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Research Article

m6A mRNA modification promotes chilling tolerance and modulates gene translation efficiency in Arabidopsis

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Abstract

N^6-methyladenosine (m6A), the most prevalent mRNA modification in eukaryotes, is an emerging player of gene regulation at transcriptional and translational levels. Here, we explored the role of m6A modification in response to low temperature in Arabidopsis (*Arabidopsis thaliana*). Knocking down mRNA adenosine methylase A (MTA), a key component of the modification complex, by RNA interference (RNAi) led to drastically reduced growth at low temperature, indicating a critical role of m6A modification in the chilling response. Cold treatment reduced the overall m6A modification level of mRNAs especially at the 3′ untranslated region. Joint analysis of the m6A methylome, transcriptome and translatome of the wild type (WT) and the *MTA* RNAi line revealed that m6A-containing mRNAs generally had higher abundance and translation efficiency than non-m6A-containing mRNAs under normal and low temperatures. In addition, reduction of m6A modification by *MTA* RNAi only moderately altered the gene expression response to low temperature but led to dysregulation of translation efficiencies of one third of the genes of the genome in response to cold. We tested the function of the m6A-modified cold-responsive gene *ACYL-COA:DIACYLGLYCEROL ACYLTRANSFERASE 1* (*DGAT1*) whose translation efficiency but not transcript level was reduced in the chilling-susceptible *MTA* RNAi plant. The *dgat1* loss-of-function mutant exhibited reduced growth under cold stress. These results reveal a critical role of m6A modification in regulating growth under low temperature and suggest an involvement of translational control in chilling responses in Arabidopsis.

Introduction

N^6-methyladenosine (m6A) is the most prevalent nucleotide modification of mRNA in eukaryotes and is extensively characterized as an epi-transcriptomic mark (Fu et al. 2014; Meyer and Jaffrey 2014). m6A modification is associated with a wide range of mRNA metabolic processes, including mRNA stability (Wang et al. 2014), splicing (Xiao et al. 2016), translation efficiency (Wang et al. 2015; Shi et al. 2017; Slobodin et al. 2017), primary-microRNA processing (Alarcon et al. 2015; Bhat et al. 2020), alternative polyadenylation (Yue et al. 2018; Hou et al. 2021; Song et al. 2021), nuclear-to-cytoplasmic export (Roundtree et al. 2017), and maintenance of transcriptome integrity (Pontier et al. 2019). m6A modification is carried out by a m6A "writer" complex containing a group of proteins that are highly conserved between animals and plants. In Arabidopsis (*Arabidopsis thaliana*), the m6A methyltransferase complex

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consists of mRNA adenosine methylase A (MTA), methyltransferase B (MTB), FKBP12 interacting protein 37 kDa (FIP37), Virilizer (VIR), and the E3 ubiquitin ligase HAKAI (Zhong et al. 2008; Shen et al. 2016; Ruzicka et al. 2017). The m6A marks in mRNAs are recognized by m6A "readers," namely "evolutionarily conserved C-terminal region" (ECT) proteins, also known as YT521-B homology (YTH) domain proteins in mammals (Arribas-Hernandez et al. 2018, 2020; Scutenaire et al. 2018; Wei et al. 2018). This modification can be deleted from transcripts by "erasers," including the AlkB homologue 9B (ALKBH9B) and ALKBH10B proteins in Arabidopsis (Duan et al. 2017; Martinez-Perez et al. 2017). Studies on these core members of writers, erasers, and readers in plants indicate that m6A plays vital roles in embryo development (Zhong et al. 2008; Bodi et al. 2012), leaf morphogenesis (Shen et al. 2016; Ruzicka et al. 2017; Arribas-Hernandez et al. 2018, 2020), trichome branching (Vespa et al. 2004; Bodi et al. 2012; Arribas-Hernandez et al. 2018; Wei et al. 2018), floral transition (Duan et al. 2017; Song et al. 2021), root growth (Ruzicka et al. 2017; Chen et al. 2018), pathogen response (Martinez-Perez et al. 2017), and salt tolerance (Hu et al. 2021; Zheng et al. 2021). These findings indicate a prevalent involvement of m6A modification in diverse processes, although the exact mechanisms underlying the regulation especially in stress tolerance are not fully understood.

Plants respond to low temperature with diverse regulatory mechanisms occurring at transcriptional and post-transcriptional levels. Over the past 2 decades, the C-REPEAT BINDING FACTOR/DEHYDRATION-RESPONSIVE ELEMENT -BINDING PROTEIN1 (CBF/DREB1)-dependent cold signaling pathway has been extensively studied. The 3 cold-induced Arabidopsis CBF proteins, CBF1-3 are APETALA2/ETHYLEN E-RESPONSIVE (AP2/ERF1)-type transcription factors that bind to the conserved CRT/DRE motifs in the promoters of *COR* (Cold-regulated) genes and activate their expression under cold conditions (Gilmour et al. 1998; Liu et al. 1998; Medina et al. 1999). The *cbf1 cbf2 cbf3* triple knockout mutant displayed compromised freezing tolerance (Jia et al. 2016; Zhao et al. 2016). The induction of the *CBF* genes by low temperature is mainly regulated by transcription factors ICE1 (Inducer of CBF expression 1), ICE2 and the 3 closely related CAMTAs (Calmodulin-binding transcription activators) (Chinnusamy et al. 2003; Doherty et al. 2009; Fursova et al. 2009; Kim et al. 2013; Tang et al. 2020). ICE1 is modified at the post-translational level, including ubiquitinated by HOS1 (high expression of osmotically responsive gene 1), sumoylated by SIZ1 (SUMO E3 ligase for SAP and Miz), and phosphorylated by OST1 (Open stomata1) and MPK3/6 (Mitogen-activated protein kinase 3/6) (Dong et al. 2006; Miura et al. 2007; Ding et al. 2015; Li et al. 2017; Zhao et al. 2017). In addition, accumulating evidence revealed the important roles of protein translation regulation in chilling growth. The Arabidopsis ribosome biogenesis factors REI1-LIKE (REIL) proteins positively regulate growth under chilling through influencing cold-induced ribosome remodeling and enhance the accumulation of cytosolic ribosome subunits after cold shift (Beine-Golovchuk et al. 2018). The *REIL2* gene was also found to enhance freezing tolerance by promoting CBFs translation under low temperature (Yu et al. 2020). The mutant of Arabidopsis cytosolic ribosome biogenesis gene *RPL9* (Ribosomal Protein L9) showed reduced hypocotyl growth under chilling but not at normal temperature (Liu et al. 2021). The chloroplast translation elongation factor SVR3 (Suppressor of variegation 3), the ribosomal large subunit protein L24 (SVR8: Suppressor of variegation 8), and the rRNA maturation factors NUS1 (N utilization substance 1) and RBD1 (RN A-binding domain 1) also have been shown to be important for chilling tolerance in Arabidopsis (Liu et al. 2010, 2013; Kusumi et al. 2011; Kupsch et al. 2012; Wang et al. 2016).

Here, we studied the role of m6A modification in growth under chilling conditions in Arabidopsis by analyzing mutants of the mRNA m6A writer components *MTA* and *FIP37*. We found that cold treatment reduces the global m6A abundance and down-methylated genes were enriched on diverse biological processes whereas up-methylated genes were mainly involved in defense response. In addition, m6A-containing genes displayed a significantly higher transcript level and a higher translation efficiency than non-m6A-containing genes. However, the alterations of m6A abundance caused by chilling were not correlated with changes of transcript level or translation efficiency in response to cold. Lack of m6A modification led to a change of the transcript level of 3,592 genes and a change of translation efficiency of 7,340 genes at 24 h after cold treatment. We further identified a chilling tolerance function for a diacylglycerol acyltransferase gene *DGAT1* (*ACYL-COA: DIACYLGLYCEROL ACYLTRANSFERASE 1*), whose m6A modification and translation efficiency, but not transcript level, were decreased in the *AmiR-mta* plants. This study uncovers a role of m6A in the regulation of translation efficiency and suggests the importance of such a regulation in chilling tolerance.

Results

The mRNA m6A writer complex is essential for chilling tolerance in Arabidopsis

To determine the biological function of m6A under chilling stress in Arabidopsis, we analyzed knockdown mutants of its writer complex as the knockout mutant of a writer subunit is lethal (Zhong et al. 2008; Ruzicka et al. 2017). Using artificial microRNA (AmiR) interference (Schwab et al. 2006), we generated *AmiR-mta* transgenic plants that had the *MTA* expression reduced to 20% of the WT Col-0 level (Fig. 1, A and B) . A reduction of function mutant of *FIP37*, *fip37-4*, was available from a T-DNA insertion mutant collection (Alonso et al. 2003), and it had a reduced expression of *FIP37* (Ruzicka et al. 2017). Although this mutant was reported to be homozygous lethal (Shen et al. 2016), it was fertile in our growth conditions as in some other studies (Ruzicka et al. 2017; Zhang et al. 2022). To assess the degree of reduction of m6A modification in these plants, we analyzed purified mRNAs from rosette leaves by dot-blot with

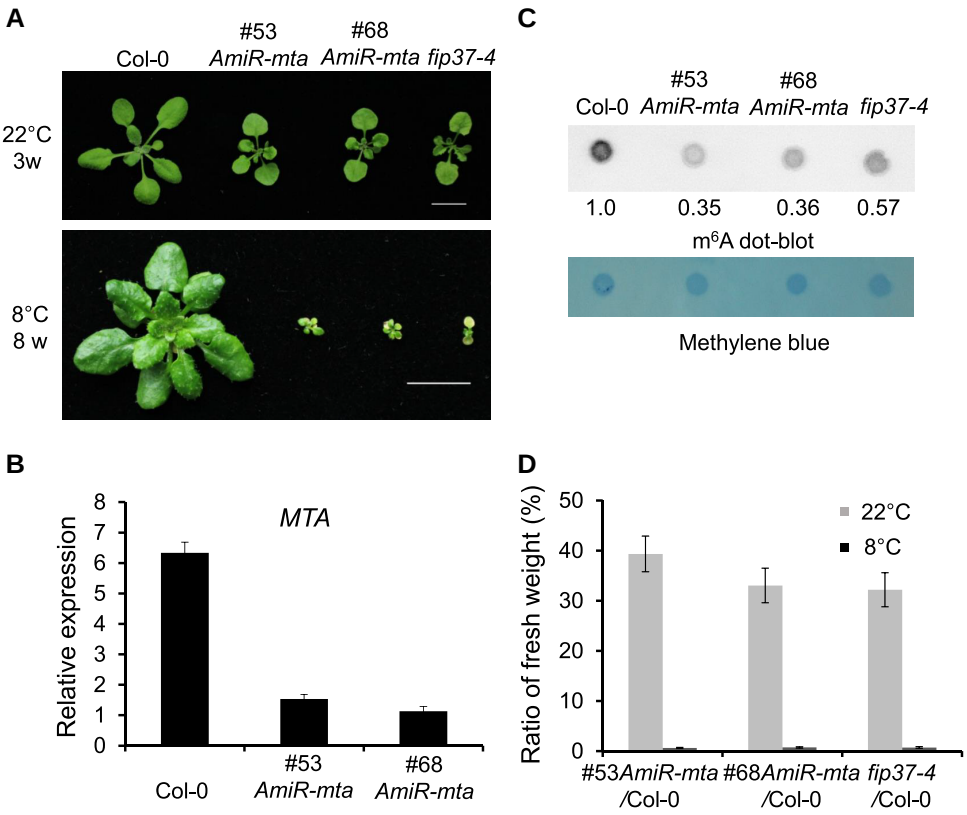

Figure 1. Mutants of the m6A writer complex showed chilling-susceptible growth phenotypes. **A)** Morphological phenotypes of the *AmiR-mta* and the *fip37-4* mutants grown at 22°C for 3 wk or at 8°C for 2 mo. Bars, 1 cm. **B)** Expression of *MTA* in *AmiR-mta* transgenic plants analyzed by RT-qPCR. The *Actin* gene was used as a normalization control and the experiments were repeated 3 times with similar results. Values represent means ± SES. **C)** m6A dot blot of mRNA samples from WT, *AmiR-mta* and *fip37-4* plants using anti-m6A antibody. mRNA samples were extracted from 3-wk-old plants grown at 22°C, and 200 ng of mRNA was loaded for each sample. Marked numbers indicate the relative amount in the mutants compared to that in Col-0 quantified by Image J. **D)** Ratio of fresh weight of *AmiR-mta* and *fip37-4* versus WT plants that were grown at 22°C for 3 wk or at 8°C for 2 mo. Values represent means ± SES ($n \geq 15$).

an anti-m6A antibody. The overall m6A level was reduced in both the *AmiR-mta* and the *fip37-4* plants, to approximately 35% and 57% of the WT, respectively (Fig. 1C), indicating a substantial reduction of modification.

Both the *AmiR-mta* and *fip37-4* mutant plants exhibited smaller size and more lobed leaves compared to the WT when grown at 22°C (Fig. 1A), similar to what was reported earlier (Shen et al. 2016; Ruzicka et al. 2017). When *AmiR-mta* and *fip37-4* plants were grown at 8°C, both exhibited a severe dwarf phenotype with very small leaves (Fig. 1A). While the fresh weights of the *AmiR-mta* and the *fip37-4* plants were each 30% to 40% of the WT at 22°C, they were <1% of the WT when grown at 8°C (Fig. 1D). These results indicate that the mRNA m6A writer complex is important for Arabidopsis to sustain growth under chilling condition.

m6A modification, especially at the 3′ untranslated region, is decreased under low temperature

To reveal how m6A modification affects chilling growth, we analyzed the properties of m6A modification under chilling condition. The overall changes of m6A methylation level after 4°C treatment was first examined by dot-blot assay for rosette leaves of 3-wk-old plants. After cold treatment, the m6A signals were reduced to 74% at 6 h and further reduced to 56% at 24 h compared to the nontreated plants (Fig. 2A). This prompted us to examine the expression of m6A modified genes under cold. Most of the members of m6A "writers," "erasers," and "readers" exhibit expression changes at 6, 12, or 24 h after 4°C treatment. The core m6A writer gene *MTA* had increased expression at 24 h, but the other writer members *MTB* and *FIP37* had decreased expression at 6 and 12 h after 4°C treatment (Supplemental Fig. S1). The m6A "erasers" *ALKBH9A* and *ALKBH10B* had decreased expression at 12 h and increased expression at 24 h while *ALKBH9B* and *ALKBH10A* had decreased expression at 24 h (Supplemental Fig. S1). Therefore, there appears to be an overall early stage (6 and 12 h) decrease of m6A "writers" and late stage (24 h) increase of writers and decrease of erasers. For "readers," most had little expression changes at 6 h and drastic changes at 24 h with *ECT1*, *2*, *3*, *4*, *8*, *10*, and *11* having increased expression and *ECT5*, *6*, *9*, and *12* had reduced expression (Supplemental Fig. S1). This indicates that

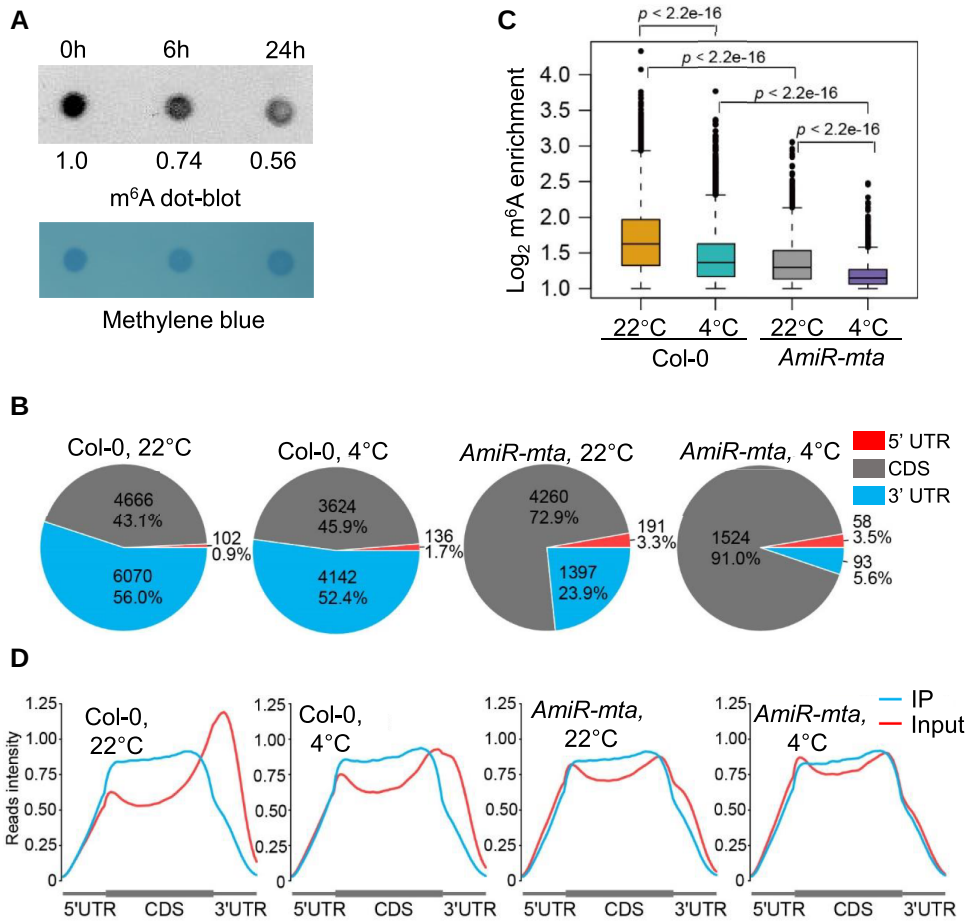

Figure 2. The overall m6A level is decreased under cold. **A)** m6A dot-blot of mRNA samples from 3-wk-old WT plants with 4°C cold treatment for 0, 6, and 24 h. Two hundred nanaograms of mRNA are loaded. Marked numbers indicate the relative amount in cold-treated samples compared to nontreated sample quantified by Image J. **B)** Number of m6A peaks and their location in gene regions (5′ UTR, CDS, and the 3′ UTR) identified for WT and *AmiR-mta* plants growth at 22°C for 3 wk or with 4°C cold treatment for 24 h. **C)** Box plot of the enrichment of m6A peaks in the WT and *AmiR-mta* plants growth at 22°C for 3 wk or with 4°C cold treatment for 24 h. The *P*-values were calculated for significant differences between 2 groups by Mann–Whitney *U* test. The box limits represent 25th and 75th percentiles; center line, 50th percentiles. Whiskers were ×1.5 interquartile range extending from the edge of the box. **D)** Distribution (density) of normalized m6A peaks along the mRNA transcripts, where each mRNA transcript is divided into the 5′ UTR, CDS, and the 3′ UTR.

all writers, erasers, and readers of m6A have a complex gene expression change in response to cold.

We subsequently analyzed m6A distribution profiles by m6A-IP (immunoprecipitation)-seq for the WT and *AmiR-mta* plants under normal and cold conditions. Two biological replicates were performed for each genotype and growth condition combination, and high Spearman correlation coefficients ($R \geq 0.96$) were observed between the 2 biological replicates (Supplemental Fig. S2, A and B). After peak calling, we identified 10,838 and 7,902 high-confidence m6A peaks in WT at 22°C and 4°C, respectively (Fig. 2B; Supplemental Table S1). Most of the m6A peaks were located in 3′ untranslated region (UTR) and CDS (coding sequences) in WT plants (Fig. 2B). Box plot analysis showed that the overall enrichment of m6A peaks was significantly reduced after cold treatment in WT (Fig. 2C). The distribution of m6A along the transcripts was analyzed by plotting the read number ratio of m6A-IP and input throughout transcripts. The m6A modification showed a higher enrichment around 3′ UTR than 5′ UTR and CDS in WT under normal condition (Fig. 2D), which is consistent with prior reports (Luo et al. 2014; Xu et al. 2021). The density of m6A around 3′ UTR was reduced under cold treatment compared to under normal growth temperature (Fig. 2D).

In *AmiR-mta* plants, a total of 5,848 and 1,675 m6A peaks were identified at 22°C and 4°C, respectively, which were lower than in WT (Fig. 2B; Supplemental Table S1). Consistently, *AmiR-mta* plants showed lower m6A density compared to the WT at both 22°C and 4°C (Fig. 2C). In addition, the global m6A abundance was reduced after cold treatment in the *AmiR-mta* mutant similarly to in the WT (Fig. 2C). The high abundance of m6A at 3′ UTR of the transcripts observed in WT was reduced in the *AmiR-mta* plants at 22°C and 4°C (Fig. 2D). We further compared the m6A peaks at 22°C and at

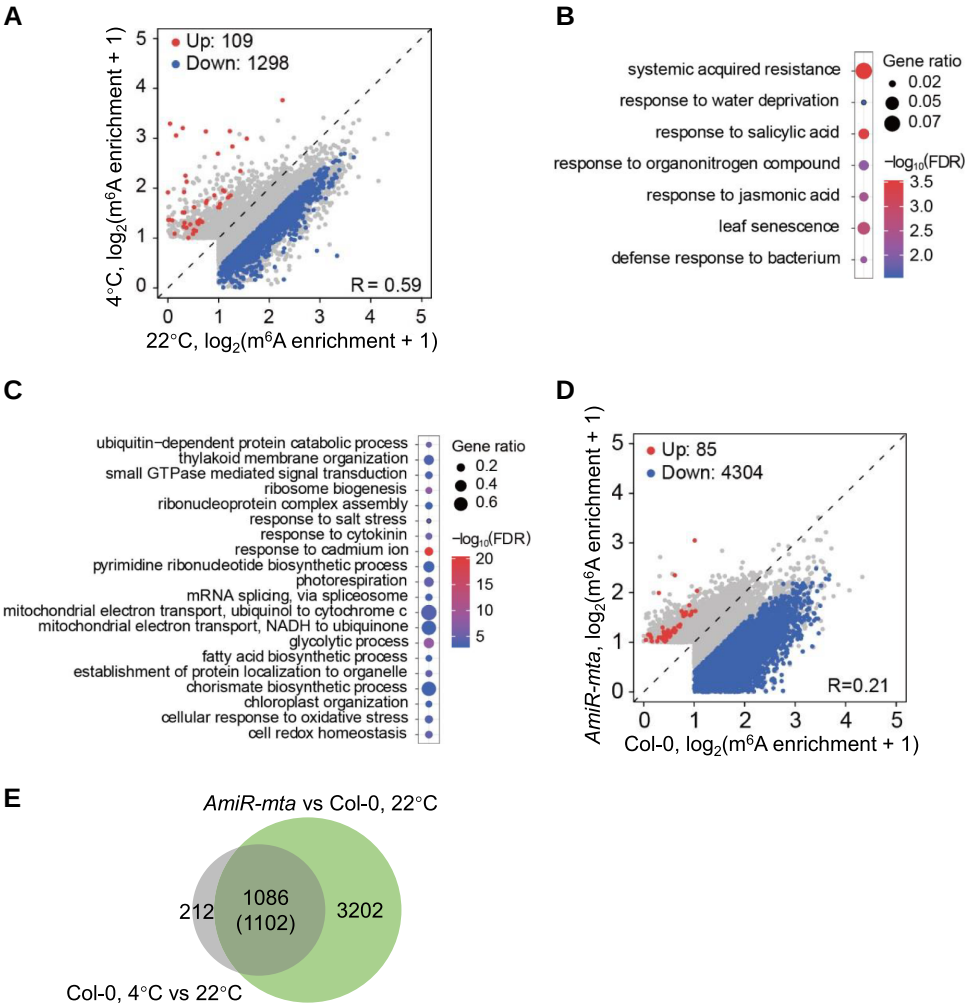

Figure 3. Identification and GO analysis of DMGs in *AmiR-mta* and WT after cold treatment. **A**) Scatter plots showing differential m6A peaks with a fold change > 1.5 in WT before and after cold treatment. GO analysis of up- **(B)** and down- **(C)** DMGs in WT after cold treatment. The top 20 significantly enriched GO terms were shown in (**C**). **D)** Scatter plots showing the differential m6A peaks with a fold change > 1.5 between *AmiR-mta* versus WT. **E)** Venn diagram showing overlaps of down-DMPs caused by chilling and by a reduction of *MTA*. In total, 1,086 DMPs of 4°C versus 22°C in WT Col-0 and 1,102 DMPs of *AmiR*-mta versus Col-0 were shared.

4°C and found that only about 10% and 3% of them were cold-specific in WT and *AmiR-mta* plants, respectively (Supplemental Fig. S2C). Collectively, these data showed that the genome-wide m6A methylation, especially at the 3′ UTRs, is decreased under low temperature.

Differentially methylated genes caused by chilling were enriched in diverse biological processes

To further analyze m6A alterations caused by chilling and the reduction of MTA, we selected differentially methylated peaks (DMPs) defined by more than 1.5-fold of m6A enrichment change and with a significant test at $q < 0.05$. A total of 109 up- and 1,298 downregulated DMPs were identified between cold versus normal temperature in WT (Fig. 3A). These peaks reside in 93 and 1,294 genes, respectively (Supplemental Table S2), and are defined as differentially methylated genes (DMGs). Gene Ontology (GO) analysis revealed that up-DMGs are enriched mostly in terms related to defense responses such as "systemic acquired resistance" and "response to salicylic acid" (Fig. 3B; Supplemental Table S3), whereas down-DMGs are enriched in more diverse terms in cadmium ion response, glycolytic process, ribosome biogenesis among others (Fig. 3C; Supplemental Table S3). Meanwhile, 85 up- and 4,304 downregulated DMPs were identified in the *AmiR-mta* plants compared to WT (Fig. 3D). Eighty-four percent of cold induced down-DMPs displayed significantly decreased methylation level by the reduction of MTA (Fig. 3E). Therefore, low temperature caused down-DMPs were dependent on MTA.

In *AmiR-mta* plants, 9 up- and 918 down-DMPs of 4°C versus 22°C were identified (Supplemental Fig. S3A), which corresponded to 9 and 900 DMGs, respectively (Supplemental Table S2). Among these cold caused DMGs, 3 up-DMGs

and 247 down-DMGs of 4°C versus 22°C were shared by WT and *AmiR-mta* plants (Supplemental Fig. S3B and Table S4). Meanwhile, 90/1,047 and 6/653 (up/down) DMGs were unique in WT and *AmiR-mta* plants, respectively (Supplemental Fig. S3B and Table S4). The enriched GO terms of the 1,047 unique down-DMGs were similar to that of down-DMGs of 4°C versus 22°C in WT (Supplemental Fig. S3C and Table S5). The most significantly enriched GO term of the unique down-DMGs in *AmiR-mta* plants was "mRNA splicing" (Supplemental Fig. S3D and Table S5), which was not enriched for other DMGs. This prompted us to analyze whether alternative splicing (AS) was affected by the reduction of MTA. A total of 1,211 differential AS events were observed between cold treatment and normal growth condition in *AmiR-mta*, more than the 912 differential AS events observed in WT (912), and 498 of them were specific for *AmiR-mta* (Supplemental Fig. S3E and Table S6). Therefore, cold induced more AS events with the reduction of MTA.

Lack of m6A altered the extent but not the quality of expression changes in response to cold

To determine if m6A modification plays a role in gene expression regulation response to low temperature, we performed RNA-seq of the WT and *AmiR-mta* plants at normal and low temperatures. The 2 RNA-seq replicates for each genotype and temperature combination showed high Spearman correlation coefficients ($R \geq 0.98$) (Supplemental Fig. S4). In order to assess the association between m6A modification and transcript abundance, genes were divided into m6A- and non-m6A-classes. In the WT, m6A-containing genes on average had a higher expression level than non-m6A-containing genes, and this was the case under normal and cold conditions (Fig. 4A). A similar phenomenon was also observed in the *AmiR-mta* mutant where the overall m6A modification was reduced. The m6A-class genes had a higher expression than the non-m6A class genes at both 22°C and 4°C in the mutant (Fig. 4A). These indicate that m6A modification is correlated with gene expression level under both normal and low temperatures.

We analyzed if the reduction of m6A would alter cold induced transcriptome changes. A total of 7,912 (4,021 up/3,891 down) and 9,713 (4,679 up/5,034 down) differentially expressed genes (DEGs) (defined by false discovery rate [FDR] < 0.05 and $|\log_2(\text{fold change [FC]})| > 1$) of 4°C versus 22°C were identified for WT and *AmiR-mta* plants, respectively (Fig. 4B; Supplemental Table S7). Among them, 3,034 up- and 3,087 down-DEGs, representing 63% of the total DEGs, were shared between WT and the *AmiR-mta* plants (Fig. 4C). Heatmap analysis revealed that the vast majority of these DEGs had the same increase or decrease of expression in WT and *AmiR-mta* plants (Fig. 4D). These data indicate that overall reduction of m6A modification did not qualitatively affect induction or repression of gene expression by low temperature but moderately affected the extent of change of gene expression. We further assessed if cold-induced change of m6A modification had an impact on cold-induced gene expression change. Association analysis revealed that the fold change of gene expression was not correlated with m6A enrichment induced by cold in WT or *AmiR-mta* (Fig. 4E). These results suggest that reduction of overall m6A altered the extent but not the quality of expression changes in response to cold and that alterations of m6A modification are not correlated with gene expression changes in response to low temperature.

Global translation efficiency level was reduced under low temperature

A previous study found that protein translation rates are proportional to temperature in Arabidopsis (Wigge et al. 2020), but the profiles of translatome changes in response to low temperature were unknown in Arabidopsis. Therefore, we applied polysome-seq to detect mRNAs that are actively translated under normal and cold conditions. The 2 biological replicates of each genotype and condition combination showed a high Spearman correlation (Supplemental Fig. S5), indicating a good reproducibility of the high throughput sequencing data. The translation efficiency of each gene was calculated by the ratio of polysome-bound mRNA to the total mRNA analyzed by RNA-seq. The overall translation efficiency level was significantly decreased after cold treatment in WT (Fig. 5A). A total of 2,034 genes showed a more than 2-fold change at 4°C versus 22°C in WT plants (Fig. 5B; Supplemental Table S8), and these genes were defined as differential translation efficiency genes (DTEGs). There were many more down-DTEGs than up-DTEGs (1,521 versus 513) in WT, suggesting a general reduction of protein translation efficiency after cold treatment. GO enrichment analysis found that down-DTEGs were enriched in terms of diverse cellular processes of DNA, RNA, histone, cytoskeleton, and organelle (Fig. 5C). Up-DTEGs were enriched in fewer terms, some of which are related to stress such as "hydrogen peroxide transmembrane transport" (Fig. 5D). These data indicate that most genes have a reduced translation efficiency while a small group of genes related to stress tolerance have an increased translation efficiency at low temperatures.

Translation efficiency was dysregulated under chilling in *AmiR-mta* plants

We next investigated the effects of m6A modification on translation efficiency. Polysome-seq was performed for *AmiR-mta* plants under normal and cold conditions. High Spearman correlation coefficients ($R \geq 0.98$) were observed between the 2 biological replicates (Supplemental Fig. S5), indicating a good reproducibility. Similarly to that WT, the global translation efficiency level was decreased in *AmiR-mta* after cold treatment (Supplemental Fig. S6A). Also similarly to that in the WT, m6A-containing genes showed a higher translation efficiency than non-m6A-containing genes in the *AmiR-mta* plants under normal and cold conditions (Fig. 6A). These results indicate that m6A has a positive role on translation efficiency. However, DMGs of 4°C versus

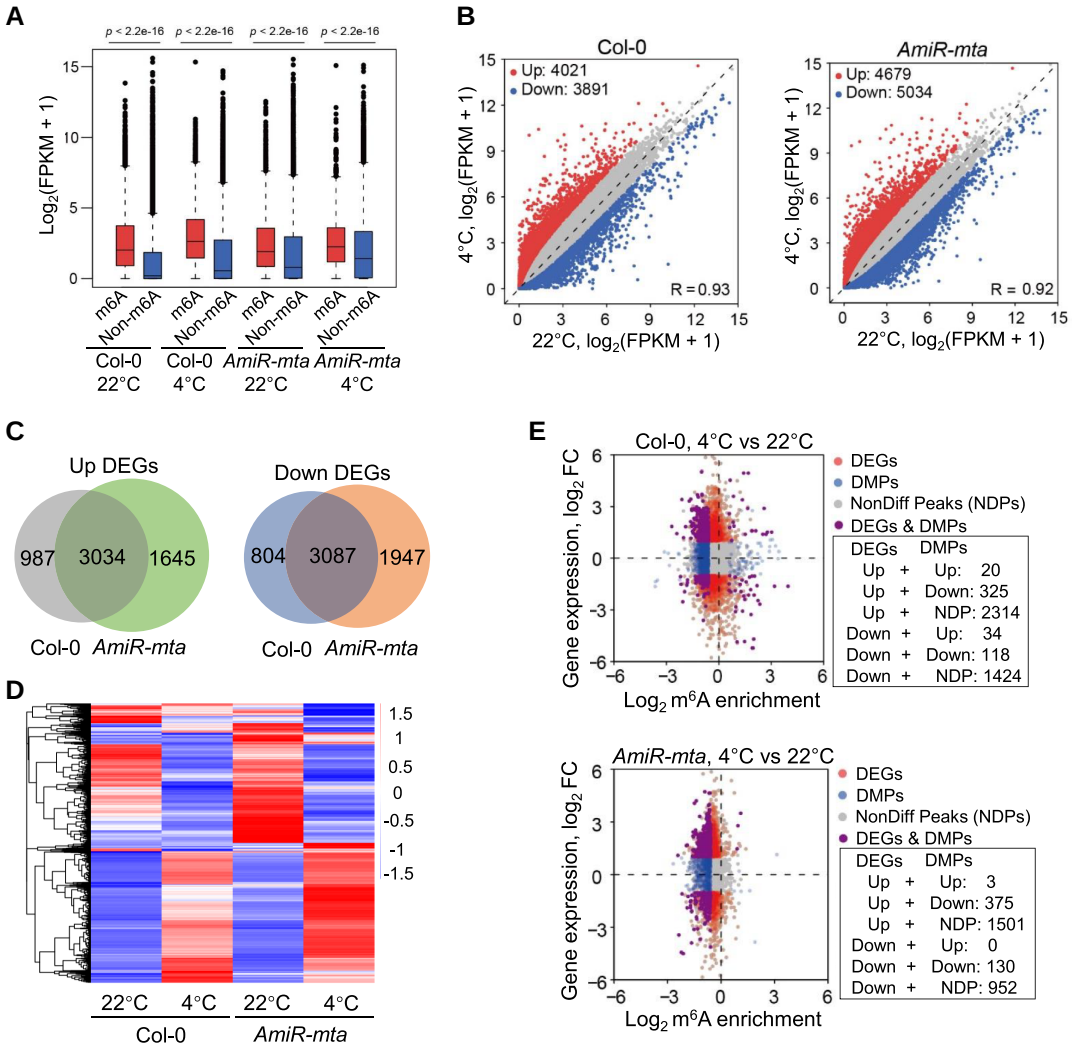

Figure 4. Loss of m6A altered the extent but not the quality of expression changes in response to cold. **A**) Box plot of mRNA expression levels (based on FPKM values) for genes with or without m6A peaks (m6A and non-m6A) in WT and *AmiR-mta* plants at 22°C for 3 wk or with 4°C cold treatment for 24 h. The *P* values were calculated for significant differences between 2 groups by the Mann–Whitney *U* test. The box limits represent 25th and 75th percentiles; center line, 50th percentiles. Whiskers were ×1.5 interquartile range extending from the edge of the box. **B**) Scatter plots showing the transcripts with differential expression levels (fold change > 2) in the WT and *AmiR-mta* plants between control- and cold-treated groups. **C**) Venn diagrams showing the overlaps of up- and down-DEGs between WT and *AmiR-mta* plants after cold treatment. **D**) Heat map showing that most of the DEGs in WT and *AmiR-mta* plants after cold treatment displayed similar trends of expression change. **E**) Correlation of fold changes between m6A modification level and the expression of m6A-containing genes in the WT and *AmiR-mta* plants in response to low temperature.

22°C could have either increased or decreased translation efficiency at 4°C versus 22°C (Supplemental Fig. S6, B and C), suggesting that a m6A modification change may not predict the translation efficiency change of a gene.

We identified a total of 8,760 (3,821 up/4,939 down) DTEGs of 4°C versus 22°C in the *AmiR-mta* plants, more than 2,034 DTEGs identified in WT (Fig. 6B; Supplemental Table S8). Only 1,151 (298 in up/1,122 in down) DTEGs were shared by WT and *AmiR-mta* plants, and 7,340 DTEGs (3,523 in up/3,817 in down) were unique in *AmiR-mta* (Fig. 6C). GO enrichment analysis found that the 3,523 up-dysregulated DTEGs were enriched in terms such as "cell redox homeostasis," "small GTPase mediated signal transduction," "protein transport," and "photosynthesis" (Fig. 6D), whereas the 3,817 down-dysregulated DTEGs were enriched in terms such as "protein phosphorylation," "mRNA processing," "embryo development," "microtubule-based movement" (Fig. 6E). Collectively, our results suggest that reduction of overall m6A modification caused a pronounced change in translation efficiency under low temperature.

A m6A modified gene promotes growth under chilling

Because the *AmiR-mta* mutant exhibited chilling susceptibility, reduced m6A of some genes in the mutant must

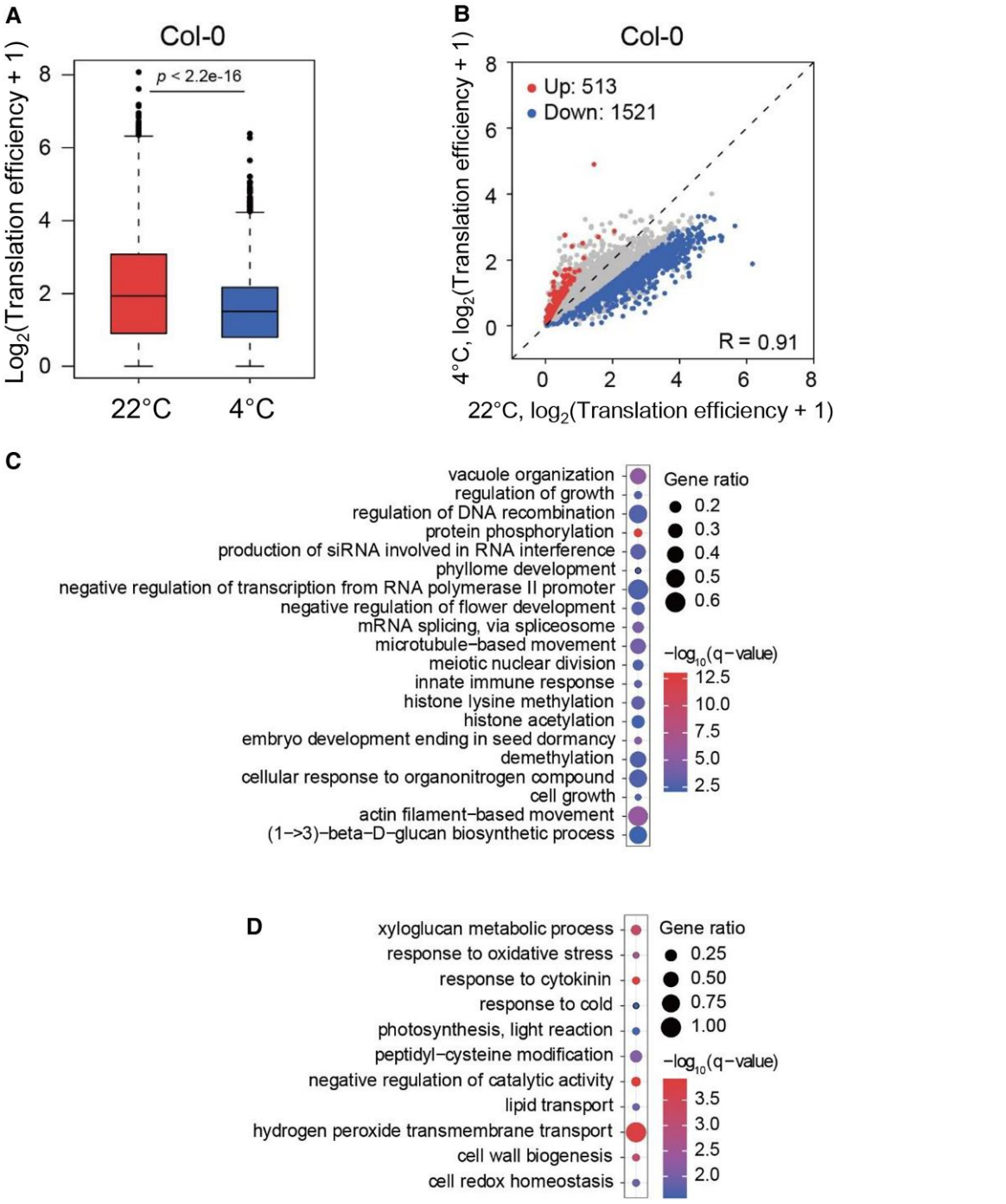

Figure 5. The global translation efficiency level was reduced under low temperature. **A**) Box plot of gene translation efficiency levels in WT under normal and cold conditions. The *P* values were calculated for significant differences between 2 groups by the Mann–Whitney *U* test. The box limits represent 25th and 75th percentiles; center line, 50th percentiles. Whiskers were ×1.5 interquartile range extending from the edge of the box. **B**) Scatter plot showing the genes with differential translation efficiency levels (fold change > 2) in WT between control- and cold-treated groups. GO analysis of down- **(C)** and up-DTEGs **(D)** in WT after cold treatment. The top 20 significantly enriched GO terms were shown in (**C**).

contribute to this chilling susceptibility. A total of 4,383 and 2,921 DMGs were found between WT and *AmiR-mta* plants at 22°C and 4°C, respectively (Fig. 7, A and B), and these genes might have function in chilling tolerance. Because translation efficiency appears to be affected by m6A modification, we analyzed the DTEGs between WT and the *AmiR-mta* plants. A total of 2,644 and 1,866 DTEGs between WT and *AmiR-mta* plants were identified at 22°C and 4°C, respectively (Fig. 7, A and B). Among these DMGs and DTEGs, 371 and 159 genes were both DMGs and DTEGs of WT versus mutant at 22°C

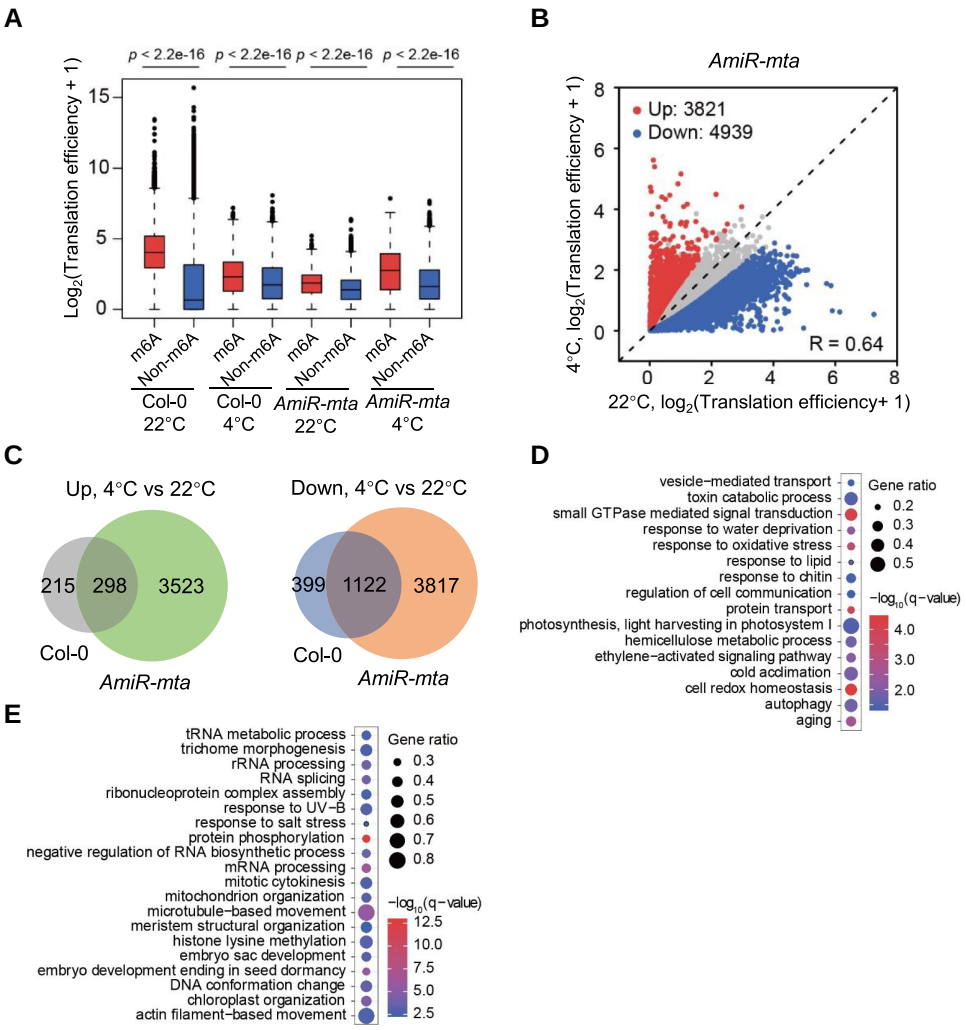

Figure 6. Translation efficiency is dysregulated under chilling in *AmiR-mta* plants. **A**) Box plot of translation efficiency levels for genes with and without m6A peaks (m6A and non-m6A) in WT and *AmiR-mta* plants under normal and cold conditions. The *P* values were calculated for significant differences between 2 groups by the Mann–Whitney *U* test. The box limits represent 25th and 75th percentiles; center line, 50th percentiles. Whiskers were ×1.5 interquartile range extending from the edge of the box. **B**) Scatter plot showing genes with differential translation efficiency levels (fold change > 2) in *AmiR-mta* between control- and cold-treated groups. **C**) Venn diagrams showing the overlaps of up- and down-DTEGs between WT and *AmiR-mta* plants after cold treatment. GO analysis of up- **D)** and down-dysregulated DTEGs **E)** in *AmiR-mta* plants after cold treatment. The top 20 significantly enriched GO terms were shown in (**E**).

and 4°C, respectively (Fig. 7, A and B). This was a small fraction of DMGs (8.3% and 5.4% in total), indicating that translation efficiency change of majority of genes may not result from the m6A modification change of themselves. This suggests that m6A modification of a mRNA might not have a direct effect on its protein translation and that reduced m6A modification in the *AmiR-mta* mutant might induce global translation defect.

To begin to reveal genes whose m6A modifications contribute to chilling tolerance, we first examined the expression, m6A modification and translation efficiency of cold-responsive genes in WT and the *AmiR-mta* mutant. Among the 248 genes that are defined as cold-response genes under the GO term 0009409, 51 and 40 genes were DMGs between WT and the *AmiR-mta* mutant at 22°C and 4°C, respectively (Fig. 7, A and B). Among these, 8 and 3 were also DTEGs at 22°C and 4°C, respectively (Fig. 7, A and B). We selected 1 gene from these for functional analysis, although it is unknown whether or not the decrease in translation efficiency in these genes results directly from the decrease of their m6A modification. This gene DGAT1 encodes diacylglycerol (DAG) acyltransferase that catalyzes the conversion of DAG to triacylglycerol in Arabidopsis (Tan et al. 2018). The m6A level of *DGAT1* was substantially decreased in *AmiR-mta* compared to WT under both normal and cold conditions (Supplemental Fig. S7A). The RNA expression of *DGAT1* was induced by cold in both WT and *AmiR-mta* mutant, and the induction fold appeared to be lower in the mutant than in the WT (Supplemental Fig. S7A). The translation efficiency of the *DGAT1* gene was

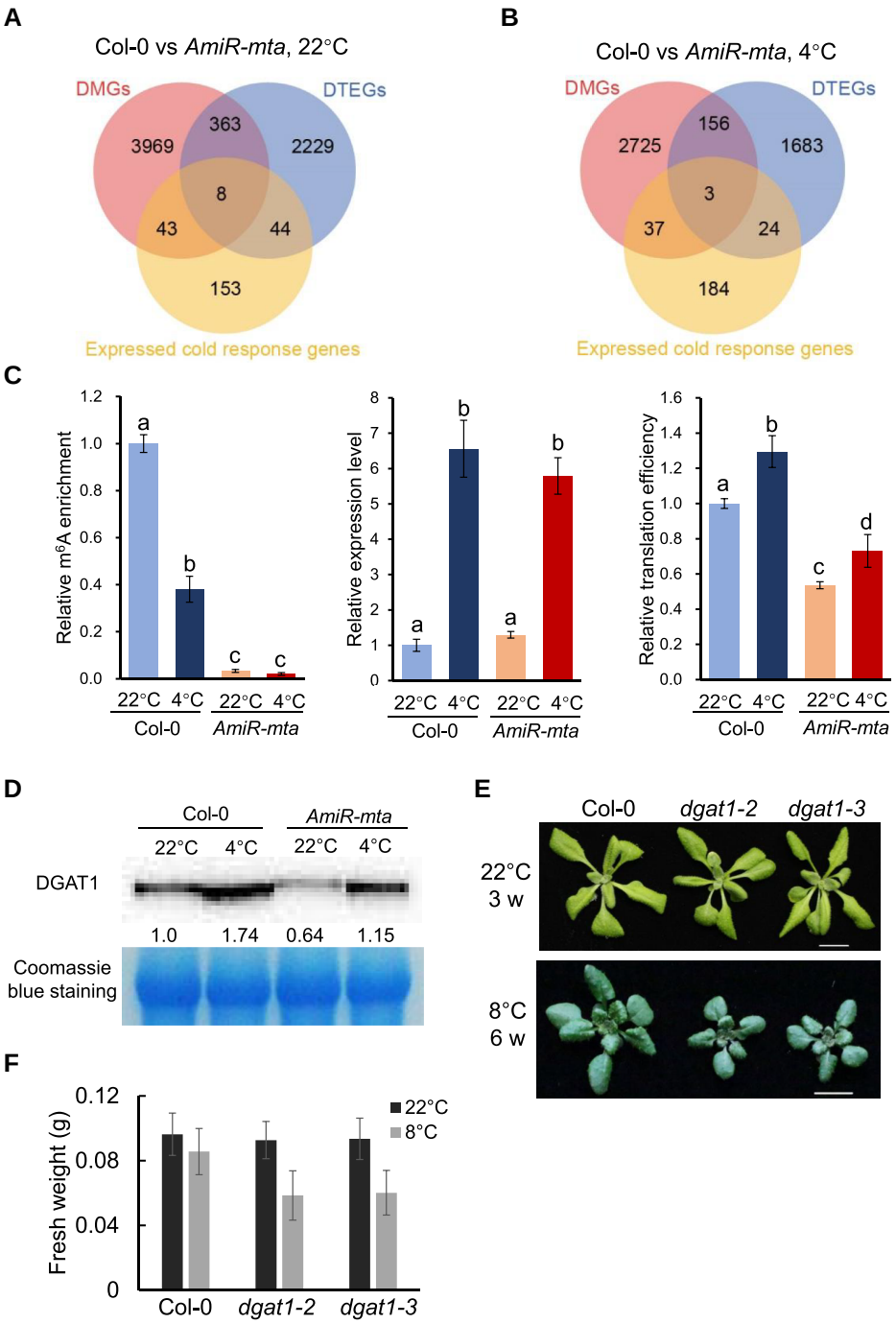

Figure 7. A m6A-modified gene *DGAT1* enhances growth under chilling condition. Venn diagrams showing the overlaps of DMGs, DTEGs and cold expressed genes between WT and *AmiR-mta* plants under normal **(A)** and cold **(B)** conditions. **C**) Validation of the transcript expression, m6A enrichment and translation efficiency of *DGAT1* in WT and *AmiR-mta* plants under normal and cold conditions. Total RNAs, m6A IPed RNAs, and polysome bound mRNAs were used for RT-qPCR of *DGAT1*. Data are means ± SD of 3 biological replicates. Different letters indicate significant differences according to Student–Newman–Keuls test ($P < 0.05$). **D**) Protein level of FLAG–DGAT1–GFP transiently expressed in protoplasts of WT and *AmiR-mta* plants under normal and cold conditions as detected by western blot assay using anti-FLAG antibody. Coomassie blue staining was used to show equal loading among samples. **E**) Growth phenotype of *dgat1* mutants at 22°C for 3 wk and at 8°C for 6 wk. Bars, 1 cm. **F**) Fresh weight of WT and *dgat1* mutants grown at 22°C for 3 wk and at 8°C for 6 wk. Values represent means ± SES ($n \geq 10$).

highly increased at 4°C compared to 22°C, and it was lower in the mutant compared to the WT under both conditions (Supplemental Fig. S7A). Similar changes were observed in additional independent biological samples using RT-qPCR to detect *DGAT1* in total RNA, m6A-IP-RNA and polysome-bound RNA from WT and *AmiR-mta* plants under normal

and cold conditions (Fig. 7C). In addition, we examined the protein level of DGAT1 using the protoplast expression system. DGAT1 exhibited higher protein level in WT than in *AmiR-mta* at both normal and cold conditions (Fig. 7D). Therefore, *DGAT1* has m6A modification under both normal and cold conditions, and it has a higher RNA expression, translation efficiency and protein level under cold. It showed reduced m6A modification, translation efficiency and protein level, but not significantly altered RNA level in the *AmiR-mta* mutant compared to the WT, which suggest that m6A increase under cold is important for the enhanced translation efficiency of *DGAT1* gene.

To assess the function of *DGAT1* in chilling growth, we obtained T-DNA insertion mutants of this gene. The *dgat1-2* and *dgat1-3* mutants had an insertion in the tenth and the last intron of *DGAT1*, respectively (Supplemental Fig. S7B). Neither of them had a detectable RNA expression of *DGAT1* by reverse transcription (RT)-PCR analysis (Supplemental Fig. S7C), indicating that they are loss of function mutants. The *dgat1-2* and *dgat1-3* mutants both had a smaller rosette size and a lower fresh weight than WT under cold condition while they grew to a similar extent as the WT plants at normal condition (Fig. 7, E and F). These data indicate that *DGAT1* has a role in promoting growth under chilling condition and that m6A modification of its transcript might contribute to chilling growth in Arabidopsis.

Discussion

Plants undergo physiological and biochemical adjustments through transcriptional and post-transcriptional regulations to minimize the negative impacts of chilling. In this study, we found that m6A writer complex is indispensable for Arabidopsis growth under chilling stress. Despite of the global reduction of m6A levels under cold, reduced m6A modification as in the *AmiR-mta* mutant and the *fip37-4* mutant leads to severely retarded growth under cold. Lack of m6A did not alter the induction or repression of cold-response genes but moderately affect the extent of their expression changes. Moreover, reduction of overall m6A resulted in aberrant translation efficiency in several thousands of genes. We further showed the importance of a m6A modified gene *DGAT1* in chilling tolerance, and its translation efficiency but not transcript level was drastically decreased in the *AmiR-mta* plants. Collectively, we identify an essential role of m6A in chilling growth and suggest translation regulation as an important process for chilling tolerance.

m6A modification was found to influence gene transcript level and translation efficiency. Accumulating evidences obtained from mammalian studies have indicated that the contrasting effects of m6A modification on gene expression regulation are dependent on different "reader" proteins (Wang et al. 2014; Shi et al. 2017). For example, YTHDF2/3 promote the degradation of m6A-containing RNA transcripts (Du et al. 2016; Shi et al. 2017), whereas IGF2BP stabilize m6A-modified mRNAs through interacting with ELAVL1, MATR3, and PABPC1 (Huang et al. 2018). In addition, m6A regulates translation, and this is through different mechanisms. YTHDF1 interacts with initiation factor eukaryotic initiation factor 3 (eIF3) to promote translation initiation and protein synthesis of m6A marked transcripts (Wang et al. 2015). Meanwhile, m6A at 5′ UTR can directly bind eIF3 in a cap-independent manner to promote translation initiation of mammalian mRNAs under heat stress (Meyer et al. 2015). In our study, we observed m6A-containing genes show higher expression and translation efficiency levels than non-m6A containing genes in WT and *AmiR-mta* plants under both normal and chilling conditions (Fig. 6A). However, the changes of m6A enrichment were not correlated with alterations of gene expression and translation efficiency levels after cold shift (Supplemental Fig. S6, B and C), suggesting complex effects of m6A on regulating RNA abundance and translation control.

Diverse cellular stresses are shown to alter the transcriptome-wide distribution of m6A. Previous studies found that 5% to 30% of m6A peaks are altered under ultraviolet light, heat shock or interferon-gamma, and some of them are treatment specific (Dominissini et al. 2012). Our findings revealed that the m6A levels especially at 3′ UTR of mRNAs were remarkably reduced after cold treatment (Fig. 2), and 13% of the m6A peaks showed more than 1.5-fold changes (Fig. 3A). The differential expressions of these m6A effectors in response to cold suggest their distinct functions in regulating m6A-containning transcripts under low temperature. For instance, the m6A reader ECT2 protein was found to associate with m6A-containing RNAs and relocate them to stress granules under heat stress (Scutenaire et al. 2018). Therefore, it is possible that the decreased methylation level in m6A-containing transcripts contributes to its escape from transporting to P-bodies or stress granules under chilling stress in Arabidopsis. The relevance of m6A in determining mRNA fates for the adaptation of environmental cues needs to be investigated in future.

Translational regulation is critical for cold adaptation as has been shown for the role of a number of protein synthesis genes in chilling growth. Here we find that m6A modification of mRNA plays a critical role in cold adaption and it may do so via translation too. Analysis of the changes of translation efficiency in WT and *AmiR-mta* plants after cold treatment revealed a dysregulation of translation efficiency of 7,000 of genes in the *AmiR-mta* plants (Figs. 5A and 6B). This suggests that m6A plays a role in translation control under chilling stress. Interestingly, we found cold induced DMGs and DTEGs were enriched on several GO terms that are related to ribosome heterogeneity and translation process, including "ribosome biogenesis," "tRNA modification," "rRNA processing," and "ribosomal small subunit biogenesis" (Supplemental Fig. S3, C and D; Figs. 3C and 6E). Previous studies have found altered ribosomal composition facilitates the translation of proteins that are important for plant growth and survival under cold stress (Yu et al. 2020). tRNA modifications can be dynamically altered in response to environmental stresses

and contribute to translate stress-response genes (Endres et al. 2015; Chan et al. 2018). Therefore, it is possible that m6A regulates translation of *COR* genes through modulating ribosomal composition or tRNA modification. How it affects translation and how translation control relates to cold adaptation remain to be characterized in plants.

In sum, we have identified an essential role of MTA-mediated mRNA m6A modification in chilling growth. m6A modification only moderately affects the extent of change of gene expression, but substantially impacts translation efficiency change in response to low temperature. An increase of translation efficiency of cold-responsive genes such as *DGAT1* may contribute to cold adaption. The role of epi-modification of mRNA in low temperature adaptation uncovered here not only expands knowledge of environment adaptation but also expands the tools for generating stress tolerant plants.

While we were preparing the manuscript, another study on the role of m6A modification of mRNA in cold tolerance was published (Govindan et al. 2022). The m6A-IP-seq data presented here is largely consistent with this previous study. Nearly 80% of m6A modified genes identified in this study were also identified in that research (Supplemental Fig. S8).

Similar to this study, they find that m6A modification is important for plant growth under cold using the mutants defective in m6A modification. They also show that cold-responsive *CBF* and *COR* genes have increased m6A enrichment and ribosome occupancy under cold. These observations are consistent with findings in this study. In addition to these above shared findings, this study identified m6A peaks under normal and cold conditions in the *AmiR-mta* plants, which enables a further analysis of the influence of loss of m6A (by comparing the *AmiR-mta* mutant and the WT) on transcriptome and translatome in response to low temperature. This study also provides analyses on the effect of cold on overall m6A modification level, the global influences of m6A on transcription and translation under chilling stress, as well as the function of a m6A modified gene *DGAT1* in cold response.

Materials and methods

Plant materials and growing conditions

The T-DNA insertion lines SALK_018636 (*fip37-4*), SK429 (*dgat1-2*), and SALK_039456 (*dgat1-3*) were obtained from the Arabidopsis Biological Resource Center. Seeds were sown onto soil, stratified at 4°C for 2 d, and then transferred to a growth chamber at 22°C or 8°C under long day conditions (16 h light/8 h dark) with a light intensity of 100 μmol m^{-2} s^{-1}. Upon cold treatment, 3-wk-old plants were transfer to 4°C for 6 and 24 h.

Plasmid construction

AmiRNA for *MTA* gene were designed using the Web MicroRNA Designer (http://wmd3.weigelworld.org) based on the parameters previously established by Schwab et al. (2006). Oligonucleotide sequences can be found in Supplemental Table S9. The miR319a precursor was amplified by PCR using pRS300 as a template. Overlapping PCR products were digested with XhoI and BamHI followed by a ligation with the pMDC32 vector digested with XhoI and BamHI. Transgenic lines were obtained via *Agrobacterium tumefaciens*-mediated transformation through the floral dip method (Clough and Bent 1998), and T1 seeds were selected on MS medium containing 50 mg L^{-1} hygromycin. To generate a FLAG:DGAT1:GFP fusion protein for transient expression in protoplasts, full-length coding region of the *DGAT1* was amplified with a forward primer containing the FLAG sequence, and then was cloned into the pSAT6-EGFP vector (Tzfira et al. 2005).

m6A-IP-seq

m6A-IP-seq was performed as previously described (Dominissini et al. 2012). Briefly, total RNA was extracted using Trizol (15596018, Ambion) according to its procedures. Two rounds of Poly(A) RNA selection were performed using the Dynabeads mRNA direct purification kit (MRN10, Sigma-Aldrich). Then, Poly(A) RNA samples were fragmented into ~100-nucleotide-long fragments by RNA reagent (AM8740, Invitrogen). One-tenth of fragmented RNA was saved as input control. The rest was rotated at 4°C for 4 h with anti-m6A polyclonal antibody (202003, Synaptic Systems) in 1× IP buffer (150 mM NaCl, 0.1% (v/v) Igepal CA-630, 10 mM Tris–HCl pH 7.4) supplemented with RNasin Plus (N2611, Promega). The antibody-bound RNA was then immunoprecipitated by incubation with preblocked protein A beads (10001D, Invitrogen) at 4°C for an additional 2 h. After extensive washing, RNA was released in elution buffer (1× IP buffer supplemented with 6.7 mM m6A [M2780], Sigma-Aldrich). All samples were precipitated using glycogen, 3 M NaOAc and 2.5 × vol/vol 100% ethanol and kept at −80°C overnight. NEBNext Ultra RNA Library Prep Kit for Illumina (E7645S, NEB) was used to construct libraries for Input and IP samples according to the product manual. The libraries were subjected to sequencing on the Illumina NovaSeq6000 platform with 150-bp paired-end reads at Annoroad Gene Technology Co., Ltd. (Beijing, China).

Gene expression analysis by RNA-seq

For expression profiling, total RNA was isolated from 3-wk-old Arabidopsis rosette leaves using Trizol for 2 biological replicates. The amount and quality of RNA were tested by Agilent Bioanalyzer 2100 system and Qubit RNA Assay Kit (Life Technologies). Ribosomal RNA was removed by using mRNA Miniprep Kit (Sigma, MRN10). NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB, E7770S) was used to construct the library, and sequencing was performed on Illumina NovaSeq6000 platform with 150-bp paired-end reads at BerryGenomics Company (http://www.berrygenomics.com/, Beijing, China). Clean reads were aligned to TAIR10 using Hisat2 v2.2.1(http://daehwankimlab.github.io/hisat2).

Polysome profiling

Polysome isolation was performed as previously described (Luo et al. 2020). In brief, 3 g of tissue powder was homogenized in 10 mL of polysome extraction buffer (200 mM of Tris–HCl, pH 9.0, 35 mM of $MgCl_2$, 200 mM of KCl, 25 mM of EGTA, 1% [v/v] TWEEN 20, 1% [v/v] Triton X-100, 2% [v/v] polyoxyethylene, 5 mM of dithiothreitol, 0.5 mg mL^{-1} of heparin, 25 mg mL^{-1} of cycloheximide, and 100 mg mL^{-1} of chloramphenicol). Crude cell lysate was centrifuged at 13,000 × *g* for 15 min at 4°C. The supernatants were layered on the top of a 1.7 M of Sucrose cushion and centrifuged at 47,000 rpm (SW41Ti Swinging Bucket Rotor in a Beckman L-100XP Ultracentrifuge) for 3 h at 4°C. The ribosome pellet was resuspended in 350 μL of resuspension buffer (200 mM of Tris–HCl, pH 9.0, 35 mM of $MgCl_2$, 200 mM of KCl, 25 mM of EGTA, 100 mg mL^{-1} of chloramphenicol, and 25 mg mL^{-1} of cycloheximide). The solution was then layered over a 20% to 60% (w/v) sucrose density gradient and centrifuged at 41,000 rpm (SW55Ti Swinging Bucket Rotor in a Beckman L-100XP Ultracentrifuge) for 2 h at 4°C. After ultracentrifugation, the gradients were fractionated into 24 fractions and detected at an absorbance of 254 nm. Fractions 14 to 24 were pooled and treated by 5% (w/v) SDS/0.2 M of EDTA, and then extracted 2 times with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1; v/v/v). The mixture was centrifuged at 12,000 rpm for 10 min at 4°C and RNA was precipitated by isopropanol followed by washed using 70% (v/v) ethanol, and eventually resuspended for the library construction. NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB, E7770S) was used to construct the library, and sequencing was performed on Illumina NovaSeq6000 platform with 150-bp paired-end reads at Jiangbei New Area Biopharmaceutical Public Service Platform Co., Ltd, Nanjing, China.

Identification of m6A peaks and DMPs

The methods of identify m6A peaks and DMPs were according to our previous reports (Xu et al. 2021). Briefly, MeTPeak (Cui et al. 2016) was used to identify m6A peaks. Furthermore, to avoid huge differences in the calculation of peak enrichment due to insufficient coverage, we performed a random sampling of genomic regions and calculated reads of all input and IP samples, we maintained high confidence peaks on peaks with input FPKM ≥ 1 and peaks enrichment ≥ 2.

The common m6A peaks between any 2 samples were defined according to whether they intersected with each other. Read counts of IP and input replicates for each m6A peak were calculated in every comparison group. A 2 × 2 contingency table was filled by IP and input normalized reads of samples, respectively. The Fisher's exact test was applied to identify DMPs, and *P*-value was adjusted by Bonferroni–Holm correction using R scripts. The DMPs should be satisfied by 2 requirements: (i) *P*adj < 0.05; (ii) the difference between any 2 samples >1.5.

RNA-seq analysis and translation efficiency calculation

HTSeq (Anders et al. 2015) and R package DESeq2 (version 1.32.0) were used to calculate the number of reads for each gene and analyze DEGs, only genes with *P*adj < 0.05 and $|\log_2(FC)| > 1$ were considered as DEGs. Translation efficiency was calculated by "FPKM (ribosome bound)/FPKM (transcript)" as described in Bazin et al. (2017), and genes with *P*adj < 0.05 and $|\log_2(FC)| > 1$ were considered as DTEGs. FPKM was calculated for the reads that mapped in CDS region of the genes.

GO analysis

The agriGO v2.0 database was used for GO enrichment analysis in this study (Tian et al. 2017). Functional enrichment was performed using the singular enrichment analysis tool and TAIR genome locus (TAIR 10) as background. The GO terms with FDR ≤ 0.05 were considered to be enriched. For GO terms with a hierarchical relationship, the child GO terms were kept and the parent terms were removed to avoid redundancy in this study.

AS analysis

rMATS (v.4.1.2) was used to detect differentially AS events (Shen et al. 2014), including skipped exon, alternative 5′ splice site, alternative 3′ splice site, mutually exclusive exons, and retained intron. The events with FDR < 0.05 were identified as significantly differential events.

RT-qPCR

Total RNA was extracted using Trizol (15596018, Ambion) according to its instructions. m6A-IP-RNA and polysome-bound RNA were isolated as described above. The Maxima H Minus cDNA Synthesis Master Mix with DNase (Thermo Fisher Scientific) was used for reverse transcription. RT-qPCR was performed using ChamQ SYBR Color qPCR Master Mix (Q441, Vazyme). *Actin* was used as an internal control gene for the normalization. All primers used in this study are listed in Supplemental Table S9.

Protoplast transfection and protein analysis

Protoplasts were isolated from 14-d-old seedlings grown on 1/2 MS plates as described previously (Zhai et al. 2009). The recombined construct was transformed into protoplasts isolated from WT and *AmiR-mta* plants. After incubation of 3 h at 22°C, protoplasts were split for additional incubation for 16 h at 22 and 4°C. Total proteins were extracted from transformed protoplasts with protein extraction buffer (50 mM Tris–HCl, 1 mM EDTA, 1 mM EGTA, 150 mM NaCl, 10% [v/v] glycerol, 5 mM DTT, 0.25% [v/v] Triton-X 100, 2% [w/v] PVPP, 1 mM PMSF), and separated by SDS-PAGE. The FLAG:DGAT1:GFP fusion protein was detected on immunoblots using an anti-FLAG antibody (Sigma-Aldrich, A8592).

Statistical analyses

Fisher's exact test, Student–Newman–Keuls test, and Mann–Whitney *U*-test was performed using Fisher.test, SNK.test, and Mann–Whitney *U* function of R package, respectively.

Accession numbers

All sequencing data generated from this study have been deposited into the NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) under the accession number GSE206292. All data are openly available.

Author contributions

YF.W. and S.W. supervised the study. YF.W., J.H., and S.W. conceived the project and analyzed data. S.W., HY.W., ZH.X., SS.J., YC.S., HR.X., and Shu.W. performed the experiments. HY.W. and ZH.X. conducted bioinformatics analysis. YF.W., J.H., and S.W. wrote the article. All authors read and approved the final manuscript.

Supplemental data

The following materials are available in the online version of this article.

Supplemental Figure S1. Expression of genes encoding m6A "writers," "erasers," and "readers" at 6, 12, and 24 h after 4°C cold treatment.

Supplemental Figure S2. Spearman correlation analysis of m6A-IP-seq read counts.

Supplemental Figure S3. GO enrichment for unique down-DMGs caused by chilling in WT and *AmiR-mta* plants.

Supplemental Figure S4. Spearman correlation analysis of RNA-seq data.

Supplemental Figure S5. Spearman correlation analysis of polysome-seq data.

Supplemental Figure S6. Association of m6A level with translation efficiency.

Supplemental Figure S7. Expression analysis of *DGAT1*.

Supplemental Figure S8. The overlaps of m6A modified genes identified in this study with Govindan et al published data at 22°C and 4°C.

Supplemental Table S1. Confident m6A peaks identified by 2 independent m6A-IP-seq experiments in *AmiR-mta* and WT plants under normal and cold conditions.

Supplemental Table S2. m6A modified genes in WT and *AmiR-mta* plants under normal and cold conditions.

Supplemental Table S3. GO analysis of up- and downregulated DMGs caused by chilling in WT.

Supplemental Table S4. Shared and unique up-/down-DMGs of 4°C versus 22°C between WT and *AmiR-mta* plants.

Supplemental Table S5. GO analysis of unique downregulated DMGs of 4°C versus 22°C in WT and *AmiR-mta* plants.

Supplemental Table S6. Summary of differential AS events in WT and *AmiR-mta* plants under normal and cold conditions.

Supplemental Table S7. Up- and downregulated DEGs caused by chilling in WT and *AmiR-mta* plants.

Supplemental Table S8. Up- and downregulated DTEGs caused by chilling in WT and *AmiR-mta* plants.

Supplemental Table S9. Primers used in this study.

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Conflict of interest statement. None declared.

Data availability

Data openly available in a public repository

References

Alarcon CR, Goodarzi H, Lee H, Liu XH, Tavazoie S, Tavazoie SF. HNRNPA2B1 is a mediator of m6A-dependent nuclear RNA processing events. Cell. 2015:**162**(6):1299–1308. https://doi.org/10.1016/j.cell.2015.08.011

Alonso JM, Stepanova AN, Leisse TJ, Kim CJ, Chen HM, Shinn P, Stevenson DK, Zimmerman J, Barajas P, Cheuk R, et al. Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. Science. 2003:**301**(5633):653–657. https://doi.org/10.1126/science.1086391

Anders S, Pyl PT, Huber W. HTSeq-a python framework to work with high-throughput sequencing data. Bioinformatics. 2015:**31**(2):166–169. https://doi.org/10.1093/bioinformatics/btu638

Arribas-Hernandez L, Bressendorff S, Hansen MH, Poulsen C, Erdmann S, Brodersen P. An m6A-YTH module controls developmental timing and morphogenesis in Arabidopsis. Plant Cell. 2018:**30**(5):952–967. https://doi.org/10.1105/tpc.17.00833

Arribas-Hernandez L, Simonini S, Hansen MH, Paredes EB, Bressendorff S, Dong Y, Ostergaard L, Brodersen P. Recurrent requirement for the m6A-ECT2/ECT3/ECT4 axis in the control of cell proliferation during plant organogenesis. Development. 2020:**147**(14):dev189134. https://doi.org/10.1242/dev.189134

Bazin J, Baerenfaller K, Gosai SJ, Gregory BD, Crespi M, Bailey-Serres J. Global analysis of ribosome-associated noncoding RNAs unveils new modes of translational regulation. Proc Natl Acad Sci U S A. 2017:**114**(46):E10018–E10027. https://doi.org/10.1073/pnas.1708433114

Beine-Golovchuk O, Firmino AAP, Dabrowska A, Schmidt S, Erban A, Walther D, Zuther E, Hincha DK, Kopka J. Plant temperature acclimation and growth rely on cytosolic ribosome biogenesis factor homologs. Plant Physiol. 2018:**176**(3):2251–2276. https://doi.org/10.1104/pp.17.01448

Bhat SS, Bielewicz D, Gulanicz T, Bodi Z, Yu X, Anderson SJ, Szewc L, Bajczyk M, Dolata J, Grzelak N, et al. mRNA adenosine methylase (MTA) deposits m6A on pri-miRNAs to modulate miRNA biogenesis in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A. 2020:**117**(35):21785–21795. https://doi.org/10.1073/pnas.2003733117

Chan C, Pham P, Dedon PC, Begley TJ. Lifestyle modifications: coordinating the tRNA epitranscriptome with codon bias to adapt translation during stress responses. Genome Biol. 2018:**19**(1):1–11. https://doi.org/10.1186/s13059-018-1611-1

Chen MJ, Urs MJ, Sanchez-Gonzalez I, Olayioye MA, Herde M, Witte CP. m6A RNA degradation products are catabolized by an evolutionarily conserved N6-methyl-AMP deaminase in plant and mammalian cells. Plant Cell. 2018:**30**(7):1511–1522. https://doi.org/10.1105/tpc.18.00236

Chinnusamy V, Ohta M, Kanrar S, Lee BH, Hong XH, Agarwal M, Zhu JK. ICE1: a regulator of cold-induced transcriptome and freezing tolerance in Arabidopsis. Genes Dev. 2003:**17**(8):1043–1054. https://doi.org/10.1101/gad.1077503.

Clough SJ, Bent AF. Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. Plant J. 1998:**16**(6):735–743. https://doi.org/10.1046/j.1365-313x.1998.00343.x

Cui XD, Meng J, Zhang SW, Chen YD, Huang YF. A novel algorithm for calling mRNA m6A peaks by modeling biological variances in MeRIP-seq data. Bioinformatics. 2016:**32**(12):378–385. https://doi.org/10.1093/bioinformatics/btw281

Ding YL, Li H, Zhang XY, Xie Q, Gong ZZ, Yang SH. OST1 Kinase modulates freezing tolerance by enhancing ice1 stability in Arabidopsis. Dev Cell. 2015:**32**(3):278–289. https://doi.org/10.1016/j.devcel.2014.12.023

Doherty CJ, Van Buskirk HA, Myers SJ, Thomashow MF. Roles for Arabidopsis CAMTA transcription factors in cold-regulated gene expression and freezing tolerance. Plant Cell. 2009:**21**(3):972–984. https://doi.org/10.1105/tpc.108.063958

Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, Cesarkas K, Jacob-Hirsch J, Amariglio N, Kupiec M, et al. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature. 2012:**485**(7397):201–206. https://doi.org/10.1038/nature11112

Dong CH, Agarwal M, Zhang YY, Xie Q, Zhu JK. The negative regulator of plant cold responses, HOS1, is a RING E3 ligase that mediates the ubiquitination and degradation of ICE1. Proc Natl Acad Sci U S A. 2006:**103**(21):8281–8286. https://doi.org/10.1073/pnas.0602874103

Du H, Zhao Y, He JQ, Zhang Y, Xi HR, Liu MF, Ma JB, Wu LG. YTHDF2 destabilizes m6A-containing RNA through direct recruitment of the CCR4-NOT deadenylase complex. Nat Commun. 2016:**7**(1):1–11. https://doi.org/10.1038/ncomms12626

Duan HC, Wei LH, Zhang C, Wang Y, Chen L, Lu ZK, Chen PR, He C, Jia GF. ALKBH10B is an RNA N6-methyladenosine demethylase affecting Arabidopsis floral transition. Plant Cell. 2017:**29**(12):2995–3011. https://doi.org/10.1105/tpc.16.00912

Endres L, Dedon PC, Begley TJ. Codon-biased translation can be regulated by wobble-base tRNA modification systems during cellular stress responses. RNA Biol. 2015:**12**(6):603–614. https://doi.org/10.1080/15476286.2015.1031947

Fu Y, Dominissini D, Rechavi G, He C. Gene expression regulation mediated through reversible m6A RNA methylation. Nat Rev Genet. 2014:**15**(5):293–306. https://doi.org/10.1038/nrg3724

Fursova OV, Pogorelko GV, Tarasov VA. Identification of ICE2, a gene involved in cold acclimation which determines freezing tolerance in *Arabidopsis thaliana*. Gene. 2009:**429**(1–2):98–103. https://doi.org/10.1016/j.gene.2008.10.016

Gilmour SJ, Zarka DG, Stockinger EJ, Salazar MP, Houghton JM, Thomashow MF. Low temperature regulation of the Arabidopsis CBF family of AP2 transcriptional activators as an early step in cold-induced COR gene expression. Plant Physiol. 1998:**16**(4):433–442. https://doi.org/10.1046/j.1365-313x.1998.00310.x

Govindan G, Sharma B, Li YF, Armstrong CD, Merum P, Rohila JS, Gregory BD, Sunkar R. mRNA N6-methyladenosine is critical for cold tolerance in Arabidopsis. Plant J. 2022:**111**(4):1052–1068. https://doi.org/10.1111/tpj.15872

Hou YF, Sun J, Wu BX, Gao YY, Nie HB, Nie ZT, Quan SX, Wang Y, Cao XF, Li SS. CPSF30-L-mediated recognition of mRNA m6A modification controls alternative polyadenylation of nitrate signaling-related gene transcripts in Arabidopsis. Mol Plant. 2021:**14**(4):688–699. https://doi.org/10.1016/j.molp.2021.01.013

Hu J, Cai J, Park SJ, Lee K, Li YX, Chen Y, Yun JY, Xu T, Kang H. N6-methyladenosine mRNA methylation is important for salt stress tolerance in Arabidopsis. Plant J. 2021:**106**(6):1759–1775. https://doi.org/10.1111/tpj.15270

Huang HL, Weng HY, Sun WJ, Qin X, Shi HL, Wu HZ, Zhao BS, Mesquita A, Liu C, Yuan CL, et al. Recognition of RNA N6-methyladenosine by IGF2BP proteins enhances mRNA stability and translation. Nat Cell Biol. 2018:**20**(3):285–295. https://doi.org/10.1038/s41556-018-0045-z

Jia YX, Ding YL, Shi YT, Zhang XY, Gong ZZ, Yang SH. The cbfs triple mutants reveal the essential functions of CBFs in cold acclimation and allow the definition of CBF regulons in Arabidopsis. New Phytol. 2016:**212**(2):345–353. https://doi.org/10.1111/nph.14088

Kim Y, Park S, Gilmour SJ, Thomashow MF. Roles of CAMTA transcription factors and salicylic acid in configuring the low-temperature transcriptome and freezing tolerance of Arabidopsis. Plant J. 2013:**75**(3):364–376. https://doi.org/10.1111/tpj.12205

Kupsch C, Ruwe H, Gusewski S, Tillich M, Small I, Schmitz-Linneweber C. Arabidopsis chloroplast RNA binding proteins CP31A and CP29A associate with large transcript pools and confer cold stress tolerance by influencing multiple chloroplast RNA processing steps. Plant Cell. 2012:**24**(10):4266–4280. https://doi.org/10.1105/tpc.112.103002

Kusumi K, Sakata C, Nakamura T, Kawasaki S, Yoshimura A, Iba K. A plastid protein NUS1 is essential for build-up of the genetic system for early chloroplast development under cold stress conditions. Plant J. 2011:**68**(6):1039–1050. https://doi.org/10.1111/j.1365-313X.2011.04755.x

Li H, Ding YL, Shi YT, Zhang XY, Zhang SQ, Gong ZZ, Yang SH. MPK3-and MPK6-mediated ICE1 phosphorylation negatively regulates ICE1 stability and freezing tolerance in Arabidopsis. Dev Cell. 2017:**43**(5):630–642. https://doi.org/10.1016/j.devcel.2017.09.025

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K. Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. Plant Cell. 1998:**10**(8):1391–1406. https://doi.org/10.1105/tpc.10.8.1391

Liu XY, Rodermel SR, Yu F. A var2 leaf variegation suppressor locus, SUPPRESSOR OF VARIEGATION3, encodes a putative chloroplast translation elongation factor that is important for chloroplast development in the cold. BMC Plant Biol. 2010:**10**(1):1–18. https://doi.org/10.1186/1471-2229-10-287

Liu HM, Zhang Y, Lu S, Chen H, Wu JW, Zhu X, Zou BH, Hua J. Hsfa1d promotes hypocotyl elongation under chilling via enhancing expression of ribosomal protein genes in Arabidopsis. New Phytol. 2021:**231**(2):646–660. https://doi.org/10.1111/nph.17413

Liu XY, Zheng MD, Wang R, Wang RJ, An LJ, Rodermel SR, Yu F. Genetic interactions reveal that specific defects of chloroplast translation are associated with the suppression of var2-mediated leaf variegation. J Integr Plant Biol. 2013:**55**(10):979–993. https://doi.org/10.1111/jipb.12078

Luo GZ, MacQueen A, Zheng GQ, Duan HC, Dore LC, Lu ZK, Liu J, Chen K, Jia GF, Bergelson J, et al. Unique features of the m6A methylome in *Arabidopsis thaliana*. Nat Commun. 2014:**5**(1):1–8. https://doi.org/10.1038/ncomms6630

Luo JH, Wang Y, Wang M, Zhang LY, Peng HR, Zhou YY, Jia GF, He Y. Natural variation in RNA m6A methylation and its relationship with translational status. Plant Physiol. 2020:**182**(1):332–344. https://doi.org/10.1104/pp.19.00987

Martinez-Perez M, Aparicio F, Lopez-Gresa MP, Belles JM, Sanchez-Navarro JA, Pallas V. Arabidopsis m6A demethylase activity modulates viral infection of a plant virus and the m6A abundance

in its genomic RNAs. Proc Natl Acad Sci U S A. 2017:**114**(40):10755–-10760. https://doi.org/10.1073/pnas.1703139114

Medina J, Bargues M, Terol J, Perez-Alonso M, Salinas J. The Arabidopsis CBF gene family is composed of three genes encoding AP2 domain-containing proteins whose expression is regulated by low temperature but not by abscisic acid or dehydration. Plant Physiol. 1999:**119**(2):463–469. https://doi.org/10.1104/pp.119.2.463

Meyer KD, Jaffrey SR. The dynamic epitranscriptome: N6-methyladenosine and gene expression control. Nat Rev Mol Cell Biol. 2014:**15**(5):313–326. https://doi.org/10.1038/nrm3785

Meyer KD, Patil DP, Zhou J, Zinoviev A, Skabkin MA, Elemento O, Pestova TV, Qian SB, Jaffrey SR. 5′ UTR m6A promotes cap-independent translation. Cell. 2015:**163**(4):999–1010. https://doi.org/10.1016/j.cell.2015.10.012

Miura K, Jin JB, Lee J, Yoo CY, Stirm V, Miura T, Ashworth EN, Bressan RA, Yun DJ, Hasegawa PM. SIZ1-mediated Sumoylation of ICE1 controls CBF3/DREB1A expression and freezing tolerance in Arabidopsis. Plant Cell. 2007:**19**(4):1403–1414. https://doi.org/10.1105/tpc.106.048397

Pontier D, Picart C, El Baidouri M, Roudier F, Xu T, Lahmy S, Llauro C, Azevedo J, Laudie M, Attina A, et al. The m6A pathway protects the transcriptome integrity by restricting RNA chimera formation in plants. Life Sci Alliance. 2019:**2**(3):e201900393. https://doi.org/10.26508/lsa.201900393

Roundtree IA, Luo GZ, Zhang ZJ, Wang X, Zhou T, Cui YQ, Sha JH, Huang XX, Guerrero L, Xie P, et al. YTHDC1 mediates nuclear export of N6-methyladenosine methylated mRNAs. Elife. 2017:**6**:e31311. https://doi.org/10.7554/eLife.31311

Ruzicka K, Zhang M, Campilho A, Bodi Z, Kashif M, Saleh M, Eeckhout D, El-Showk S, Li HY, Zhong SL, et al. Identification of factors required for m6A mRNA methylation in Arabidopsis reveals a role for the conserved E3 ubiquitin ligase HAKAI. New Phytol. 2017:**215**(1):157–172. https://doi.org/10.1111/nph.14586

Schwab R, Ossowski S, Riester M, Warthmann N, Weigel D. Highly specific gene silencing by artificial microRNAs in Arabidopsis. Plant Cell. 2006:**18**(5):1121–1133. https://doi.org/10.1105/tpc.105.039834

Scutenaire J, Deragon JM, Jean V, Benhamed M, Raynaud C, Favory JJ, 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**(5):986–1005. https://doi.org/10.1105/tpc.17.00854

Shen LS, Liang Z, Gu XF, Chen Y, Teo ZWN, Hou XL, Cai WM, Dedon PC, Liu L, Yu H. N6-methyladenosine RNA modification regulates shoot stem cell fate in Arabidopsis. Dev Cell. 2016:**38**(2):186–200. https://doi.org/10.1016/j.devcel.2016.06.008

Shen S, Park JW, Lu ZX, Lin L, Henry MD, Wu YN, Zhou Q, Xing Y. rMATS: robust and flexible detection of differential alternative splicing from replicate RNA-seq data. Proc Natl Acad Sci U S A. 2014:**111**(51): E5593–E5601. https://doi.org/10.1073/pnas.1419161111

Shi HL, Wang X, Lu ZK, Zhao BXS, Ma HH, Hsu PJ, Liu C, He C. YTHDF3 facilitates translation and decay of N6-methyladenosine-modified RNA. Cell Res. 2017:**27**(3):315–328. https://doi.org/10.1038/cr.2017.15

Slobodin B, Han RQ, Calderone V, Vrielink JAFO, Loayza-Puch F, Elkon R, Agami R. Transcription impacts the efficiency of mRNA translation via co-transcriptional N6-adenosine methylation. Cell. 2017:**169**(2):326–337. https://doi.org/10.1016/j.cell.2017.03.031

Song PZ, Yang JB, Wang CL, Lu Q, Shi LQ, Tayier S, Jia GF. Arabidopsis N6-methyladenosine reader CPSF30-L recognizes FUE signals to control polyadenylation site choice in liquid-like nuclear bodies. Mol Plant. 2021:**14**(4):571–587. https://doi.org/10.1016/j.molp.2021.01.014

Tan WJ, Yang YC, Zhou Y, Huang LP, Xu L, Chen QF, Yu LJ, Xiao S. DIACYLGLYCEROL ACYLTRANSFERASE and DIACYLGLYCEROL KINASE modulate triacylglycerol and phosphatidic acid production in the plant response to freezing stress. Plant Physiol. 2018:**177**(3):1424–1425. https://doi.org/10.1104/pp.18.00402

Tang K, Zhao L, Ren YY, Yang SH, Zhu JK, Zhao CZ. The transcription factor ICE1 functions in cold stress response by binding to the promoters of CBF and COR genes. J Integr Plant Biol. 2020:**62**(3):258–263. https://doi.org/10.1111/jipb.12918

Tian T, Liu Y, Yan HY, You Q, Yi X, Du Z, Xu WY, Su Z. agriGO v2.0: a GO analysis toolkit for the agricultural community, 2017 update. Nucleic Acids Res. 2017:**45**(W1):W122–W129. https://doi.org/10.1093/nar/gkx382

Tzfira T, Tian GW, Lacroix B, Vyas S, Li JX, Leitner-Dagan Y, Krichevsky A, Taylor T, Vainstein A, Citovsky V. pSAT vectors: a modular series of plasmids for autofluorescent protein tagging and expression of multiple genes in plants. Plant Mol Biol. 2005:**57**(4):503–516. https://doi.org/10.1007/s11103-005-0340-5

Vespa L, Vachon G, Berger F, Perazza D, Faure JD, Herzog M. The immunophilin-interacting protein AtFIP37 from Arabidopsis is essential for plant development and is involved in trichome endoreduplication. Plant Physiol. 2004:**134**(4):1283–1292. https://doi.org/10.1104/pp.103.028050

Wang S, Bai G, Wang S, Yang L, Yang F, Wang Y, Zhu JK, Hua J. Chloroplast RNA-binding protein RBD1 promotes chilling tolerance through 23S rRNA processing in Arabidopsis. PLoS Genet. 2016:**12**(5):e1006027. https://doi.org/10.1371/journal.pgen.1006027

Wang X, Lu ZK, Gomez A, Hon GC, Yue YN, Han DL, Fu Y, Parisien M, Dai Q, Jia GF, et al. N6-methyladenosine-dependent regulation of messenger RNA stability. Nature. 2014:**505**(7481):117–120. https://doi.org/10.1038/nature12730

Wang X, Zhao BS, Roundtree IA, Lu ZK, Han DL, Ma HH, Weng XC, Chen K, Shi HL, He C. N6-methyladenosine modulates messenger RNA translation efficiency. Cell. 2015:**161**(6):1388–1399. https://doi.org/10.1016/j.cell.2015.05.014

Wei LH, Song PZ, Wang Y, Lu ZK, Tang Q, Yu Q, Xiao Y, Zhang X, Duan HC, Jia GF. The m6A reader ECT2 controls trichome morphology by affecting mRNA stability in Arabidopsis. Plant Cell. 2018:**30**(5):968–985. https://doi.org/10.1105/tpc.17.00934

Wigge PA, Guillaume-Schoepfer D, Jaeger KE, Geng F, Doccula FG, Costa A, Webb AA. Ribosomes act as cryosensors in plants. bioRxiv; 2020. https://doi.org/10.1101/2020.12.07.414789

Xiao W, Adhikari S, Dahal U, Chen YS, Hao YJ, Sun BF, Sun HY, Li A, Ping XL, Lai WY, et al. Nuclear m6A reader YTHDC1 regulates mRNA splicing. Mol Cell. 2016:**61**(4):507–519. https://doi.org/10.1016/j.molcel.2016.01.012

Xu ZH, Shi XB, Bao MM, Song XQ, Zhang YX, Wang HY, Xie HR, Mao F, Wang SA, Jin HM, et al. Transcriptome-wide analysis of RNA m6A methylation and gene expression changes among two Arabidopsis ecotypes and their reciprocal hybrids. Front Plant Sci. 2021:**12**:685189. https://doi.org/10.3389/fpls.2021.685189

Yu HS, Kong XF, Huang H, Wu WW, Park J, Yun DJ, Lee BH, Shi HZ, Zhu JK. STCH4/REIL2 confers cold stress tolerance in Arabidopsis by promoting rRNA processing and CBF protein translation. Cell Rep. 2020:**30**(1):229–242. https://doi.org/10.1016/j.celrep.2019.12.012

Yue YA, Liu J, Cui XL, Cao J, Luo GZ, Zhang ZZ, Cheng T, Gao MS, Shu X, Ma HH, et al. VIRMA mediates preferential m6A mRNA methylation in 3′ UTR and near stop codon and associates with alternative polyadenylation. Cell Discov. 2018:**4**(1):1–17. https://doi.org/10.1038/s41421-018-0019-0

Zhai Z, Jung HI, Vatamaniuk OK. Isolation of protoplasts from tissues of 14-day-old seedlings of *Arabidopsis thaliana*. J Vis Exp. 2009:**30**:e1149. https://doi.org/10.3791/1149

Zhang M, Bodi Z, Mackinnon K, Zhong SL, Archer N, Mongan NP, Simpson GG, Fray RG. Two zinc finger proteins with functions in m(6)A writing interact with HAKAI. Nat Commun. 2022:**13**(1):1127. https://doi.org/10.1038/s41467-022-28753-3

Zhao CZ, Wang PC, Si T, Hsu CC, Wang L, Zayed O, Yu ZP, Zhu YF, Dong J, Tao WA, et al. MAP kinase cascades regulate the cold

response by modulating ICE1 protein stability. Dev Cell. 2017:**43**(5): 618–629. https://doi.org/10.1016/j.devcel.2017.09.024

Zhao CZ, Zhang ZJ, Xie SJ, Si T, Li YY, Zhu JK. Mutational evidence for the critical role of CBF transcription factors in cold acclimation in Arabidopsis. Plant Physiol. 2016:**171**(4):2744–2759. https://doi.org/10.1104/pp.16.00533

Zheng HX, Sun X, Li JL, Song YS, Song J, Wang F, Liu LN, Zhang XS, Sui N. Analysis of N6-methyladenosine reveals a new important mechanism regulating the salt tolerance of sweet sorghum. Plant Sci. 2021:**304**:110801. https://doi.org/10.1016/j.plantsci.2020.110801

Zhong SL, Li HY, Bodi Z, Button J, Vespa L, Herzog M, Fray RG. MTA is an Arabidopsis messenger RNA adenosine methylase and interacts with a homolog of a sex-specific splicing factor. Plant Cell. 2008:**20**(5):1278–1288. https://doi.org/10.1105/tpc.108.058883