signaling in plants and pathogenic bacteria.[16,17] Nonetheless, the highly conserved arginine-dependent nitric oxide synthases (NOS)[18] found in diverse animal and bacteria, can not be detected with significant homology in any genome from a photosynthetic organism reported to date.[19]

In order to dissect the signaling pathway involved in mediating sensing of this aldehyde to regulate diatom cell fate, we took a functional genomics approach to study the role of a DD-induced gene from the marine diatom *Phaeodactylum tricornutum* designated PtNOA1 (nitric oxide-associated protein). This protein belongs to the highly conserved YqeH subfamily of GTP-binding proteins, and is thought to play a role in ribosome biogenesis,[20] sporulation and nitric oxide (NO) generation.[21]

Overexpressing this gene in *P. tricornutum* led to higher NO production, reduced growth and impaired photosynthetic efficiency

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compared to wild-type cells. Cells expressing PtNOA-YFP displayed localized signal within the plastid, differentiating it from a mitochondrial-localized plant orthologue.[22,23] PtNOA transformants were hypersensitive to sublethal levels of this aldehyde, manifesting through altered expression of superoxide dismutase, metacaspases and caspase activity, key components of stress and cell death pathways (Fig. 1).[23] The critical function of metacaspases was recently revealed in diatoms response to iron starvation and in the coccolithophore response to viral infection.[24,25] Widespread documentation of programmed cell death (PCD) in diverse classes of phytoplankton in response to environmental stresses[26] raises fundamental ecological and evolutionary questions for its origin and function in a population composed of unicellular cells. The occurrence of PCD in unicells can be explained when coupled to developmental changes (cyst/spore formation),[27] when it acts as a selective mechanism in intra-species competition[28] or as a viral exclusion strategy.[25] Differential susceptibility to infochemicals may be a way to eliminate propagation of deleterious mutations in stressed-cells with high reactive oxygen species.[27]

Microbial mats and biofilm are important ecological niches where diatoms cells are likely to operate cell-cell chemical signaling. Changes in environmental conditions such as salinity and nutrients availability can shift diatom cells between pelagic and benthic forms to facilitate diatom biofilms. Adhesion of diatoms to surfaces, mediated by cell surface properties and secretion of extracellular polymeric substances (e.g., biofouling), is an important ecological strategy for growth and survival. Recent work in diatoms showed how cells can selectivity sense and adhere to surfaces via NO signaling.[29] Consistent with this finding, PtNOA transformants were compromised in their ability to form biofilm by a reduced attachment strength compare to wild-type cells. This suggests NO plays an important role not only in diatom's perception of chemical cues but also in sensing optimal substrata and regulation of biofouling production.

We also detected NOA-like sequences from diverse oceanic samples, suggesting this novel NO-based system may be widespread among phytoplankton. These data also provide a biological context for NO production in the upper ocean, where it was suggested to originate solely from abiotic nitrite photolysis or bacterial (de) nitrification.[30]

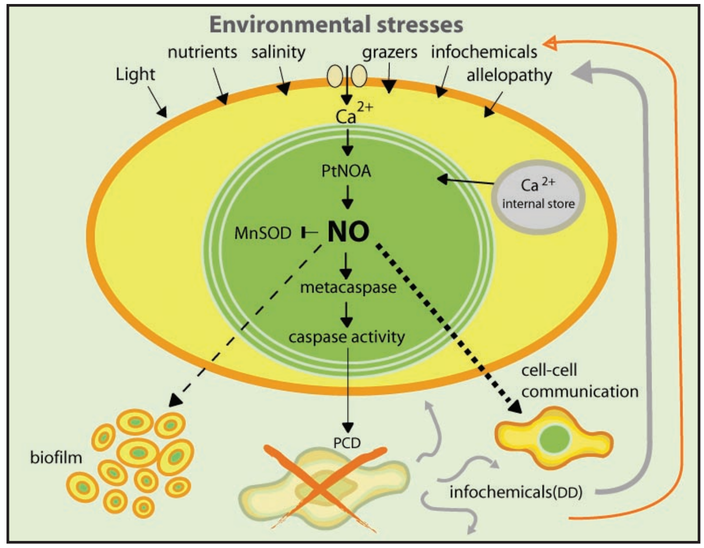

Figure 1. Chemical signaling and programmed cell death in diatoms. Diatom cells can perceive environmental stress conditions and chemical cues (infochemicals) by intracellular calcium transients. The source of calcium depends on nature of the environmental signal. The formation of infochemicals (e.g., diatom-derived aldehydes-DD) by stressed cells, regulate intercellular NO signalling, stress surveillance and defence against grazers. Accurate sensing of these aldehydes is mediated by Ca^{2+} and NO signaling. Integration of these signals in a spatio-temporal context would determine the fate of the cells and their adhesion to surfaces. Under low levels, (orange arrow) DD can act as a signal that immunizes cells against subsequent stress conditions while in high doses (gray arrow) it will induce PCD and initiate bloom termination. Nitric-oxide-associated protein (NOA) plays a key role in linking the initial perception of infochemical to cellular functions such as biofilm formation, cell-cell communication and cell death.

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