DNA Methylation in Algae and Abiotic Stress Responses

DNA methylation is one of the most conserved epigenetic mechanisms, playing a pivotal role in organism response to several biotic and abiotic stressors. Indeed, stress can induce changes in gene expression through hypo- or hyper-methylation of DNA at specific loci and/or in DNA methylation at the genome-wide level, which has an adaptive significance and can direct genome evolution. Exploring DNA methylation in responses to abiotic stress could have important implications for improving stress tolerance in algae.

1. DNA Methylation

DNA methylation is a conserved epigenetic modification crucial for various biological processes, including gene and transposon silencing, imprinting and X chromosome inactivation. Together with other epigenetic marks, such as the histone code, post-translational modifications and small RNA interference, DNA methylation contributes to chromatin remodelling processes, and it is involved in the regulation of gene expression without altering the primary DNA sequence [1][2].

Although all nucleotides can be methylated, DNA methylation generally refers to an addition of a methyl group through a covalent modification onto the C5-cytosine to form 5-methylcytosine (5-mC) [3], as well as onto the N6-adenine to form 6-methyladenine (6mA) [4].

DNA methylation can be found both in prokaryotes and eukaryotes. In bacteria, 6mA is a prevalent form of DNA methylation that plays an important role in DNA repair and replication. Bacterial DNA methylation can also act as a defence mechanism against invading phage; in fact, methylation differentiates the host from the phage genome, which becomes the preferential target of the cleavage action of host restriction enzymes [5].

In eukaryotes, DNA methylation is a fundamental mechanism for the maintenance of genome stability and the regulation of gene expression in response to both external and internal stimuli, thus playing a relevant role in plant diversity and development [6][7][8][9]. In plants, DNA methylation is more extensive and affects a wider sequence diversity than in animal genomes. In animals, cytosine methylation is mainly restricted to the symmetric CpG dinucleotide, except for the embryonic stem cells [10], the adult mouse cortex and the human brain [11], where CpH methylation was found. By contrast, plants have relatively high concentrations of 5-mC compared to non-plant species because cytosine methylation occurs in three sequence contexts: symmetric CG and asymmetric CHG and CHH (where H = A, T or C) [12].

The main studies of mechanisms on DNA methylation derive from the model species *Arabidopsis thaliana.* The dynamic of DNA methylation is controlled by specific DNA methyltransferases that mediate cytosine methylation in the different sequence contexts [1][13].

Cytosine-5 DNA methyltransferases recognise a specific DNA sequence and catalyse the transfer of a methyl group from the cofactor *S*-adenosyl-l-methionine (AdoMet) to carbon 5 in the pyrimidine ring of cytosine residues [14]. Different C5-cytosine methyltransferases have been characterised in prokaryotes and eukaryotes. All methyltransferases share a catalytic domain containing 10 conserved small motifs (annotated I to X), suggesting a common origin [15].

DNA methylation can be distinguished in maintenance and de novo methylation. Maintenance methylation preserves the methylation status of symmetric (palindromic) sites after DNA replication through the recognition of hemi-methylated sites and methylation of the newly synthesised filament [16][17]. On the other hand, de novo methylation consists in the methylation of DNA sequences not previously methylated; in plants, an RNA-directed DNA methylation pathway is crucial

for this process [18]. De novo methylation is involved in the rearrangement of methylation patterns during embryogenesis and in the cell differentiation processes during development [19].

In land plants, three DNA methyltransferase subfamilies have been implicated in the establishment and/or maintenance of DNA methylation [20][21], namely the DNA METHYLTRANSFERASEs (METs), CHROMOMETHYLASEs (CMTs) and DOMAINS-REARRANGED METHYLTRANSFERASEs (DRMs) subfamilies.

The plant homolog of mammalian DNA methyltransferase 1 (DNMT1), namely MET1, is the major CG maintenance methyltransferase. In plants DNMT2 homologs are also present that, like mammalian DNMT2, possess transfer RNA (tRNA) methylase activity [22]. Recent in vitro research has provided evidence that DNMT2 can efficiently methylate DNA when DNA fragments are presented as covalent DNA–RNA hybrids in the structural context of tRNAs [23].

The plant DRMs and their mammalian homologs, the DNMT3 group, are predominantly de novo methyltransferases. The domains of amino- and carboxy-terminal halves of DRM proteins are arranged in reverse order when compared with DNMT3 (domains VI–X are followed by I–V). DRM2 catalyses cytosine methylation in all sequence contexts and is the prominent cytosine methyltransferase in the RNA-directed DNA methylation pathway [24].

CMTs, identified exclusively in plants, are characterised by the presence of an amino acid chromodomain motif, between the conserved motifs II and IV, which binds to methylated histones. CMT3 is the enzyme primarily responsible for CHG maintenance methylation [13][21]. CMT2 plays a role in maintaining CHH methylation in specific genomic contexts, such as the central regions of large transposable elements, presumably via cross-talk with histone modifications like its paralog, CMT3 [25].

1.1. Maintenance of DNA Methylation

Maintenance of plant DNA methylation depends on the cytosine sequence context and it is catalysed by DNA methyltransferases, which, in turn, are regulated by different mechanisms. The maintenance of already established DNA methylation patterns occurs in all the different sequence contexts: CG, CHG and CHH. The maintenance of CG methylation in plants requires the activity of MET1, the VARIANT IN METHYLATION (VIM) and the DDM1 chromatin remodelling factor. VIMs are a family of SRA (SET and RING-associated) domain proteins orthologs of mammalian ubiquitin-like PHD and RING finger domains 1 (UHRF1) [1], suggesting that both plants and mammals maintain CG methylation in a similar manner. MET1 is associated with DNA replication sites, it recognizes hemi-methylated CG dinucleotides following DNA replication and methylates the unmodified cytosine in the daughter strand. MET1 has been proposed to be recruited to DNA by VIM proteins in a similar way to how DNMT1 is recruited by UHRF1 [6].

The maintenance of methylation at CHG sites does not seem to depend on the palindromic symmetry of the sequence and appears to involve histone methylation [26]. Genome-wide analyses showed that lysine 9 on histone 3 (H3K9) dimethylation and DNA methylation are highly correlated [27]. Indeed CMT3 is largely responsible for maintaining CHG methylation [28] and its activity is strongly associated with the dimethylation of H3K9, where the dual recognition of H3K9me2 by BAH (bromo adjacent homology) and CHROMO domains of CMT3 leads to the methylation of CHG sites [21].

Methylation in the CHH asymmetric context is maintained by DRM2 and RNA-directed DNA methylation (RdDM), which is involved in de novo methylation. However, at some loci, this kind of methylation is provided by CMT3 and DRM2 [29].

1.2. De Novo DNA Methylation

In plants, de novo DNA methylation is mediated through the RNA-directed DNA methylation (RdDM) pathway, which involves small interfering RNAs (siRNAs) and scaffold RNAs, in addition to an array of proteins. The mechanism for RdDM has been divided into the canonical RNA Polymerase (RNA Pol IV) and non-canonical (RNA Pol II) pathways. Both rely on the generation of siRNA molecules that direct the activity of DRM methyltransferases towards targets through sequence homology [18].

The canonical RdDM pathway relies on the activity of two plant-specific RNA Pol II paralogs, RNA Pol IV and RNA Pol V [30]. Canonical RdDM initiates with the recruitment of RNA Pol IV, preferentially towards heterochromatic regions by SAWADEE HOMEODOMAIN HOMOLOG 1 (SHH1) [31] and by RNA-DIRECTED RNA POLYMERASE 2 (RDR2) to produce double-stranded RNA (dsRNA) [32]. The putative chromatin remodelled CLASSY 1 (CLSY1) is also required for a correct RNA Pol IV and RDR2 recruitment and generation of the corresponding transcripts [33]. The dsRNA is processed by DICER-LIKE 3 (DCL3) into 24-nucleotide siRNAs that are subsequently methylated at their 3' ends by HUA

ENHANCER 1 (HEN1) and incorporated into ARGONAUTE 4 (AGO4). Pol V, in the nucleus, transcribes a scaffold RNA that base-pairs with AGO4-bound siRNAs. A key role is played by RNA-DIRECTED DNA METHYLATION 1 (RDM1), the only protein that interacts with both AGO4 and DRM2, thus creating a bridge between them [34]. Through this action, the canonical RdDM machinery acts to maintain methylation levels, predominantly in the CHH context, through de novo methylation at heterochromatic regions for continued silencing [33].

The non-canonical pathway could be considered to act as a surveillance mechanism aimed to identify and target actively transcribed regions for silencing, such as at active TE elements, potentially due to the loss of silencing factors or recent transposition [35].

1.3. DNA Methyltransferase in Algae

DNA methylation machinery contributes to the biological diversity of algae as they contain several DNA methyltransferases responsible for the methylation of different sequence contexts [36]. DNA cytosine methyltransferases are widely distributed among Archaeplastida but in a patchy pattern, with specific subfamily enzymes largely limited to subgroups of organisms [37]. The analysis of both phylogenesis and domain organisation of DNA cytosine methyltransferases in Archaeplastida have been conducted by Ma et al. (2017) [37] through a survey on 12 complete or near-complete algal genomes. These authors have observed that among the species analysed, only *Klebsormidium flaccidum* appears to have a set of DNA cytosine methyltransferases similar to that found in land plants, whereas homologues from other Archaeplastida algae tended to cluster independently within each subfamily. In particular, DNMT1/MET1 homologues are the methyltransferases with the widest distribution, including species belonging to Trebouxiophyceae (*Chlorella sorokiniana* and *Chlorella variabilis*) and Chlorophyceae (*Chlamydomonas reinhardtii* and *Volvox carteri*), as well as to the Charophyta division (*K. flaccidum*). Algal MET1 proteins share high sequence similarity in the DNA methyltransferase catalytic domain with the land plant polypeptides. Additionally, most of these algal proteins possess a D-RFD (DNA methyltransferase replication foci domain) and BAH motifs in their N-terminal extensions, as observed in the canonical enzyme. Furthermore, in *C. reinhardtii*, two additional DNMT1-related polypeptides, localised in the chloroplasts and influencing plastid DNA methylation, were found. These paralogues, lacking conserved motifs in the N-terminal region, have been termed DMT1a and DMT1b as novel DNA methyltransferase with nonselective de novo cytosine methylation activity [38][39].

According to Ma et al. (2017) [37], chromomethylase-like methyltransferases, structurally similar to land plant CMT3 except for the lack of the chromodomain in the *Chlorella* enzymes, seemed to be restricted to the chlorophytes *C. sorokiniana* and *C. variabilis* and to the charophyte *K. flaccidum*. Moreover, the homologues of DNMT3/DRM proteins, implicated in de novo DNA methylation, were found exclusively in the red alga *Cyanidioschyzon merolae* and the charophyte *K. flaccidum*. Intriguingly, the *C. merolae*-predicted polypeptide shows a structural organisation similar to the vertebrate DNMT3s, whereas the *K. flaccidum* proteins appeared more closely related to land plant DRMs. In contrast, DNMT5-related enzymes appeared limited to the Mamiellophyceae (*Bathycoccus prasinos*, *Micromonas pusilla* and *Ostreococcus lucimarinus*). The glaucophyte *Cyanophora paradoxa* contains a single DNA methyltransferase that cannot be unequivocally categorised, maybe because this alga genome has not been completely characterised and some proteins may be missing in the database [37].

Among the DNA methyltransferases encoded by microalgae, there are also other enzymes that cannot be clearly categorised. These predicted proteins contain catalytic domains somewhat related to those of the MET1 and/or the CMT subfamilies but lacking either N-terminal extensions or conserved domains in the N-terminal extensions [37]. *Chlamydomonas* DMT4 belongs to this group and it is tempting to speculate that some of these enzymes might be responsible for DNA methylation processes unique to microalgae [39].

Some species of *Chlorella* exhibit a 5-mC pattern of the nuclear genome and a complement of DNA methyltransferases (except for the lack of a DNMT3/DRM homologue) very similar to those observed in land plants. The divergence of DNA cytosine methyltransferases in other Chlorophyceae, i.e., *C. reinhardtii* and *V. carteri* from other land plants, reflect the preferential methylation of transposons and repeats in the CG, rather than in the CHG/CHH context.

Regarding Stramenopile algae, DNMT2 in *Saccharina japonica* potentially mediates the methylation in GC, CHG and CHH contexts as there is no other DNA methyltransferase encoded in the genome of this alga [40]. Instead, in the genome of diatom *Phaeodactylum tricornutum*, a peculiar set of DNMTs, as compared with other eukaryotes, was found. In this diatom, DNMT1 appears to be absent and, in addition to DNMT3, the *P. tricornutum* genome also encodes a DNMT5 protein, as well as a bacterial-like DNMT [41].

Dinoflagellates have a set of DNMTs unlike that of any other eukaryote, which is simplified in protein domain architecture but diversified in copy number [42]. *Symbiodinium* species are two orders of magnitude more abundant in copy number than observed in any other eukaryote. Moreover, well-characterised members of the DNMT1 and DNMT3 families were not found, but several paralogues of the DNMT5, DNMT6 and tRNA methyltransferase DNMT2 families were found in *Symbiodinium* genome [42].

2. DNA Methylation in Algae Response to Abiotic Stress

Plants have evolved a variety of physiological and biochemical mechanisms to cope with environmental hazards. Lipid production, extreme temperatures, lighting, the amount of carbon dioxide, UV exposure, salt content and nutrient starvation are the typical abiotic stress factors that significantly affect the biochemical composition of algal cells [43][44][45][46][47][48].

Several studies describe the involvement of DNA methylation in abiotic stress response. However, the response varies for different stresses in different plant species. Most related studies have been performed on plant model organisms, especially *A. thaliana* [49] and on crops [50]. Few algal epigenetic mechanisms related to stress are known, although it has been reported that epigenetic regulation could play a positive role in stress adaptation in algae [51][52]. In this section, a summary of the recent advances in the research of algal DNA methylation in response to some abiotic stress was provided.

1.1. DNA Methylation and Heavy Metal Stress

Heavy metals (HMs) are significant environmental pollutants, and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons [53][54]. Despite low concentrations of iron (Fe), vanadium (V), zinc (Zn) and molybdenum (Mo) are essential to carry out cellular functions of algae [55][56]; the same metals at high concentrations, also including the unessential HMs, such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb) and mercury (Hg), cause blockages of the cell division, reduction in photosynthesis and inhibition of various thiol-group-containing enzyme activities [57][58][59].

An increasing number of studies are highlighting the role of epigenetic mechanisms in the regulation of plant HMs stress responses [60][61][62]. However, the minimal data in the literature about algal DNA methylation in response to HMs stress, concern only Cd and Cr [51][58][59][63].

Regarding Cr, one of the most diffused and toxic metals in the environment, the exposure of the green alga *Scenedesmus acutus* to Cr (VI), induces specific DNA methylation changes [51]. In detail, the two *S. acutus* strains with different Cr sensitivity showed a very different methylation pattern, as revealed by whole genome bisulfite sequencing (WGBS) [51]. It was supposed that in *S. acutus,* the DNA methylation pattern might be of particular importance in reprogramming primary metabolism during prolonged Cr exposure and might define signal specificity associated with the resistance mechanism to metal stress [51][58]. Thus, under prolonged HMs exposure, algae activity is directed to enhance and/or maintain the signal levels and responses that are relevant during stress to sustain the growth through a different DNA methylation pattern as "stress memory" [51][58].

Moreover, through the immunolocalization of 5-mC, an initial lower methylation level in the Cr-tolerant strain, with respect to the wild type, was found; after Cr treatments, methylation level strongly decreased in the wild type, mainly in the heterochromatic fraction, whereas it increased in the Cr-tolerant strain [58]. Upon Cr exposure, the demethylation and the increase in euchromatin/heterochromatin ratio observed in the wild type suggest a massive gene activation in response to Cr, whereas in the tolerant strain, weaker gene activation and/or gene silencing might be involved in the response to metal exposure [58].

Cd is an unessential trace element ubiquitous in the environment [64]. After 5 days of Cd treatment, a slight increase in total 5-mC (%) level in both *C. reinhardtii* and *Scenedesmus quadricauda* was found, as revealed by global DNA methylation (5-mC) ELISA assay [59].

On the contrary, Cd exposure stimulates DNA demethylation in the red seaweed *Gracilaria dura* genome, resulting in 18.1% of hypomethylation [63]. In this alga, it was also observed that the exogenous putrescine application, either alone or in combination with selenium (Se) during Cd exposure, induced remarkable demethylation events, whereas the addition of Se and spermine (Spm), individually or in combination, significantly reduced the hypomethylation level [63]. As the induction of demethylation under Cd stress may have mediated through oxygen radicals, Se and Spm could be allied

either with the elimination of Cd from enzymes that are active in metabolic processes or with the removal of ROS, stabilising the DNA methylation patterns [63].

1.2. DNA Methylation and Nutrient Stress

The biochemical composition of algae often responds strongly to nutrient stress. For instance, nitrogen (N), sulphur (S) and phosphorus (P) are essential macronutrients needed to promote algal growth, and they regulate metabolic activities if supplied in an acceptable form [65][66]. In algae, nutrient limitation and/or enrichment can induce significant differences in stress response and biochemical composition [67]. Nutrient stress causes the generation of free radical species in the cell and can thus result in changes in antioxidant content [68]. Several studies have investigated the influence of nutrient stress on metabolic changes in microalgae, such as the production enhancing of lipids (reviewed in [48]), carbohydrates [69][70] and/or secondary metabolites [71].

DNA methylation in plants plays a vital role in the response to nutrient changes and is involved in controlling nutrient homeostasis [72]. Although studies on nutrient deprivation in the model organism *C. reinhardtii* are primarily focused on the transcriptional programs underlying these processes [73][74][75][76], the manipulation of DNA methylation in *C. reinhardtii* cultured for many asexual generations in different environments (salt stress, phosphate starvation, and high CO_2), affected its adaptive evolution [52].

The effects of nutrient stress, such as N, P, Fe, Zn and S stress, on DNA methylation are widely provided for plants [50]. The minimal data in the literature regarding algal DNA methylation in response to nutritional stress mainly concern nitrogen deprivation.

DNA methylation plays a role in N responses in the Chlorophyta *Picochlorum soloecismus* and potentially regulates genes that are involved in stress responses and lipid accumulation [77]. Indeed, the genome of this alga becomes hypomethylated during the growth cycle in response to N starvation. The greatest DNA hypomethylation occurs after 10 days in culture under severe N depletion conditions. During N starvation, several of the hypomethylated CG sites of *P. soloecismus* genome are annotated as genes in pathways involved in lipid biosynthesis, including diacylglycerol acyltransferase 2 (*DGAT2*) [77]. Fei et al. (2017) [78] explored the relationship between DNA methylation and transgenic silence upon N deprivation in *C. reinhardtii*. The promoter region of a diacylglycerol acyltransferase (*DGTT3*) was fused to the arylsulfatase (*ARS*) reporter gene, and it was observed that the DNA methylation rate of the transformed insertion region was high. Thus, under N deprivation, although the mRNA of the endogenous DGTT3 was significantly increased, the ARS activity of the chimeric gene expression was significantly decreased [78].

DNA methylation may also be implicated in N metabolism in diatom *P. tricornutum* [41]. The methylation profile of genes that were both methylated in normal conditions and induced in response to nitrate limitation were assessed by bisulfite sequencing in N-limiting conditions. A total of 33 genes were found to be demethylated and displayed higher expression levels than in the normal N-replete conditions [41].

According to Traller et al. (2016) [79], silicon (Si) starvation does not produce as severe phenotypes on diatoms as the nitrogen deprivation, which can cause severe cellular damage and detrimental effects. For this reason, in *P. tricornutum* DNA methylation does not control the response of Si-deplete conditions, as is reported with nitrate metabolism upon N starvation [79]. Indeed, in *Cyclotella cryptica* there is no significant change in the methylation of genes belonging to Si metabolism and/or lipid accumulation under Si-deplete and -replete conditions; nonetheless, a globally significant correlation of methylation is observed between the two growing conditions in this alga [79].

Regarding S deprivation stress, in the Cr-tolerant strain of *S. acutus* it was observed that epigenetic mechanisms play an important role in the modulation of the sulphate pathway [51]. The hypomethylation of the *SaSULTR1* promoter (encoding for a H^+/SO_4^{2-} transporter) is linked to its overexpression in this strain [51] and S starvation induced strong *SaSULTR1* expression [80]. Thus, Ferrari et al. (2022) [80] hypothesised that DNA methylation is also involved in response to S availability in *S. acutus*.

1.3. DNA Methylation and Temperature Stress

Fluctuations in temperature affect numerous metabolic activities in algae. For instance, high temperature stimulates the production of active oxygen species that damage photosynthetic machinery due to the suppression of activities of antioxidant enzymes [81]. Heat stress can also induce changes in the composition and production of lipids [82], as well as many other macromolecules of algae [83]. Low temperature also shows a decrease in growth due to photo-oxidative damage of several macromolecules [84].

Increasing evidence has indicated that DNA methylation plays important roles in the response to temperature stress in land plants [78][85]. Nevertheless, minimal data for algae are reported, which only regard some rhodophytes and symbiotic dinoflagellates [42][86].

By using the MSAP (methylation-sensitive amplified polymorphism) technique, it was observed that both high and low temperature stress induced some changes in the methylation pattern of the red alga *Gracilariopsis lemaneiformis* [86]. Furthermore, the high temperature could induce more cytosine methylation/demethylation events than low-temperature treatment [86]. The increase in DNA methylation level under temperature stress may inhibit the expression of some genes contributing to the temperature adaptation in *G. lemaneiformis* [86].

In the thermoacidophile red alga *G. sulphuraria*, after continuous cold stress (14 °C below its optimal growth temperature) for more than 100 generations, CpG islands located in the intergenic region accumulated a significant number of variants, which is likely a sign of epigenetic remodelling [87]. Moreover, the cold-adapted samples grew ~30% faster than the starting population. The significant growth enhancement of *Galdieria sulphuraria* grown at low temperatures is driven mainly by mutations in genes involved in the cell cycle, gene regulation and signal transfer, as well as mutations that occurred in the intergenic regions, possibly changing the epigenetic methylation pattern and altering the binding specificity to *cis*-regulatory elements [87].

WGBS was performed in the dinoflagellates *Symbiodinium kawagutii* and *Symbiodinium minutum* at different temperature conditions, and global cytosine methylation levels provided similar results across samples of the same species, indicating the minimal effect of thermal stress on global methylation levels [42]. However, through a transcriptomic approach, it was observed that in the dinoflagellate *Biecheleriopsis adriatica* under cold stress, the "methylation" category had the highest difference between up- and down-regulated genes in gene ontology analysis, suggesting that cold stress may affect DNA methylation and gene expression in this microalga [88].

References

1. Law, J.A.; Jacobsen, S.E. Establishing, Maintaining and Modifying DNA Methylation Patterns in Plants and Animals. Nat. Rev. Genet. 2010, 11, 204–220.

2. He, X.-J.; Chen, T.; Zhu, J.-K. Regulation and Function of DNA Methylation in Plants and Animals. Cell Res. 2011, 21, 442–465.

3. Kalisz, S.; Purugganan, M.D. Epialleles via DNA Methylation: Consequences for Plant Evolution. Trends Ecol. Evol. 2004, 19, 309–314.

4. Bochtler, M.; Fernandes, H. DNA Adenine Methylation in Eukaryotes: Enzymatic Mark or a Form of DNA Damage? Bio Essays 2021, 43, 2000243.

5. Chinnusamy, V.; Zhu, J.-K. Epigenetic Regulation of Stress Responses in Plants. Curr. Opin. Plant Biol. 2009, 12, 133–139.

6. Zhang, H.; Lang, Z.; Zhu, J.-K. Dynamics and Function of DNA Methylation in Plants. Nat. Rev. Mol. Cell Biol. 2018, 19, 489–506.

7. Becker, C.; Hagmann, J.; Müller, J.; Koenig, D.; Stegle, O.; Borgwardt, K.; Weigel, D. Spontaneous Epigenetic Variation in the Arabidopsis thaliana Methylome. Nature 2011, 480, 245–249.

8. Lauria, M.; Rossi, V. Epigenetic Control of Gene Regulation in Plants. Biochim. Biophys. Acta (BBA) Gene Regul. Mech. 2011, 1809, 369–378.

9. Schmitz, R.J.; Schultz, M.D.; Lewsey, M.G.; O'Malley, R.C.; Urich, M.A.; Libiger, O.; Schork, N.J.; Ecker, J.R. Transgenerational Epigenetic Instability Is a Source of Novel Methylation Variants. Science 2011, 334, 369–373.

10. Ramsahoye, B.H.; Biniszkiewicz, D.; Lyko, F.; Clark, V.; Bird, A.P.; Jaenisch, R. Non-CpG Methylation Is Prevalent in Embryonic Stem Cells and May Be Mediated by DNA Methyltransferase 3a. Proc. Natl. Acad. Sci. USA 2000, 97, 5237–5242.

11. Lister, R.; Mukamel, E.A.; Nery, J.R.; Urich, M.; Puddifoot, C.A.; Johnson, N.D.; Lucero, J.; Huang, Y.; Dwork, A.J.; Schultz, M.D.; et al. Global Epigenomic Reconfiguration During Mammalian Brain Development. Science 2013, 341, 1237905.

12. Henderson, I.R.; Jacobsen, S.E. Epigenetic Inheritance in Plants. Nature 2007, 447, 418–424.

13. Stroud, H.; Do, T.; Du, J.; Zhong, X.; Feng, S.; Johnson, L.; Patel, D.J.; Jacobsen, S.E. Non-CG Methylation Patterns Shape the Epigenetic Landscape in Arabidopsis. Nat. Struct. Mol. Biol. 2014, 21, 64–72.

14. Kumar, S.; Cheng, X.; Klimasauskas, S.; Sha, M.; Posfai, J.; Roberts, R.J.; Wilson, G.G. The DNA (Cytosine-5) Methyltransferases. Nucleic Acids Res. 1994, 22, 1–10.

15. Pavlopoulou, A.; Kossida, S. Plant Cytosine-5 DNA Methyltransferases: Structure, Function, and Molecular Evolution. Genomics 2007, 90, 530–541.

16. Finnegan, E.J.; Dennis, E.S. Isolation and Identification by Sequence Homology of a Putative Cytosine Methyltransferase from Arabidopsis thaliana. Nucleic Acids Res. 1993, 21, 2383–2388.

17. Saze, H.; Scheid, O.M.; Paszkowski, J. Maintenance of CpG Methylation Is Essential for Epigenetic Inheritance during Plant Gametogenesis. Nat. Genet. 2003, 34, 65–69.

18. Matzke, M.A.; Mosher, R.A. RNA-Directed DNA Methylation: An Epigenetic Pathway of Increasing Complexity. Nat. Rev. Genet. 2014, 15, 394–408.

19. Markulin, L.; Škiljaica, A.; Tokić, M.; Jagić, M.; Vuk, T.; Bauer, N.; Leljak Levanić, D. Taking the Wheel—De Novo DNA Methylation as a Driving Force of Plant Embryonic Development. Front. Plant Sci. 2021, 12, 764999.

20. Goll, M.G.; Bestor, T.H. Eukaryotic Cytosine Methyltransferases. Annu. Rev. Biochem. 2005, 74, 481–514.

21. Du, J.; Johnson, L.M.; Jacobsen, S.E.; Patel, D.J. DNA Methylation Pathways and Their Crosstalk with Histone Methylation. Nat. Rev. Mol. Cell Biol. 2015, 16, 519–532.

22. Goll, M.G.; Kirpekar, F.; Maggert, K.A.; Yoder, J.A.; Hsieh, C.-L.; Zhang, X.; Golic, K.G.; Jacobsen, S.E.; Bestor, T.H. Methylation of TRNAAsp by the DNA Methyltransferase Homolog Dnmt2. Science 2006, 311, 395–398.

23. Kaiser, S.; Jurkowski, T.P.; Kellner, S.; Schneider, D.; Jeltsch, A.; Helm, M. The RNA Methyltransferase Dnmt2 Methylates DNA in the Structural Context of a TRNA. RNA Biol. 2017, 14, 1241–1251.

24. Pikaard, C.S.; Mittelsten Scheid, O. Epigenetic Regulation in Plants. Cold Spring Harb. Perspect. Biol. 2014, 6, a019315.

25. Zemach, A.; McDaniel, I.E.; Silva, P.; Zilberman, D. Genome-Wide Evolutionary Analysis of Eukaryotic DNA Methylation. Science 2010, 328, 916–919.

26. Johnson, L.M.; Bostick, M.; Zhang, X.; Kraft, E.; Henderson, I.; Callis, J.; Jacobsen, S.E. The SRA Methyl-Cytosine-Binding Domain Links DNA and Histone Methylation. Curr. Biol. 2007, 17, 379–384.

27. Bernatavichute, Y.V.; Zhang, X.; Cokus, S.; Pellegrini, M.; Jacobsen, S.E. Genome-Wide Association of Histone H3 Lysine Nine Methylation with CHG DNA Methylation in Arabidopsis thaliana. PLoS ONE 2008, 3, e3156.

28. Lindroth, A.; Cao, X.; Jackson, J.; Zilberman, D.; McCallum, C.; Henikoff, S.; Jacobsen, S. Requirement of CHROMOMETHYLASE3 for Maintenance of CpXpG Methylation. Science 2001, 292, 2077–2080.

29. Cao, X.; Aufsatz, W.; Zilberman, D.; Mette, M.F.; Huang, M.S.; Matzke, M.; Jacobsen, S.E. Role of the DRM and CMT3 Methyltransferases in RNA-Directed DNA Methylation. Curr. Biol. 2003, 13, 2212–2217.

30. Erdmann, R.M.; Picard, C.L. RNA-Directed DNA Methylation. PLoS Genet. 2020, 16, e1009034.

31. Law, J.A.; Du, J.; Hale, C.J.; Feng, S.; Krajewski, K.; Palanca, A.M.S.; Strahl, B.D.; Patel, D.J.; Jacobsen, S.E. Polymerase IV Occupancy at RNA-Directed DNA Methylation Sites Requires SHH1. Nature 2013, 498, 385–389.

32. Blevins, T.; Podicheti, R.; Mishra, V.; Marasco, M.; Wang, J.; Rusch, D.; Tang, H.; Pikaard, C.S. Identification of Pol IV and RDR2-Dependent Precursors of 24 Nt SiRNAs Guiding De Novo DNA Methylation in Arabidopsis. eLife 2015, 4, e09591.

33. Li, S.; Vandivier, L.E.; Tu, B.; Gao, L.; Won, S.Y.; Li, S.; Zheng, B.; Gregory, B.D.; Chen, X. Detection of Pol IV/RDR2-Dependent Transcripts at the Genomic Scale in Arabidopsis Reveals Features and Regulation of SiRNA Biogenesis. Genome Res. 2015, 25, 235–245.

34. Liu, W.; Duttke, S.H.; Hetzel, J.; Groth, M.; Feng, S.; Gallego-Bartolome, J.; Zhong, Z.; Kuo, H.Y.; Wang, Z.; Zhai, J.; et al. RNA-Directed DNA Methylation Involves Co-Transcriptional Small-RNA-Guided Slicing of Polymerase V Transcripts in Arabidopsis. Nat. Plants 2018, 4, 181–188.

35. Panda, K.; Ji, L.; Neumann, D.A.; Daron, J.; Schmitz, R.J.; Slotkin, R.K. Full-Length Autonomous Transposable Elements Are Preferentially Targeted by Expression-Dependent Forms of RNA-Directed DNA Methylation. Genome Biol. 2016, 17, 170.

36. Rastogi, A.; Lin, X.; Lombard, B.; Loew, D.; Tirichine, L. Probing the Evolutionary History of Epigenetic Mechanisms: What Can We Learn from Marine Diatoms. AIMS Genet. 2015, 2, 173–191.

37. Ma, X.; Kim, E.J.; Cerutti, H. Gene Silencing in Archaeplastida Algae. In Plant Gene Silencing; CABI International: Wallingford, UK, 2017; pp. 75–93.

38. Nishiyama, R.; Wada, Y.; Mibu, M.; Yamaguchi, Y.; Shimogawara, K.; Sano, H. Role of a Nonselective De Novo DNA Methyltransferase in Maternal Inheritance of Chloroplast Genes in the Green Alga, Chlamydomonas reinhardtii. Genetics 2004, 168, 809–816.

39. Lopez, D.; Hamaji, T.; Kropat, J.; De Hoff, P.; Morselli, M.; Rubbi, L.; Fitz-Gibbon, S.; Gallaher, S.D.; Merchant, S.S.; Umen, J.; et al. Dynamic Changes in the Transcriptome and Methylome of Chlamydomonas reinhardtii throughout Its Life Cycle. Plant Physiol. 2015, 169, 2730–2743.

40. Fan, X.; Han, W.; Teng, L.; Jiang, P.; Zhang, X.; Xu, D.; Li, C.; Pellegrini, M.; Wu, C.; Wang, Y.; et al. Single-Base Methylome Profiling of the Giant Kelp Saccharina Japonica Reveals Significant Differences in DNA Methylation to Microalgae and Plants. New Phytol. 2019, 225, 234–249.

41. Veluchamy, A.; Lin, X.; Maumus, F.; Rivarola, M.; Bhavsar, J.; Creasy, T.; O'Brien, K.; Sengamalay, N.A.; Tallon, L.J.; Smith, A.D.; et al. Insights into the Role of DNA Methylation in Diatoms by Genome-Wide Profiling in Phaeodactylum tricornutum. Nat. Commun. 2013, 4, 2091.

42. de Mendoza, A.; Bonnet, A.; Vargas-Landin, D.B.; Ji, N.; Li, H.; Yang, F.; Li, L.; Hori, K.; Pflueger, J.; Buckberry, S.; et al. Recurrent Acquisition of Cytosine Methyltransferases into Eukaryotic Retrotransposons. Nat. Commun. 2018, 9, 1341.

43. Morgan-Kiss, R.M.; Priscu, J.C.; Pocock, T.; Gudynaite-Savitch, L.; Huner, N.P.A. Adaptation and Acclimation of Photosynthetic Microorganisms to Permanently Cold Environments. Microbiol. Mol. Biol. Rev. 2006, 70, 222–252.

44. Metsoviti, M.N.; Papapolymerou, G.; Karapanagiotidis, I.T.; Katsoulas, N. Effect of Light Intensity and Quality on Growth Rate and Composition of Chlorella vulgaris. Plants 2020, 9, 31.

45. Varshney, P.; Beardall, J.; Bhattacharya, S.; Wangikar, P.P. Effect of Elevated Carbon Dioxide and Nitric Oxide on the Physiological Responses of Two Green Algae, Asterarcys quadricellulare and Chlorella sorokiniana. J. Appl. Phycol. 2020, 32, 189–204.

46. Takagi, M.; Karseno; Yoshida, T. Effect of Salt Concentration on Intracellular Accumulation of Lipids and Triacylglyceride in Marine Microalgae Dunaliella Cells. J. Biosci. Bioeng. 2006, 101, 223–226.

47. Mishra, S.; Liu, Y.-J.; Chen, C.-S.; Yao, D.-J. An Easily Accessible Microfluidic Chip for High-Throughput Microalgae Screening for Biofuel Production. Energies 2021, 14, 1817.

48. Magoni, C.; Bertacchi, S.; Giustra, C.M.; Guzzetti, L.; Cozza, R.; Ferrari, M.; Torelli, A.; Marieschi, M.; Porro, D.; Branduardi, P.; et al. Could Microalgae Be a Strategic Choice for Responding to the Demand for Omega-3 Fatty Acids? A European Perspective. Trends Food Sci. Technol. 2022, 121, 142–155.

49. Thiebaut, F.; Hemerly, A.S.; Ferreira, P.C.G. A Role for Epigenetic Regulation in the Adaptation and Stress Responses of Non-Model Plants. Front. Plant Sci. 2019, 10, 246.

50. Fan, X.; Peng, L.; Zhang, Y. Plant DNA Methylation Responds to Nutrient Stress. Genes 2022, 13, 992.

51. Ferrari, M.; Torelli, A.; Marieschi, M.; Cozza, R. Role of DNA Methylation in the Chromium Tolerance of Scenedesmus acutus (Chlorophyceae) and Its Impact on the Sulfate Pathway Regulation. Plant Sci. 2020, 301, 110680.

52. Kronholm, I.; Bassett, A.; Baulcombe, D.; Collins, S. Epigenetic and Genetic Contributions to Adaptation in Chlamydomonas. Mol. Biol. Evol. 2017, 34, 2285–2306.

53. Nagajyoti, P.C.; Lee, K.D.; Sreekanth, T.V.M. Heavy Metals, Occurrence and Toxicity for Plants: A Review. Environ. Chem. Lett. 2010, 8, 199–216.

54. Emamverdian, A.; Ding, Y.; Mokhberdoran, F.; Xie, Y. Heavy Metal Stress and Some Mechanisms of Plant Defense Response. Sci. World J. 2015, 2015, 756120.

55. Bothe, H.; Schmitz, O.; Yates, M.G.; Newton, W.E. Nitrogen Fixation and Hydrogen Metabolism in Cyanobacteria. Microbiol. Mol. Biol. Rev. 2010, 74, 529–551.

56. Sunda, W. Feedback Interactions between Trace Metal Nutrients and Phytoplankton in the Ocean. Front. Microbiol. 2012, 3, 204.

57. Monteiro, C.M.; Castro, P.M.L.; Malcata, F.X. Metal Uptake by Microalgae: Underlying Mechanisms and Practical Applications. Biotechnol. Prog. 2012, 28, 299–311.

58. Cozza, D.; Torelli, A.; Veltri, A.; Ferrari, M.; Marieschi, M.; Cozza, R. Ultrastructural Features, Chromium Content and in Situ Immunodetection of 5-Methyl-Cytosine Following Cr (VI) Treatment in Two Strains of Scenedesmus acutus M. (Chlorophyceae) with Different Chromium Sensitivity. Eur. J. Phycol. 2016, 51, 294–306.

59. Bacova, R.; Klejdus, B.; Ryant, P.; Cernei, N.; Adam, V.; Huska, D. The Effects of 5-Azacytidine and Cadmium on Global 5-Methylcytosine Content and Secondary Metabolites in the Freshwater Microalgae Chlamydomonas reinhardtii and Scenedesmus quadricauda. J. Phycol. 2019, 55, 329–342.

60. Pacenza, M.; Muto, A.; Chiappetta, A.; Mariotti, L.; Talarico, E.; Picciarelli, P.; Picardi, E.; Bruno, L.; Bitonti, M.B. In Arabidopsis thaliana Cd Differentially Impacts on Hormone Genetic Pathways in the Methylation Defective Ddc Mutant Compared to Wild Type. Sci. Rep. 2021, 11, 10965.

61. Labra, M.; Grassi, F.; Imazio, S.; Di Fabio, T.; Citterio, S.; Sgorbati, S.; Agradi, E. Genetic and DNA-Methylation Changes Induced by Potassium Dichromate in Brassica napus L. Chemosphere 2004, 54, 1049–1058.

62. Aina, R.; Sgorbati, S.; Santagostino, A.; Labra, M.; Ghiani, A.; Citterio, S. Specific Hypomethylation of DNA Is Induced by Heavy Metals in White Clover and Industrial Hemp. Physiol. Plant. 2004, 121, 472–480.

63. Kumar, M.; Bijo, A.J.; Baghel, R.S.; Reddy, C.R.K.; Jha, B. Selenium and Spermine Alleviate Cadmium Induced Toxicity in the Red Seaweed Gracilaria dura by Regulating Antioxidants and DNA Methylation. Plant Physiol. Biochem. 2012, 51, 129–138.

64. Haider, F.U.; Liqun, C.; Coulter, J.A.; Cheema, S.A.; Wu, J.; Zhang, R.; Wenjun, M.; Farooq, M. Cadmium Toxicity in Plants: Impacts and Remediation Strategies. Ecotoxicol. Environ. Saf. 2021, 211, 111887.

65. Yang, L.; Chen, J.; Qin, S.; Zeng, M.; Jiang, Y.; Hu, L.; Xiao, P.; Hao, W.; Hu, Z.; Lei, A.; et al. Growth and Lipid Accumulation by Different Nutrients in the Microalga Chlamydomonas reinhardtii. Biotechnol. Biofuels 2018, 11, 40.

66. Salbitani, G.; Perrone, A.; Rosati, L.; Laezza, C.; Carfagna, S. Sulfur Starvation in Extremophilic Microalga Galdieria sulphuraria: Can Glutathione Contribute to Stress Tolerance? Plants 2022, 11, 481.

67. Kaur, M.; Saini, K.C.; Ojah, H.; Sahoo, R.; Gupta, K.; Kumar, A.; Bast, F. Abiotic Stress in Algae: Response, Signaling and Transgenic Approaches. J. Appl. Phycol. 2022, 34, 1843–1869.

68. Goiris, K.; Van Colen, W.; Wilches, I.; León-Tamariz, F.; De Cooman, L.; Muylaert, K. Impact of Nutrient Stress on Antioxidant Production in Three Species of Microalgae. Algal Res. 2015, 7, 51–57.

69. Dragone, G.; Fernandes, B.D.; Abreu, A.P.; Vicente, A.A.; Teixeira, J.A. Nutrient Limitation as a Strategy for Increasing Starch Accumulation in Microalgae. Appl. Energy 2011, 88, 3331–3335.

70. Harun, R.; Danquah, M.K. Influence of Acid Pre-Treatment on Microalgal Biomass for Bioethanol Production. Process. Biochem. 2011, 46, 304–309.

71. Skjånes, K.; Rebours, C.; Lindblad, P. Potential for Green Microalgae to Produce Hydrogen, Pharmaceuticals and Other High Value Products in a Combined Process. Crit. Rev. Biotechnol. 2013, 33, 172–215.

72. Huang, X.-Y.; Chao, D.-Y.; Koprivova, A.; Danku, J.; Wirtz, M.; Müller, S.; Sandoval, F.J.; Bauwe, H.; Roje, S.; Dilkes, B.; et al. Nuclear Localised MORE SULPHUR ACCUMULATION1 Epigenetically Regulates Sulphur Homeostasis in Arabidopsis thaliana. PLoS Genet. 2016, 12, e1006298.

73. Nguyen, A.V.; Thomas-Hall, S.R.; Malnoë, A.; Timmins, M.; Mussgnug, J.H.; Rupprecht, J.; Kruse, O.; Hankamer, B.; Schenk, P.M. Transcriptome for Photobiological Hydrogen Production Induced by Sulfur Deprivation in the Green Alga Chlamydomonas reinhardtii. Eukaryot. Cell 2008, 7, 1965–1979.

74. González-Ballester, D.; Casero, D.; Cokus, S.; Pellegrini, M.; Merchant, S.S.; Grossman, A.R. RNA-Seq Analysis of Sulfur-Deprived Chlamydomonas Cells Reveals Aspects of Acclimation Critical for Cell Survival. Plant Cell 2010, 22, 2058–2084.

75. Toepel, J.; Albaum, S.P.; Arvidsson, S.; Goesmann, A.; la Russa, M.; Rogge, K.; Kruse, O. Construction and Evaluation of a Whole Genome Microarray of Chlamydomonas reinhardtii. BMC Genom. 2011, 12, 579.

76. Schmollinger, S.; Mühlhaus, T.; Boyle, N.R.; Blaby, I.K.; Casero, D.; Mettler, T.; Moseley, J.L.; Kropat, J.; Sommer, F.; Strenkert, D.; et al. Nitrogen-Sparing Mechanisms in Chlamydomonas Affect the Transcriptome, the Proteome, and Photosynthetic Metabolism. Plant Cell 2014, 26, 1410–1435.

77. Steadman, C.R.; Banerjee, S.; Kunde, Y.A.; Sanders, C.K.; Marrone, B.L.; Twary, S.N. Inhibition of DNA Methylation in Picochlorum soloecismus Alters Algae Productivity. Front. Genet. 2020, 11, 560444.

78. Fei, X.; Li, P.; Li, X.; Deng, X. Low-Temperature- and Phosphate Deficiency-Responsive Elements Control DGTT3 Expression in Chlamydomonas Reinhardtii. J. Eukaryot. Microbiol. 2018, 65, 117–126.

79. Traller, J.C.; Cokus, S.J.; Lopez, D.A.; Gaidarenko, O.; Smith, S.R.; McCrow, J.P.; Gallaher, S.D.; Podell, S.; Thompson, M.; Cook, O.; et al. Genome and Methylome of the Oleaginous Diatom Cyclotella cryptica Reveal Genetic Flexibility toward a High Lipid Phenotype. Biotechnol. Biofuels 2016, 9, 258.

80. Ferrari, M.; Cozza, R.; Marieschi, M.; Torelli, A. Role of Sulfate Transporters in Chromium Tolerance in Scenedesmus acutus M. (Sphaeropleales). Plants 2022, 11, 223.

81. Ma, D.; Li, Y.; Fu, H. Effect of High Temperature on the Balance between Photosynthetic Light Absorption and Energy Utilization in Chlorella pyrenoidosa (Chlorophyceae). J. Oceanol. Limnol. 2020, 38, 186–194.

82. Calhoun, S.; Bell, T.A.S.; Dahlin, L.R.; Kunde, Y.; LaButti, K.; Louie, K.B.; Kuftin, A.; Treen, D.; Dilworth, D.; Mihaltcheva, S.; et al. A Multi-Omic Characterization of Temperature Stress in a Halotolerant Scenedesmus Strain for Algal Biotechnology. Commun. Biol. 2021, 4, 333.

83. Zhao, T.; Han, X.; Cao, H. Effect of Temperature on Biological Macromolecules of Three Microalgae and Application of FT-IR for Evaluating Microalgal Lipid Characterization. ACS Omega 2020, 5, 33262–33268.

84. Zheng, Y.; Xue, C.; Chen, H.; He, C.; Wang, Q. Low-Temperature Adaptation of the Snow Alga Chlamydomonas Nivalis Is Associated With the Photosynthetic System Regulatory Process. Front. Microbiol. 2020, 11, 1233.

85. Qian, Y.; Hu, W.; Liao, J.; Zhang, J.; Ren, Q. The Dynamics of DNA Methylation in the Maize (Zea mays L.) Inbred Line B73 Response to Heat Stress at the Seedling Stage. Biochem. Biophys. Res. Commun. 2019, 512, 742–749.

86. Peng, C.; Sui, Z.; Zhou, W.; Hu, Y.; Mi, P.; Jiang, M.; Li, X.; Ruan, X. Analysis of DNA Methylation of Gracilariopsis Lemaneiformis Under Temperature Stress Using the Methylation Sensitive Amplification Polymorphism (MSAP) Technique. J. Ocean Univ. China 2018, 17, 623–631.

87. Rossoni, A.W.; Weber, A.P.M. Systems Biology of Cold Adaptation in the Polyextremophilic Red Alga Galdieria sulphuraria. Front. Microbiol. 2019, 10, 927.

88. Kang, H.C.; Jeong, H.J.; Park, S.A.; Ok, J.H.; You, J.H.; Eom, S.H.; Park, E.C.; Jang, S.H.; Lee, S.Y. Comparative Transcriptome Analysis of the Phototrophic Dinoflagellate Biecheleriopsis adriatica Grown Under Optimal Temperature and Cold and Heat Stress. Front. Mar. Sci. 2021, 8, 761095.