

Deciphering the epitranscriptome: A green perspective

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Abstract

The advent of high-throughput sequencing technologies coupled with new detection methods of RNA modifications has enabled investigation of a new layer of gene regulation – the epitranscriptome. With over 100 known RNA modifications, understanding the repertoire of RNA modifications is a huge undertaking. This review summarizes what is known about RNA modifications with an emphasis on discoveries in plants. RNA ribose modifications, base methylations and pseudouridylation are required for normal development in *Arabidopsis*, as mutations in the enzymes modifying them have diverse effects on plant development and stress responses. These modifications can regulate RNA structure, turnover and translation. Transfer RNA and ribosomal RNA modifications have been mapped extensively and their functions investigated in many organisms, including plants. Recent work exploring the locations, functions and targeting of N⁶-methyladenosine (m⁶A), 5-methylcytosine (m⁵C), pseudouridine (Ψ), and additional modifications in mRNAs and ncRNAs are highlighted, as well as those previously known on tRNAs and rRNAs. Many questions remain as to the exact mechanisms of targeting and functions of specific modified sites and whether these modifications have distinct functions in the different classes of RNAs.

Key words: RNA modifications; epitranscriptome; RNA 5-methylcytosine (m⁵C); N⁶-methyladenosine (m⁶A); Pseudouridine (Ψ); *Arabidopsis*

INTRODUCTION

Chemical modifications of DNA and proteins such as histones have been established as important regulators of gene expression, eukaryotic development and stress responses (Suzuki and Bird 2008; Lawrence et al. 2016). More recently, a new level of gene regulation, the epitranscriptome, or RNA modifications has gained interest and momentum. There are over 100 different RNA modifications found in different RNA species, the most abundant and most intensively studied are transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs). Loss of modifications on tRNAs and rRNAs is linked to multiple human diseases (Blanco and Frye 2014; Torres et al. 2014) and detrimental effects on development and stress responses in other organisms, including plants, underscoring their vital roles (Motorin and Helm 2011; El Yacoubi et al. 2012). However, new functions and interactions are also being discovered for RNA modifications in mRNAs and other non-coding RNAs (ncRNAs) such as long non-coding RNAs (lncRNAs), micro RNAs (miRNAs) and other small RNAs.

Although the presence of RNA modifications such as the 5' cap structure and internal N⁶-methyladenosine (m⁶A) in mRNAs has been known for decades (Desrosiers et al. 1974; Perry and Kelley 1974; Dubin and Taylor 1975; Shatkin 1976), the flood gates have only just been opened for a new wave of research describing other modifications and their impact on gene regulation. Several recently developed high-throughput sequencing methods for detecting RNA modifications have allowed investigation of low abundance mRNA and ncRNAs on an unprecedented scale thereby enabling deciphering of their functions in RNA metabolism, gene regulation, translation, development and stress responses (methods are reviewed in Shafik et al. 2016). Interestingly, many of the RNA modifications and the enzymes responsible for 'writing' and 'reading' the modifications are conserved across the three domains of life (Jackman and Alfonzo 2013), suggesting important, conserved biological functions of this added layer of complexity and flexibility for RNA regulation.

In this article, we provide an update on the research on tRNAs and rRNAs, highlighting the discoveries in plants before discussing the recent studies investigating the dynamic role of RNA modifications in regulating the function of tRNAs and rRNAs, and the epitranscriptomic landscape of other classes of RNA, including mRNAs and lncRNAs. Continuing on from Fray and Simpson (2015), this review extends and discusses recent developments utilizing transcriptome-wide sequencing to explore the RNA modification landscapes of N⁶-methyladenosine (m⁶A), 5-methylcytosine (m⁵C), pseudouridine (Ψ) and other modifications which perturb Watson-Crick base pairing. Finally, we will discuss conclusions and future perspectives to shed light on the *Arabidopsis* epitranscriptome.

PLANT TRANSFER RNA MODIFICATIONS AND THEIR FUNCTIONS

Transfer RNAs are considered the most heavily modified types of RNA, and these modifications are highly conserved in bacteria, yeast, mammals and plants, consistent with their central role in translation. At least 92 unique chemical modifications have been identified in tRNAs with varied chemical properties and effects on the stability and function of tRNAs (Machnicka et al. 2013). Transfer RNA modifications include RNA editing of adenosine to inosine (A-I), methylation or acetylation of RNA bases, isomerization or reduction of uridine to pseudouridine (Ψ) or dihydrouridine (D) to name a few. These post-transcriptional modifications can occur on the base, or on the ribose sugar backbone of the RNA molecule.

Transfer RNAs can vary in length from 70-90 nucleotides long with RNA modifications occurring at different positions on the iconic clover-leaf secondary structure. The functional roles of tRNA modifications are determined by their position on the clover leaf structure and by the chemical properties of the RNA modification. The functions of these modifications affecting tRNA biogenesis can be divided into three major groups, (1) modifications that affect amino-acylation on the acceptor stem, (2) modifications on or near the anticodon loop can affect anti-codon binding, wobble base pairing and frame shifting and (3) other positions on D-stem, TΨC stem and variable loop/junction affecting stability, structure, translation

and tRNA cleavage/degradation. The positions and types of modifications and the enzymes responsible for mediating them throughout different domains of life have been reviewed extensively in (Phizicky and Hopper 2010; El Yacoubi et al. 2012; Towns and Begley 2012). Here we focus on tRNA modifications and tRNA modifying enzymes investigated in plants.

The identities of RNA modifications present in tRNAs of several plant species (*Arabidopsis*, tobacco, maize, hybrid aspen and wheat) have been investigated using a combination of chromatography and mass spectroscopy techniques on purified tRNAs (Shugart 1972; Jones and Scott 1981; Chen et al. 2010; Hienzschi et al. 2013). In two independent studies on *Arabidopsis* tRNAs, a total of 26 known tRNA RNA modifications were identified and 4 novel, potentially plant specific RNA modifications (Chen et al. 2010; Hienzschi et al. 2013). In addition, bioinformatics approaches have been utilized to predict hundreds of RNA base modification sites in *Arabidopsis* miRNAs and tRNAs, based on the ability of certain RNA modifications to introduce mismatches in sequences after reverse transcription (Iida et al. 2009). Evidence has also shown dynamic regulation of tRNA modifications. When comparing different plant tissues, differences in the abundance and types of tRNA modifications were found when comparing different plant tissues and cell cultures (Jones and Scott 1981; Hienzschi et al. 2013) and new and old leaves (Shugart 1972). Recently, the tRNA modification 2'-O-cytosine methylation (Cm) was shown to be increased in response to pathogen infection in *Arabidopsis* (Ramirez et al. 2015). In other organisms, several tRNA modifications were shown to be induced under stress conditions such as oxidative stress (Chan et al. 2010; Chan et al. 2012), nutrient starvation (Preston et al. 2013) and toxins (Hertz et al. 2014).

Identification of RNA modifications present in tRNAs is only half the story – the tRNA modifying enzymes, or ‘writers’ are just beginning to be characterized in plants. A combination of bioinformatics and reverse genetics approaches have been utilized to predict and identify tRNA modifying enzymes in *Arabidopsis* (Golovko et al. 2002; Chen et al. 2006; Miyawaki et al. 2006; Pavlopoulou and Kossida 2009; Zhou et al. 2009; Chen et al. 2010;

Hu et al. 2010; Mehlgarten et al. 2010; Leihne et al. 2011; Zhou et al. 2013; Burgess et al. 2015; Ramirez et al. 2015). Transfer RNA modifying enzymes have been characterized for mediating base methylations of guanine and cytosine residues, and modifying 2'-O-ribose methylations.

RNA methylation of guanine and cytosine residues commonly occurs in *Arabidopsis* tRNAs and have roles in mediating RNA structure and stabilization through for example Mg^{2+} binding (Chen et al. 1993; David et al. 2016). Based on homologous genes in yeast, three guanosine transfer RNA methyltransferase (TRM) enzymes have been identified in *Arabidopsis*, namely AtTRM10 (At5g47680), AtTRM11 (At3g26410) and AtTRM82 (At1g03110), which mediate m^1G , m^2G and m^7G in tRNAs, respectively (Chen et al. 2010). Of these, a biological role in plant development was only identified for AtTRM11, as the mutant showed an early-flowering phenotype. 5-methylcytosine in tRNAs is mediated by *Arabidopsis* transfer RNA aspartic acid methyltransferase 1 (TRDMT1, At5g25480) at position 38 and by tRNA specific methyltransferase 4B (TRM4B, At2g22400) at the variable loop/T Ψ C stem junction (Goll et al. 2006; Burgess et al. 2015). Loss of both TRDMT1 and TRM4B results in increased sensitivity to the antibiotic Hygromycin B, suggesting roles for these modifications in translation (Burgess et al. 2015). Similar functions were found for TRM4 in yeast (Wu et al. 1998) and translation efficiency was reduced in mammals (Tuorto et al. 2012). Another tRNA methylation modification is 2'-O-ribose methylation. Recently, an *Arabidopsis* homolog of yeast TRM7 (At5g01230), a 2'-O-ribose methyltransferase, was identified to be required for efficient immune response to *Pseudomonas syringae* (Ramirez et al. 2015).

Several modifications in the anticodon loop fine-tune translation by reducing frame shift mutations and mediating codon binding stringency at the third 'wobble' base pair position. RNA editing of adenosine to inosine (A-I) at the first position of the anticodon allows a single tRNA to decode multiple codons for the same amino acid, because I can base pair with A, C or U. RNA editing (A-I) by AtTAD1 (homologous to yeast Tad1p tRNA-specific adenosine deaminase) (At1g01760) at the position 3'-adjacent to the anticodon in

nuclear tRNA^{Ala(AGC)} has been shown to be required for efficient translation under stress conditions, as *Arabidopsis attad1* mutants have reduced biomass when exposed to heat and cold stress treatments (Zhou et al. 2013). The molecular function of this specific RNA editing event is unclear. A conserved multi-protein Elongator complex mediates acetylation of histones and tRNA wobble uridine modifications (Mehlgarten et al. 2010). Four components of the Elongator complex have been characterized in plants, demonstrating roles for the Elongator complex in ABA and oxidative stress response in *Arabidopsis* (Chen et al. 2006; Zhou et al. 2009). In addition, Elongator mutants such as *atelp1* (At5g13680) display pleiotropic growth defects (Chen et al. 2010).

RIBOSOMAL RNA MODIFICATIONS AND FUNCTIONS IN *ARABIDOPSIS*

Ribosomes are multi-subunit complexes of non-coding ribosomal RNAs and proteins. In eukaryotes, three of the four rRNAs present in the small and large rRNA subunits are encoded in a single, polycistronic, pre-rRNA transcript. Multiple processing steps involving cleavage and RNA modifications are required for maturation and assembly of the rRNAs with ribosomal proteins (Henras et al. 2015). Ribosomal RNA modifications tend to be clustered around conserved structural and functional regions of the ribosomes such as the peptidyl transferase center (PTC) and are required for efficient translation (Decatur and Fournier 2002). Ribosomal RNAs contain three broad types of RNA modifications, ribose methylation, pseudouridylation and several types of base methylations (e.g m⁵C, m³U, m⁶A) reviewed in (Decatur and Fournier 2002; Baxter-Roshek et al. 2007).

The most abundant rRNA modifications are Ψ and 2'-O-ribose methylations. The majority of these rRNA modifications are mediated by small nucleolar ribonucleoprotein complexes (snoRNPs) composed of multiple conserved proteins and a small nucleolar RNA (snoRNA), which directs sequence-specific targeting. These two modifications are guided by two different classes of snoRNAs, (1) box-C/D snoRNAs which guide 2'-O-ribose methylations mediated by the methyltransferase NOP1 (yeast)/Fibrillarin (human) and (2) box-H/ACA snoRNAs which direct conversion of uridine to pseudouridine by

Cbf5/NAP57/Dyskerin (human) (Kiss 2001; Brown et al. 2003). Three genes encoding homologues of the essential yeast and human Fibrillarin 2'-O- ribose methyltransferase were identified in *Arabidopsis*, AtFIB1 (At5g52470), AtFIB2 (At4g25630) and AtFIB3 (At5g52490) (Barneche et al. 2000; Pih et al. 2000). Of these three genes, transcripts were only detected from AtFIB1 and AtFIB2, and both proteins are able to partially complement a conditional yeast NOP1/Fibrillarin mutant. This suggests that the *Arabidopsis* 2'-O-ribose methyltransferase snoRNPs might be heterogeneous, and contain either AtFIB1 or AtFIB2, and these different snoRNPs may have specialized functions in plants. Similarly, removal of rRNA pseudouridylation in yeast and *Arabidopsis* by deletion of CBF5, is also lethal (Lermontova et al. 2007) while defects in the human homolog Dyskerin result in dyskeratosis congenita, a disease characterized by abnormal skin pigmentation and bone marrow failure (Heiss et al. 1998). Moreover, patients with this condition were recently found to have reduced Ψ in rRNA and the ncRNA telomerase component TERC (Telomerase RNA component) (Schwartz et al. 2014a). Another chloroplast specific rRNA Ψ synthase was identified in *Arabidopsis* in a suppressor screen for mutants complementing a chloroplast variegation mutation, SUPPRESSOR OF VARIAGATION1 (SVR1, At2g39140) (Yu et al. 2008). *Arabidopsis* *svr1* mutants are small and pale green, with defects in chloroplast rRNA processing and translation. SVR1 is predicted to target chloroplast rRNA Ψ independently of a snoRNA guide in a similar manner to other tRNA and mitochondrial rRNA Ψ synthases from yeast and bacteria (Ansmant et al. 2000).

Unlike most of the rRNA Ψ and 2'-O-ribose methylations, which are catalyzed by snoRNPs, the rRNA base methylations are all performed by site-specific base methyltransferases. The nuclear large subunit 25S rRNA in yeast and *Arabidopsis* contains two m⁵C sites, which are methylated by RNA methyltransferases RCM1 (rRNA cytosine methyltransferase 1) and NOP2 (nucleolar protein 2) (Sharma et al. 2013; Gigova et al. 2014). The two methylation sites have roles in antibiotic sensitivity and rRNA biogenesis and processing in yeast, respectively (Hong et al. 1997; Sharma et al. 2013). In *Arabidopsis* the RCM1 homolog, NOP2/Sun domain protein 5 (NSUN5), was

found to methylate the orthologous position in 25S rRNA (Burgess et al. 2015). The second m⁵C site unexpectedly remained unchanged in single mutants for all three *Arabidopsis* NOP2 homologs, NOP2A (At5g55920), NOP2B (At4g26600) and NOP2C (At1g06560), as *nop2a* mutants have a leaf phenotype (Fujikura et al. 2009; Burgess et al. 2015). The unchanged methylation level in the single mutants may suggest functional redundancy (Burgess et al. 2015). Two adjacent adenosines are N-6 dimethylated (m₂⁶A) in small subunit rRNAs of eukaryotes and prokaryotes by adenosine dimethyl transferase 1 (DIM1) homologs. Similar to the case of NOP2, the *Arabidopsis* genome encodes three rRNA dimethyl transferase enzymes: DIM1A (At2g47420), the nuclear 18S rRNA dimethyl transferase required for organized root growth and epidermal patterning (Wieckowski and Schiefelbein 2012), DIM1B (At5g66360), the mitochondrial rRNA dimethyl transferase (Richter et al. 2010) and DIM1C/PALEFACE1 (At1g01860), which is located in the chloroplast and is required for chloroplast development in the cold (Tokuhisa et al. 1998).

In organisms such as yeast, with only one copy of DIM1 and NOP2, loss of either of these enzymes results in lethality (Lafontaine et al. 1994; Hong et al. 1997). Surprisingly, the presence of catalytically inactivated modifying enzymes rescues the phenotype in several organisms, suggesting other important roles for DIM1 and NOP2 in ribosome biogenesis (Lafontaine et al. 1995; King and Redman 2002; Zorbas et al. 2015). *Arabidopsis dim1a* and *nop2a* mutants both display small, malformed leaves, slow growth and other phenotypes (Fujikura et al. 2009; Wieckowski and Schiefelbein 2012), reminiscent of many other *Arabidopsis* mutants with roles in rRNA biogenesis and of ribosomal protein mutants (Nishimura et al. 2005; Byrne 2009; Abbasi et al. 2010), pointing to additional functions besides RNA methylation for these proteins in plants. Another predicted rRNA m⁵C methyltransferase is *Arabidopsis* RNA methyltransferase (RNMT, At3g13180), which is related to the bacterial Fmu 16S rRNA methyltransferase (Pavlopoulou and Kossida 2009). *Arabidopsis rnmt* mutants have reduced global cytosine methylation, however, the specific nucleotide position is yet to be identified (Hebrard et al.

2013). In addition to m⁵C and m²⁶A, *Arabidopsis* rRNA also contains several m⁶A base methylations (Wan et al. 2015).

MESSENGER RNA AND OTHER NON-CODING RNA MODIFICATIONS

In the following sections we review and discuss the RNA modifications discovered in *Arabidopsis*, animal, yeast and bacterial epitranscriptomes to date and their diverse functions. Recently, two modifications have been identified transcriptome-wide using direct detection methods, m⁶A (Immunoprecipitation and next generation sequencing) and m⁵C (RNA Bisulfite sequencing) in plants (Li et al. 2014b; Luo et al. 2014; Wan et al. 2015; David et al. 2016). The first discovered and globally most abundant RNA modification, pseudouridine (Ψ) has been mapped transcriptome-wide by several recent studies in mammals and yeast (Carlile et al. 2014; Lovejoy et al. 2014; Schwartz et al. 2014a; Li et al. 2015). Although Ψ sites have not been mapped in plants transcriptome-wide to date, the enzymatic functions required for transcriptome-wide Ψ have been investigated in *Arabidopsis* (Lermontova et al. 2007; Yu et al. 2008; Chen et al. 2010). Interestingly, these high throughput studies have revealed m⁶A, m⁵C and Ψ to show distinct distribution patterns along mRNA transcripts and are associated with specific functions as discussed in the following sections (Figure 1). Additional modifications in the *Arabidopsis* epitranscriptome such as 3-methyl cytosine (m³C) and 1-methyl guanosine (m¹G) have been computationally predicted transcriptome-wide based on common nucleotide substitution and reverse transcription errors caused by these modifications during RNA-seq library preparation (Ryvkin et al. 2013; Vandivier et al. 2015). The modifications m⁶A, m⁵C and Ψ are unable to be detected using this method, as they do not alter Watson-Crick base pairing.

N⁶-METHYLADENOSINE (m⁶A)

m⁶A 'writers'

Although the presence of m⁶A in mRNAs was first discovered in the 1970's (Desrosiers et al. 1974; Perry and Kelley 1974), many questions still remain unanswered about the roles of m⁶A in protein coding transcripts. While Ψ is

the globally most abundant RNA modification, m⁶A is the most highly abundant RNA modification in mRNAs and is enriched in poly-adenylated RNA fractions in plants and animals and has recently also been identified in bacterial mRNAs (Zhong et al. 2008; Meyer et al. 2012; Deng et al. 2015). A multi-protein complex mediates these m⁶A sites. The catalytic core is composed of a heterodimer of methyltransferase like 3 (METTL3) and methyltransferase like 14 (METTL14) in mammals (Bokar et al. 1994; Liu et al. 2014). Recently, the mammalian splicing factor Wilm's tumor 1 associating protein (WTAP) and KIAA1429 were identified as additional components of the m⁶A 'writer' complex (Ping et al. 2014; Schwartz et al. 2014b). WTAP may have roles in targeting the m⁶A activity of METTL3 and METTL14, in a site-specific manner, as m⁶A sites were mediated by WTAP dependent or independent mechanisms (Schwartz et al. 2014b).

Likewise, the *Arabidopsis* m⁶A 'writer' complex contains the adenosine methyltransferase MTA (At4g10760), which is predicted to form a heterodimer with MTB (At4g09980) (Bujnicki et al. 2002; Zhong et al. 2008). The *Arabidopsis* homologue of mammalian WTAP is known as *Arabidopsis thaliana* FKBP12 interacting protein 37 (AtFIP37, At3g54170), and was identified as a binding partner of MTA several years prior to similar studies in mammals (Faure et al. 1998; Zhong et al. 2008). This is shown in Figure 2A. Further studies are required to identify additional components and interacting proteins. All three known components of the *Arabidopsis* m⁶A writer complex are essential, as loss results in embryo lethality (Bujnicki et al. 2002; Vespa et al. 2004; Zhong et al. 2008). The lethality of *mta* mutants in *Arabidopsis* can be rescued by expressing MTA during embryo development using the *ABI3* promoter (Bodi et al. 2012). Use of this system allowed investigation of the requirement for m⁶A in vegetative development, floral architecture and cell specification. The importance of m⁶A methylation for gene regulation is underscored by disorders caused by loss of m⁶A 'writer' complex components in human, yeast, mouse and fly (Clancy et al. 2002; Hongay and Orr-Weaver 2011; Bodi et al. 2012; Wang et al. 2014b; Chen et al. 2015b).

In order to elucidate why m⁶A is essential to plant development, three independent studies have mapped m⁶A epitranscriptomes in *Arabidopsis* and rice (Li et al. 2014b; Luo et al. 2014; Wan et al. 2015). As reported in the earlier studies in mammalian mRNAs, m⁶A sites were found to occur all along transcripts, with low signals observed across coding sequences and high enrichment in 3'UTRs and around stop codons (Dominissini et al. 2012; Meyer et al. 2012). Specifically in plants, there was a slight enrichment for m⁶A peaks at the start codon (Li et al. 2014b; Luo et al. 2014). However, a more recent study with greater sequencing depth and stringency conditions for m⁶A antibody binding, did not detect enrichment at start codons in *Arabidopsis* (Wan et al. 2015). Thousands of methylated transcripts were detected in different tissue types in *Arabidopsis* and rice and even in different *Arabidopsis* ecotypes. While many sites were specific to a particular tissue or ecotype, a large number of these sites were also conserved, even between animals and plants (Luo et al. 2014; Wan et al. 2015). As the deposition patterns and even specific m⁶A sites are conserved, the targeting mechanisms of the m⁶A 'writer' complex and functions of m⁶A are also likely conserved (Figure 2A).

In support of this, transcriptome-wide mapping studies of m⁶A have confirmed earlier reports that the m⁶A 'writer' complex methylates sites within a highly conserved consensus sequence 'RRACH', (R = A/G and H = A/C/U) and this mostly occurs in GAC or less commonly in the AAC context (Wei and Moss 1977; Csepany et al. 1990). This consensus sequence is conserved in yeast, mammals and plants (Dominissini et al. 2012; Meyer et al. 2012; Schwartz et al. 2013; Li et al. 2014b; Luo et al. 2014; Wan et al. 2015). Interestingly, this is not the case for prokaryotes as unique distribution patterns and potential targeting were discovered in bacteria, as unlike animals and plants, m⁶A is enriched in coding sequences and at a novel 'GCCAG' consensus sequence (Deng et al. 2015). The mechanism of targeting and the functional significance of the distributions of m⁶A methylation on mRNA remain to be elucidated. While the highly conserved eukaryotic consensus sequence 'RRACH' is present many times in the transcriptome, the mechanism for determining which of these sites are methylated, remains unknown.

m⁶A ‘erasers’ – reversible RNA methylation

One intriguing mechanism for regulating m⁶A deposition is through the active removal of m⁶A in mRNAs. Two m⁶A demethylases have been characterized in mammals, namely Fat mass and obesity associated protein (FTO) and Alkylation repair homologue protein 5 (ALKBH5) (Jia et al. 2011; Zheng et al. 2013). These demethylases are part of the *Escherichia coli* ALKB dioxygenase homologues (ALKBH) family. The founding member, *E. coli* ALKB mediates oxidative demethylation of nucleic acid bases in DNA and RNA (Aas et al. 2003). Based on sequence homology, thirteen ALKBH family proteins were predicted in *Arabidopsis* (Mielecki et al. 2012). These proteins showed diverse subcellular localizations, suggesting specialized functions in different cell compartments and hence potential layers of regulation for m⁶A demethylation in plants (Mielecki et al. 2012). While the identity of the *Arabidopsis* m⁶A demethylase(s) are still undetermined, they are expected to cause gross development defects, reminiscent of their animal homologs. In mice, loss of FTO leads to defects in alternative splicing and adipogenesis (Zhao et al. 2014), while loss of ALKBH5 affects mRNA processing in human cells and leads to male infertility in mice (Zheng et al. 2013). While the m⁶A ‘writer’ complex and demethylase ‘erasers’ act in concert to dynamically regulate m⁶A, additional RNA binding proteins or ‘readers’ are thought to decide the fate of m⁶A methylated transcripts.

m⁶A ‘readers’ – consequences for m⁶A on RNAs

The presence of m⁶A can influence RNA metabolism by regulating binding of modified RNA with proteins (m⁶A ‘readers’) and can also alter local RNA structure, leading to alternate outcomes for methylated and non-methylated transcripts. Several classes of m⁶A ‘reader’ proteins have been identified in animals, such as YTH domain proteins, serine/arginine-rich (SR) proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs) (Dominissini et al. 2012; Schwartz et al. 2013; Schwartz et al. 2014b; Wang et al. 2014a; Zhao et al. 2014; Alarcon et al. 2015a). Potential homologs of these m⁶A ‘readers’ have been identified in plants, suggesting conserved functions (Lorkovic and Barta 2002).

YTH domain containing proteins have been identified as a class of RNA binding proteins that preferentially bind m⁶A methylated RNA in mammals and yeast (Dominissini et al. 2012; Schwartz et al. 2013; Schwartz et al. 2014b; Wang et al. 2014a). The first m⁶A ‘reader’ to be characterized was YTH Domain Family 2 (YTHDF2), which was shown to bind thousands of m⁶A containing mRNAs in the cytoplasm and deliver them to processing bodies for degradation (Wang et al. 2014a). This discovery explains the negative correlation of m⁶A with mRNA abundance in both plants and animals as m⁶A is used as a mark for rapid turn-over of mRNAs (Li et al. 2014b; Schwartz et al. 2014b; Wang et al. 2014a; Wan et al. 2015). The sub-cellular locations of m⁶A readers is crucial for the outcome of m⁶A on RNAs and this is clearly demonstrated by YTHDF2 (Zhou et al. 2015). While cytoplasmic YTHDF2 leads to mRNA decay, heat shock induces YTHDF2 to re-localize to the nucleus. YTHDF2 is then able to compete with the nuclear ‘eraser’ FTO for binding of m⁶A sites, leading to increased 5’UTR methylation of newly transcribed, heat stress responsive mRNAs. The increased methylation leads to increased translation initiation independent of the 5’ cap, allowing selective mRNA translation under heat shock stress. In addition, the m⁶A reader YTHDF1 has also been shown to increase translation initiation of transcripts harboring m⁶A sites (Wang et al. 2015). This occurs in the cytoplasm, leading to competition with the mRNA degrading cytoplasmic YTHDF2. This competition is thought to allow fast responses and regulation of mRNA abundance and translation through m⁶A methylation.

Plant proteins containing this conserved YTH domain are expected to mediate similar functions for m⁶A in RNA. The *Arabidopsis* genome encodes thirteen predicted YTH domain containing proteins, which may be responsible for ‘reading’ the m⁶A code and regulating RNA metabolism (Li et al. 2014a). One such protein is the *Arabidopsis* homologue of Cleavage and Polyadenylation Specificity Factor 30 (CPSF30, At1g30460), which is required in plants and mammals for polyadenylation and 3’end formation (Thomas et al. 2012; Chan et al. 2014). Intriguingly, while the presence of the YTH domain of *Arabidopsis* AtCPSF30 is dependent on alternative splicing, the YTH domain is completely

absent in yeast and mammalian CPSF30 homologues (Delaney et al. 2006; Hunt et al. 2012; Chakrabarti and Hunt 2015). Furthermore, AtCPSF30 has also been shown to be involved in oxidative stress responses (Zhang et al. 2008) and is required for programmed cell death and immunity in *Arabidopsis* (Bruggeman et al. 2014). These functions are independent of the YTH domain and raise questions about the possible roles of YTH domain-containing AtCPSF30 in regulating m⁶A containing RNAs.

In addition to YTH domain proteins, two other classes of potential m⁶A 'readers', namely SR proteins and hnRNPs, have been investigated. One SR protein, SR Splicing Factor 2 (SRSF2), was shown to preferentially bind mRNAs containing m⁶A sites, leading to increased inclusion of target exons during splicing when the 'eraser' FTO is depleted (Zhao et al. 2014). SRSF2 RNA binding sites tend to overlap with m⁶A sites, however, it is unclear if SRSF2 binds m⁶A directly, or indirectly through interactions with other proteins. Recently, SRSF3 and SRSF10 were found to competitively bind YTHDC1, which directly binds m⁶A, to regulate mRNA splicing (Xiao et al. 2016). While the interaction between SRSF3 and YTHDC1 promotes SRSF3 binding to RNA target sites, YTHDC1 binding of SRSF10 inhibits SRSF10 binding to RNA target sites. In combination, these events result in exon inclusion, while successful SRSF10 binding to RNA results in exon exclusion. Similarly, over expressing the predicted *Arabidopsis* SRSF2 orthologue AtSRp30 (At1g09140) demonstrated its function in regulating splicing (Lopato et al. 1999). Further studies are required to determine if m⁶A deposition directly or indirectly affects the activities of the eighteen SR proteins in *Arabidopsis* (Lorkovic and Barta 2002). Other m⁶A 'readers' include hnRNPs, which have diverse roles in RNA processing and export (Lorkovic et al. 2000).

Recently, m⁶A methylation was shown to be required for the biogenesis and function of a subset of miRNAs, and this is mediated in part through the m⁶A 'reader' HNRNPA2B1 and m⁶A 'anti-reader' human antigen R (HuR) in animals. miRNA processing and abundance is deregulated when either the m⁶A 'writer' METTL3 or the m⁶A 'eraser' FTO were perturbed, demonstrating a role for m⁶A in miRNA biogenesis (Alarcon et al. 2015b; Berulava et al.

2015). The m⁶A mark in miRNAs is important, as nuclear HNRNPA2B1 binds a subset of m⁶A containing pri-miRNAs and recruits the Microprocessor complex to cleave pri-miRNAs into pre-miRNAs (Alarcon et al. 2015a). In addition, m⁶A methylation is required for efficient regulation of a sub-set of miRNA target transcripts. m⁶A methylation can aid miRNA driven degradation of mRNAs, as m⁶A blocks binding of the m⁶A ‘anti-reader’ HuR, allowing miRNAs access to their target sites in mRNAs (Wang et al. 2014b). While m⁶A methylation regulates the biogenesis of a subset of miRNAs, some miRNAs are also able to affect targeting of m⁶A methylation. Artificial and endogenous miRNAs were recently shown to target m⁶A deposition and increase m⁶A abundance by guiding and modulating METTL3 binding to mRNAs (Chen et al. 2015b). This proposed mechanism of miRNAs guiding m⁶A sites is supported as animal miRNA target sites are highly enriched at m⁶A methylated regions, however, no such correlation was identified in *Arabidopsis* (Luo et al. 2014; Chen et al. 2015b). Is another class of small guide RNAs mediating m⁶A targeting in plants? These intriguing findings raise many questions about possible roles of m⁶A in regulating miRNAs in plants, and how the plant m⁶A ‘writer’ complex is targeted to mRNAs.

Another interesting example of an hnRNP m⁶A ‘reader’ is HNRNPC. HNRNPC does not bind m⁶A, however, it requires m⁶A methylation of mRNA and lncRNA targets such as Metastasis Associated Lung Adenocarcinoma Transcript 1 (MALAT1) to alter local RNA structure in order to facilitate RNA binding (Liu et al. 2015). This RNA remodeling, or ‘m⁶A-switch’, is achieved through the ability of m⁶A to disrupt adenosines from forming non-Watson-Crick G:A base pairs and also destabilizes A:U base pairs (Roost et al. 2015). It remains to be seen if the nine hnRNPs in *Arabidopsis* genome also show such diverse interactions with m⁶A as a dance partner (Lorkovic et al. 2000).

Additional functions for m⁶A methylation in reprogramming, organ differentiation and cell division functions are conserved, as indicated by the *Arabidopsis mta* phenotype and the roles of m⁶A in inducing pluripotent cells in mammals and sporulation in yeast (Clancy et al. 2002; Bodi et al. 2012; Wang et al. 2014b; Chen et al. 2015b). Furthermore, m⁶A was shown to

regulate the mammalian circadian clock, as loss of RNA m⁶A methylation slows RNA processing resulting in delayed release of mature clock transcripts from the nucleus (Fustin et al. 2013). m⁶A may also play a role in regulating the plant circadian clock, as several transcripts regulating the *Arabidopsis* clock were highly methylated (Wan et al. 2015). Functions for m⁶A in splicing intron retention, polyadenylation, microRNA regulation, reprogramming and stress responses in plants warrants further investigation. Recently developed, single nucleotide resolution approaches to mapping m⁶A epitranscriptomes will enable further characterization of the functions of this mark in plants (Chen et al. 2015a; Linder et al. 2015).

5-METHYLCYTOSINE (m⁵C)

m⁵C ‘writers’

While the functions of m⁵C as an epigenetic mark in DNA have been studied intensively, the role of m⁵C in RNA is less well studied. The importance of m⁵C has been established for tRNAs and rRNAs (Motorin et al. 2010), but functions are still being investigated for other RNAs such as mRNAs and lncRNAs. m⁵C was first identified transcriptome-wide using RNA Bisulfite sequencing (bsRNA-seq) in human (HeLa) cells, uncovering over 10,000 m⁵C sites (Squires et al. 2012). This prompted the development of additional techniques that enrich the direct RNA targets of specific RNA methyltransferases using RNA immunoprecipitation (Hussain et al. 2013a; Khoddami and Cairns 2013). Recently, the *Arabidopsis* m⁵C landscape was mapped using bsRNA-seq in several tissue types and RNA methyltransferase mutants, identifying hundreds of m⁵C sites (David et al. 2016).

Together, these studies identified two m⁵C ‘writers’ that catalyze methylation in mRNAs and other classes of RNAs; the first RNA methyltransferase is tRNA specific methyltransferase 4 (TRM4) otherwise known as NOP2/Sun domain protein 2 (NSUN2), in yeast and animals respectively. NSUN2 plays broad roles in many organisms for mediating oxidative stress tolerance and balancing stem cell self-renewal and differentiation. This is demonstrated in *nsun2* mutant mice presenting with epidermal differentiation defects, male

infertility and small size, which is thought to be due to a reduction in stem cell proliferation (Blanco et al. 2011; Hussain et al. 2013b). Furthermore, NSUN2 depletion in humans leads to mild microcephaly, short stature and neurological disorders (Abbasi-Moheb et al. 2012; Khan et al. 2012; Martinez et al. 2012; Fahiminiya et al. 2014). Loss of *nsun2* leads to increased tRNA cleavage under oxidative stress and these cleavage products are thought to cause these neuro-developmental disorders (Blanco et al. 2014). These roles are also conserved in plants, as *Arabidopsis trm4b* mutants display shorter primary roots, which is linked to a reduced capacity for cells to divide in the root meristem (David et al. 2016). Furthermore, *trm4b* mutants are also more sensitive to oxidative stress and have reduced stability of non-methylated tRNAs. However, it is difficult to tease apart the contributions of tRNA and mRNA methylations to these biological functions, as NSUN2/TRM4B methylates both these classes of RNAs.

The second m⁵C ‘writer’ shown to target mRNAs is Transfer RNA aspartic acid methyltransferase 1 (TRDMT1) also known as DNA methyltransferase 2 (DNMT2). TRDMT1 was previously thought to methylate DNA, due to its structural similarity to DNA methyltransferases, however it is now regarded as an RNA methyltransferase (Goll et al. 2006). In plants and animals, depletion of TRDMT1 is not phenotypically evident under controlled conditions (Goll et al. 2006). However, the functions of TRDMT1 become apparent under stress conditions such as oxidative and heat stress in *Drosophila* (Schaefer et al. 2010). Stress induced cleavage of tRNAs in TRDMT1 mutants also leads to inhibition of Dicer-2 functions (Durdevic et al. 2013b). Furthermore, TRDMT1 is required for efficient immune response against viruses in *Drosophila* (Durdevic et al. 2013a). In contrast, depletion of TRDMT1 in zebra fish leads to gross morphological defects (Rai et al. 2007). NSUN2 mediates many more m⁵C sites in the transcriptome than TRDMT1. Only two TRDMT1 mRNA targets were identified in human cells, type I cytokeratin *KRT18* mRNA and *KRT18* pseudogene mRNA and this methylation was not conserved in mouse (Khoddami and Cairns 2013). This minor role for TRDMT1 in mediating m⁵C transcriptome-wide seems to be conserved in plants, as only tRNA targets were identified (Burgess et al. 2015; David et al. 2016).

Locations, functions and targeting of m⁵C

In order to determine potential functions of m⁵C in RNA, transcriptome-wide deposition patterns of this mark were analyzed in human cancer cells. Methylated sites are statistically enriched in ncRNAs compared to mRNAs (Squires et al. 2012). Within mRNAs, m⁵C sites are observed in higher numbers than expected for untranslated regions and are relatively depleted in coding regions, when normalized for length and sequence coverage (Squires et al. 2012). Moreover, m⁵C candidate sites in 3'UTRs are associated with binding regions for the Argonaute I-IV proteins, which are involved in miRNA mediated decay and translational inhibition, suggesting possible roles for m⁵C in mediating miRNA activity (Squires et al. 2012). Although further experiments are required to clearly determine the m⁵C and Argonaute association. Additional functions for m⁵C in increasing mRNAs half-life have been proposed, as synthetic m⁵C methylated mRNAs exhibit increased stability (Warren et al. 2010). This does not seem to be the case for the majority of mRNAs, as methylation levels do not strongly correlate with gross changes in transcript abundance in mammals or plants (Hussain et al. 2013a; David et al. 2016). Furthermore, no major changes in global mRNA abundance were observed in mouse *nsun2* mutants (Tuorto et al. 2012; Hussain et al. 2013b).

In contrast to mRNAs, several functions for m⁵C have been investigated in ncRNAs. Vault ncRNAs were identified as NSUN2-specific m⁵C targets (Hussain et al. 2013a). Loss of m⁵C in vault ncRNAs leads to processing into small RNAs which can be incorporated into Argonaute complexes to regulate genes, in a manner similar to miRNAs. In addition to roles for m⁵C in small ncRNAs, functions for this modification have been elucidated for long ncRNAs. The 5' A-region of the lncRNA X-inactive specific transcript (XIST) contains five m⁵C sites, which were shown to inhibit binding of the Polycomb repressive complex 2 (PRC2) *in vitro* (Amort et al. 2013). It remains to be determined if the PRC2 complex acts globally as an m⁵C 'anti-reader' in both plants and animals.

Analysis of m⁵C transcriptome-wide has shown that only approximately 0.4 % of cytosines are methylated in mRNA, suggesting precise targeting of m⁵C to select target sites (Squires et al. 2012). In archaea, m⁵C was located in a consensus motif of AUCGANGU in mRNAs, providing a potential targeting mechanism for archaeal m⁵C ‘writers’ (Edelheit et al. 2013). In contrast, no such consensus target sequences were identified for m⁵C sites in animals or plants (Squires et al. 2012; Hussain et al. 2013a; David et al. 2016). As a general consensus sequence has not been identified, it is hypothesized that additional factors such as local RNA structure and RNA binding proteins may regulate the site selection of TRM4 and TRDMT1 (Figure 2B).

Many questions remain unanswered such as the targeting mechanism of m⁵C ‘writers’ and the functions of m⁵C in mRNAs and other non-coding RNAs. The identification of potential m⁵C ‘readers’ and ‘erasers’ using techniques such as m⁵C RNA bait to immuno-precipitate m⁵C binding proteins, similar to those performed for m⁶A should lead to future insights into how m⁵C sculpts the epitranscriptome.

PSEUDOURIDYLATION (Ψ)

Isomerization of uridine to Ψ was the first RNA modification to be discovered, and is also the most abundant (Charette and Gray 2000; Ge and Yu 2013). As discussed earlier, Ψ is common in tRNAs and rRNAs and also in spliceosomal snRNAs, however, it is an open question whether Ψ is present on *Arabidopsis* mRNAs. Recently, four research groups independently investigated Ψ transcriptome wide at single-nucleotide resolution in yeast, human and mouse cells using modified approaches to Ψ-sequencing (Carlile et al. 2014; Lovejoy et al. 2014; Schwartz et al. 2014a; Li et al. 2015). Using these transcriptome-wide approaches, they were able to confirm known Ψ sites and cognate Ψ synthases in tRNAs, rRNAs, snRNAs and snoRNAs and extend the known sites to mRNAs and lncRNAs such as XIST and MALAT1. As the components required for Ψ are conserved in *Arabidopsis*, it seems more than likely that Ψ also occurs in plant mRNAs (Lermontova et al. 2007; Yu et al. 2008; Chen et al. 2010) (Figure 2C).

Ψ synthases are targeted to specific sites in RNAs through two mechanisms (1) snoRNA guided H/ACA snoRNPs containing CBF5/Dyskerin and (2) snoRNA independent Pseudouridine synthases (PUS). Using a combination of deletion and knock down mutants for PUS proteins and CBF5/Dyskerin in yeast and human, mRNA Ψ sites were found to be dependent on Ψ synthases using both snoRNA dependent and independent mechanisms.

While the targeting mechanism for Ψ by H/ACA snoRNPs is based on the snoRNA guide, and synthetic snoRNAs have been successfully designed to target Ψ at novel sites (Karijovich and Yu 2011), the targeting mechanisms of PUS proteins to RNA are less understood. Transcriptome-wide identification of Ψ sites in several yeast PUS deletion mutants allowed analysis and confirmation of sequence consensus sites preferred by specific PUS enzymes. In particular, yeast PUS4 mediated Ψ occurs at 'GUΨC/NANNC' consensus sites, while yeast PUS7 Ψ sites generally occur at the consensus 'UGΨA/R'. Not all sites with these consensus sequences are modified, suggesting other, additional factors mediating targeting. For example, the structure, as opposed to the sequence, of the tRNA^{Ser} anticodon and TΨC stem loops were required for human PUS1 targeting (Sibert and Patton 2012).

Ψ sites mediated by these enzymes were located all along mRNA transcripts, with no positional bias found in coding sequences in any of the four transcriptome-wide studies (Carlile et al. 2014; Lovejoy et al. 2014; Schwartz et al. 2014a; Li et al. 2015). However, while Ψ sites were under represented in 3'UTRs of yeast and human cervical cancer (HeLa) cells (Carlile et al. 2014), a chemical pulldown method, which enriched for Ψ sites prior to sequencing found that Ψ sites were under represented in 5'UTRs of mouse and human (H36KT) cells (Li et al. 2015). Prior enrichment of Ψ sites enabled the identification of thousands of sites transcriptome-wide, compared to other studies finding only hundreds of sites.

Dynamic regulation of Ψ sites was conserved across species, as tissue specific and stress responsive Ψ sites were identified in animals and yeast.

Strong, stimuli-specific patterns of Ψ were induced for heat shock, addition of a viral mimic and oxidative stress (Schwartz et al. 2014a; Li et al. 2015). Ψ sites were also regulated by different cellular growth rates and nutrient availability (Carlile et al. 2014). Interestingly, Schwartz et al. (2014) show that PUS7 Ψ mediates heat sensitivity in yeast, as yeast mutants have increased heat sensitivity and >200 PUS7 dependent Ψ sites are induced by heat shock. Ψ transcripts were expressed at higher levels in wild type than in PUS7 mutants during heat shock, suggesting a role for Ψ in stabilizing specific mRNAs in stress conditions.

The function of Ψ in mRNAs is unclear, however, Ψ is thought to help stabilize RNAs by promoting base stacking, pairing, and conformational stability. Ψ may also affect the translation of modified mRNAs (Davis 1995). For example, Ψ has been shown to convert nonsense codons into sense codons, thus 'rewiring' the genetic code (Karijovich and Yu 2011). However, the precise role of Ψ in translation is controversial as Ψ has been shown to both aid and inhibit translation in eukaryotic and bacterial systems, respectively (Kariko et al. 2012; Hoernes et al. 2015). The locations and roles of this modification in *Arabidopsis* mRNAs is yet to be discovered.

OTHER MODIFICATIONS IN THE *ARABIDOPSIS* EPITRANSCRIPTOME

Of the over 100 RNA modifications discovered in RNA, high-throughput methods of detection have been limited to detecting only a small subset of these modifications. Recently, a new hybrid method has been introduced, referred to as HAMR (High-throughput Annotation of Modified Ribonucleotides), which is able to detect and predict modifications that affect Watson-Crick base pairing transcriptome-wide (Ryvkin et al. 2013). HAMR was trained using data from well characterized yeast tRNA modifications to predict the identity of several RNA modifications. Subsequently, HAMR was put to use on the *Arabidopsis* epitranscriptome, and several types of RNA modifications that perturb reverse transcription were predicted in all types of RNA classes (Vandivier et al. 2015).

Three types of RNA-seq data sets were tested and compared in this study, (1) polyadenylated, (2) small RNAs and (3) degrading RNA. RNA modifications were enriched in *Arabidopsis* exons and 3'UTR's of uncapped, degrading mRNA and lncRNA transcripts and the same enrichment pattern was detected in two human cell lines, suggesting broad conservation and possible regulatory functions of these RNA modifications. It remains to be determined if RNA modifications are targeted to degrading transcripts, or if the RNA modifications serve as signals to mark transcripts for degradation. In addition, RNA modifications predicted by HAMR in stable mRNAs from the polyadenylated RNA-seq data sets were enriched within introns that were annotated to be alternatively spliced in both plants and humans.

Distributions of specific RNA modifications were specific to different types of RNAs and depended on whether the transcripts were undergoing degradation. For example, degrading mRNA transcripts had much higher predicted levels of dihydrouridylation (D), N⁶-isopentenyladenosylation (i⁶A) and threonylcarbamoyladenosylation (t⁶A) than stable mRNAs. Uncapped, degrading transcripts involved in various stress responses were enriched for HAMR-predicted modifications, suggesting possible roles in gene regulation and stress responses for these mRNA modifications in *Arabidopsis*.

FUTURE DIRECTIONS AND CONCLUSIONS

The four base constituents of RNA are modified by over 100 different RNA modifications. This additional complexity of RNA is essential for basic functions, such as gene regulation and translation. The *Arabidopsis* epitranscriptome has now been mapped for several RNA modifications, which occur in different locations across transcripts, are inducible in response to abiotic and biotic stresses and have diverse roles in plant development, ranging from subtle (m⁵C) to dramatic (m⁶A) effects on plant growth. While the RNA modifying 'writers' have been investigated in plants, studies on potential 'erasers' and 'readers' are lacking. The *Arabidopsis* genome encodes over 200 RNA binding proteins which serve as potential readers and effectors of outcomes for RNA modifications (Lorkovic and Barta 2002). Furthermore, potential *Arabidopsis* 'erasers' from the ALKBH family are yet to be explored

for roles in plant development and mediating dynamic regulation of RNA modifications (Mielecki et al. 2012). The ALKBH family of dioxygenases has diverse substrate specificities and are not limited to demethylation of adenosine (Aas et al. 2003; Jia et al. 2011). Specific *Arabidopsis* ALKBH family proteins may also remove additional RNA modifications. Further research is needed to elucidate the mechanisms and functional roles of mRNA modifications such as alternative splicing, and stress responses. Using small RNA guides, it is possible to artificially induce and block m⁶A and Ψ in mRNAs (Karijovich and Yu 2011; Chen et al. 2015b). This should enable the study of the specific functions of individual RNA modifications. There are many different ways that RNA modifications can affect RNA structure and interactions between RNA, RNA and proteins and even potentially RNA-DNA interactions (Figure 3). The next steps for deciphering the *Arabidopsis* epitranscriptome include Ψ-seq, mapping 2'-O-ribose methylations (Karijovich and Yu 2011; Birkedal et al. 2015), mapping N1-methyladenosine (m¹A) (Dominissini et al. 2016), single-nucleotide resolution mapping of m⁶A (Ke et al. 2015), and determining potential reversibility, and the elusive targeting mechanism(s) for RNA modifications.

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REFERENCES

- Aas PA, Otterlei M, Falnes PO, Vagbo CB, Skorpen F, Akbari M, Sundheim O, Bjoras M, Slupphaug G, Seeberg E, Krokan HE (2003) Human and bacterial oxidative demethylases repair alkylation damage in both RNA and DNA. **Nature** 421: 859-863.
- Abbasi-Moheb L, Mertel S, Gonsior M, Nouri-Vahid L, Kahrizi K, Cirak S, Wieczorek D, Motazacker MM, Esmaeeli-Nieh S, Cremer K, Weissmann R, Tzschach A, Garshasbi M, Abedini SS, Najmabadi H, Ropers HH, Sigrist SJ, Kuss AW (2012) Mutations in NSUN2 cause autosomal-recessive intellectual disability. **Am J Hum Genet** 90: 847-855.
- Abbasi N, Kim HB, Park NI, Kim HS, Kim YK, Park YI, Choi SB (2010) APUM23, a nucleolar Puf domain protein, is involved in pre-ribosomal RNA processing and normal growth patterning in *Arabidopsis*. **Plant J** 64: 960-976.
- Alarcon CR, Goodarzi H, Lee H, Liu XH, Tavazoie S, Tavazoie SF (2015a) HNRNPA2B1 is a mediator of m(6)A-dependent nuclear RNA processing events. **Cell** 162: 1299-1308.
- Alarcon CR, Lee H, Goodarzi H, Halberg N, Tavazoie SF (2015b) N6-methyladenosine marks primary microRNAs for processing. **Nature** 519: 482-485.
- Amort T, Souliere MF, Wille A, Jia XY, Fiegl H, Worle H, Micura R, Lusser A (2013) Long non-coding RNAs as targets for cytosine methylation. **RNA Biol** 10: 1003-1008.
- Ansmant I, Massenet S, Grosjean H, Motorin Y, Branlant C (2000) Identification of the *Saccharomyces cerevisiae* RNA:pseudouridine synthase responsible for formation of psi(2819) in 21S mitochondrial ribosomal RNA. **Nucleic Acids Res** 28: 1941-1946.
- Barneche F, Steinmetz F, Echeverria M (2000) Fibrillarin genes encode both a conserved nucleolar protein and a novel small nucleolar RNA involved in ribosomal RNA methylation in *Arabidopsis thaliana*. **J Biol Chem** 275: 27212-27220.
- Baxter-Roshek JL, Petrov AN, Dinman JD (2007) Optimization of ribosome structure and function by rRNA base modification. **PLoS One** 2: e174.
- Berulava T, Rahmann S, Rademacher K, Klein-Hitpass L, Horsthemke B (2015) N6-adenosine methylation in MiRNAs. **PLoS One** 10: e0118438.
- Birkedal U, Christensen-Dalsgaard M, Krogh N, Sabarinathan R, Gorodkin J, Nielsen H (2015) Profiling of ribose methylations in RNA by high-throughput sequencing. **Angew Chem Int Ed Engl** 54: 451-455.

Blanco S, Dietmann S, Flores JV, Hussain S, Kutter C, Humphreys P, Lukk M, Lombard P, Treps L, Popis M, Kellner S, Holter SM, Garrett L, Wurst W, Becker L, Klopstock T, Fuchs H, Gailus-Durner V, de Angelis MH, Karadottir RT, Helm M, Ule J, Gleeson JG, Odom DT, Frye M (2014) Aberrant methylation of tRNAs links cellular stress to neuro-developmental disorders. **EMBO J** 33: 2020-2039.

Blanco S, Frye M (2014) Role of RNA methyltransferases in tissue renewal and pathology. **Curr Opin Cell Biol** 31: 1-7.

Blanco S, Kurowski A, Nichols J, Watt FM, Benitah SA, Frye M (2011) The RNA-methyltransferase Misu (NSun2) poises epidermal stem cells to differentiate. **PLoS Genet** 7: e1002403.

Bodi Z, Zhong SL, Mehra S, Song J, Graham N, Li HY, May S, Fray RG (2012) Adenosine methylation in *Arabidopsis* mRNA is associated with the 3' end and reduced levels cause developmental defects. **Front Plant Sci** 3: 48.

Bokar JA, Rath-Shambaugh ME, Ludwiczak R, Narayan P, Rottman F (1994) Characterization and partial purification of mRNA N6-adenosine methyltransferase from HeLa cell nuclei. Internal mRNA methylation requires a multisubunit complex. **J Biol Chem** 269: 17697-17704.

Brown JW, Echeverria M, Qu LH (2003) Plant snoRNAs: Functional evolution and new modes of gene expression. **Trends Plant Sci** 8: 42-49.

Bruggeman Q, Garmier M, de Bont L, Soubigou-Taconnat L, Mazubert C, Benhamed M, Raynaud C, Bergounioux C, Delarue M (2014) The polyadenylation factor subunit CLEAVAGE AND POLYADENYLATION SPECIFICITY FACTOR30: A key factor of programmed cell death and a regulator of immunity in *Arabidopsis*. **Plant Physiol** 165: 732-746.

Bujnicki JM, Feder M, Radlinska M, Blumenthal RM (2002) Structure prediction and phylogenetic analysis of a functionally diverse family of proteins homologous to the MT-A70 subunit of the human mRNA:m(6)A methyltransferase. **J Mol Evol** 55: 431-444.

Burgess AL, David R, Searle IR (2015) Conservation of tRNA and rRNA 5-methylcytosine in the kingdom Plantae. **BMC Plant Biol** 15: 199.

Byrne ME (2009) A role for the ribosome in development. **Trends Plant Sci** 14: 512-519.

Carlile TM, Rojas-Duran MF, Zinshteyn B, Shin H, Bartoli KM, Gilbert WV (2014) Pseudouridine profiling reveals regulated mRNA pseudouridylation in yeast and human cells. **Nature** 515: 143-146.

Chakrabarti M, Hunt AG (2015) CPSF30 at the interface of alternative polyadenylation and cellular signaling in plants. **Biomolecules** 5: 1151-1168.

Chan CT, Dyavaiah M, DeMott MS, Taghizadeh K, Dedon PC, Begley TJ (2010) A quantitative systems approach reveals dynamic control of tRNA modifications during cellular stress. **PLoS Genet** 6: e1001247.

Chan CTY, Pang YLJ, Deng WJ, Babu IR, Dyavaiah M, Begley TJ, Dedon PC (2012) Reprogramming of tRNA modifications controls the oxidative stress response by codon-biased translation of proteins. **Nat Commun** 3: 937.

Chan SL, Huppertz I, Yao C, Weng L, Moresco JJ, Yates JR, 3rd, Ule J, Manley JL, Shi Y (2014) CPSF30 and Wdr33 directly bind to AAUAAA in mammalian mRNA 3' processing. **Genes Dev** 28: 2370-2380.

Charette M, Gray MW (2000) Pseudouridine in RNA: What, where, how, and why. **IUBMB Life** 49: 341-351.

Chen K, Lu Z, Wang X, Fu Y, Luo GZ, Liu N, Han D, Dominissini D, Dai Q, Pan T, He C (2015a) High-resolution N(6)-methyladenosine (m(6)A) map using photo-crosslinking-assisted m(6)A sequencing. **Angew Chem Int Ed Engl** 54: 1587-1590.

Chen P, Jager G, Zheng B (2010) Transfer RNA modifications and genes for modifying enzymes in *Arabidopsis thaliana*. **BMC Plant Biol** 10: 201.

Chen T, Hao YJ, Zhang Y, Li MM, Wang M, Han W, Wu Y, Lv Y, Hao J, Wang L, Li A, Yang Y, Jin KX, Zhao X, Li Y, Ping XL, Lai WY, Wu LG, Jiang G, Wang HL, Sang L, Wang XJ, Yang YG, Zhou Q (2015b) m(6)A RNA methylation is regulated by microRNAs and promotes reprogramming to pluripotency. **Cell Stem Cell** 16: 289-301.

Chen Y, Sierzputowskagracz H, Guenther R, Everett K, Agris PF (1993) 5-methylcytidine is required for cooperative binding of Mg^{2+} and a conformational transition at the anticodon stem-loop of yeast phenylalanine transfer-RNA. **Biochemistry** 32: 10249-10253.

Chen Z, Zhang H, Jablonowski D, Zhou X, Ren X, Hong X, Schaffrath R, Zhu JK, Gong Z (2006) Mutations in ABO1/ELO2, a subunit of holo-Elongator, increase abscisic acid sensitivity and drought tolerance in *Arabidopsis thaliana*. **Mol Cell Biol** 26: 6902-6912.

Clancy MJ, Shambaugh ME, Timpert CS, Bokar JA (2002) Induction of sporulation in *Saccharomyces cerevisiae* leads to the formation of N6-methyladenosine in mRNA: A potential mechanism for the activity of the IME4 gene. **Nucleic Acids Res** 30: 4509-4518.

Csepany T, Lin A, Baldick CJ, Jr., Beemon K (1990) Sequence specificity of mRNA N6-adenosine methyltransferase. **J Biol Chem** 265: 20117-20122.

David R, Burgess AL, Parker BJ, Pulsford KE, Sibbritt T, Preiss T, Searle IR (2016) Transcriptome-wide mapping of RNA 5-methylcytosine in *Arabidopsis* mRNAs and ncRNAs. **Under submission to Nature Commun.**

Davis DR (1995) Stabilization of RNA stacking by pseudouridine. **Nucleic Acids Res** 23: 5020-5026.

Decatur WA, Fournier MJ (2002) rRNA modifications and ribosome function. **Trends Biochem Sci** 27: 344-351.

Delaney KJ, Xu R, Zhang J, Li QQ, Yun KY, Falcone DL, Hunt AG (2006) Calmodulin interacts with and regulates the RNA-binding activity of an *Arabidopsis* polyadenylation factor subunit. **Plant Physiol** 140: 1507-1521.

Deng X, Chen K, Luo GZ, Weng X, Ji Q, Zhou T, He C (2015) Widespread occurrence of N6-methyladenosine in bacterial mRNA. **Nucleic Acids Res** 43: 6557-6567.

Desrosiers R, Friderici K, Rottman F (1974) Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells. **Proc Natl Acad Sci USA** 71: 3971-3975.

Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, Cesarkas K, Jacob-Hirsch J, Amariglio N, Kupiec M, Sorek R, Rechavi G (2012) Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. **Nature** 485: 201-206.

Dominissini D, Nachtergaele S, Moshitch-Moshkovitz S, Peer E, Kol N, Ben-Haim MS, Dai Q, Di Segni A, Salmon-Divon M, Clark WC, Zheng G, Pan T, Solomon O, Eyal E, HersHKovitz V, Han D, Dore LC, Amariglio N, Rechavi G, He C (2016) The dynamic N1-methyladenosine methylome in eukaryotic messenger RNA. **Nature** 530: 441-446.

Dubin DT, Taylor RH (1975) The methylation state of poly A-containing messenger RNA from cultured hamster cells. **Nucleic Acids Res** 2: 1653-1668.

Durdevic Z, Hanna K, Gold B, Pollex T, Cherry S, Lyko F, Schaefer M (2013a) Efficient RNA virus control in *Drosophila* requires the RNA methyltransferase Dnmt2. **EMBO Rep** 14: 269-275.

Durdevic Z, Mobin MB, Hanna K, Lyko F, Schaefer M (2013b) The RNA methyltransferase Dnmt2 is required for efficient dicer-2-dependent siRNA pathway activity in *Drosophila*. **Cell Rep** 4: 931-937.

Edelheit S, Schwartz S, Mumbach MR, Wurtzel O, Sorek R (2013) Transcriptome-wide mapping of 5-methylcytidine RNA modifications in bacteria, archaea, and yeast reveals m5C within archaeal mRNAs. **PLoS Genet** 9: e1003602.

El Yacoubi B, Bailly M, de Crecy-Lagard V (2012) Biosynthesis and function of posttranscriptional modifications of transfer RNAs. **Annu Rev Genet** 46: 69-95.

Fahiminiya S, Almuriekhi M, Nawaz Z, Staffa A, Lepage P, Ali R, Hashim L, Schwartzentruber J, Abu Khadija K, Zaineddin S, Gamal H, Majewski J, Ben-Omran T (2014) Whole exome sequencing unravels disease-causing genes in consanguineous families in Qatar. **Clin Genet** 86: 134-141.

Faure JD, Gingerich D, Howell SH (1998) An *Arabidopsis* immunophilin, AtFKBP12, binds to AtFIP37 (FKBP interacting protein) in an interaction that is disrupted by FK506. **Plant J** 15: 783-789.

Fray RG, Simpson GG (2015) The *Arabidopsis* epitranscriptome. **Curr Opin Plant Biol** 27: 17-21.

Fujikura U, Horiguchi G, Ponce MR, Micol JL, Tsukaya H (2009) Coordination of cell proliferation and cell expansion mediated by ribosome-related processes in the leaves of *Arabidopsis thaliana*. **Plant J** 59: 499-508.

Fustin JM, Doi M, Yamaguchi Y, Hida H, Nishimura S, Yoshida M, Isagawa T, Morioka MS, Kakeya H, Manabe I, Okamura H (2013) RNA-methylation-dependent RNA processing controls the speed of the circadian clock. **Cell** 155: 793-806.

Ge JH, Yu YT (2013) RNA pseudouridylation: New insights into an old modification. **Trends Biochem Sci** 38: 210-218.

Gigova A, Duggimpudi S, Pollex T, Schaefer M, Kos M (2014) A cluster of methylations in the domain IV of 25S rRNA is required for ribosome stability. **RNA** 20: 1632-1644.

Goll MG, Kirpekar F, Maggert KA, Yoder JA, Hsieh CL, Zhang XY, Golic KG, Jacobsen SE, Bestor TH (2006) Methylation of tRNA(Asp) by the DNA methyltransferase homolog Dnmt2. **Science** 311: 395-398.

Golovko A, Sitbon F, Tillberg E, Nicander B (2002) Identification of a tRNA isopentenyltransferase gene from *Arabidopsis thaliana*. **Plant Mol Biol** 49: 161-169.

Hebrard C, Trap-Gentil MV, Lafon-Placette C, Delaunay A, Joseph C, Lefebvre M, Barnes S, Maury S (2013) Identification of differentially methylated regions during vernalization revealed a role for RNA methyltransferases in bolting. **J Exp Bot** 64: 651-663.

Heiss NS, Knight SW, Vulliamey TJ, Klauck SM, Wiemann S, Mason PJ, Poustka A, Dokal I (1998) X-linked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions. **Nat Genet** 19: 32-38.

Henras AK, Plisson-Chastang C, O'Donohue MF, Chakraborty A, Gleizes PE (2015) An overview of pre-ribosomal RNA processing in eukaryotes. **Wiley Interdiscip Rev RNA** 6: 225-242.

Hertz R, Tovy A, Kirschenbaum M, Geffen M, Nozaki T, Adir N, Ankri S (2014) The entamoeba histolytica Dnmt2 homolog (ehmeth) confers resistance to nitrosative stress. **Eukaryotic Cell** 13: 494-503.

Hienzsich A, Deiml C, Reiter V, Carell T (2013) Total synthesis of the hypermodified RNA bases wybutosine and hydroxywybutosine and their quantification together with other modified RNA bases in plant materials. **Chemistry** 19: 4244-4248.

Hoernes TP, Clementi N, Faserl K, Glasner H, Breuker K, Lindner H, Huttenhofer A, Erlacher MD (2015) Nucleotide modifications within bacterial messenger RNAs regulate their translation and are able to rewire the genetic code. **Nucleic Acids Res** 44: 852-862.

Hong B, Brockenbrough JS, Wu P, Aris JP (1997) Nop2p is required for pre-rRNA processing and 60S ribosome subunit synthesis in yeast. **Mol Cell Biol** 17: 378-388.

Hongay CF, Orr-Weaver TL (2011) Drosophila Inducer of MEiosis 4 (IME4) is required for Notch signaling during oogenesis. **Proc Natl Acad Sci USA** 108: 14855-14860.

Hu Z, Qin Z, Wang M, Xu C, Feng G, Liu J, Meng Z, Hu Y (2010) The *Arabidopsis* SMO2, a homologue of yeast TRM112, modulates progression of cell division during organ growth. **Plant J** 61: 600-610.

Hunt AG, Xing D, Li QQ (2012) Plant polyadenylation factors: Conservation and variety in the polyadenylation complex in plants. **BMC Genomics** 13: 641.

Hussain S, Sajini AA, Blanco S, Dietmann S, Lombard P, Sugimoto Y, Paramor M, Gleeson JG, Odom DT, Ule J, Frye M (2013a) NSun2-mediated cytosine-5 methylation of vault noncoding RNA determines its processing into regulatory small RNAs. **Cell Rep** 4: 255-261.

Hussain S, Tuorto F, Menon S, Blanco S, Cox C, Flores JV, Watt S, Kudo NR, Lyko F, Frye M (2013b) The mouse cytosine-5 RNA methyltransferase NSun2 is a component of the chromatoid body and required for testis differentiation. **Mol Cell Biol** 33: 1561-1570.

Iida K, Jin HL, Zhu JK (2009) Bioinformatics analysis suggests base modifications of tRNAs and miRNAs in *Arabidopsis thaliana*. **BMC Genomics** 10: 155.

Jackman JE, Alfonzo JD (2013) Transfer RNA modifications: Nature's combinatorial chemistry playground. **Wires RNA** 4: 35-48.

Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, Yi C, Lindahl T, Pan T, Yang YG, He C (2011) N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. **Nat Chem Biol** 7: 885-887.

Jones LH, Scott TK (1981) Transfer ribonucleic acid modification and its relationship to tumorous and nontumorous plant growth. **Plant Physiol** 67: 535-538.

Karjilovich J, Yu YT (2011) Converting nonsense codons into sense codons by targeted pseudouridylation. **Nature** 474: 395-399.

Kariko K, Muramatsu H, Keller JM, Weissman D (2012) Increased erythropoiesis in mice injected with submicrogram quantities of pseudouridine-containing mRNA encoding erythropoietin. **Mol Ther** 20: 948-953.

Ke S, Alemu EA, Mertens C, Gantman EC, Fak JJ, Mele A, Haripal B, Zucker-Scharff I, Moore MJ, Park CY, Vagbo CB, Kussnierczyk A, Klungland A, Darnell JE, Jr., Darnell RB (2015) A majority of m6A residues are in the last exons, allowing the potential for 3' UTR regulation. **Genes Dev** 29: 2037-2053.

Khan MA, Rafiq MA, Noor A, Hussain S, Flores JV, Rupp V, Vincent AK, Malli R, Ali G, Khan FS, Ishak GE, Doherty D, Weksberg R, Ayub M, Windpassinger C, Ibrahim S, Frye M, Ansar M, Vincent JB (2012) Mutation in NSUN2, which encodes an RNA methyltransferase, causes autosomal-recessive intellectual disability. **Am J Hum Genet** 90: 856-863.

Khoddami V, Cairns BR (2013) Identification of direct targets and modified bases of RNA cytosine methyltransferases. **Nat Biotechnol** 31: 458-464.

King MY, Redman KL (2002) RNA methyltransferases utilize two cysteine residues in the formation of 5-methylcytosine. **Biochemistry** 41: 11218-11225.

Kiss T (2001) Small nucleolar RNA-guided post-transcriptional modification of cellular RNAs. **EMBO J** 20: 3617-3622.

Lafontaine D, Delcour J, Glasser AL, Desgres J, Vandenhoute J (1994) The DIM1 gene responsible for the conserved m6(2)Am6(2)A dimethylation in the 3'-terminal loop of 18 S rRNA is essential in yeast. **J Mol Biol** 241: 492-497.

Lafontaine D, Vandenhoute J, Tollervey D (1995) The 18S rRNA dimethylase Dim1p is required for pre-ribosomal RNA processing in yeast. **Genes Dev** 9: 2470-2481.

Lawrence M, Daujat S, Schneider R (2016) Lateral thinking: How histone modifications regulate gene expression. **Trends Genet** 32: 42-56.

Leihne V, Kirpekar F, Vagbo CB, van den Born E, Krokan HE, Grini PE, Meza TJ, Falnes PO (2011) Roles of Trm9-and ALKBH8-like proteins in the formation of modified wobble uridines in *Arabidopsis* tRNA. **Nucleic Acids Res** 39: 7688-7701.

Lermontova I, Schubert V, Bornke F, Macas J, Schubert I (2007) *Arabidopsis* CBF5 interacts with the H/ACA snoRNP assembly factor NAF1. **Plant Mol Biol** 65: 615-626.

Li D, Zhang H, Hong Y, Huang L, Li X, Zhang Y, Ouyang Z, Song F (2014a) 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** 32: 1169-1186.

Li X, Zhu P, Ma S, Song J, Bai J, Sun F, Yi C (2015) Chemical pulldown reveals dynamic pseudouridylation of the mammalian transcriptome. **Nat Chem Biol** 11: 592-597.

Li Y, Wang X, Li C, Hu S, Yu J, Song S (2014b) Transcriptome-wide N(6)-methyladenosine profiling of rice callus and leaf reveals the presence of tissue-specific competitors involved in selective mRNA modification. **RNA Biol** 11: 1180-1188.

Linder B, Grozhik AV, Olarerin-George AO, Meydan C, Mason CE, Jaffrey SR (2015) Single-nucleotide-resolution mapping of m6A and m6Am throughout the transcriptome. **Nat Methods** 12: 767-772.

Liu J, Yue Y, Han D, Wang X, Fu Y, Zhang L, Jia G, Yu M, Lu Z, Deng X, Dai Q, Chen W, He C (2014) A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. **Nat Chem Biol** 10: 93-95.

Liu N, Dai Q, Zheng G, He C, Parisien M, Pan T (2015) N(6)-methyladenosine-dependent RNA structural switches regulate RNA-protein interactions. **Nature** 518: 560-564.

Lopato S, Kalyna M, Dorner S, Kobayashi R, Krainer AR, Barta A (1999) atSRp30, one of two SF2/ASF-like proteins from *Arabidopsis thaliana*, regulates splicing of specific plant genes. **Genes Dev** 13: 987-1001.

Lorkovic ZJ, Barta A (2002) Genome analysis: RNA recognition motif (RRM) and K homology (KH) domain RNA-binding proteins from the flowering plant *Arabidopsis thaliana*. **Nucleic Acids Res** 30: 623-635.

Lorkovic ZJ, Wieczorek Kirk DA, Lambermon MH, Filipowicz W (2000) Pre-mRNA splicing in higher plants. **Trends Plant Sci** 5: 160-167.

Lovejoy AF, Riordan DP, Brown PO (2014) Transcriptome-wide mapping of pseudouridines: Pseudouridine synthases modify specific mRNAs in *S. cerevisiae*. **PLoS One** 9: e110799.

Luo GZ, MacQueen A, Zheng G, Duan H, Dore LC, Lu Z, Liu J, Chen K, Jia G, Bergelson J, He C (2014) Unique features of the m6A methylome in *Arabidopsis thaliana*. **Nat Commun** 5: 5630.

Machnicka MA, Milanowska K, Osman Oglou O, Purta E, Kurkowska M, Olchowik A, Januszewski W, Kalinowski S, Dunin-Horkawicz S, Rother KM, Helm M, Bujnicki JM, Grosjean H (2013) MODOMICS: A database of RNA modification pathways--2013 update. **Nucleic Acids Res** 41: D262-267.

Martinez FJ, Lee JH, Lee JE, Blanco S, Nickerson E, Gabriel S, Frye M, Al-Gazali L, Gleeson JG (2012) Whole exome sequencing identifies a splicing mutation in NSUN2 as a cause of a Dubowitz-like syndrome. **J Med Genet** 49: 380-385.

Mehlgarten C, Jablonowski D, Wrackmeyer U, Tschitschmann S, Sondermann D, Jager G, Gong Z, Bystrom AS, Schaffrath R, Breunig KD (2010) Elongator function in tRNA wobble uridine modification is conserved between yeast and plants. **Mol Microbiol** 76: 1082-1094.

Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, Jaffrey SR (2012) Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. **Cell** 149: 1635-1646.

Mielecki D, Zugaj DL, Muszewska A, Piwowarski J, Chojnacka A, Mielecki M, Nieminiusz J, Grynberg M, Grzesiuk E (2012) Novel AlkB dioxygenases--alternative models for in silico and in vivo studies. **PLoS One** 7: e30588.

Miyawaki K, Tarkowski P, Matsumoto-Kitano M, Kato T, Sato S, Tarkowska D, Tabata S, Sandberg G, Kakimoto T (2006) Roles of *Arabidopsis* ATP/ADP isopentenyltransferases and tRNA isopentenyltransferases in cytokinin biosynthesis. **Proc Natl Acad Sci USA** 103: 16598-16603.

Motorin Y, Helm M (2011) RNA nucleotide methylation. **Wiley Interdiscip Rev RNA** 2: 611-631.

Motorin Y, Lyko F, Helm M (2010) 5-methylcytosine in RNA: Detection, enzymatic formation and biological functions. **Nucleic Acids Res** 38: 1415-1430.

Nishimura T, Wada T, Yamamoto KT, Okada K (2005) The *Arabidopsis* STV1 protein, responsible for translation reinitiation, is required for auxin-mediated gynoecium patterning. **Plant Cell** 17: 2940-2953.

Pavlopoulou A, Kossida S (2009) Phylogenetic analysis of the eukaryotic RNA (cytosine-5)-methyltransferases. **Genomics** 93: 350-357.

Perry RP, Kelley DE (1974) Existence of methylated messenger RNA in mouse L cells. **Cell** 1: 37-42.

Phizicky EM, Hopper AK (2010) tRNA biology charges to the front. **Genes Dev** 24: 1832-1860.

Pih KT, Yi MJ, Liang YS, Shin BJ, Cho MJ, Hwang I, Son D (2000) Molecular cloning and targeting of a fibrillarin homolog from *Arabidopsis*. **Plant Physiol** 123: 51-58.

Ping XL, Sun BF, Wang L, Xiao W, Yang X, Wang WJ, Adhikari S, Shi Y, Lv Y, Chen YS, Zhao X, Li A, Yang Y, Dahal U, Lou XM, Liu X, Huang J, Yuan WP, Zhu XF, Cheng T, Zhao YL, Wang XQ, Danielsen JMR, Liu F, Yang YG (2014) Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. **Cell Res** 24: 177-189.

Preston MA, D'Silva S, Kon Y, Phizicky EM (2013) tRNA^{His} 5-methylcytidine levels increase in response to several growth arrest conditions in *Saccharomyces cerevisiae*. **RNA** 19: 243-256.

Rai K, Chidester S, Zavala CV, Manos EJ, James SR, Karpf AR, Jones DA, Cairns BR (2007) Dnmt2 functions in the cytoplasm to promote liver, brain, and retina development in zebrafish. **Genes Dev** 21: 261-266.

Ramirez V, Gonzalez B, Lopez A, Castello MJ, Gil MJ, Zheng B, Chen P, Vera P (2015) Loss of a conserved tRNA anticodon modification perturbs plant immunity. **PLoS Genet** 11: e1005586.

Richter U, Kuhn K, Okada S, Brennicke A, Weihe A, Borner T (2010) A mitochondrial rRNA dimethyladenosine methyltransferase in *Arabidopsis*. **Plant J** 61: 558-569.

Roost C, Lynch SR, Batista PJ, Qu K, Chang HY, Kool ET (2015) Structure and thermodynamics of N6-methyladenosine in RNA: A spring-loaded base modification. **J Am Chem Soc** 137: 2107-2115.

Ryvkin P, Leung YY, Silverman IM, Childress M, Valladares O, Dragomir I, Gregory BD, Wang LS (2013) HAMR: High-throughput annotation of modified ribonucleotides. **RNA** 19: 1684-1692.

Schaefer M, Pollex T, Hanna K, Tuorto F, Meusburger M, Helm M, Lyko F (2010) RNA methylation by Dnmt2 protects transfer RNAs against stress-induced cleavage. **Genes Dev** 24: 1590-1595.

Schwartz S, Agarwala SD, Mumbach MR, Jovanovic M, Mertins P, Shishkin A, Tabach Y, Mikkelsen TS, Satija R, Ruvkun G, Carr SA, Lander ES, Fink GR, Regev A (2013) High-resolution mapping reveals a conserved, widespread, dynamic mRNA methylation program in yeast meiosis. **Cell** 155: 1409-1421.

Schwartz S, Bernstein DA, Mumbach MR, Jovanovic M, Herbst RH, Leon-Ricardo BX, Engreitz JM, Guttman M, Satija R, Lander ES, Fink G, Regev A

(2014a) Transcriptome-wide mapping reveals widespread dynamic-regulated pseudouridylation of ncRNA and mRNA. **Cell** 159: 148-162.

Schwartz S, Mumbach MR, Jovanovic M, Wang T, Maciag K, Bushkin GG, Mertins P, Ter-Ovanesyan D, Habib N, Cacchiarelli D, Sanjana NE, Freinkman E, Pacold ME, Satija R, Mikkelsen TS, Hacohen N, Zhang F, Carr SA, Lander ES, Regev A (2014b) Perturbation of m6A writers reveals two distinct classes of mRNA methylation at internal and 5' sites. **Cell Rep** 8: 284-296.

Shafik A, Schumann U, Evers M, Sibbritt T, Preiss T (2016) The emerging epitranscriptomics of long noncoding RNAs. **Biochim Biophys Acta** 1859: 59-70.

Sharma S, Yang J, Watzinger P, Kotter P, Entian KD (2013) Yeast Nop2 and Rcm1 methylate C2870 and C2278 of the 25S rRNA, respectively. **Nucleic Acids Res** 41: 9062-9076.

Shatkin AJ (1976) Capping of eucaryotic mRNAs. **Cell** 9: 645-653.

Shugart L (1972) A possible age-related modification of phenylalanine transfer RNA from wheat tissue. **Exp Gerontol** 7: 251-262.

Sibert BS, Patton JR (2012) Pseudouridine synthase 1: A site-specific synthase without strict sequence recognition requirements. **Nucleic Acids Res** 40: 2107-2118.

Squires JE, Patel HR, Nusch M, Sibbritt T, Humphreys DT, Parker BJ, Suter CM, Preiss T (2012) Widespread occurrence of 5-methylcytosine in human coding and non-coding RNA. **Nucleic Acids Res** 40: 5023-5033.

Suzuki MM, Bird A (2008) DNA methylation landscapes: Provocative insights from epigenomics. **Nat Rev Genet** 9: 465-476.

Thomas PE, Wu X, Liu M, Gaffney B, Ji G, Li QQ, Hunt AG (2012) Genome-wide control of polyadenylation site choice by CPSF30 in *Arabidopsis*. **Plant Cell** 24: 4376-4388.

Tokuhisa JG, Vijayan P, Feldmann KA, Browse JA (1998) Chloroplast development at low temperatures requires a homolog of DIM1, a yeast gene encoding the 18S rRNA dimethylase. **Plant Cell** 10: 699-711.

Torres AG, Batlle E, Ribas de Pouplana L (2014) Role of tRNA modifications in human diseases. **Trends Mol Med** 20: 306-314.

Towns WL, Begley TJ (2012) Transfer RNA methyltransferases and their corresponding modifications in budding yeast and humans: Activities, predilections, and potential roles in human health. **DNA Cell Biol** 31: 434-454.

Tuorto F, Liebers R, Musch T, Schaefer M, Hofmann S, Kellner S, Frye M, Helm M, Stoecklin G, Lyko F (2012) RNA cytosine methylation by Dnmt2 and NSun2 promotes tRNA stability and protein synthesis. **Nat Struct Mol Biol** 19: 900-905.

Vandivier LE, Campos R, Kuksa PP, Silverman IM, Wang LS, Gregory BD (2015) Chemical modifications mark alternatively spliced and uncapped messenger RNAs in *Arabidopsis*. **Plant Cell** 27: 3024-3037.

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

Wan Y, Tang K, Zhang D, Xie S, Zhu X, Wang Z, Lang Z (2015) Transcriptome-wide high-throughput deep m(6)A-seq reveals unique differential m(6)A methylation patterns between three organs in *Arabidopsis thaliana*. **Genome Biol** 16: 272.

Wang X, Lu Z, Gomez A, Hon GC, Yue Y, Han D, Fu Y, Parisien M, Dai Q, Jia G, Ren B, Pan T, He C (2014a) N6-methyladenosine-dependent regulation of messenger RNA stability. **Nature** 505: 117-120.

Wang X, Zhao BS, Roundtree IA, Lu Z, Han D, Ma H, Weng X, Chen K, Shi H, He C (2015) N(6)-methyladenosine modulates messenger RNA translation efficiency. **Cell** 161: 1388-1399.

Wang Y, Li Y, Toth JI, Petroski MD, Zhang Z, Zhao JC (2014b) N6-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. **Nat Cell Biol** 16: 191-198.

Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, Ebina W, Mandal PK, Smith ZD, Meissner A, Daley GQ, Brack AS, Collins JJ, Cowan C, Schlaeger TM, Rossi DJ (2010) Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. **Cell Stem Cell** 7: 618-630.

Wei CM, Moss B (1977) Nucleotide sequences at the N6-methyladenosine sites of HeLa cell messenger ribonucleic acid. **Biochemistry** 16: 1672-1676.

Wieckowski Y, Schiefelbein J (2012) Nuclear ribosome biogenesis mediated by the DIM1A rRNA dimethylase is required for organized root growth and epidermal patterning in *Arabidopsis*. **Plant Cell** 24: 2839-2856.

Wu P, Brockenbrough JS, Paddy MR, Aris JP (1998) NCL1, a novel gene for a non-essential nuclear protein in *Saccharomyces cerevisiae*. **Gene** 220: 109-117.

Xiao W, Adhikari S, Dahal U, Chen YS, Hao YJ, Sun BF, Sun HY, Li A, Ping XL, Lai WY, Wang X, Ma HL, Huang CM, Yang Y, Huang N, Jiang GB, Wang HL, Zhou Q, Wang XJ, Zhao YL, Yang YG (2016) Nuclear m(6)A reader YTHDC1 regulates mRNA splicing. **Mol Cell** 61: 507-519.

Yu F, Liu X, Alsheikh M, Park S, Rodermel S (2008) Mutations in SUPPRESSOR OF VARIEGATION1, a factor required for normal chloroplast translation, suppress var2-mediated leaf variegation in *Arabidopsis*. **Plant Cell** 20: 1786-1804.

Zhang J, Addepalli B, Yun KY, Hunt AG, Xu R, Rao S, Li QQ, Falcone DL (2008) A polyadenylation factor subunit implicated in regulating oxidative signaling in *Arabidopsis thaliana*. **PLoS One** 3: e2410.

Zhao X, Yang Y, Sun BF, Shi Y, Yang X, Xiao W, Hao YJ, Ping XL, Chen YS, Wang WJ, Jin KX, Wang X, Huang CM, Fu Y, Ge XM, Song SH, Jeong HS, Yanagisawa H, Niu Y, Jia GF, Wu W, Tong WM, Okamoto A, He C, Rendtlew Danielsen JM, Wang XJ, Yang YG (2014) FTO-dependent demethylation of N6-methyladenosine regulates mRNA splicing and is required for adipogenesis. **Cell Res** 24: 1403-1419.

Zheng GQ, Dahl JA, Niu YM, Fedorcsak P, Huang CM, Li CJ, Vagbo CB, Shi Y, Wang WL, Song SH, Lu ZK, Bosmans RPG, Dai Q, Hao YJ, Yang X, Zhao WM, Tong WM, Wang XJ, Bogdan F, Furu K, Fu Y, Jia GF, Zhao X, Liu J, Krokan HE, Klungland A, Yang YG, He C (2013) ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. **Mol Cell** 49: 18-29.

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

Zhou J, Wan J, Gao X, Zhang X, Jaffrey SR, Qian SB (2015) Dynamic m(6)A mRNA methylation directs translational control of heat shock response. **Nature** 526: 591-594.

Zhou WB, Karcher D, Bock R (2013) Importance of adenosine-to-inosine editing adjacent to the anticodon in an *Arabidopsis* alanine tRNA under environmental stress. **Nucleic Acids Res** 41: 3362-3372.

Zhou X, Hua D, Chen Z, Zhou Z, Gong Z (2009) Elongator mediates ABA responses, oxidative stress resistance and anthocyanin biosynthesis in *Arabidopsis*. **Plant J** 60: 79-90.

Zorbas C, Nicolas E, Wacheul L, Huvelle E, Heurgue-Hamard V, Lafontaine DL (2015) The human 18S rRNA base methyltransferases DIMT1L and WBSCR22-TRMT112 but not rRNA modification are required for ribosome biogenesis. **Mol Biol Cell** 26: 2080-2095.

FIGURE LEGENDS

Figure 1. RNA modifications in messenger RNAs have distinct deposition patterns.

Shown is a pictorial representation of relative abundance of the RNA modifications m^6A , m^5C and Ψ along mRNA transcripts. These representations are based on transcriptome-wide RNA bisulfite sequencing data for m^5C and antibody data for m^6A in animals and plants. The Ψ abundance is based on a combination of Ψ -seq and antibody enrichment data from animals. m^6A is lowly abundant along coding sequences and enriched at long last exons and at the start of 3'UTR's. While the majority of m^5C sites are detected in the coding sequence of mRNA transcripts, m^5C sites are statistically enriched in 3'UTR's. For Ψ , the modified sites are evenly distributed along the coding sequence, but are statistically underrepresented in 5' UTRs.

Figure 2. Distinct catalytic and targeting mechanisms of different RNA modifications in *Arabidopsis*

(A) The predicted *Arabidopsis* m^6A 'writer' complex is composed of a heterodimer of MTA and MTB, bound to AtFIP37 and potentially other, uncharacterized proteins. In mammals, miRNAs are able to guide the m^6A 'writer' complex, however, it is not known if this targeting mechanism is conserved in plants. Potential m^6A 'erasers' and 'readers' have been predicted in *Arabidopsis* and await further characterization. (B) A model for targeting of m^5C methylation by TRM4B, based on RNA structure and potentially the presence of other RNA modifications. (C) Proposed H/ACA snoRNP Ψ 'writer' complex in *Arabidopsis* contains a guide H/ACA box snoRNA and the proteins GAR1 (At3g03920/At5g18180) or NAF1 (At1g03530), NHP2 (At5g08180), NOP10 (At2g20490) and the Ψ synthase AtCBF5 (At3g57150).

Figure 3. Potential functions of RNA modifications in mediating interactions between nucleic acids and nucleic acids and proteins.

RNA modifications have diverse chemical properties and can have different effects on RNA interactions. A spikey, pink ball is used to represent a generic RNA modification. RNA modifications can regulate protein binding to RNA through remodeling of local RNA structure (e.g. 'm⁶A switches'), increasing or inhibiting protein binding. In addition, RNA modifications could potentially affect other types of interactions, such as R-loops, which are RNA-DNA hybrids.

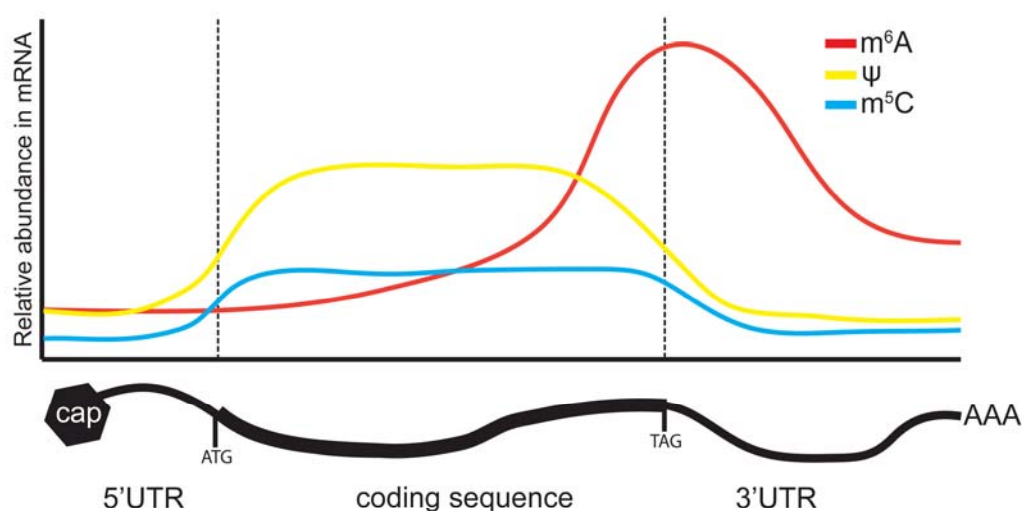


Figure 1

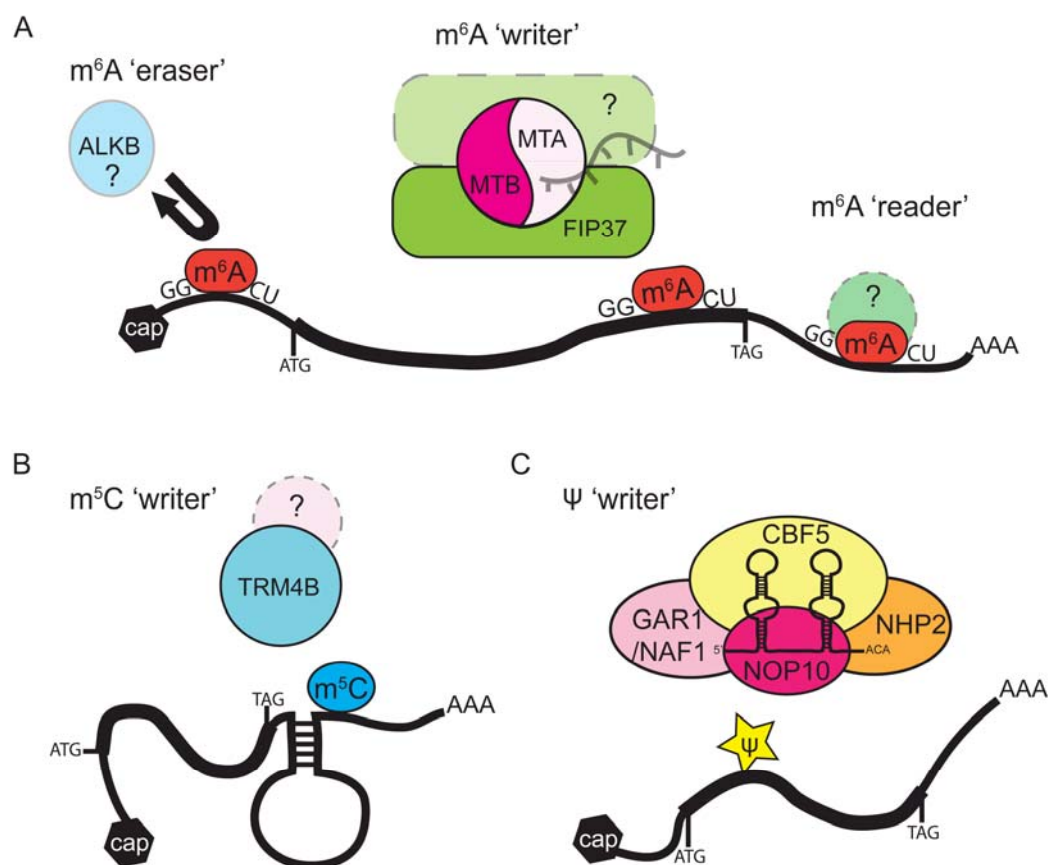


Figure 2

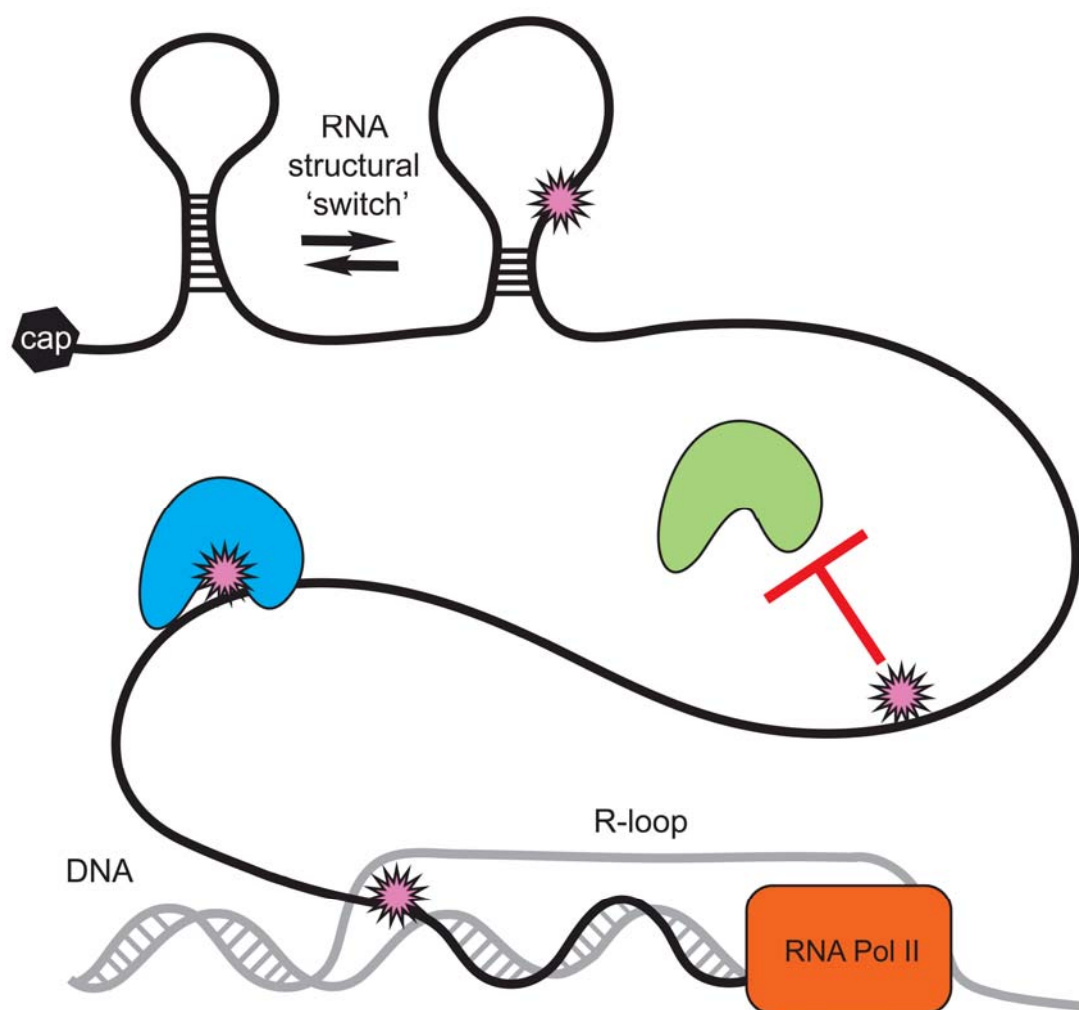


Figure 3



Iain