RNA Methylations

RNA methylations, such as N6-methyladenosine (m6A) and 5-methylcytidine (m5C), are the most common mRNA modifications in eukaryotes, with m6A being more prevalent in both plants and animals.

1. Overview

Unpredictable climate changes cause plants to often be exposed to various abiotic stresses that mainly include extreme temperature, dehydration, high salinity, low nutrition, ultraviolet radiation, and heavy metal toxicity, which all affect the productivity of plants. The dynamics of epigenetic codes play an important role in regulating genes in response to environmental stresses [1]. For example, histone modification and DNA methylation are known to alter the expression of stress-responsive genes at the transcriptional and post-transcriptional levels by changing the chromatin status of those genes [2]. For instance, transcription factors and RNA-directed DNA methylation (RdDM) play a key role in the regulation of gene expression under abiotic stress in plants. Some known heat stress genes are regulated through RdDM pathway-mediated DNA methylation [3]. As another example, the MYB74 transcription factor in the Arabidopsis plant is silenced by RdDM in normal conditions, and when the plant experiences salinity stress, it will become desilenced [4]. In addition, some epigenetic changes such as histone modifications play a key role in stress memory, which can be passed on to offspring [5].

How epigenetic marks affect gene expressions depends not only on the type of modification but also on their position on the genes, meaning, for example, if they are placed on the promoter region or gene body. For instance, when DNA methylation and H3K9me2 are positioned within the promoter region, they may prevent transcription and therefore repress the expression of genes, whereas there are cases where these heterochromatin epigenetic marks are located in the gene body and then help with the full-length transcription of the gene [6]. Transposable elements (TEs) often insert into the gene body of stress-related genes in plants' genomes, and therefore, it is very likely that intragenic epigenetic modifications regulate the expression of stress-responsive genes, for example by affecting the alternative splicing of the transcripts [7]. Epigenetic changes usually return to their prestress state after the stimulus that was causing the stress is gone. However, it has been reported that in some cases, the epigenetic changes (or part of them) remain even after the withdrawal of stress and act as stress memory for plants. This stress memory then helps with their adaptation and even evolution in the longer term. Possible usage of this trait in plants can help to advance epigenome engineering in order to improve plants' tolerance to environmental stresses and climate changes.

When genetically identical plants are exposed to various stresses, they show changes in DNA methylation. For example, apomictic *Taraxacum officinale* plants were exposed to some abiotic stresses, and all plants in all types of stresses showed significant variations in DNA methylation [8]. The results indicated that DNA methylation variation caused by stress is common and highlighted that epigenetic inheritance in the adaptation of plants can be independent of genetic variation among individuals. Many studies have also reported that stress-induced methylation patterns depend on the type of stress, the genotype, the tissue, and the organism, which also affect the regulation of a wide range of stress-responsive genes [9][10][11]. Another example is a comparative analysis of the methylome and gene expression in sixty annual clones of a stress-tolerant poplar genotype (*Populus simonii*), where the authors investigated the effect of four abiotic stress treatments (salinity, osmotic, heat, and cold) from 3 h to 24 h [12]. The DNA methylation level was higher after three hours of all stress treatments and was especially the highest for heat stress. The heat stress raised the methylation level to its maximum at six hours and maintained it unchanged for the rest of the treatment, whereas other stresses gradually raised the methylation levels up to 24 h. At the end of the treatment time (at 24 h), the DNA methylation levels were the highest for osmotic and cold treatment samples. Therefore, the patterns of methylated fragments were stress-specific, and different stress treatments showed different impacts on the expression of DNA methylation genes. In addition, some

methylated fragments mapped to miRNAs and long noncoding RNAs. These noncoding RNAs and their putative target genes showed different expression patterns to stresses which could imply the effect of epigenetic regulation of noncoding RNA expression in the stress response of plants.

2. RNA Methylation and Abiotic Stress Responses in Plants

The level of m6A varies according to the activity of the cellular factors and enzymatic machinery, named "writers (methyltransferase)", which catalyze the methylation process; "erasers (demethylase)", mediating adenosine demethylation; and "readers (RNA-binding protein)", introducing, deleting, and interpreting specific methylation marks on mRNAs, respectively [13]. Our knowledge of writers, readers, and erasers in plants is far behind that of their animal counterparts, and the identity and functions of these factors in plants are currently not very clear. Although recent studies have reported about the roles of m6A writers in plant growth and development [14][15], deep studies about their impact on plant response to abiotic stresses are lacking. Hu et al. [13] systematically identified potential m6A writers, readers, and erasers in *A. thaliana* and rice (*Oryza sativa*) by searching their homologous sequences against animal databases. They analyzed publicly available microarray data and reported that expressions levels of writers in Arabidopsis and rice are differently affected by diverse abiotic stresses. In Arabidopsis, levels of most m6A writer components were not significantly modulated by abiotic stresses or in some cases were only marginally increased by only cold and heat stress. In rice, the level of some writers was increased by cold stress, whereas the levels of others were decreased by cold, drought, or salt stress. The expression of m6A writer components under both normal and stress conditions led to the conclusion that m6A methylation in plants may affect both the development and stress responses. In another study, Anderson et al. [16] reported differential mRNA m6A methylation in Arabidopsis upon salt treatment and identified a strong association between m6A methylation and salinity stress response. A study using Arabidopsis mutants of *m6A* writer components showed the important role of m6A methylation in salt tolerance [17]. It was reported that one of the m6A writer components named VIRILIZER (VIR) modulates the expression of many salt-stress-response genes. It was also reported that VIR-mediated m6A mRNA methylation is associated with the mRNA stability of salt-stress-negative regulators. The findings suggested a link between m6A methylation and mRNA stability during adaptation to stress [17].

Erasers are the least studied among RNA methylation factors in plants. However, new information is being collected [18]. The α-ketoglutarate-dependent dioxygenase homolog (ALKBH) protein family is one of the known erasers in plants. Thirteen members of them have been identified by bioinformatic analysis in Arabidopsis [19], and only a few of them have been studied. It has been shown that ALKBH9A is highly expressed in roots under salt stress but not in response to ABA, and its level of expression is much lower than ALKBH9 and ALKBH10 under normal conditions [20]. ALKBH10A is downregulated under heat stress [21], whereas ALKBH10B is upregulated in response to karrikins [22]. These studies imply a potential role for ALKBHs in stress responses. In their analyses, Hu et al. [13] reported that expression levels of ALKBH members were marginally up- or downregulated under different abiotic stress factors. For example, ALKBH1 was upregulated under drought, cold, or ABA treatment in rice, whereas ALKBH6, ALKBH8B, and ALKBH10A were all downregulated by drought, ABA, or cold. They concluded that ALKBHs could potentially be important for abiotic stress responses, although this requires more investigations.

Adenosine methylation of mRNA results in its remodeling and therefore increases the chances for its binding with specific reader proteins, which are usually members of the YTH family [23]. Although several RNA methylation reader proteins (interpreting m6A marks) have been reported in animals, only three m6A reader proteins (from YTHD family) are identified in Arabidopsis [14][24][25]. Different expression levels of proteins belonging to the YTH domain family in response to stress factors are reported that suggest their role in plants' reaction to stressful conditions (e.g., YTHD09). Cytoplasmic-localized YTHD09 relocates to stress granules upon heat stress [25][26][27]. This is also supported by studies that introduced some YTH domain proteins from apple into Arabidopsis plants, and as a result, higher tolerance to salinity and drought in Arabidopsis was found [28]. It has also been shown that the expression of different members of the YTHD family in Arabidopsis and rice is either increased or decreased under different abiotic stresses [13][29]. The fact that m6A reader proteins are more responsive to abiotic stresses than writers and erasers, or at least this is what appears to be based on our knowledge at the time of writing this review, implies that under stress conditions, the decoding and interpreting of methylation marks are much more important than methylation and demethylation, which might help with the adaptation of plants to stresses. Future studies are needed to identify the function of reader proteins in RNA metabolism and its impact on stress tolerance in plants.

Even though m6A is the most common mRNA methylation, other methylated ribonucleotides might also have significant effects on the functioning of plant cells [30]. In fact, m5C in mRNA has been reported in *A. thaliana*, *Zea mays*, *Oryza sativa*, *foxtail millet*, and *Medicago truncatula*. This modification occurs with the activity of tRNA-specific methyltransferase 4 (TRM4) and various external factors, such as drought, heat, and treatment with phytohormones. A reduced level of m5C

is correlated with reduced root length, inhibited cell proliferation, and higher sensitivity to oxidative stress, which suggests the role of m5C in the regulation of both plant development and oxidative responses in plants [31][32]. Although cytosine methylation (m5C) in DNA has been studied for many years, its functions in RNAs are just starting to be noticed. Overall, it is a less common modification of mRNA than m6A methylation. In their analyses, Hu et al. [13] found two enzymes responsible for m5C RNA methylation in Arabidopsi. Even though their expression patterns suggested the potential roles of m5C writers in abiotic stress response, the relevance of m5C methylation to abiotic stress responses was not clear and needed more investigation.

3. Conclusions

In summary, epigenetic marks on stress-induced genes dynamically change and therefore affect the accessibility of chromatin and the expression of those genes at the transcriptional or translational level. Epigenetic changes such as DNA methylation, histone modifications, chromatin remodeling, histone variants, and long noncoding RNAs (lncRNAs) may all be involved in the various regulatory mechanisms of abiotic stress responses. The important role of epigenetic modifications in regulating gene expression, and also their ability to transfer to the next generation, makes them a unique adaptation tool for plants. The phenotypic plasticity caused by epigenetic variation, which, in turn, is through changes in gene expression, will affect fitness and eventually natural selection in plants. Unlike classic DNA sequence mutations, epimutations can happen at much shorter times, and even though they are stable, they are also mostly reversible, which makes them a perfect tool for a quick emergency response to unpredictable environmental stresses. It should also be noted that epigenetic variations usually depend on the underlying genetic variation, and these two aspects need to be studied in parallel. Future studies are needed for a deeper understanding of the epigenetic mechanisms behind chromatin alterations and the subsequent transcriptional regulations that affect plants' response to environmental stresses. The mechanism of inherited stress memory also needs more attention.

References

1. Zhang, H.; Lang, Z.; Zhu, J.K. Dynamics and function of DNA methylation in plants. Nat. Rev. Mol. Cell Biol. 2018, 19, 489–506.

2. Luo, X.; He, Y. Experiencing winter for spring flowering: A molecular epigenetic perspective on vernalization. J. Integr. Plant Biol. 2020, 62, 104–117.

3. Popova, O.V.; Dinh, H.Q.; Aufsatz, W.; Jonak, C. The RdDM pathway is required for basal heat tolerance in Arabidopsis. Mol. Plant 2013, 6, 396–410.

4. Xu, R.; Wang, Y.; Zheng, H.; Lu, W.; Wu, C.; Huang, J.; Yan, K.; Yang, G.; Zheng, C. Salt-induced transcription factor MYB74 is regulated by the RNA-directed DNA methylation pathway in Arabidopsis. J. Exp. Bot. 2015, 66, 5997–6008.

5. Friedrich, T.; Faivre, L.; Bäurle, I.; Schubert, D. Chromatin-based mechanisms of temperature memory in plants. Plant Cell Environ. 2019, 42, 762–770.

6. Lei, M.; La, H.; Lu, K.; Wang, P.; Miki, D.; Ren, Z.; Duan, C.-G.; Wang, X.; Tang, K.; Zeng, L.; et al. Arabidopsis EDM2 promotes IBM1 distal polyadenylation and regulates genome DNA methylation patterns. Proc. Natl. Acad. Sci. USA 2014, 111, 527.

7. Chang, Y.-N.; Zhu, C.; Jiang, J.; Zhang, H.; Zhu, J.-K.; Duan, C.-G. Epigenetic regulation in plant abiotic stress responses. J. Integr. Plant Biol. 2020, 62, 563–580.

8. Verhoeven, K.J.; Jansen, J.J.; van Dijk, P.J.; Biere, A. Stress-induced DNA methylation changes and their heritability in asexual dandelions. New Phytol. 2010, 185, 1108–1118.

9. Bonasio, R.; Tu, S.; Reinberg, D. Molecular signals of epigenetic states. Science 2010, 330, 612–616.

10. Wang, W.-S.; Pan, Y.-J.; Zhao, X.-Q.; Dwivedi, D.; Zhu, L.-H.; Ali, J.; Fu, B.-Y.; Li, Z.-K. Drought-induced site-specific DNA methylation and its association with drought tolerance in rice (Oryza sativa L.). J. Exp. Bot. 2011, 62, 1951–1960.

11. Mastan, S.G.; Rathore, M.S.; Bhatt, V.D.; Yadav, P.; Chikara, J. Assessment of changes in DNA methylation by methylation-sensitive amplification polymorphism in Jatropha curcas L. subjected to salinity stress. Gene 2012, 508, 125–129.

12. Song, Y.; Ci, D.; Tian, M.; Zhang, D. Stable methylation of a non-coding RNA gene regulates gene expression in response to abiotic stress in Populus simonii. J. Exp. Bot. 2016, 67, 1477–1492.

13. Hu, J.; Manduzio, S.; Kang, H. Epitranscriptomic RNA Methylation in Plant Development and Abiotic Stress Responses. Front. Plant Sci. 2019, 10, 500.

14. Wei, L.-H.; Song, P.; Wang, Y.; Lu, Z.; Tang, Q.; Yu, Q.; Xiao, Y.; Zhang, X.; Duan, H.-C.; Jia, G. The m6A Reader ECT2 Controls Trichome Morphology by Affecting mRNA Stability in Arabidopsis. Plant Cell 2018, 30, 968–985.

15. Shen, L.; Liang, Z.; Gu, X.; Chen, Y.; Teo, Z.W.N.; Hou, X.; Cai, W.M.; Dedon, P.C.; Liu, L.; Yu, H. N(6)-Methyladenosine RNA Modification Regulates Shoot Stem Cell Fate in Arabidopsis. Dev. Cell 2016, 38, 186–200.

16. Anderson, S.J.; Kramer, M.C.; Gosai, S.J.; Yu, X.; Vandivier, L.E.; Nelson, A.D.L.; Anderson, Z.D.; Beilstein, M.A.; Fray, R.G.; Lyons, E.; et al. N(6)-Methyladenosine Inhibits Local Ribonucleolytic Cleavage to Stabilize mRNAs in Arabidopsis. Cell Rep. 2018, 25, 1146–1157.e1143.

17. Hu, J.; Cai, J.; Park, S.J.; Lee, K.; Li, Y.; Chen, Y.; Yun, J.-Y.; Xu, T.; Kang, H. N6-Methyladenosine mRNA methylation is important for salt stress tolerance in Arabidopsis. Plant J. 2021, n/a.

18. Mielecki, D.; Zugaj, D.Ł.; Muszewska, A.; Piwowarski, J.; Chojnacka, A.; Mielecki, M.; Nieminuszczy, J.; Grynberg, M.; Grzesiuk, E. Novel AlkB Dioxygenases—Alternative Models for In Silico and In Vivo Studies. PLoS ONE 2012, 7, e30588.

19. Ma, S.; Gong, Q.; Bohnert, H.J. Dissecting salt stress pathways. J. Exp. Bot. 2006, 57, 1097–1107.

20. Duan, H.-C.; Wei, L.-H.; Zhang, C.; Wang, Y.; Chen, L.; Lu, Z.; Chen, P.R.; He, C.; Jia, G. ALKBH10B Is an RNA N 6-Methyladenosine Demethylase Affecting Arabidopsis Floral Transition. Plant Cell 2017, 29, 2995–3011.

21. Merret, R.; Nagarajan, V.K.; Carpentier, M.-C.; Park, S.; Favory, J.-J.; Descombin, J.; Picart, C.; Charng, Y.-y.; Green, P.J.; Deragon, J.-M.; et al. Heat-induced ribosome pausing triggers mRNA co-translational decay in Arabidopsis thaliana. Nucleic Acids Res. 2015, 43, 4121–4132.

22. Nelson, D.C.; Flematti, G.R.; Riseborough, J.-A.; Ghisalberti, E.L.; Dixon, K.W.; Smith, S.M. Karrikins enhance light responses during germination and seedling development in Arabidopsis thaliana. Proc. Natl. Acad. Sci. USA 2010, 107, 7095.

23. Liu, N.; Dai, Q.; Zheng, G.; He, C.; Parisien, M.; Pan, T. N6-methyladenosine-dependent RNA structural switches regulate RNA–protein interactions. Nature 2015, 518, 560–564.

24. Arribas-Hernández, L.; Bressendorff, S.; Hansen, M.H.; Poulsen, C.; Erdmann, S.; Brodersen, P. An m6A-YTH Module Controls Developmental Timing and Morphogenesis in Arabidopsis. Plant Cell 2018, 30, 952–967.

25. Scutenaire, J.; Deragon, J.-M.; Jean, V.; Benhamed, M.; Raynaud, C.; Favory, J.-J.; Merret, R.; Bousquet-Antonelli, C. The YTH Domain Protein ECT2 Is an m6A Reader Required for Normal Trichome Branching in Arabidopsis. Plant Cell 2018, 30, 986–1005.

26. Wang, N.; Guo, T.; Sun, X.; Jia, X.; Wang, P.; Shao, Y.; Liang, B.; Gong, X.; Ma, F. Functions of two Malus hupehensis (Pamp.) Rehd. YTPs (MhYTP1 and MhYTP2) in biotic- and abiotic-stress responses. Plant Sci. 2017, 261, 18–27.

27. Zhou, Y.; Hu, L.; Jiang, L.; Liu, S. Genome-wide identification and expression analysis of YTH domain-containing RNA-binding protein family in cucumber (Cucumis sativus). Genes Genom. 2018, 40, 579–589.

28. Wang, N.; Guo, T.; Wang, P.; Sun, X.; Shao, Y.; Jia, X.; Liang, B.; Gong, X.; Ma, F. MhYTP1 and MhYTP2 from Apple Confer Tolerance to Multiple Abiotic Stresses in Arabidopsis thaliana. Front. Plant Sci. 2017, 8, 1367.

29. Li, D.; Zhang, H.; Hong, Y.; Huang, L.; Li, X.; Zhang, Y.; Ouyang, Z.; Song, F. Genome-Wide Identification, Biochemical Characterization, and Expression Analyses of the YTH Domain-Containing RNA-Binding Protein Family in Arabidopsis and Rice. Plant Mol. Biol. Rep. 2014, 32, 1169–1186.

30. Traube, F.R.; Carell, T. The chemistries and consequences of DNA and RNA methylation and demethylation. RNA Biol. 2017, 14, 1099–1107.

31. Cui, X.; Liang, Z.; Shen, L.; Zhang, Q.; Bao, S.; Geng, Y.; Zhang, B.; Leo, V.; Vardy, L.A.; Lu, T.; et al. 5-Methylcytosine RNA Methylation in Arabidopsis thaliana. Mol. Plant 2017, 10, 1387–1399.

32. David, R.; Burgess, A.; Parker, B.; Li, J.; Pulsford, K.; Sibbritt, T.; Preiss, T.; Searle, I.R. Transcriptome-Wide Mapping of RNA 5-Methylcytosine in Arabidopsis mRNAs and Noncoding RNAs. Plant Cell 2017, 29, 445–460.