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UTR dependent control of gene expression in plants

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Abstract

Throughout their lives, plants sense many developmental and environmental stimuli, and activation of optimal responses against these stimuli requires extensive transcriptional reprogramming. To facilitate this activation, plant mRNA contains “UTRs” or untranslated regions that significantly increase the coding capacity of the genome by producing multiple mRNA variants from the same gene. In this review we compare UTRs of arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativum*) at genome scale to highlight their complexity in crop plants. We discuss different modes of UTR-based regulation with emphasis on genes that regulate multiple plant processes, including flowering, stress responses, and nutrient homeostasis. We demonstrate functional specificity in genes with variable UTR length and propose future research directions.

Keywords

Adenosine methylation; alternative splicing; epigenetics; nonsense-mediated decay; polyadenylation; riboswitch

Significance of UTRs in Plants: Genome-Scale Evidence

Because plants are sessile, they have to tolerate many abiotic and biotic stresses. Besides, plants undergo major growth transitions during their life cycle, from seed germination to vegetative growth, flowering and seed development. Both the activation of stress tolerance mechanisms and developmental transitions require extensive transcriptional reprogramming, which is controlled at transcriptional and post-transcriptional levels. Transcriptional control is mediated by transcription factors, while post-transcriptional control is regulated by a complex set of cis- and trans-acting elements [1–3]. Cis-acting elements for posttranscriptional control, which are generally present in untranslated regions (UTRs, see

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Glossary) of mRNAs, can regulate various mRNA properties like stability, transport and translation efficiency and also the functioning and sub-cellular localization of the translated proteins [4]. Thus, UTRs represent important genetic component that regulate processes at the interface of mRNAs and proteins. In addition, owing to their characteristics of having multiple variants from the same gene, UTRs can increase the coding capacity of the genome. This provides versatility to the gene expression system and thereby helps plants adapt to environmental fluctuations. In the genomes of both *Arabidopsis thaliana* (a model dicot; genome size 129 Mb) and *Oryza sativa* (a model monocot; genome size 430 Mb), UTRs represent approximately 18–19% of the transcribed sequence, which further substantiates their significance in plants. Although *O. sativa* genome is three-times larger than that of *A. thaliana*, the proportion of 5' and 3' UTRs is almost the same in both species (Fig. 1A); however, their length-wise distribution indicates higher complexity in rice. In general, UTRs are longer in rice than in Arabidopsis, with average lengths of 5' and 3' UTRs in rice being 259 bp and 469 bp, but only 155 bp and 242 bp, respectively in arabidopsis (Figure 1B). In arabidopsis, using reciprocal best hits that compare cross species protein sequences, we identified a total of 7,933 genes having orthologs in the rice genome. Since orthologs have common ancestors and diverged during speciation, they have high sequence similarity in the protein coding region. However, rice UTRs (especially 3'UTR) are comparatively longer than arabidopsis UTRs, even for orthologous genes (Figure 1B). Thus, inspite of the conserved nature of many UTR-associated cis-regulatory elements across plant families [5], the existence of plant species-specific mechanisms is expected. Here we provide a comprehensive overview of UTR-dependent regulatory mechanisms that control gene expression as well as the fate of translated proteins in plants. Using enrichment analysis of UTR-containing genes at the whole-genome level (Box 1), we highlight the need to include UTR-related regulatory mechanisms in gene function studies, especially in crops. We also discuss future research directions that will increase the application of UTR-based research to crop improvement.

Box 1

UTR Length-Dependent Functional Specificity in Arabidopsis and Rice

Arabidopsis and rice are the model plants for two major angiosperm subclasses, dicots and monocots, respectively. To date, most genes that have been characterized for UTR-based regulation belong to arabidopsis (Table 1). The cis-elements and also up- and downstream sequence contexts for most UTR-based regulatory mechanisms are more or less conserved in plants [45, 49, 74, 75]. However, UTR-based regulatory processes proposed for arabidopsis genes may not hold true for rice. In both arabidopsis and rice, distinct functional categories are enriched in genes with short (1–500 bp), medium (501–1,000 and 1,001–2,000 bp), and long (> 2,000 bp) UTRs (Figure I). This suggests that apart from regulating gene expression, UTRs may also provide functional specificity to genes in a length-dependent manner (Figure 3). Recently, a subset of dehydration stress related genes has been demonstrated to undergo 3' UTR extensions for regulating the expression of neighbouring genes through long noncoding RNAs. This further substantiates the significance of UTR length in plant adaptation to stress conditions [76]. Furthermore, the functional enrichments within UTR length categories differ between

arabidopsis and rice. For instance, genes with 5′ or 3′ UTRs in the 1 to 500-bp category mediate stress responses in arabidopsis, but genes in this category mediate transport and metabolism in rice. This further strengthens the notion that conclusions derived from UTR-related functional studies in arabidopsis cannot be directly applied to rice or other crops. Thus, large-scale projects like “RICE 2020”, which aims to understand the function of all proteins derived from the primary gene pool in rice [77], should be complemented with parallel studies that determine the UTR-dependent regulation of all genes. Such a holistic approach is needed to ensure effective utilization of functional genomics studies for rice genetic improvement.

Figure I. Functional enrichment analysis of UTR-containing genes in Arabidopsis and rice UTR-containing genes of Arabidopsis and rice genomes were sorted according to their 3′ and 5′ UTR length before functional enrichment analysis was independently performed for genes with short (1–500 bp), medium (501–1000 and 1001–2000 bp), and long (> 2000 bp) UTRs using Bioconductor package topGO in R software (version 3.1.1). On the basis of *p* value, top five GO terms related to biological process are represented, independently for 3′ and 5′ UTRs. The colour of bars such as blue (1–500 bp), black (501–1,000 bp), green (1,001–2,000 bp) and red (>2,000 bp) signifies variable UTR length. In rice, relatively fewer GO terms were enriched, especially in medium (1,001–2,000 bp) and long (>2,000 bp) UTR categories.

Different Modes of UTR-Mediated Control of Gene Expression

UTRs are known to control gene expression and protein function via a wide range of mechanisms (Figure 2). The major mechanisms and their involvement in the regulations of multiple plant processes are briefly discussed below. Important genes that have been functionally characterized to be regulated by UTRs are listed in Table 1.

Alternative polyadenylation

In alternative polyadenylation (**APA**), an mRNA transcript is processed to produce multiple isoforms that generally differ in their 3'-UTR length. If the alternative polyA signal falls upstream of the stop codon, APA can also produce a nonfunctional protein or a protein with an entirely different function [6]. More than 80% of the genes in humans [7] and more than 75% of the genes in arabidopsis [8] are known to produce multiple mRNA isoforms through APA. APA regulates multiple plant processes, and a well-studied example is flowering-time regulation through *FCA*, *FPA* and *Flowering Locus C (FLC)*-mediated autonomous pathway [9]. *FCA* and *FPA* are RNA-binding proteins that promote proximal polyadenylation of *FLC* antisense RNA to decrease *FLC* expression and induce flowering [10]. In addition, *FCA* and *FPA* mRNAs undergo APA to generate either full-length coding transcripts (using distal polyA site) or truncated transcripts (using proximal polyA site). *FCA* and *FPA* provide feedback control to their own levels by favouring the usage of proximal polyA [10, 11]. The recently characterized *HLP1A* regulates distal-to-proximal polyA site shift in *FCA*, which in turn upregulates *FLC* to delay flowering [12]. *FLC* expression is repressed by a group of antisense, long, noncoding transcripts (collectively termed **COOLAIR**) that undergo APA. Promotion of proximal polyadenylation of *COOLAIR* is directly linked to reduced *FLC* expression [13]. Apart from flowering, APA also regulates mRNA transport to the surface of organelles for facilitating co-translational import of the protein into organelles. This can be observed in the case of *VDAC3*, which is a member of voltage-dependent anion channel family in arabidopsis and produces two mRNA variants with short or long UTRs. The most distal 140-nt 3' sequence of long UTR is essential and sufficient for targeting *VDAC3* mRNA or any other unrelated mRNA to mitochondrial surface for efficient co-translational import of the protein into mitochondria [14]. APA also regulates agriculturally important traits. A naturally occurring mutant, *sdt* (**SEMI**DWARF AND **HIGH**-TILLERING), has been identified in which *OsmiR156h* precursor expression is upregulated via APA to promote tillering capacity and grain yield in rice [15].

APA represents an important link between DNA methylation- and histone modification-dependent regulation of gene expression. Transposons and other repetitive elements are silenced by DNA methylation and heterochromatic histone modifications such as H3K9 dimethylation. *IBM1* (Increase in Bonsai Methylation 1) is a histone demethylase that regulates the expression of many genes by removing the repressive H3K9me2 mark from gene bodies [16]. Arabidopsis *ibm1* mutant plants display a wide range of developmental defects including a decreased production of stomatal lineage cells, which is associated with reduced expression of *ERECTA*-family genes [17]. *IBM1* has a large intron that harbors a heterochromatic repeat element [20]. *AS11* (Anti-silencing 1), a bromodomain-containing RNA-binding protein and *EDM2* (Enhanced Downy Mildew 2), a chromatin regulator that

recognizes both repressive and activation histone marks in the intronic repeat, are required for the production of functional *IBM1* transcript [20, 22]. In wild type arabidopsis plants, a distal polyA signal in *IBM1* pre-mRNA is used, leading to the formation of functional *IBM1* transcript [18]. In *asl1* and *edm2* loss-of-function mutants, a proximal polyA signal upstream of the intronic heterochromatic repeat is used, resulting in truncated *IBM1* transcripts. Besides this epigenetic regulation, APA is also regulated genetically via Cleavage and Polyadenylation Specificity Factor 30 (CPSF30). Because of the variation in polyA signal selection in genes related to stress and defence, arabidopsis *ox16* mutant (defective in CPSF30) has an oxidative stress-sensitive phenotype [19, 20]. Thus, APA is regulated by both genetic and epigenetic mechanisms, and is important for plant processes like flowering, grain yield and stress tolerance.

Riboswitching and other UTR-mediated regulation by small molecules

Riboswitches are metabolite-sensing gene-control elements that are typically located in noncoding portions of mRNAs. In eukaryotes, a well-characterized riboswitch senses the vitamin B1 derivative TPP (thiamine pyro-phosphate). TPP biosynthesis is regulated through *THIC*, which catalyses the conversion of 5-aminoimidazole ribotide to hydroxymethyl pyrimidine phosphate, a key step in the synthesis of thiamine and ultimately TPP [21]. *THIC* represents a typical example of gene expression control through the combination of riboswitch and APA. An intron element outside the stop-codon of *THIC* contains a primary polyA site followed by a riboswitch element that can bind TPP, and then a secondary polyA site at the 3'-end of the transcript. The primary polyA site is also flanked by a splicing signal whose accessibility is regulated through TPP binding with the riboswitch. In the absence of TPP, the splicing site is inaccessible, hence the primary polyA site remains activated. The resulting transcript has a shorter 3'UTR that is more stable; as a consequence, *THIC* expression is increased, which facilitates TPP biosynthesis. When TPP is available, it binds with the riboswitch and, because of conformational change, the splice site becomes accessible. As a result, the primary polyA site is spliced out, which extends transcription till the secondary polyA site located 1 kb away from the primary polyA site. The resulting transcript with a longer 3'UTR is relatively unstable and consequently *THIC* expression is decreased [22, 23].

A UTR element was recently found to be involved in regulating ethylene-dependent signalling through translational repression. The mechanism of ethylene signaling has been well described in both arabidopsis and rice [24]. In brief, the ethylene signal is propagated through the ER-located membrane protein ETHYLENE INSENSITIVE 2 (EIN2). Upon activation, the C-terminal domain of EIN2 (CEND) is cleaved and transported to the nucleus where it stabilizes two master transcription factors, ETHYLENE INSENSITIVE 3 (EIN3) and its paralog EIN3-LIKE 1 (EIL1), by repressing their proteasomal degradation caused by two F-box proteins, EIN3-BINDING F-BOX 1 (EBF1) and EBF2. EIN3 and EIL1 are mainly responsible for ethylene-responsive gene expression. In addition to the EIN2-EBF1/2 interaction at the protein level in the nucleus, an independent mode of regulation also exists wherein EIN2 interacts with the 3'UTR of *EBF1/2* to modulate their translation. The first clue for this came from a study in which an ectopic expression of *EBF1* or *EBF2* 3'UTR generated strong ethylene-insensitive phenotypes by promoting the translation of

endogenous *EBF1/2* mRNAs [25]. When EIN2 binds with *EBF1/2* 3'UTR, the complex directs the transcripts to cytoplasmic processing-bodies; therefore, a higher expression of the 3'UTR element alone is sufficient to deregulate EIN2-mediated translational repression of *EBF1/2* [25].

By using a combination of genome and transcriptome sequencing approaches, 31 new riboswitches (including TPP) were identified from a marine phytoplankton [26]. Given the large proportion of UTR elements in the model plants *Arabidopsis* and rice (Fig. 1), it is likely that many more metabolites and small molecules that can be sensed in a UTR-dependent manner remain to be identified in higher plants.

Adenosine methylation

Adenosine methylation (**m6A**) was recently identified as a conserved epi-transcriptomic modification of mRNAs. In the 5'UTR, m6A facilitates CAP-independent protein translation. The translation of mRNAs begins with the binding of eukaryotic initiation factor 4E (eIF4E) with the 5' 7-methylguanosine (**m7G**) cap, which in turn recruits the small ribosomal subunit (40S) to enable efficient translation. Using a combination of *in vitro* reconstitution and translation assays, it has been shown that a single 5'-UTR m6A can directly bind with eukaryotic initiation factor 3 (eIF3), which is sufficient to recruit 43S ribosomal complex to initiate translation even in the absence of eIF4E [27]. Due to its dramatic increase under cellular stresses, m6A modification in 5'UTRs is considered as a defence strategy to promote CAP-independent protein translation [27]. Using the Hsp70 mRNA, m6A effect has been shown to be sequence dependent. Hence, not all m6A modifications are equally efficient in stimulating protein translation [28].

How m6A regulates plant gene expression is an active area of research. MTA (*At4g10760*), a METTL3 ortholog in *Arabidopsis*, is an active component of the m6A methyltransferase complex [29]. MTA is expressed at high levels in dividing tissues, such as developing seeds, shoot meristems, and emerging lateral roots. MTA disruption causes multiple growth defects, including reduced apical dominance, organ abnormality, and increased trichome branching [30]. FIP37 (FKBP12 INTERACTING PROTEIN37 KD) is another core component of the m6A methyltransferase complex, and *fip37* mutant exhibits massive over proliferation of shoot meristems and a transcriptome-wide loss of m6A [31]. Using comparative transcriptome-wide m6A profiling in two *Arabidopsis* accessions (Can-0 and Hen-16), it has been confirmed that m6A modification is enriched in both 5' and 3' UTRs, as was previously observed in mammals [32]. However, little information is available about how m6A modification regulates plant development and responses to various abiotic/biotic stress conditions. Thus, additional research involving more precise measurement of m6A is required to clarify the function of this dynamic epi-modification in regulating various aspects of plant growth and development.

Short-peptide translation

The 5'UTR region of many eukaryotic mRNAs contains a short open-reading frame (**ORF**), termed an upstream ORF (**uORF**). Initiation of translation from uORF can repress translation from downstream ORF by stalling ribosome movement. Also, if the uORF stop-

codon is located upstream of exon junction complex (EJC), it can be recognized as a pre-mature termination codon to activate mRNA degradation through the nonsense-mediated decay (NMD) pathway [33]. In Arabidopsis, ~35% genes (>9,000) have at least one uORF [34]. Recent research using high-resolution ribosome profiling confirmed that 187 genes with an uORF having AUG start codon are translated [35]. However, this number might be an underestimate, because uORF translation can also start from unconventional start codons. The translation efficiency of uORFs is sequence-dependent and is also regulated under stress conditions. In maize (*Zea mays*), uORF translation is globally enhanced under drought stress, which helps to shape the proteome under stress [36]. The functional relevance of uORFs is well demonstrated for sucrose-induced repression of translation (SIRT) of basic leucine zipper (bZIP)-type transcription factor family genes. For instance, the arabidopsis *ATB2/AtbZIP11* has a highly conserved uORF within its exceptionally long 5'-UTR which is translated in response to sucrose, resulting in the reduction of AtbZIP11 protein [37]. Based on this information, SIRT deregulation was attempted in tomato and uUTR-free versions of *SibZIP1* and *SibZIP2* were over-expressed for increasing sweetness [38]. Similar uORFs have been identified in other genes, like *NAC096* [39], *Polyamine oxidase-2* [40], *SAC51* (*suppressor-of-ACAULIS5*)-family members [41], *TBF1* (*TL1*-binding factor-1) [42] and *AtHB1* (homeodomain-leucine zipper transcription factor) [43], that can generate regulatory peptides to repress the translation of main ORFs. The translation arrest can also be coupled with mRNA decay through the NMD pathway. This has been demonstrated using the polyamine-responsive AdoMetDC1 (S-adenosylmethionine decarboxylase) uORF-based reporter system [44]. Recently, a role has been assigned for a minimal possible uORF termed as "AUG-stop", under the context of boron (B) deficiency. In Arabidopsis, *NIP5;1*, which encodes a boric acid channel required for normal growth under B-deficient conditions, contains an AUG-stop-like uORF. Under normal conditions, ribosomes halt at AUG-stop, which suppresses translation and promotes mRNA degradation. A conserved sequence at the 5' edge of the stalled ribosome is required for mRNA degradation. Under B-deficient conditions, however, translation re-initiation is promoted, which leads to the synthesis of functional NIP5;1 [45]. Thus, translation of short-peptides from uORF provides versatility to the translation machinery and thereby helps plants to adapt to changing environmental conditions.

Nonsense-mediated decay

NMD is a surveillance mechanism that detects and degrades transcripts with a pre-mature termination codon (PTC) to prevent the formation of non-functional or aberrant proteins. Although the molecular mechanism of NMD is conserved between mammals and plants [46, 47], some plant-specific features have also been reported [48]. Various models have been proposed to explain NMD activation [49, 50]. In brief, during steady-state translation (which follows the first or pioneer round of translation), the cap-binding proteins eIF4E and eIF4G form a complex with the poly(A)-binding protein cytoplasmic 1 (PABPC1), which results in a circular conformation of the mRNA. In a circular state, when a ribosome encounters a stop codon, eukaryotic release factors like eRF1 and eRF3 are recruited; these interact with eIF4G-PABPC1 and terminate translation. This interaction, however, is inhibited by long 3'UTRs, and consequently eRF3 interacts with UPF1 (up-frameshift1) and other NMD factors to make the SURF (SMG1-Upf1-eRF1-eRF3) complex, which triggers translation

repression and mRNA decay. Introns located 50–55 nt downstream of termination codons (TCs) can also activate NMD, even in the first round of translation. After intron splicing, EJC proteins are deposited about 24 nt upstream of exon–exon junctions. If the termination occurs more than 50–55 nt upstream of EJC, terminating ribosomes cannot physically remove EJC, which therefore interacts with UPF1 via UPF3 and UPF2 and trigger NMD. UPF1 can also directly bind with mRNA before translation in a 3'UTR length-dependent manner and activate NMD. Thus, multiple signals can activate NMD. Other mechanisms, like translation read-through (translation elongation until the next in-frame stop codon) causes mRNA to escape NMD. In Arabidopsis, eRF1 is the only gene with all the cis-elements required for read-through. A low eRF1 level promotes read-through, which masks NMD signal at the 3'UTR of *eRF1* mRNA and hence increases eRF1 protein level [51]. Thus, an autoregulatory feed-back mechanism of eRF1 is important for regulating NMD and overall protein translation.

NMD plays a critical role in integrating plant responses to different environmental cues [52–56]. An interesting example concerns high temperature-induced flowering, which is controlled by the MADS-box transcription factors FLOWERING LOCUS M (FLM) and SHORTVEGETATIVE PHASE (SVP). *FLM* produces two main FLM protein splice variants, FLM- β and FLM- δ , which compete with the floral repressor SVP. At normal growth temperatures, SVP–FLM- β complex predominates and prevents flowering. At high temperatures, FLM- δ increases at the expense of FLM- β , and because the SVP–FLM- δ complex is impaired in DNA binding, flowering is accelerated [57]. This model has not been supported, however, by recent findings suggesting that temperature differences in flowering time can be explained by FLM- β only rather than the FLM- β /FLM- δ ratio [58]. Another study has demonstrated that thermal induction of flowering is abolished in *upf* mutants, which suggests active involvement of NMD [59]. Novel thermosensitive splice sites have been identified in the *FLM* locus and temperature-dependent alternative splicing was shown to produce NMD-sensitive variants of *FLM* which can be degraded through the NMD pathway and thereby reducing *FLM* expression to accelerate flowering. The temperature sensitivity of various arabidopsis natural accessions was also correlated with NMD sensitivity of their *FLM* 3'UTR sequences [59]. Many more examples of NMD-mediated regulation of various plant processes are likely to be discovered in the future.

Alternative splicing in UTR elements

Alternative splicing (AS) refers to the process by which a pre-mRNA is processed differentially to generate multiple isoforms that differ in biological activity [60]. AS in UTRs, especially in 5'UTRs, can regulate translation efficiency by producing mRNA variants that differ in terms of uORFs or riboswitches [61]. For instance, the arabidopsis *Phytoene synthase (PSY)* gene, which catalyses the rate-limiting step of carotene biosynthesis, produces AS variants with long (ASV1) and short (ASV2) 5'UTRs. The long 5'UTR of ASV1 forms riboswitch-like conformations that can sense carotene flux to attenuate translation. This structure is absent in ASV2 which facilitates rapid carotene synthesis in response to salt stress or changes in light intensity [62]. A similar mechanism was reported for ZIF2 (Zinc-Induced Facilitator 2)-mediated control of Zn uptake in Arabidopsis; in this case, however, intron retention in the 5'UTR favours translation [63].

5'UTRs also may contain introns that affect translation through a process called intron-mediated enhancement (**IME**), a conserved but poorly understood mechanism [64, 65]. In the arabidopsis Mg^{2+}/H^{+} exchanger (MHX) gene, an internal intronic element has been identified that considerably increases translation efficiency [66]. AS in UTRs can also affect protein localization. For example, the transcription factor BRI1-EMS-SUPPRESSOR1 (BES1), which promotes brassinosteroid signalling, has short (BES1-S) and long (BES1-L) isoforms. BES1-L, which is produced from an alternative transcriptional start site located upstream of BES1-S, under goes AS such that it includes the 5'-UTR of BES1-S. This adds 22 amino acid residues to the N-terminus of BES1-L, which provides it a high nuclear localization activity. BES1-L is considered as a recently evolved isoform that increases plant fitness [67]. 3'UTR-mediated control of translation is well-studied in animals [68], but little information is currently available in plants, except for the few studies in which transgene effectiveness is increased when expressed with a 3'UTR [69]. AS in UTRs can also produce variants that are insensitive to miRNA-mediated cleavage [70]. To prove this notion, osa-miR7695 has been recently identified in rice that specifically cleaves an *OsNramp6* (Natural resistance-associated macrophage protein 6) variant having miRNA cleavage site within its 3'UTR region to provide resistance against fungal infection; other variants with short 3'UTRs are immune to osa-miR7695-mediated cleavage [71]. In addition, AS in UTRs can also affect miRNA biogenesis. An interesting example of this is miR400, which is an intronic miRNA from the 5'UTR of its host gene. Under control conditions, the entire 306-bp intron harbouring the miR400 hairpin is excised and processed to generate mature miR400. Under heat stress, only a small 100-bp fragment is excised, and the remaining 206-bp portion is retained within the host gene. This negatively affects miR400 processing and consequently reduces the expression of mature miR400 [72]. Thus, AS in UTRs can fine tune gene expression as well as protein levels in multiple ways that help plants adapt to changing environmental conditions.

Concluding Remarks and Future Outlook

We have evaluated UTR-containing genes in arabidopsis and rice at genome-scale and found that, although the relative proportions of coding sequence (**CDS**) and UTR are similar in arabidopsis and rice, UTRs of rice genes are longer than that in Arabidopsis. UTR length-dependent functional specialization of genes is also demonstrated. We have reviewed a broad range of UTR-dependent regulatory mechanisms, by looking at genes with multiple mRNA variants that differ in UTR length and that are involved in the regulation of flowering, stress responses, nutrient homeostasis, and other processes. Given these findings, we propose a holistic approach to crop functional genomics wherein protein-coding CDS and regulatory UTR elements are studied in parallel. For newly sequenced complex plant genomes like those of chickpea (*Cicer arietinum*), tomato (*Solanum lycopersicum*), Chenopodium and other crop plants, genome-wide searches are needed to understand how UTRs code their regulatory functions. Although the mechanisms described here can be studied in more detail, it is also important to discover new mechanisms. For example, a range of 3'UTR-binding proteins in animals are known to modulate protein translation, but this area has been little explored in plants. Also needed are in-depth studies concerning m6A function in regulating specific genes. Overall, it is obvious that our understanding of UTR function is still

inadequate and that future research, especially in crop plants, will provide many new angles by which UTRs modulate gene expression and protein function.

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GLOSSARY

7-methylguanosine (m7G)

a methylation of guanosine in the 5' terminus of mRNAs.

Adenosine methylation (m6A)

a conserved epi-transcriptomic modification of mRNAs that is directed by a methyltransferase complex containing METTL3 as the SAM-binding subunit.

Alternative polyadenylation (APA)

a process in which one pre-mRNA transcript is processed to produce multiple mRNAs that generally differ in their 3'-UTR length.

Alternative splicing (AS)

a process by which pre-mRNAs are processed differentially to generate multiple isoforms that differ in biological activity.

Coding sequence (CDS)

the sequence of a transcript that is actually translated into protein. CDS starts with a start codon (AUG) and stops at one of the three STOP codons (UAA, UGA or UAG).

COOLAIR

a group of antisense long noncoding transcripts collectively is termed as COOLAIR that undergoes alternative polyadenylation to repress the expression of the flowering time regulator FLC.

Exon junction complex (EJC)

a protein complex formed at the junction of two exons on a pre-mRNA strand joined together during RNA splicing.

Intron-mediated enhancement (IME)

the ability of an intron sequence to enhance the expression of a gene containing that intron.

Nonsense-mediated decay (NMD)

a translation-coupled mechanism that eliminates mRNAs containing premature translation-termination codons (PTCs). Its main function is to reduce errors in gene expression.

Open-reading frame (ORF)

a part of the reading frame that has potential to be translated, and usually contains start (AUG) and stop (UAA, UAG or UGA) codons.

Termination codon (TC)

a stop codon (UAA, UAG or UGA) is a nucleotide triplet within messenger RNA that signals the termination of translation into proteins.

Untranslated region (UTR)

refers to the two sections, on each side of a coding sequence, on a mRNA strand.

Upstream Open Reading Frame (uORF)

an ORF within the 5'UTR of an mRNA.

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Trends

- Plant mRNAs contain untranslated regions (UTRs) which significantly enhance the coding capacity of the genome by producing multiple mRNA variants from the same gene.
- Approximately 18–19% of transcribed sequence In *Arabidopsis thaliana* and *Oryza sativa* genomes is UTR. The length-wise distribution of UTRs indicates higher complexity in rice than in *A. thaliana*. In general, UTRs are longer in rice than in *Arabidopsis*.
- At whole-genome level, UTRs provide functional specificity to genes in a length-dependent manner.
- In spite of the conserved nature of many UTR-associated cis-regulatory elements across plant families, existence of plant-specific mechanisms are expected.
- There exists multiple ways by which UTR element can exert its control over gene expression and also, on the functioning of translated proteins.

Outstanding questions

- Whether UTR length is important for the activation of stress tolerance mechanisms and developmental transitions? If yes, what are the mechanistic links which connects UTR length with gene expression?
- Can we extend the knowledge gained from UTR related studies in *Arabidopsis thaliana* to other crop plants like rice?
- Why plants spend energy to transcribe such a large proportion of UTR elements and do not translate it? Is it possible that UTR function can be extended beyond gene expression control?
- What is the physiological relevance of epi-transcriptomic modifications like m6A at the level of single gene?
- At the whole-genome level, can we identify a subset of genes having all the required features to undergo multiple modes of UTR dependent regulation?

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Figure 2. Various modes of UTR-dependent regulation of genes

(A) Alternative polyadenylation: either more than one polyA site is utilized to produce mRNA variants that differ in 3'UTR length or, if the polyA is located upstream of the stop codon, truncated transcripts are produced. (B) Riboswitching: 3' or 5'UTRs form folding structures that sense a particular metabolite and modulate transcript stability. (C) Adenosine methylation (m6A): The presence of m6A in 5'UTR promotes CAP-independent translation. (D) Short-peptide translation: A short open reading frame in 5'UTR (uORF) can either repress the translation of the main ORF or induce mRNA decay via the NMD pathway. (E) Nonsense-mediated decay (NMD): A pre-mature termination codon (PTC) upstream of the regular termination codon (TC) recruits NMD factors that mediate mRNA decay. (F) Alternative splicing: Retention of intronic elements in 5'UTR can either promote or repress translation, while retention of intronic elements in 3'UTR can modulate miRNA-mediated cleavage.

Table 1

Summary of genes functionally characterized for UTR-dependent regulation

Gene	Organism	UTR dependent regulation	Refs.
Alternative polyadenylation			
Antisense <i>FLC</i>	<i>Arabidopsis thaliana</i>	Proximal polyA in antisense FLC RNAs represses <i>FLC</i> expression	[10]
<i>FCA</i> and <i>FPA</i>	<i>Arabidopsis thaliana</i>	Activation of distal polyA in <i>FCA</i> and <i>FPA</i> produces functional FCA and FPA to suppress <i>FLC</i> expression	[10, 11]
COOLAIR	<i>Arabidopsis thaliana</i>	Proximal polyA in COOLAIR reduces <i>FLC</i> expression	[13]
<i>VDAC3</i>	<i>Arabidopsis thaliana</i>	Most distal 140-nt sequence in 3'UTR is essential and sufficient for targeting VDAC3 mRNA into mitochondria	[14]
OsmiR156h precursor	<i>Oryza sativa</i>	Shortening of 3'UTR induces expression of both precursor and mature forms of OsmiR156h	[15]
<i>IBM1</i>	<i>Arabidopsis thaliana</i>	AS11-mediated activation of distal polyA produces functional <i>IBM1</i> transcripts	[18]
Riboswitching			
<i>THIC</i>	<i>Arabidopsis thaliana</i>	Activation of proximal polyA produces stable <i>THIC</i> transcripts with short 3'UTRs that promote vitamin B1 biosynthesis	[22]
<i>EBF1</i> and <i>EBF2</i>	<i>Arabidopsis thaliana</i>	Binding of EIN2 with 3'UTRs of <i>EBF1</i> and <i>EBF2</i> represses translation to promote ethylene signaling	[25]
Short-peptide translation			
<i>bZIP11</i>	<i>Arabidopsis thaliana</i>	Translation of uORF in response to sucrose bZIP11 reduces protein level	[37]
<i>NAC096</i>	<i>Arabidopsis thaliana</i>	uORF represses the expression of the main ORF	[39]
<i>Polyamine oxidase-2</i>	<i>Arabidopsis thaliana</i>	uORF mediates translational repression of the main ORF	[40]
<i>SAC51</i>	<i>Arabidopsis thaliana</i>	uORF mediates translational repression of the main ORF	[41]
AdoMetDC1	<i>Arabidopsis thaliana</i>	uORF causes ribosomal arrest and promotes mRNA through NMD	[44]
<i>NIP5;1</i>	<i>Arabidopsis thaliana</i>	Translation of minimum AUG-Stop uORF affects translation initiation in a boron-dependent manner	[45]
Nonsense-mediated decay			
<i>FLM</i>	<i>Arabidopsis thaliana</i>	High temperature reduces <i>FLM</i> expression by producing sensitive variants	[59]
Alternative splicing			
<i>Phytoene synthase</i>	<i>Arabidopsis thaliana</i>	<i>PSY</i> variant with long 5'UTR can sense carotene flux, attenuates translation to reduce <i>PSY</i> protein level	[62]
<i>ZIF2</i>	<i>Arabidopsis thaliana</i>	Intron retention in 5'UTR favours the translation of transcripts	[63]
<i>FRD3</i>	<i>Arabidopsis thaliana</i>	Alternative transcription initiation sites are utilized to produce <i>FRD3</i> transcripts with shorter 5'UTR for increased translation efficiency	[73]
<i>MHX</i>	<i>Arabidopsis thaliana</i>	An intronic element in 5'UTR of <i>MHX</i> considerably increases translation efficiency	[66]
<i>BES1</i>	<i>Arabidopsis thaliana</i>	Alternative transcriptional start site is used to produce long variant with high NLS (nuclear localization signal) activity	[67]
Osa-miR7695	<i>Oryza sativa</i>	AS in the target gene <i>OsNramp6</i> produces variants with short 3'UTRs that are immune to Osa-miR7695-mediated cleavage	[71]
Ath-miR400	<i>Arabidopsis thaliana</i>	AS in 5'UTR controls the removal of miR400 hairpin from the host gene and thereby affects expression of mature miR400	[72]