Proteomics studies on stress responses in diatoms

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Diatoms are a highly diverse group of eukaryotic phytoplankton that are distributed throughout marine and freshwater environments and are believed to be responsible for approximately 40% of the total marine primary productivity. The ecological success of diatoms suggests that they have developed a range of strategies to cope with various biotic and abiotic stress factors. It is of great interest to understand the adaptive responses of diatoms to different stresses in the marine environment. Proteomic technologies have been applied to the adaptive responses of marine diatoms under different growth conditions in recent years such as nitrogen starvation, iron limitation and phosphorus deficiency. These studies have provided clues to elucidate the sophisticated sensing mechanisms that control their adaptive responses. Although only a very limited number of proteomic studies were conducted in diatoms, the obtained data have led to a better understanding of the biochemical processes that contribute to their ecological success. This review presents the current status of proteomic studies of diatom stress responses and discusses the novel developments and applications for the analysis of protein post-translational modification in diatoms. The potential future application of proteomics could contribute to a better understanding of the physiological mechanisms underlying diatom acclimation to a given stress and the acquisition of an enhanced diatom stress tolerance. Future challenges and research opportunities in the proteomics studies of diatoms are also discussed.

Keywords:
Cell biology / Diatom / Mass spectrometry / Post-translational modifications / Stress response

1 Introduction

Diatoms are a diverse group of unicellular, photosynthetic eukaryotes that inhabit marine and fresh waters worldwide. It has been estimated that approximately 40% of the total carbon fixation in the oceans and 20% of the global primary productivity are accomplished by the photosynthetic activity of diatoms [1, 2]. They are widely distributed and highly successful organisms in aquatic habitats and play a central role in the biogeochemical cycling of important nutrients such as carbon (C), nitrogen (N) and silica (Si). In addition to the ecological importance in aquatic environments, diatoms can accumulate high levels of lipids (i.e. triacylglycerols, TAGs) under N stress, suggesting their innate ability to produce clean and renewable biofuel [3].

Diatoms have developed an array of sophisticated mechanisms to appropriately detect and adapt to various biotic and abiotic stress factors [4, 5]. Several signalling pathways that exist in both plants and animals have been predicted or verified in diatoms [4–6]. For example, Ca2+ and nitric oxide (NO)-based signalling pathways are involved in aldehyde(2E,4E/Z)-decadienal-triggered diatom cell death [5]. An ornithine-urea cycle known to have facilitated key physiological and life-history adaptations in vertebrates has also been investigated and proved to be essential for diatom growth and metabolism [7]. Although major advances have been made to elucidate the adaptation mechanisms of diatoms, it is necessary to investigate the biochemical processes that contribute to their ecological success and the mechanisms underlying their adaptive responses to different stresses on the system level.

Whole-genome expression analysis has recently been utilized to identify key genes in stress acclimation of diatoms [8–14]. These transcriptomic results have revealed extensive

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changes at the cellular transcription level in response to stress and provided novel insights into the molecular mechanisms of stress acclimation in diatoms. Although informative, the correlation between transcript and protein abundance is generally modest because of a range of post-transcriptional regulatory mechanisms [15, 16]. Both abiotic and biotic stresses induce profound changes in diatom proteomes aimed at an adjustment of their metabolism to the ever-changing environment and an enhancement of stress tolerance. Therefore, it is necessary to analyse the protein expression profiles at the proteome level in an effort to gain system-level information with regards to stress responses in diatoms.

During the last decade, major technical breakthroughs and improvements in proteomics have enabled researchers to study diatom responses to various stresses at the proteome level. At present, the cutting-edge proteomic technologies are capable of identifying and measuring several thousands of proteins, with high sensitivity and high accuracy, within a few hours [17–19]. Numerous studies have used different proteomic approaches to investigate the underlying mechanisms of stress responses in diatoms and these studies have provided novel insights into the adaptive responses of diatoms to different environmental and growth conditions [20–23]. Moreover, studies on the roles of post-translational modifications (PTMs), such as phosphoproteomics [24] and redoxome [25], in diatoms exposed to stress have been published recently. The continued development and application of these proteomic techniques will provide new insights into the underlying mechanisms of stress response in diatoms. A wide range of proteomic strategies have been used to study the stress responses in diatoms (Table 1).

As a result of the recent increase in available proteomic study data, it is necessary to review and summarise published data from these proteomic studies to identify key proteins involved in diatom responses to various stress factors. The aim of this review is to provide an overview of proteomic-related studies of diatom stress responses with emphasis on nutrient limitation. Moreover, the challenges, limitations and future direction of MS-based proteomics in understanding diatom stress acclimation on the molecular level will be discussed.

2 Proteomic studies of diatom response to stresses

2.1 Major nutrient fluctuations

2.1.1 Nitrogen starvation

Nitrogen (N) is essential for all organisms and is one of the major constituent of cellular macromolecules, such as proteins, nucleic acids, lipids and chlorophyll. The availability of N varies dramatically in the ocean, on spatial and temporal scales, due to biological and physical processes. The impact of N deprivation on pigments, photosynthesis, carbon fixation and N assimilation, has been studied in diatoms [26–28]. In response to N deprivation, diatoms reprogram several metabolic pathways, for example, reduced primary carbon metabolism and accumulated lipid content, in certain diatom species [29].

Hockin et al. compared the proteome of the diatom *Thalassiosira pseudonana* at the onset of N starvation with that of N-replete cells using a two-dimensional gel electrophoresis (2-DE)-based proteomic strategy [30]. Sixty-five proteins with altered expression were identified and proteins involved in the metabolism of N, amino acids, proteins, carbohydrates, photosynthesis and chlorophyll biosynthesis were represented [30]. These results suggest that the central carbon metabolism response of *T. pseudonana* to N starvation differs considerably from that of other eukaryotic photoautotrophs and bears a closer resemblance to the response of cyanobacteria [30].

Another proteomic study systematically investigated the molecular shift towards neutral lipid accumulation in *Phaeodactylum tricornutum*, during N deprivation [31]. The results revealed that N starvation led to an upregulation of the genes involved in N assimilation and fatty acid biosynthesis, accompanied by a downregulation of the genes involved in photosynthesis and lipid catabolism, resulting in the diatom metabolic network shifting from a carbon flux towards lipid accumulation [31].

2.1.2 Phosphorus stress

Phosphorus (P) supply is increasingly recognized as a critical driver of phytoplankton growth in marine ecosystems [32]. P concentrations are low in many marine systems, and there is growing evidence that P limits marine primary production [33–35]. However, diatom physiological responses to P deficiency are poorly understood. Dyhrman et al. combined deep sequencing of transcript tags and quantitative proteomics to analyse the diatom *Phaeodactylum tricornutum* grown under P-replete and P-deficient conditions [36]. The diatom’s response to P deficiency involved a specific and multi-faceted remodelling of the transcriptome, which is strongly linked to the response of the proteome. There were also clear physiological changes in the cellular P allocation patterns, enzyme activity and lipid composition that are consistent with the transcriptome and proteome patterns. The coordination in the transcriptome and proteome revealed that *T. pseudonana* has evolved a sophisticated response to P deficiency involving the following multiple biochemical strategies: (i) change in cellular P allocation, (ii) increased P transport, (iii) switch to the utilization of dissolved organic phosphorus, (iv) remodelling of the cell-surface and (v) modulation of glycolysis and translation [36]. The strong induction of a diesterase at the protein level suggests the importance of this enzyme to *T. pseudonana* cells experiencing P deficiency and provides new insight into the nature of P stress responses in phytoplankton [36].
Table 1. Summary table of publications on proteomic studies of the stress responses in diatom

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<td>[51]</td>
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<td>Silicon limitation</td>
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<tr>
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2.1.3 Silicon response

Silicon (Si) is the most critical element for diatom growth and is important for the generation of the siliceous cell wall (frustule) through a controlled formation process [37]. Genomic and transcriptomic approaches have recently been used to identify a number of candidate proteins that are potentially involved in Si metabolism in diatoms [13, 38–41]. These studies have increased our knowledge of the molecular mechanism of Si response and revealed the complexity of the molecular basis of diatom biosilification.

Du et al. employed an iTRAQ-based quantitative proteomic strategy coupled with an established synchrony technique to reveal the global metabolic Si-response in the model diatom *T. pseudonana* when subjected to Si starvation and replenishment [20]. Four samples, corresponding to the time of Si starvation, girdle-band synthesis, valve formation and immediately after daughter cell separation, were collected for proteomic analysis [20]. Approximately 16% of the predicted proteins encoded by the *T. pseudonana* genome were identified, of which 165 were differentially expressed proteins (DEPs) [20]. DEPs are involved in multiple biochemical pathways,
particularly Si transport, cell wall synthesis and cell-cycle progression [20]. These investigations not only characterize new candidate proteins, which are potentially involved in the Si response, but also provide valuable insights into the intracellular metabolic mechanisms related to the Si response.

2.2 Micronutrient fluctuations

2.2.1 Iron (Fe) limitation

The solubility of Fe in oceans is low and phytoplankton growth in marine habitats is often limited by Fe availability [42, 43], particularly in high-nitrate low-chlorophyll regions [44, 45]. Fe limitation is one of the most common stresses affecting diatoms because Fe has a critical role in diatom growth and primary production [46]. Diatoms have multi-level strategies to adapt to long-term Fe limitation, including lowering the cellular Fe requirements and increasing Fe uptake. Several studies have indicated the unique high tolerance to chronic Fe-limitation stress and the rapid response to the replenishment for some low Fe-supplemented diatom species, including P. tricornutum, Thalassiosira oceanica and Pseudo-nitzschia multiseries [14, 47–50].

Complementary to previous studies, quantitative proteomics have been employed to obtain a global view of the cellular response of diatoms to Fe deficiency [21, 47, 51]. Lommer et al. explored the cellular response of T. oceanica to low-Fe conditions by a combination of genomic, transcriptomic and proteomic approaches [47]. They established the [15]N metabolic isotope labelling-based quantitative proteomic approach for T. oceanica and showed the response to iron limitation at protein level in marine diatom for the first time. Their data reveal an unexpected metabolic flexibility in response to iron availability for T. oceanica, which comprises cellular retrenchment as well as remodelling of bioenergetic pathways [47]. It was emphasized that the photosynthetic electron transfer complexes were generally decreased to adjust the light energy utilization [47].

Nunn et al. performed a label-free quantitative proteomic analysis to investigate the long-term acclimation responses of the diatom T. pseudonana to steady-state Fe limitation [21]. They constructed comprehensive metabolic maps of proteins expressed and identified in Fe-limited and Fe-replete T. pseudonana. Many proteins involved in intracellular protein recycling, photosynthesis and pentose phosphate pathways were found to be up-regulated when T. pseudonana was acclimated to Fe limitation. The results provide novel insights into the mechanisms utilized by diatoms to survive under low-Fe conditions [21].

Using isobaric tags for relative and absolute quantitation (iTRAQ)-based proteomic analysis, Luo et al. explored the cellular responses associated with reactive oxygen species (ROS) production and cell fate decision during the early response to Fe limitation in the centric diatom T. Pseudonana [51]. The results indicated that Fe limitation caused a significant deficiency in the diatom’s photosystems and respiratory chain, leading to excess ROS accumulation [51]. Programmed cell death (PCD), in some Fe-limited cells, was thought to be triggered by the increase in ROS through the synthesis of a series of proteins involved in the delicate balance between pro-survival and pro-PCD factors [51]. These studies provide molecular-level insights into the major strategies that T. pseudonana employs in response to Fe-limitation, i.e. the reduction of cell population density through PCD to reduce competition for available Fe, the reallocation of intracellular Fe and N to avoid death, as well as an upregulation of anti-PCD and antioxidant proteins [51].

2.2.2 Cobalamin scarcity

Although diatom growth in the oceans is controlled primarily by N and Fe availability [52, 53], there is increasing evidence [54–57] that cobalamin availability can influence marine phytoplankton growth and community composition [58, 59]. Quantitative proteomic and transcriptomic approaches were used to investigate diatom gene products involved in cobalamin metabolism and to assess the global impact of cobalamin deficiency on diatom molecular physiology [12]. Diatoms can adopt the following three distinct strategies to cope with low cobalamin levels: increased cobalamin acquisition machinery, decreased cobalamin demand and management of reduced methionine synthase activity through changes in folate and S-adenosyl methionine metabolism [12]. Moreover, a previously uncharacterized protein, cobalamin acquisition protein 1 (CBA1), was implicated in cobalamin acquisition [12].

2.3 Polycyclic aromatic hydrocarbons (PAHs)

PAHs are persistent organic pollutants generated by the incomplete combustion of organic materials such as fossil fuels or wood [60]. They are ubiquitously distributed in aquatic environments and regarded as a major threat to marine and freshwater ecosystems [60]. PAHs have aroused the attention of regulatory organizations and researchers due to their mutagenic and carcinogenic effects. Carvalho and Lettieri studied protein expression changes in the marine diatom T. pseudonana upon exposure to benzo(a)pyrene (BaP), a common PAH compound, using an iTRAQ quantitative proteomic approach [61]. Researchers selected 19 proteins with altered expression post-exposure and discussed their potential use as early indicators of environmental exposure to PAHs [61]. In particular, SIT1 is considered to be a promising biomarker. Novel pathways, which may be involved in diatom exposure to BaP, such as DNA methylation and photosynthesis, were also revealed in this article [61]. This data, combined with gene expression analysis, will help to elucidate the pathway or metabolic processes involved in diatoms reacting to
2.4 Allelopathic compounds

Allelopathy is a biological phenomenon by which an organism produces or releases chemical compounds (allelopathic compounds) that influence the growth, survival and reproduction of competitor species [62]. Phytoplankton allelopathy can alter aquatic community structure, including patterns of species dominance [63, 64]. To understand the physiological impacts of the red tide dinoflagellate, Karenia brevis, allelopathy on two diatom competitors, Asterionellopsis glacialis and T. pseudonana, Poulson-Ellestad et al. performed a pioneering whole-cell metabolomic and proteomic analysis simultaneously [65]. In T. pseudonana, major metabolic processes such as glycolysis, photosynthesis, cell membrane maintenance and osmoregulation were found to be greatly impacted by exposure to K. brevis allelopathy [65]. This work provides novel insights into the physiological mechanisms by which allelopathy alters large-scale ecosystem processes and community composition in plankton.

2.5 Photosynthesis, lipid synthesis and dimethylsulphonioiopropionate (DMSP) accumulation

The studies discussed earlier have shown that environmental stresses have a drastic influence on biosynthesis in diatoms such as photosynthesis, lipid synthesis and DMSP synthesis. Several proteomic studies regarding the influence of environmental stresses on diatom biosynthesis or biosynthetic organelles have been performed.

Thylakoid organization and composition of the pigment–protein complexes in diatoms differ from those in other algae groups and higher plants. Thus, under naturally fluctuating light intensities, diatoms require specific regulatory mechanisms to guarantee equal distribution of excitation energy between photosystems (PS) I and II. Grouneva et al. identified the thylakoid protein complexes of two marine diatoms, T. Pseudonana and P. tricornutum, at the proteome level by using two-dimensional blue native (BN)/SDS-PAGE coupled with MS analysis [66]. Importantly, they identified a novel diatom specific PS I-associated protein and provided evidence for a possible PSI localization of Lb18-like light-harvesting proteins (Lhcx) in diatoms. This data enhances our understanding of the light-harvesting antenna composition and Lhcx expression and provides novel insights into the composition and function of the photosynthetic apparatus in diatoms [66].

Proteomic analyses were also performed on P. tricornutum [3, 31] and Fistulifera sp. [67, 68] in order to understand the molecular mechanisms involved in stress-induced TAGs accumulation in diatoms. The proteins involved in carbohydrate metabolic and branched-chain amino acid (BCAA) catabolic processes were found to contribute to TAG biosynthesis, storage and degradation in diatoms [3, 31]. These studies provide valuable information concerning the dynamics and regulation of diatom photosynthesis and lipid synthesis.

Marine phytoplankton play a fundamental role in the global sulphur (S) cycle and climate regulation through the synthesis of DMSP, the major precursor of the volatile sulphur compound dimethylsulphide (DMS) [69, 70]. The intracellular concentration of DMSP increases with increased salinity, increased light intensity and N starvation in diatoms. Kettles et al. investigated DMSP synthesis under different conditions at the cellular level via the analysis of enzyme activity, gene expression and proteome comparison [71]. Their findings suggest that increased S assimilation is not required for increased DMSP synthesis, which differs from the regulation of S metabolism in higher plants [71]. Lyon et al. utilized a 2-DE-based quantitative proteomic approach to investigate protein changes associated with salinity-induced DMSP increases in the model sea-ic diatom Fragilariopsis cylindrus [22]. This study demonstrated that DMSP accumulation was one of the acclimation mechanisms utilized by F. cylindrus to cope with salinity-stress [22]. Proteomic results revealed the upregulation of proteins associated with the Met-DMS biosynthesis pathway, the synthesis of multiple osmolytes and antioxidants, along with the degradation of fucoxanthin-chlorophyll binding proteins, which have been suggested to be important cellular responses during salinity acclimation [22]. Identification of candidate proteins that may regulate DMSP synthesis will enable targeted studies on biological controls of DMSP production, which may then lead to an improvement in environmental and biological feedback models on potential DMS climate effects [22].

2.6 Proteomic analysis of PTMs in diatoms

PTMs are complex and fundamental mechanisms modulating diverse protein properties and functions and are involved in almost all known cellular pathways and biological processes [72, 73]. Hence, the analysis of PTMs is a powerful tool to deduce regulatory mechanisms in cells under varied stress conditions. To date, two studies have used a proteomic approach to investigate PTMs in diatoms [24, 25].

Reversible protein phosphorylation at serine (S), threonine (T) and tyrosine (Y) residues is the most widespread type of PTM in cells, regulating crucial cellular functions, including signal transduction, cell proliferation and stress responses [74]. Phosphorylation is catalysed by specific protein kinases and phosphatases and more than 1000 protein kinases and phosphatases with a specificity for S/T/Y residues have been predicted in diatoms [24]. Thus, phosphorylation-based signalling is regulated by the antagonizing catalytic activities of protein kinases and protein phosphatases in all eukaryotic cells [75, 76]. Almost all cell signalling processes are dependent on protein phosphorylation and
dephosphorylation. Recent advances in phosphopeptide enrichment, followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS), have made it possible to study proteins that are regulated by phosphorylation at the system level, and a large number of phosphorylation events have been identified in many model organisms including Saccharomyces cerevisiae, Synechococcus, Drosophila melanogaster, mice and humans [77–81]. Our group has recently accomplished the phosphoproteomic analysis of a model diatom, P. tricornutum [24]. In this study, we used a separation strategy involving protein/peptide fractionation, titanium dioxide (TiO₂) enrichment and LC—MS/MS analyses. This analysis led to the identification of 436 phosphorylation sites, from 264 unique phosphopeptides, corresponding to 245 proteins. The phosphorylated proteins were implicated in the regulation of diverse biological processes, including stress responses. We propose that there are two major mechanisms in diatoms to cope with adverse environmental conditions: the PCD process induced by intracellular ROS and Ca²⁺-dependent or NO signalling pathways and ROS scavenging, which coincides with TAGs accumulation mediated by the ubiquitin-proteasome pathway and the amino acid degradation pathway. The phosphoproteomic analysis of P. tricornutum provides new clues on how diatoms respond and adapt to different stresses in the marine environment.

ROS are chemically reactive molecules containing oxygen formed as a toxic by product of oxygen-based metabolic pathways [82]. Perturbations in oxygenic metabolism, under various stress conditions, can induce oxidative stress from the overproduction of ROS [83]. ROS play important roles in cell signalling and homeostasis and are recognized as central secondary messengers involved in cellular signalling networks [84]. ROS signalling regulates various cellular processes by post-translational thiol oxidation of targeted proteins [85], and the redox states of these proteins contain crucial information on the mechanisms of cell acclimation to stress conditions [86]. Thus, proteome-wide identification and quantification of redox-sensitive proteins (e.g. redoxome) is the key to understanding the redox-based mechanisms that mediate cell sensing and acclimation to environmental stress [87]. The research article by Vardi et al. can be considered the ‘father’ of redoxome study on diatoms [25]. In this study, an elegant MS-based approach (OxICAT) was used to identify the diatom redoxome and quantify its degree of oxidation under oxidative stress conditions. OxICAT is a recently developed quantitative thiol trapping technique used to assess the ROS-sensitive proteome in vivo [87]. It can identify thiol groups that undergo stable and reversible oxidative modification based on differential labelling of reduced and oxidized cysteines with isotopically light (¹³C) and heavy (¹⁵C) versions of the isotope-coded affinity tag reagent followed by LC-MS/MS analysis [87]. Using this method, they demonstrated that diatom cells can sense and respond to oxidative stress through a redox-sensitive protein network and provided compelling evidence for organelle-specific oxidation patterns under N stress conditions. Based on an in-depth functional analysis, they proposed that the redox regulation of metabolic rates in response to stress provides a mechanism to ensure cellular homeostasis and may be the key factor responsible for the ecological success of diatoms in marine environment.

3 Concluding remarks and outlook

Efficient regulation of the proteome is essential for diatoms to optimally respond to the ever-changing environment. Studying the diatom’s response to stress at the proteome level could contribute to a better understanding of physiological mechanisms underlying diatom acclimation to a given stress and gain valuable insight into diatom stress tolerance. Major cellular processes and pathways of diatoms in response to a variety of exogenous and endogenous stresses were summarized in Fig. 1. It is already evident from this review of the literature that progress has been and is being made in this area but much remains to be done.

To date, most published diatom proteomic studies have mainly focused on the effects and responses to individual stresses. Proteomic changes in response to multiple stresses remain to be elucidated. Information on proteome changes under stress is often fragmental, comparing only non-stressed (control) and stressed diatoms. There is quite a lot of available information on changes in the diatom proteome under stress, whilst there is little known about low-abundance regulatory proteins involved in perception and signalling of stress. Therefore, more emphasis needs to be given to the study of low-abundance proteins with regulatory functions, such as transcription factors or protein kinases. Newer generation mass spectrometers, as well as MS-based quantitative approaches such as iTRAQ, tandem mass tags and label-free quantitation, will provide enhanced sensitivity and accuracy for the detection and quantification of low-abundance signalling and regulatory protein complexes. In particular, selected reaction monitoring (SRM, or multiple reaction monitoring, MRM) has recently proven exceptionally useful in the accurate multiplexed quantification of low-abundance proteins and modified peptides in complex biological mixtures [88, 89]. It could be foreseen that SRM techniques will contribute to a detailed protein functional characterization and further advance our understanding of diatom physiology under stress.

No proteomic technique is currently able to reveal the complete diatom proteome; therefore, the choice of the technique should be guided by specific scientific demand and a combination of complementary techniques should be applied. In particular, subcellular proteomics and functional targeting of signalling complexes of diatoms can offer improved chances of experimental success. Affinity tagging of cell-surface proteins with biotin and glycosylation techniques can also be used to identify the numbers of cell-surface or transmembrane proteins. In this respect, the increasingly sophisticated label-free quantification approaches that are coupled with
sub-cellular fractionation and targeting of signalling complexes allow the possibility that critical protein changes will be identified in diatoms. The combination of these proteomic approaches will facilitate the characterization of regulatory proteins and aid in addressing fundamental questions regarding diatom physiology under stress.

Protein PTM has an intrinsically important role in processes such as stress signalling, and response and so forth [90]. Due to their crucial functions in cells, protein PTMs have become one of the major focuses in proteomic technologies (collectively called ‘modificomics’) [91]. Therefore, the development of effective strategies for proteomic analysis requires an extensive understanding of the functions of PTMs, the cross-talk between these PTMs and their associated metabolic pathways in the diatom metabolic network. Although there have been a limited number of PTM studies in diatoms [24, 25], those performed to date have provided valuable information with regards to stress perception and response in diatoms. Lysine acylations, such as acetylation, succinylation, crotonylation, malonylation, propionylation and butyrylation play a crucial role in the functional regulation of many prokaryotic and eukaryotic proteins [92, 93]. Accumulating evidence suggests that lysine acylations provide an elegant mechanism to respond to changes in the energy status of the cell and coordinate different metabolic pathways in diverse organisms [92, 94]. It is the author’s belief that lysine acylations may play an important regulatory role in stress response in diatoms. Thus far, there are no reports on the proteomic-wide analysis of lysine acylations in diatoms. We expect that there will be a surge in the publications related to systematic studies of the identities and functional roles of the acylated proteins in diatoms in the subsequent years. In addition, with the rapidly developing quantification methods by MS, such as iTRAQ, label-free and SRM, changes in the degree of the modification sites could be measured over time in response to stress. These results will help us gain a better understanding of the protein function and signalling cascades in the processes of diatom stress acclimation and stress tolerance acquisition. However, it is important to note that we are still lacking efficient molecular tools for the functional characterization of identified PTM(s) [95]. Without a high throughput method for determining the effects of PTM(s) on protein structure and function, potential discrepancy or misinterpretation of complex PTM proteomics data may occur. Major advances need to be made towards developing superior molecular tools that can be used to validate and functionally characterize modified sites arising from PTM proteomic experiments.

Finally, we believe that the integration of multiple levels of ‘Omics’ data, including proteomics, transcriptomics, metabolomics, modificomics and interactomics with rapidly evolving databases and bioinformatics software, will enable a holistic and comprehensive view of molecular mechanisms underlying diatom performance under stress. Many of the present studies can be considered as proof-of-principle, but they provide a glimpse of methods that may become standard in the subsequent years.

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4 References


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