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Published in:
Journal of Experimental Botany

DOI:
10.1093/jxb/erae073

Publication date:
2024

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Document Version
Peer reviewed version

Link to publication in Discovery Research Portal

Citation for published version (APA):
Untranslated yet indispensable – UTRs act as key regulators in the environmental control of gene expression

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Highlight

Transcripts’ untranslated regions (UTRs) act as molecular hubs for the post-transcriptional control of gene expression and thereby help to tailor a plant’s development and physiology to its surroundings.

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Abstract

To survive and thrive in a dynamic environment, plants must continuously monitor their surroundings and adjust their development and physiology accordingly. Changes in gene expression underlie these developmental and physiological adjustments and are traditionally attributed to widespread transcriptional reprogramming. Growing evidence, however, suggests that post-transcriptional mechanisms also play a vital role in tailoring gene expression to a plant’s environment. Untranslated regions (UTRs) act as regulatory hubs for post-transcriptional control, harbouring cis elements that affect an mRNA’s processing, localisation, translation and stability and thereby tune the abundance of the encoded protein. Here, we review recent advances made in understanding the critical function UTRs exert in the post-transcriptional control of gene expression in the context of a plant’s abiotic environment. We summarise the molecular mechanisms at play, present examples of UTR-controlled signalling cascades and discuss the potential that resides within UTRs to render plants more resilient to a changing climate.

Key Words

Abiotic stress; alternative splicing; gene expression; post-transcriptional regulation; RNA-binding protein (RBP); RNA processing; RNA structure; translation; untranslated region (UTR)
Introduction

Plants are highly sensitive to the environment in which they grow. Sunlight, temperature, water availability, soil composition, and a plethora of other environmental factors affect plant growth, development and fitness. Plants have evolved intricate mechanisms to sense these environmental cues and trigger appropriate responses at the developmental and physiological level to maximise chances for survival and reproduction. These responses are driven by major changes in gene expression. Exposure to light, heat, cold or drought triggers vast transcriptional reprogramming in plants, which relies on shifts in the abundance and activity of transcription factors, epigenetic marks and chromatin structure (Baulcombe and Dean, 2014; Kim et al., 2015; Lämke and Bäurle, 2017; Strader et al., 2022). Yet, effects of these environmental signals reach far beyond transcription: post-transcriptional processes such as mRNA processing, transport, localisation, translation and turnover (Box 1; Fig. 1) are also tightly regulated in accordance with the plant’s surroundings.

Post-transcriptional control of mRNAs is mediated by trans-acting factors such as proteins and non-coding RNAs, but also relies on cis-acting elements in the mRNA, many of which reside in its untranslated regions (UTRs). UTRs, defined as the transcribed sequences 5' and 3' of an mRNA's main coding region, serve vital functions in controlling gene expression at multiple levels. In eukaryotes, preinitiation complexes (PICs) form at an mRNA’s 5’ end and scan along the 5’ UTR to identify translation initiation sites; the 5' UTR thus governs the initiation of protein synthesis. In yeast, for instance, 5’ UTR polymorphisms and isoforms can result in up to 1,000-fold differences in translation activity (Niederer et al., 2022), although such dramatic changes have so far not been observed in other eukaryotic systems (Zhong et al., 2023). 3’ UTRs can also affect translation rates, and are main determinants of mRNA localisation and stability (Barrett et al., 2012; Mayr, 2017).

Together, these regions act as a regulatory interface, influencing the fate of mRNA molecules and ultimately shaping the abundance of the encoded proteins (Fig. 2A).

In this review, we present an overview of UTR features that affect gene expression post transcription in the context of a plant’s abiotic environment; we discuss specific examples of how such features regulate development and stress responses in accordance with the plant’s surroundings; and we highlight the largely untapped potential of UTRs to customise these responses for the breeding of climate-resilient crops.
UTR length and nucleotide composition

Average 5’ UTR length of 100-200 nucleotides is roughly constant across diverse taxonomic groups, while 3’ UTR length is more variable, ranging from an average of 200 nucleotides in many plants and fungi to 800 nucleotides in humans and other vertebrates (Mignone et al., 2002). In the model plant species Arabidopsis thaliana (Arabidopsis) and Oryza sativa (rice), UTRs constitute 18-19% of the entire transcriptome; considerable differences are seen in UTR length between the two species, with Arabidopsis harbouring generally shorter 5’ (155 bp v 259 bp) and 3’ UTRs (242 bp v 469 bp), even when only orthologous genes are considered (Srivastava et al., 2018). In Arabidopsis, GO terms related to abiotic signalling (including responses to salt and cold) are enriched among genes with short 5’ and 3’ UTR sequences, suggesting that UTR length can provide some level of functional specificity. A similar relationship however has not been observed in rice, suggesting that this is not a universal phenomenon (Srivastava et al., 2018).

High GC content of the 5’ UTR, and consequently a highly negative normalised free energy of folding, has also been associated with gene function. In Arabidopsis, transcripts with GC-rich 5’ UTRs are poorly translated under environmental stresses such as darkness, hypoxia and dehydration, a process that may be linked to energy conservation (Branco-Price et al., 2005; Kawaguchi and Bailey-Serres, 2005; Juntawong and Bailey-Serres, 2012). Reduced translation of these transcripts is generally attributed to the 5’ UTRs’ higher potential for secondary structure formation, which may in turn interfere with 5’ UTR scanning by the PIC (Leppek et al., 2018). However, regulatory effects depend on the structures that are formed, and we will discuss specific examples further below.

UTR-binding proteins

RNA-binding proteins (RBPs) represent a large and highly diverse set of proteins and constitute crucial regulators of mRNA function. In Arabidopsis, over 2,700 potential RBPs have been identified, with 836 being detected as bona fide RNA-binding (Marondedze, 2020). The RNA-binding proteome is tightly regulated in response to environmental factors; multiple RBPs have been found to coordinate gene expression in response
to abiotic stimuli (Muthusamy et al., 2021), and several of these RBPs, which we discuss further below, bind preferentially or exclusively to UTRs.

The 5' UTR acts as a hub for the control of translation initiation through eukaryotic initiation factors (eIFs): the eIF3 complex mediates binding of the PIC to the mRNA; eIF4A, eIF4B and the cap-binding complex eIF4F assist in binding and have pivotal roles in mRNA unwinding during the scanning process; and eIF1, eIF1A and eIF5B are crucial factors for start site selection (Browning and Bailey-Serres, 2015; Merchante et al., 2017). Given that initiation is considered the rate-limiting step of the translation process (Dutt et al., 2015), many of these factors are likely to underpin the dramatic changes in global translation rates observed upon exposure to stimuli such as light (Juntawong and Bailey-Serres, 2012; Liu et al., 2012, 2013b), heat (Yánguez et al., 2013; Merret et al., 2015; Lukoszek et al., 2016; Chung et al., 2020), hypoxia and submergence (Branco-Price et al., 2005; Juntawong and Bailey-Serres, 2012; Lee and Bailey-Serres, 2019). However, given their importance for translation in general, few initiation factors have been assigned specific roles during environmental responses. A missense mutation in eIF5B1 results in impaired heat acclimation (Zhang et al., 2017), while a loss-of-function allele of eIFiso4G1 (which encodes a component of the eIF4F complex) displays increased sensitivity to submergence (Cho et al., 2019). The phosphorylation state of several initiation factors is also environmentally controlled: eIFiso4G1 undergoes hypoxia-induced phosphorylation by Snf1-RELATED PROTEIN KINASE 1 (SnRK1), a process thought to promote translation of hypoxia-induced transcripts (Cho et al., 2019), while light and CO2, presumably through their effect on photosynthetic assimilation, have been implicated in regulating the phosphorylation status of eIF3b, eIF4A, eIF4B and eIF4G (Boex-Fontvieille et al., 2013). In case of eIF4B and eIF4G, phosphorylation is likely catalysed by the TARGET OF RAPAMYCIN (TOR) kinase, a pivotal sensor of the cell’s energy status (Van Leene et al., 2019). The role of these phosphorylation events in the context of environmental responses, however, remains unclear.

Not all regulation at the 5' UTR is mediated through translation initiation factors. The cytosolic zinc finger protein PENTA 1 (PNT1) binds to the 5' UTR of the mRNA encoding PROTOCHLOROPHYLLIDE REDUCTASE A (PORA), an enzyme in the chlorophyll biosynthesis pathway that operates primarily in darkness. PNT1 recruits the active form of the red/far-red light phytochrome photoreceptor to the PORA mRNA and thereby prevents its translation after the dark-to-light transition (Fig. 2B) (Paik et al., 2012).

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3' UTRs are also targeted by multiple sets of RBPs including those mediating 3' processing and modification discussed further below; here, we focus on direct regulation of mRNA decay or translation. PUF/PUMILIO (PUM) proteins are a family of highly conserved eukaryotic RBPs that bind to specific 3' UTR motifs containing a UGUA core element and negatively regulate gene expression at the post-transcriptional level, affecting mRNA stability or translation (Joshna et al., 2020). Abiotic stresses such as salinity and dehydration induce \textit{ARABIDOPSIS PUM 5} (APUM5) expression, and APUM5 itself reduces abiotic stress resistance by downregulating the expression of stress tolerance genes (Huh and Paek, 2014). APUM9 and APUM23 on the other hand have been implicated in promoting heat and salt tolerance, respectively (Huang et al., 2018; Nyikó et al., 2019).

Bruno-like proteins constitute another group of RBPs that exert post-transcriptional control via 3' UTRs. Three members have been identified in Arabidopsis: FLOWERING CONTROL LOCUS A (FCA), BRUNO-LIKE 1 (BRN1) and BRN2 (Good et al., 2000). While FCA is involved in polyadenylation of mRNAs (see section below), BRN1 and BRN2 bind to specific response elements in the 3' UTR of the mRNA encoding the floral promoter SUPPRESSOR OF CONSTANS 1 (SOC1); their binding triggers decay of the SOC1 mRNA and thereby delays flowering (Kim et al., 2013). Expression of BRN1 and BRN2 is induced upon prolonged exposure to cold and hence contributes to the vernalisation-dependent control of flowering in Arabidopsis.

Other 3' UTR-binding RBPs are involved in the formation of stress granules, cytoplasmic biomolecular condensates of RNA and proteins that form when cells experience acute environmental stress. RNAs sequestered in stress granules are translationally repressed; they may later undergo targeted degradation but may also re-enter the translational cycle to ensure coordinated synthesis of selected proteins (Chantarachot and Bailey-Serres, 2018; Yan et al., 2022). OLGOURIDYLATED BINDING PROTEIN 1 (UBP1) is a core component of stress granules; it is uniformly distributed throughout the cytoplasm and nucleus during control conditions, but rapidly relocates into stress granules upon exposure to heat and hypoxia (Yan et al., 2022). UBP1c specifically appears to act as a molecular switch that interacts with selected target mRNAs depending on the environment: it interacts with U-rich 3' UTRs under non-stress conditions, but under hypoxia it binds preferentially to non-U-rich mRNAs and sequesters them into stress granules. These RNAs are rapidly released upon reoxygenation, allowing for a rapid transition between storage and translation (Sorenson and Bailey-Serres, 2014).
ACETYLMATION LOWERS BINDING AFFINITY (ALBA) proteins are another family of RBPs known to phase-separate into stress granules upon heat exposure (Náprstková et al., 2021; Tong et al., 2022). ALBA5 binding in Arabidopsis is enriched in 3' UTRs: it binds almost 3,000 transcripts under both control and heat stress conditions, but only two thirds of these transcripts were shared among the two RNA interactomes. Several mRNAs encoding heat shock factors and other components of the heat shock response are selectively bound by ALBA5 under heat stress; these mRNAs are destabilised in alba mutants, suggesting that ALBA proteins promote thermotolerance by stabilising these transcripts (Tong et al., 2022). How this change in ALBA5 binding specificity is controlled remains unknown; no RNA motifs were selectively enriched under stress versus control conditions (Tong et al., 2022).

UTR-binding RNAs

Proteins are not the only molecules acting in trans on UTRs: several non-coding RNAs exert regulatory function through binding of an mRNA’s 3’ or 5’ UTR. MiRNAs are small, approximately 21 nucleotide-long, non-coding RNAs that bind target mRNAs to interfere with their translation or to trigger their decay (Fabian et al., 2010). In animals, miRNA target sites are enriched in 3’ UTRs, and target sites in the 5’ UTR or coding region are generally considered to be less effective (Bartel, 2009). In plants, however, miRNA target sites appear to be equally effective in the 5’ UTR, coding region and 3’ UTR when translational repression is considered (Iwakawa and Tomari, 2013).

Cleavage of the PHOSPHATE 2 (PHO2) transcript is induced by binding of miR399 to its 5’ UTR (Chiou et al., 2006; Lin et al., 2008). PHO2 encodes an E2 ubiquitin-conjugating enzyme that maintains low levels of Pht1;4 and other phosphate transporters under control conditions (Huang et al., 2013; Park et al., 2014). MiR399 is induced under phosphate starvation, reduces PHO2-dependent degradation of these transporters and thereby raises intracellular phosphate concentrations (Bari et al., 2006; Chiou et al., 2006). MiR399 expression is also responsive to ambient temperature, and the miR399/PHO2 module was found to regulate flowering time in a temperature-dependent manner (Kim et al., 2011), suggesting that this signalling circuit has been rewired to control both physiological and developmental responses.
MiR156/157 represent another family of miRNAs that control flowering time, mainly through regulation of their direct targets, the mRNAs encoding SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) transcription factors (Wang et al., 2009). In case of SPL3, SPL4 and SPL5, the miRNA target site is located in the transcript’s 3’ UTR, and binding of miR156/157 causes cleavage and translational inhibition of the SPL3, and likely also SPL4 and SPL5, transcripts (Fig. 2F) (Wu and Poethig, 2006; Gandikota et al., 2007). While the miR156/157-SPL pathway constitutes foremost an age-dependent pathway (Wu et al., 2009; He et al., 2018), it also integrates environmental factors to regulate flowering time: a warming from 16 to 23 °C is sufficient to reduce miR156 and increase SPL3 levels, an effect that accelerates flowering at high temperature (Kim et al., 2012). Similarly, a low red:far-red light ratio, such as observed under a canopy or in dense vegetation, reduces miR156 levels and triggers early flowering (Xie et al., 2017).

While control of translation through miRNAs has been known for decades, recent evidence suggests that long non-coding RNAs (lncRNAs) can also regulate gene expression by interaction with a transcript’s UTR region: the rice cis-NATPHO1.2 antisense transcript is induced under phosphate starvation and enhances translation of its target mRNA; this process depends on structural rearrangements in the transcript’s 5’ UTR (Reis et al., 2021) and is discussed in detail further below.

Polyadenylation

While the mRNA binding partners discussed above directly control translation and decay of their target mRNAs, others specifically modify their targets’ UTRs and thereby regulate mRNA function. 3’ polyadenylation is a key modification of mRNAs: it is required for proper mRNA localisation, nuclear export, stability control and translation (Box 1). Consequently, alternative polyadenylation (APA) can affect mRNA metabolism and function (Xing and Li, 2011). Environmental stresses such as dehydration (Sun et al., 2017), hypoxia (de Lorenzo et al., 2017), salinity (Chakrabarti et al., 2020; Ma et al., 2022), nitrogen starvation (Conesa et al., 2020) and elevated temperature (Wu et al., 2020; Yan et al., 2021) globally change APA profiles of the plant’s transcriptome. Consistent with these findings, mutations in components of the cleavage and polyadenylation specificity factor (CPSF) complex, a core regulator of APA, result in altered responses to various abiotic stresses.
(Liu et al., 2014; Téllez-Robledo et al., 2019; Yu et al., 2019). Together, these observations suggest that APA may be a widespread mechanism to tailor gene expression to a plant’s surroundings.

Whereas global APA changes in response to external stimuli have been widely observed, specific examples that establish a causal link between environmentally controlled APA and defined developmental or physiological outputs remain scarce. Arabidopsis transcripts encoding AT3G47610 (a putative transcriptional regulator) and ANKYRIN REPEAT-CONTAINING PROTEIN 2 (AKR2) are alternatively polyadenylated under salt stress, and their APA requires a functional CPSF complex. T-DNA insertions that preclude the use of distal polyadenylation sites in these two genes increase seed germination under salt stress, suggesting that polyadenylation site selection affects salt tolerance (Yu et al., 2019). In the salt-tolerant monocot Spartina alterniflora, exposure to high salinity induces the use of distal polyadenylation sites in several ion transporter transcripts including HIGH-AFFINITY K⁺ TRANSPORTER 1 (SaHKT1), and extension of SaHKT1's 3’ UTR increases the transcript’s stability and translation (Fig. 2I) (Wang et al., 2023b). Similarly, several Arabidopsis transcripts that encode core regulators of the heat shock response such as HEAT SHOCK PROTEIN 70 (HSP70) and HSP17.6C display increased 3’ UTR length under heat stress, and selection of their distal polyadenylation sites appears to be required for protein synthesis (Wu et al., 2020).

APA also represents a core mechanism in the post-transcriptional control of flowering time through the floral repressor FLOWERING LOCUS C (FLC). FCA and FPA, two RBPs in the autonomous pathway – a flowering time pathway distinct from the well-defined day length-, temperature- and age-dependent pathways – regulate APA of FLC antisense transcripts collectively termed COOLAIR. FCA and FPA promote proximal polyadenylation of COOLAIR, and these proximally polyadenylated COOLAIR isoforms in turn downregulate FLC expression (Liu et al., 2007; Swiezewski et al., 2009; Hornyik et al., 2010). While their function was initially thought to be completely independent of the environment, we now know that FCA and FPA signalling intersects with temperature-dependent regulation of flowering time: COOLAIR expression is induced by cold (Swiezewski et al., 2009; Leon et al., 2023) and a switch from distal to proximal polyadenylation of COOLAIR occurs in response to vernalisation (i.e., prolonged cold exposure) (Csorba et al., 2014). Loss of FCA function also disrupts the flowering time response to elevated temperature (Blázquez et al., 2003), but whether this effect relies on RNA processing is yet unknown.
RNA modifications

RNA undergoes modifications at single nucleotides that alter RNA form and function, and over 160 different modifications have been reported (Boccaletto et al., 2018). Several of these modifications are present in plant mRNAs, but specific roles have been assigned to few of them: N7-methylguanosine (m\(^7\)G) is part of the mRNA’s 5’ cap structure and hence a key determinant of mRNA stability and translation (Box 1), while N6-methyladenosine (m\(^6\)A) and 5-methylcytosine (m\(^5\)C) are found across the entire mRNA molecule and affect a variety of processes including mRNA transport, processing, translation and decay (Chmielowska-Bąk et al., 2019; Shen et al., 2019; Liang et al., 2020). In Arabidopsis, most m\(^5\)C sites have been detected in the coding region (Cui et al., 2017; David et al., 2017), while m\(^6\)A sites are enriched in 3’ UTRs and, arguably, around start and stop codons (Luo et al., 2014; Shen et al., 2016; Anderson et al., 2018; Parker et al., 2020). In plants, m\(^6\)A interferes with site-specific RNA cleavage and promotes transcript stability (Anderson et al., 2018; Parker et al., 2020). In vitro studies using mammalian translation components revealed that m\(^6\)A in a transcript’s 5’ UTR promotes cap-independent translation through direct recruitment of eIF3 (Meyer et al., 2015), but whether this process occurs in plants is yet unknown.

Global m\(^6\)A patterns in plant transcriptomes are highly dynamic and change in response to light (Artz et al., 2023, Preprint), temperature (Liu et al., 2020; Wang et al., 2023a), salinity (Anderson et al., 2018; Kramer et al., 2020) and drought (Zhang et al., 2021). In agreement with these findings, mutants in many m\(^6\)A writers, readers and erasers – proteins that incorporate, recognise and remove m\(^6\)A, respectively – impact the plant’s sensitivity to these environmental signals (Dhingra et al., 2023). FIONA1 (FIO1) is an Arabidopsis m\(^6\)A methyltransferase that installs m\(^6\)A in U6 small nuclear RNAs, but also in a small number of polyadenylated RNAs (Wang et al., 2022). A knock-out of FIO1 results in increased hypocotyl elongation under red and far-red light and in early flowering (Kim et al., 2008; Wang et al., 2022). These phenotypic changes are underpinned by altered methylation rates in the mRNAs encoding the blue light photoreceptor CRYPTOCHROME 2 (CRY2), the transcription factor PHYTOCHROME INTERACTING FACTOR 4 (PIF4, a core component of phytochrome-dependent light and temperature signalling), the floral promoter CONSTANS (CO) and the floral repressor FLC, and FIO1 was shown to directly methylate these mRNAs. Interestingly, methylation leads to a stabilisation of
FLC mRNA, but destabilises the mRNAs of CRY2, PIF4 (Fig. 2G) and CO (Wang et al., 2022), contrary to the notion that m^6^A modifications generally promote transcript stability.

In Arabidopsis, the majority of mRNAs are m^6^A-methylated by a writer complex consisting of the mRNA adenosine methyltransferase MTA, its closest homolog MTB, FKBP12 INTERACTING PROTEIN 37KD (FIP37), VIRLIZER (VIR) and HAKAI (Shen et al., 2019). The blue light receptors cry1 and cry2 interact with MTA and MTA, MTB and FIP37, respectively, and approximately 10% of mRNAs are m^6^A-modified in response to blue light in a cry-dependent manner (Wang et al., 2021a). While their interaction is light-independent, blue light promotes co-condensation of the cry/writer complex into photobodies – biomolecular condensates formed by many components of the light signalling pathway – thereby potentially increasing local concentration and activity of the writer complex (Wang et al., 2021a).

MTA also contributes to cold acclimation: while m^6^A modification decreases in the cold, this effect is exacerbated in an MTA RNAi line and alters translation efficiency for approximately one third of cold-regulated transcripts. One of these transcripts encodes ACYL-CoA:DIACYLGLYCEROL ACYLTRANSFERASE 1 (DGAT1), and dgat1 loss-of-function mutants, like the MTA RNAi line, show reduced growth in the cold (Wang et al., 2023a). Heat, on the other hand, increases overall m^6^A modification in flowers, but not leaves, of Arabidopsis. This increase negatively impacts variability in gene expression, which has been suggested to underpin reduced fertility rates at elevated temperature (Wang et al., 2022). While changes in m^6^A modification are clearly relevant for heat and cold sensitivity, it remains unknown how the observed transcriptome-wide changes are brought about.

**RNA secondary structures**

RNA has the capacity to fold into a variety of secondary and tertiary structures such as hairpins, apical and internal loops, junctions and pseudoknots (Cruz and Westhof, 2009). Folding depends directly on a structure’s thermodynamic free energy, and only the most energetically favourable conformations will be realised under any given condition. Consequently, RNA structure is highly sensitive to its environment, and changes in temperature and osmolarity directly impart wide structural changes across plant transcriptomes (Su et al., 2018; Tack et al., 2020; Yang et al., 2022). In vivo structure probing revealed that heat shock triggers global transcript unfolding in rice, and unfolding is inversely correlated with transcript abundance (Su et al., 2018).
Arabidopsis, the formation of RNA G-quadruplexes – non-canonical secondary structures formed by G-rich nucleic acid sequences – is globally enhanced in response to cold, and their formation promotes mRNA stability (Yang et al., 2022). Notably, the correlation between structure formation and mRNA abundance found in these studies was strongest in 3’ UTRs (Su et al., 2018; Yang et al., 2022), emphasising that the 3’ UTR is a strong determinant of mRNA stability. Yang et al. (2022) were also able to link a G-quadruplex in the transcript encoding the translocase subunit protein COLD RESPONSIVE RNA G-QUADRUPLEX 1 (CORG1) to cold-responsive growth: presence of the G-quadruplex is essential for CORG1-mediated repression of root growth at 4 °C as well as for the expression of cold-induced genes (Fig. 2H).

RNA structural changes under salt stress appear to be more diverse, with structure probing in Arabidopsis suggesting a slight overall increase in RNA structure formation in the shoot, but a decrease in the root. As with temperature effects, unfolded transcripts exhibit reduced abundance under salt stress, but in this case structures in coding sequences were found to be most relevant for transcript stability, followed by the 5’ UTR and the 3’ UTR (Tack et al., 2020). Transcript unfolding under salt stress has been linked to increased m⁶A methylation (Kramer et al., 2020), although this study did not observe a correlation between structure formation and transcript abundance. In any case, a direct link between salinity-induced structural changes and the salt stress response has yet to be established.

Besides their effect on mRNA abundance, structural rearrangements can also affect mRNA translation. This type of regulation is well established in prokaryotes, where dynamic structures such as RNA thermometers and riboswitches have been described to regulate translation initiation (Winkler and Breaker, 2005; Kortmann and Narberhaus, 2012), but similar examples are rare in plants. In the unicellular green alga Chlamydomonas reinhardtii, an RNA thermometer controls translation of the chloroplastic psaA mRNA: This structure forms across and thereby masks the transcript’s Shine-Dalgarno sequence (the prokaryotic ribosome binding site) at standard temperatures, but melts at elevated temperatures of 40 °C, enhancing protein synthesis (Chung et al., 2023). A thermosensitive hairpin has also been identified in the 5’ UTR of the Arabidopsis transcript encoding the transcription factor PIF7, but appears to operate differently from prokaryotic RNA thermometers: this RNA thermoswitch adopts a more relaxed conformation at elevated temperature that enhances PIF7 translation, although the underlying mechanism is not fully understood (Fig. 2E) (Chung et al., 2020). Elevated PIF7 protein levels subsequently trigger cell elongation and a more open growth habit to
promote the plant’s cooling capacity (Chung et al., 2020; Fiorucci et al., 2020). Similar hairpin structures enhance the translation of transcription factors WRKY22 and HEAT SHOCK FACTOR 2A (HSFA2) in vitro, but their function in vivo has not been tested (Chung et al., 2020).

RNA structural rearrangements can also be tailored to the plant’s light environment: translation of the plastidic psbA RNA, which encodes the photosystem protein D1, increases under high light due to structural changes in the 5’ UTR (Gawroński et al., 2021). It has been proposed that these changes are brought about by an RBP, but experimental evidence for this has yet to be obtained.

Binding of other molecules can trigger structural rearrangements to promote translation. Expression of the non-coding natural antisense transcript (NAT) cis-NATPHO1.2 in rice is induced under phosphate starvation and enhances translation of the OsPHO1.2 sense transcript (Jabnoune et al., 2013). OsPHO1.2 encodes the main root transporter loading phosphate into the xylem for distribution across the plant and is essential for phosphate homeostasis (Secco et al., 2010). In the absence of cis-NATPHO1.2, an GC-rich inhibitory region 350 bp downstream of the initiation codon was found to restrict translation, likely through local interactions with upstream regions in the 5’ UTR. Binding of cis-NATPHO1.2 triggers rearrangements that enhance access for the large ribosomal subunit to the initiation codon (Fig. 2D), increasing the formation of 80S ribosomal complexes (Reis et al., 2021). These observations present a first mechanistic insight into the activity of plant antisense transcripts on translation.

**Upstream open reading frames**

Upstream open reading frames (uORFs) are short translated sequences found in a transcript’s 5’ UTR (Zhang et al., 2020). Present in eukaryotic organisms ranging from yeast (Lin et al., 2019) to humans (McGillivray et al., 2018), uORFs have been identified in over 30% of mRNAs in Arabidopsis, 29% in maize, and 6% in tomato (Zhao et al, 2020). The vast majority of uORF-encoded amino acid sequences are not evolutionarily conserved, suggesting that the peptide sequences of translated uORFs serve little regulatory function (Hayden and Jorgensen, 2007). However, so-called Conserved Peptide uORFs (CPuORFs), estimated at less than 1% of all uORFs, are significantly enriched in the UTRs of transcription factor-encoding mRNAs and may thus present important regulators of transcription (Hayden and Jorgensen, 2007).
Acting as cis-regulatory elements, uORFs can alter the translation of main ORFs (mORFs), thereby impacting gene expression. Some uORFs cause ribosomes to stall while others trigger a complete dissociation of the ribosome once the uORF’s stop codon is reached, both effectively reducing the translation of a transcript’s mORF. Additionally, stalling or early termination may be recognised as a sign of abnormal translation, triggering nonsense-mediated decay (NMD) of the mRNA (von Arnim et al., 2014; Zhang et al., 2020). Though rarer, uORFs can also increase the rate of mORF translation (Lin et al., 2019). In many cases, uORFs do not act as an on/off switch for translation, but represent a fine-tuning mechanism for the control of gene expression (McManus et al., 2014; Moro et al., 2021) and were recently shown to contribute to noise reduction in gene expression (Wu et al., 2022).

Translation of uORF-containing transcripts can be regulated by abiotic stresses: expression of Arabidopsis HEAT SHOCK FACTOR B1 (HSFB1) is suppressed by a uORF under control temperatures, and this suppression is lost under heat stress, boosting HSFB1 protein levels and the heat shock response (Zhu et al., 2012). Additionally, uORFs have been found in 7 of the 21 members of the Arabidopsis HSF family, though their functional relevance is not yet known (Zhu et al., 2012; Guo et al., 2016). CPuORFs have also been implicated in heat and drought responses: when incorporated in luciferase reporter constructs, Arabidopsis CPuORF19 and CPuORF47 strongly increase reporter gene expression under water limitation, while CPuORF46 promotes translation in response to heat (Causier et al., 2022). CPuORF47 is part of an mRNA encoding a putative methyltransferase that promotes drought tolerance when overexpressed; however, whether CPuORF47 or the other two CPuORFs are required for the regulation of drought or heat tolerance in planta remains unknown (Causier et al., 2022).

In addition to the regulation of abiotic stress responses, uORFs impact the uptake and distribution of nutrients. A uORF in NOD26-LIKE INTRINSIC PROTEIN 5;1 (NIP5;1), which codes for a boron transporter, suppresses the transcript’s translation and promotes its degradation at high boron concentrations to minimise toxic effects of boron within the cell (Tanaka et al., 2016). Multiple uORFs also directly or indirectly control the abundance of phosphate transporters and thereby impact phosphate acquisition and distribution in Arabidopsis (Reis et al., 2020), rice (Yang et al., 2020) and soybean (Guo et al., 2022).
Several uORFs influence the expression of developmental regulators in response to light. Genes encoding the photomorphogenesis-promoting transcription factors ELONGATED HYPOCOTYL (HY5) and HYS HOMOLOGUE (HYH) are transcribed at a higher rate from a downstream transcriptional start site under blue light compared to dark conditions. This leads to the inclusion of uORFs in the respective transcripts, causing an overall reduction of mORF translation (Kurihara et al., 2018). While the importance of HY5 and HYH uORFs has not been investigated at the phenotypic level, CPuORF33 in ARABIDOPSIS THALIANA HOMEBOX 1 (ATHB1) was shown to control multiple aspects of plant development. ATHB1 is a transcription factor with known roles in light signalling and leaf development (Aoyama et al., 1995), and Arabidopsis plants expressing an ATHB1 transcript with a mutated CPuORF33 displayed a phenotype similar to plants overexpressing ATHB1, including serrated leaves, delayed bolting and senescence as well as shorter siliques and reduced seed set (Romani et al., 2016; Ribone et al., 2017). The effect of CPuORF33 is tissue-specific: it represses ATHB1 translation selectively in aerial tissues in response to light, and this process involves a yet unknown signal from the chloroplast (Ribone et al., 2017).

How environmental signals can affect uORF (and thereby mORF) translation remains an open question. Metabolites such as polyamines, sucrose and ascorbate regulate gene expression via uORFs, and it has been proposed that these molecules are directly sensed by the ribosome and act as cofactors for ribosomal stalling (van der Horst et al., 2020). The rate of reinitiation after uORF translation is also tightly regulated: initiation factor eIF3h is essential for reinitiation (Zhou et al., 2010) and its phosphorylation status is controlled by the TOR pathway (Schepetilnikov et al., 2013), which balances plant growth and stress responses. Stress also promotes phosphorylation of eIF2α, which results in ribosomal read-through of uORFs (Young and Wek, 2016), a mechanism controlling the growth-to-defense transition in Arabidopsis (Pajerowska-Mukhtar et al., 2012). Causal links between any of these phosphorylation events and the perception of abiotic cues however have yet to be established.

**Alternative splicing**

Alternative splicing (AS) due to the selection of alternative splice sites, intron retention or exon skipping can generate multiple mRNA isoforms from a single gene and thereby contributes to transcriptome and proteome diversity (Reddy et al., 2013). AS in UTRs often has profound consequences for a transcript’s fate within the...
cell: intron retention in the 5’ UTR can insert uORFs and other cis elements that impact translational efficiency (Jacob and Smith, 2017). In Arabidopsis, intron retention events in the 5’ UTR are increased under biotic and abiotic stress and are particularly enriched in genes harbouring uORFs, suggesting this as a common mechanism to fine-tune translation in response to the plant’s environment (Martín et al., 2021). While AS events in the 3’ UTR can also affect translation, they have a particularly strong effect on transcript stability: unusually long 3’ UTRs (> 300-350 bp) and the presence of splice junctions more than 50 bp downstream of the stop codon act as quality control signals for cytosolic mRNAs, triggering NMD of the respective mRNA (Shaul, 2015).

Changes in the light environment trigger substantial changes in AS: two transcriptome-wide studies detected 700 and 2,200 AS events, respectively, when dark-grown Arabidopsis seedlings were exposed to red, blue or white light (Shikata et al., 2014; Hartmann et al., 2016), while an acute light pulse during the night changed AS for almost 400 transcripts (Mancini et al., 2016). In the moss Physcomitrium patens, exposure of dark-adapted protonemata to red or blue light leads to AS in almost 50% of transcripts in either condition (Wu et al., 2014). In monochromatic light, AS strongly depends on activity of phytochrome A (phyA) and phyB photoreceptors, while under white light, control of AS may be more strongly coupled to energy supply (Shikata et al., 2014; Hartmann et al., 2016).

Several light signalling components are regulated by light-dependent AS in their 5’ UTR: under continuous red light, phyB induces retention of an intron in the 5’ UTR of the mRNA encoding the Arabidopsis transcription factor PIF3. In turn, uORF sequences within this intron attenuate PIF3 translation and thereby reduce PIF3-mediated repression of light signalling, resulting in decreased hypocotyl elongation (Fig. 2C) (Dong et al., 2020). In the Physcomitrium transcript encoding transcription factor HYH2 (PpHYH2), a positive regulator of light responses, blue and red light reduce intron retention in its 5’ UTR; the effect of these retention events is unclear, but may involve production of an N-terminally extended protein as well as uORF-mediated repression of translation (Wu et al., 2014). How AS events are controlled by light is not completely understood, but involves the splicing factors REDUCED RED-LIGHT RESPONSES IN CRY1 CRY2 BACKGROUND 1 (RRC1) and SPlicing Factor for Phytochrome Signalling (SFPS). Both proteins interact with phyB and promote phyB-mediated AS (Shikata et al., 2012; Xin et al., 2017, 2019). Interestingly, RRC1 undergoes light-dependent AS itself: inclusion of the third exon produces a transcript with a premature stop codon and a very long 3’ UTR.
that is subject to NMD. Light reduces inclusion of exon 3 and hence increases the levels of functional RRC1 protein (Shikata et al., 2014).

Temperature changes also induce wide-spread AS. Cold triggers AS in more than 2,400 Arabidopsis genes when compared to 20 °C levels (Calixto et al., 2018). One of the transcripts undergoing AS in response to cold encodes REGULATOR OF CBF EXPRESSION 1 (RCF1), an RNA helicase required for cold tolerance (Guan et al., 2013). Its transcript isoforms differ in their 3’ UTRs, and consequences may involve retention of transcripts in the nucleus as well as NMD, but they have not been investigated experimentally (Calixto et al., 2018). In some cases, a drop in temperature by as little as 2 °C can trigger AS events: intron retention in the 5’ UTR of the circadian clock component LATE ELONGATED HYPOCOTYL (LHY) is scalable between 20 and 4 °C and has been suggested to function as a molecular thermostat (James et al., 2018a). Decreasing temperatures result in a gradual increase in intron retention in the LHY 5’ UTR, contributing to an overall reduction in LHY transcript levels via NMD (James et al., 2012, 2018a).

Mildly elevated temperature triggers comparatively fewer AS events, which include several key flowering time regulators, the most prominent being FLOWERING LOCUS M (FLM) (Verhage et al., 2017). FLM produces two competing protein splice variants, FLM-β and FLM-δ, both of which bind to the floral repressor SHORT VEGETATIVE PHASE (SVP). Under control temperatures, an SVP–FLM-β complex prevents flowering by repressing the floral integrator gene SOC1. Elevated temperature increases the ratio of FLM-δ to FLM-β; FLM-δ however is impaired in DNA binding and was thus thought to accelerate flowering by forming an inactive complex with SVP (Posé et al., 2013). Later observations however challenged the role of FLM-δ because plants solely expressing FLM-δ were undistinguishable from an fml knock-out (Capovilla et al., 2017). Sureshkumar et al. (2016) showed that high temperature induces formation of multiple splice variants that undergo NMD and hence accelerate flowering simply by reducing levels of FLM-β. Several variants display AS selectively in the 3’ UTR, and this agrees with the observation that naturally occurring polymorphisms in the FLM 3’ UTR affect the temperature sensitivity of flowering (Sureshkumar et al., 2016).

Other abiotic factors influence AS patterns, but specific examples of functionally relevant AS events in the 5’ or 3’ UTR are limited. Salt stress as well as sudden illumination cause a shift in PHYTOENE SYNTHASE (PSY) splicing in Arabidopsis, favouring the accumulation of a variant with a shorter 5’ UTR. This variant lacks a translation-
inhibitory structure, increases PSY protein levels and thus allows for fast production of carotenoids under stress (Álvarez et al., 2016). A reverse mechanism operates in the transcript encoding the Zn transporter ZINC-INDUCED FACILITATOR 2 (ZIF2): here, zinc induces intron retention in the 5' UTR, and the retained sequence forms a stable stem loop immediately upstream of the start codon, which enhances translation. Consequently, selectively expressing the long ZIF2 splice variant enhances plant zinc tolerance (Remy et al., 2014). These examples highlight that post-transcriptional mechanisms can act cooperatively in the control of gene expression.

**UTRs as regulatory hubs for the environmental control of gene expression**

In the preceding sections, we highlighted the important role 5’ and 3’ UTR sequences play in the control of gene expression, and we discussed multiple post-transcriptional mechanisms operating via UTRs to tune the amount of protein made from the respective transcripts. 5’ UTRs are major regulators of translation, providing a platform for the binding of translation initiation factors, but also affecting ribosomal scanning and translation initiation through cis elements such as uORFs and RNA secondary structures. 3’ UTRs, on the other hand, harbour binding sites for many RBPs involved in mRNA sequestration and turnover as well as cis elements that define the site of polyadenylation, another strong determinant of mRNA stability.

Providing evidence for the functional relevance of UTR regulatory elements also remains a challenging task. Recent advancements in next generation sequencing techniques revealed that changes in the environment can trigger post-transcriptional processes that affect hundreds of transcripts. Examples for which environmental regulation through UTRs has been causally linked to a developmental or physiological output, however, are far and few between (Table 1) when compared with examples of transcriptional control. Transcriptome-wide studies thus need to be supplemented by detailed gene-focused analyses to prove functional relevance of post-transcriptional effects, but these require investment of additional time and resources. The issue is further complicated by the fact that some post-transcriptional mechanisms fine-tune or buffer gene expression against external influences and do not act as major on/off switches of protein production (Guerra et al., 2015; Prall et al., 2019; Moro et al., 2021; Zhong et al., 2023); therefore, removing components of these regulatory systems may have minor consequences at the developmental or physiological level. In such cases,
computational modelling may be required to fully appreciate the complexity and importance of gene expression regulation (Becker et al., 2018; Furlan et al., 2021).

Post-transcriptional mechanisms operating via UTRs are highly sensitive to the plant’s environment, but how environmental signals selectively affect processes such as RNA modification, splicing or translation in a transcript-specific manner often remains enigmatic. In some cases, trans-acting components such as RBPs or miRNAs are regulated by environmental factors and confer this regulation onto their target mRNAs, which are recognised via specific binding sites. In other cases, the UTR itself exhibits environmental responsiveness: this is best exemplified by the effects of RNA secondary structures such as hairpins or G-quadruplexes, whose formation is directly dependent on temperature and osmolarity and can in turn affect accessibility of binding sites or enzymatic processing of the RNA. Understanding the relationship between environmental input and molecular output is essential to exploit these mechanisms for improving plants’ resilience to a changing environment.

**UTRs – a means to promote environmental resilience in crops?**

Beyond elucidating the regulatory mechanisms through which UTR elements operate, a major goal is to harness these mechanisms for crop improvement. With the progression of climate change, crops are exposed to hostile environments that include cold and heat waves, drought, high salinity and nutrient scarcity. While changing the expression of key development and stress regulators is at the core of many efforts to render plants more resilient to such conditions, they largely rely on modifying gene transcription. Yet, employing post-transcriptional mechanisms represents a promising expansion of the plant breeding toolbox – these mechanisms act directly on existing transcripts and thus allow for rapid and dynamic responses to the plant’s surroundings, and modification in UTR elements can nowadays be easily accomplished using gene editing techniques (Zhang et al., 2018a).

A direct way to exploit UTR function for crop improvement is to take advantage of natural variation present in UTRs. In Arabidopsis, UTR polymorphisms have been linked to temperature adjustments within the circadian clock (James et al., 2018b), thermo-responsiveness of growth (Wang et al., 2021b) and root hydraulic conductivity in the context of drought tolerance (Tang et al., 2018). Natural variation in UTRs is also a widespread phenomenon in crop species, and several polymorphisms have been linked to stress- and yield-related
traits: in maize, a 4 bp deletion in the 3’ UTR of Na⁺ CONTENT UNDER SALINE-ALKALINE CONDITION 1 (ZmNSA1) promotes saline-alkaline tolerance (Cao et al., 2020) while deletion of an ER stress response element in the 5’ UTR of the protein phosphatase-encoding transcript ZmPP2C-A and a single nucleotide polymorphism in the 5’ UTR of DEHYDRATION RESPONSIVE ELEMENT BINDING PROTEIN 27 (ZmdREB27) enhanced drought tolerance (Liu et al., 2013a; Xiang et al., 2017). Polymorphisms in uORFs have also been linked to changes in phosphate acquisition in Arabidopsis (Reis et al., 2020) and soybean (Guo et al., 2022) and globally to whole-plant phenotypic diversity in maize (Gage et al., 2022). Thus, UTR polymorphisms represent a promising genetic resource for conventional breeding, and increasing our understanding of post-transcriptional regulation through UTRs will facilitate selection for specific cis-regulatory UTR elements that convey desired crop traits.

Gene editing represents an alternative approach to exploit UTRs for crop improvement as it allows for the precise modification of cis-regulatory elements in situ (Zhang et al., 2018a). Engineering uORF sequences has already been used to improve vitamin C content in lettuce (Zhang et al., 2018b) and sugar content in strawberry (Xing et al., 2020), to increase variation in height and tiller number (Xue et al., 2023) and to boost broad-spectrum disease resistance in rice (Xu et al., 2017). Other UTR modifications include the editing of splice sites in the WAXY 5’ UTR to improve rice grain quality (Zeng et al., 2020) and efforts to implement artificial riboswitches to control gene expression in planta (Bocobza and Aharoni, 2014). Cis-regulatory elements of viruses have also been employed to alter gene expression in plants; the Omega element from tobacco mosaic virus has been widely used to boost translation of transgenes (Gallie, 2002), while recently, a targeted knock-in of a translational enhancer from alfalfa mosaic virus has been used to enhance salt tolerance in rice (Shen et al., 2023). The approaches discussed above aimed at constitutive changes in gene expression, and evidence that genetic modification of UTRs can tailor such changes to specific environmental conditions has yet to be obtained. Nevertheless, as our mechanistic insight into the function of cis-regulatory UTR elements increases, we expect many more of these to be targeted by breeding and gene editing efforts in the future, with particular focus on environmental resilience.
Author contributions

MB: conceptualisation; ECH, MB: writing; MB: figure design.

Conflict of Interest

The authors declare no conflict of interest.

Funding

MB and his lab are supported by the Royal Society (URF\R1\211672) and the BBSRC (BB/Y001672/1). ECH is supported by the BBSRC EASTBIO Doctoral Training Partnership.

Data availability

There are no primary data associated with this manuscript.
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Ribone PA, Capella M, Arce AL, Chan RL. 2017. A uORF Represses the Transcription Factor AtHB1 in Aerial Tissues to Avoid a Deleterious Phenotype. Plant Physiology 175, 1238–1253.


Table 1. Transcripts whose function is environmentally controlled via their UTRs. This table only contains transcripts for which functional relevance of UTR-mediated regulation in response to specific environmental signals has been shown.

<table>
<thead>
<tr>
<th>Transcript</th>
<th>Regulatory mechanism</th>
<th>Environmental stimulus</th>
<th>Developmental/physiological output</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKR2</td>
<td>APA</td>
<td>Salinity</td>
<td>Salt tolerance</td>
<td>Yu et al. (2019)</td>
</tr>
<tr>
<td>AT3G47610</td>
<td>APA</td>
<td>Salinity</td>
<td>Salt tolerance</td>
<td>Yu et al. (2019)</td>
</tr>
<tr>
<td>ATHB1</td>
<td>uORF</td>
<td>Light</td>
<td>Leaf development</td>
<td>Ribone et al. (2017)</td>
</tr>
<tr>
<td>CO</td>
<td>m^6Ä methylation</td>
<td>Light</td>
<td>Flowering</td>
<td>Wang et al. (2022)</td>
</tr>
<tr>
<td>COOLAIR</td>
<td>APA</td>
<td>Temperature</td>
<td>Flowering</td>
<td>Csorba et al. (2014)</td>
</tr>
<tr>
<td>CORG1</td>
<td>RNA structure</td>
<td>Temperature</td>
<td>Root growth</td>
<td>Yang et al. (2022)</td>
</tr>
<tr>
<td>CRY2</td>
<td>m^6Ä methylation</td>
<td>Light</td>
<td>Hypocotyl elongation; flowering</td>
<td>Wang et al. (2022)</td>
</tr>
<tr>
<td>DGAT1</td>
<td>m^6Ä methylation</td>
<td>Cold</td>
<td>Growth</td>
<td>Wang et al. (2023a)</td>
</tr>
<tr>
<td>FLC</td>
<td>m^6Ä methylation</td>
<td>Light</td>
<td>Flowering</td>
<td>Wang et al. (2022)</td>
</tr>
<tr>
<td>FLM</td>
<td>AS</td>
<td>Temperature</td>
<td>Flowering</td>
<td>Posé et al. (2013); Sureshkumar et al. (2016)</td>
</tr>
<tr>
<td>HSFB1</td>
<td>uORF</td>
<td>Temperature</td>
<td>Heat shock response</td>
<td>Zhu et al. (2012)</td>
</tr>
<tr>
<td>LHY</td>
<td>AS</td>
<td>Temperature</td>
<td>Circadian clock</td>
<td>James et al. (2018a)</td>
</tr>
<tr>
<td>NIP5:1</td>
<td>uORF</td>
<td>Boron</td>
<td>Boron tolerance</td>
<td>Tanaka et al. (2016)</td>
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<tr>
<td>OsPHO1.2</td>
<td>Interaction with cis-NATPHO1.2; RNA structure</td>
<td>Phosphate</td>
<td>Phosphate homeostasis</td>
<td>Reis et al. (2021)</td>
</tr>
<tr>
<td>PHO2</td>
<td>Interaction with miR399</td>
<td>Phosphate; temperature</td>
<td>Phosphate acquisition; flowering</td>
<td>Bari et al. (2006); Chiou et al. (2006); Kim et al. (2011)</td>
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<tr>
<td>PIF3</td>
<td>AS, uORF</td>
<td>Light</td>
<td>Hypocotyl elongation</td>
<td>Dong et al. (2020).</td>
</tr>
<tr>
<td>PIF4</td>
<td>m^6Ä methylation</td>
<td>Light</td>
<td>Hypocotyl elongation; flowering</td>
<td>Wang et al. (2022)</td>
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<tr>
<td>PIF7</td>
<td>RNA structure</td>
<td>Temperature</td>
<td>Elongation growth; stomata formation</td>
<td>Chung et al. (2023)</td>
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<td>PORA</td>
<td>Interaction with PNT1 and phytochrome</td>
<td>Light</td>
<td>Chlorophyll biosynthesis</td>
<td>Paik et al. (2012)</td>
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<tr>
<td>PSY</td>
<td>AS</td>
<td>Light; salinity</td>
<td>Carotenoid biosynthesis</td>
<td>Alvarez et al. (2016)</td>
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<tr>
<td>SaHKT1</td>
<td>APA</td>
<td>Salinity</td>
<td>Salt tolerance</td>
<td>Wang et al. (2023b)</td>
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<tr>
<td>SOC1</td>
<td>Interaction with BRN1/BRN2</td>
<td>Temperature</td>
<td>Flowering</td>
<td>Kim et al. (2013)</td>
</tr>
<tr>
<td>SPL3</td>
<td>Interaction with miR156/157</td>
<td>Light; temperature</td>
<td>Flowering</td>
<td>Gandikota et al. (2007); Kim et al. (2012); Xie et al. (2017)</td>
</tr>
<tr>
<td>ZIF2</td>
<td>AS, RNA structure</td>
<td>Zinc</td>
<td>Zinc tolerance</td>
<td>Remy et al. (2014)</td>
</tr>
</tbody>
</table>

*The prefix Os refers to Oryza sativa, the prefix Sa refers to Spartina alterniflora; all other transcripts stem from Arabidopsis thaliana.*
Boxes

Box 1. Post-transcriptional control of gene function in plants

Gene expression is tightly controlled at the transcriptional and post-transcriptional level, with the term “post-transcriptional” usually referring to regulatory processes occurring at the level of mRNA (Fig. 1). They are controlled by a plethora of RNA-binding proteins (RBPs) that associate with the nascent RNA as soon as it emerges from RNA polymerase II; in fact, some RNA processing steps occur while the transcript is still associated with RNA polymerase II and can thus be considered co-transcriptional (Marquardt et al., 2023).

5' capping: The 7-methylguanosine (m^7G) cap structure, linked to the first RNA nucleoside through a 5'-5' triphosphate bridge, is formed through the subsequent activity of RNA 5' triphosphatase, RNA guanylyltransferase and RNA (guanine-N7) methyltransferase; it protects the RNA from degradation by 5' exonucleases and facilitates translation initiation (Shuman, 2002).

3' polyadenylation: Cis-acting elements (the far and near upstream elements and the cleavage element) in the 3' UTR define the site at which the cleavage and polyadenylation complex cleaves the mRNA and adds a stretch of adenosine nucleotides. This poly(A) tail is critical for nuclear export, translation and stability of the mRNA (Bernardes and Menossi, 2020; Yang et al., 2021).

Splicing: The removal of introns and ligation of exons is essential to produce a translation-competent mRNA. It is catalysed by a macromolecular ribonucleoprotein complex, the spliceosome, which is assembled anew for each splicing event upon recognition of core cis-acting elements including the 3' splice site with a conserved GU and the 5' splice site with a conserved AG nucleotide pair (Lorković et al., 2000; Reddy et al., 2013).

RNA modification and editing: The term RNA modification denotes chemical modifications of single nucleotides, collectively referred to as the epitranscriptome. Known modifications in Arabidopsis include m^7G, N6-methyladenosine (m^6A), N1-methyladenosine (m^1A), 5-methylcytosine (m^5C), 5-hydroxymethylcytosine (hm^5C) and uridylation. These modifications can affect most aspects of mRNA metabolism including splicing, nuclear export, translation and turnover (Shen et al., 2019). The term RNA editing refers to enzymatic changes of the primary nucleotide sequence; in flowering plants, it appears to be restricted to U-to-C editing in mitochondria and plastids (Takenaka et al., 2013).
**Nuclear export:** Mature mRNAs are exported from the nucleus as a tightly packaged mRNA-ribonucleoprotein complex. Translocation through nuclear pore complexes, mediated by transport receptor proteins, represents a major route of nuclear export, but mechanisms involving vesicular transport also exist (Natalizio and Wente, 2013).

**RNA localisation:** Once exported from the nucleus, mRNA localisation is determined by cis-acting “zipcode” elements; many mRNAs are targeted to the endoplasmatic reticulum for translation, but also to mitochondria, plastids or plasmodesmata (Tian et al., 2020). Additionally, RBPs can sequester mRNAs into biomolecular condensates such as processing bodies and stress granules, where translationally inactive mRNAs may be stored for rerelease or subjected to degradation (Kearly et al., 2022).

**RNA turnover:** Degradation of messenger RNAs (mRNAs) is required for both mRNA quantity and quality control. Several pathways contribute to mRNA decay: (1) bulk degradation via exoribonucleases (XRNs) or the exosome complex after deadenylation; (2) co-translational decay by XRN4; (3) mRNA quality control, which triggers RNA decay upon recognising transcripts with premature stop codons (nonsense-mediated decay, NMD), without stop codons (no-stop decay) or with stalled ribosomes (no-go decay); (4) miRNA-mediated cleavage (Zhang and Guo, 2017).

**Translation:** The process of translation is divided into steps of initiation, elongation and termination, with the main regulatory processes thought to occur during initiation. Eukaryotic initiation factor 4 (eIF4) recognises the 5’ cap structure and, aided by poly(A) binding proteins, circularises the mRNA molecule. Subsequently, the preinitiation complex (PIC), consisting of the small ribosomal subunit, initiation factors eIF1, eIF1A, eIF3, eIF5 and a ternary complex of eIF2, GTP and the initiator Met-tRNA, is recruited to the mRNA and starts scanning along the 5’ UTR to identify the correct translation start site (Dutt et al., 2015). Once the start codon is identified, initiation factors are released and the large ribosomal subunit is recruited to form a translationally active ribosome, which catalyses the formation of the first peptide bond upon accepting an elongator tRNA and thereby enters the elongation phase. Ribosome-catalysed polypeptide formation proceeds as the mRNA is translocated codon by codon until a stop codon is reached, upon which the polypeptide chain is detached from the ribosome. This is followed by ribosome release, and ribosomal subunits can subsequently be recycled for multiple rounds of translation (Browning and Bailey-Serres, 2015; Merchante et al., 2017).
Figure Legends

Figure 1. Post-transcriptional mechanisms controlling gene expression in plants. After transcription by RNA polymerase II (RNA Pol II), a pre-mRNA undergoes processing, which includes attachment of the m7G cap structure at the 5’ end, cleavage and polyadenylation at the 3’ end as well as splicing and nucleotide modifications such as m6A (1). The resulting mature mRNA is then tightly packed into a ribonucleoprotein complex and exported into the cytoplasm through nuclear pore complexes (2). Some cytoplasmic mRNAs will immediately undergo translation (2), which is tightly regulated at the level of initiation (and includes recruitment of the pre-initiation complex, scanning of the 5’ UTR and assembly of the full ribosome at the AUG initiation codon). RNAs can also be sequestered into biomolecular condensates, from which they are later released (4). Finally, mRNAs are subjected to degradation (5), which can be a result of mRNA quality control or specific RNA modifications; it can also occur co-translationally or within biomolecular condensates. Solid arrows indicate movement of molecular components during translation; dotted arrows indicate the mRNA’s progression through consecutive stages of gene expression. Blue spheres represent proteins or (ribonucleo)protein complexes; numbers within the spheres indicate specific eukaryotic initiation factors (eIFs).

Figure 2. Regulatory features of 5’ and 3’ UTRs and their role in environmental responses. (A) UTRs act as hubs for post-transcriptional regulation of mRNA function and thereby ultimately determine the abundance of the encoded protein. Regulatory features of UTRs include binding sites for RBPs, miRNAs and IncRNAs, uORFs, RNA secondary structures such as hairpins and RNA G-quadruplexes (RG4s) as well as sites for splicing, nucleotide modification and polyadenylation, the latter determined with the help of far (FUE) and near upstream elements (NUE). (B-I) Examples of UTR-mediated regulatory processes that operate in response to environmental signals. (B) PNT1 recruits the active phyB photoreceptor under red light, inhibiting translation of PORA mRNA and thereby regulating plant greening. (C) PhyB, together with the splicing factors RRC1 and SFPS, triggers intron retention in the PIF3 5’ UTR; the retained intron contains a uORF that downregulates PIF3 translation in the light and thereby promotes seedling de-etiolation. (D) Binding of the IncRNA cis-NATPHO1.2 triggers structural rearrangements in the 5’ UTR and coding region of the rice PHO1.2 mRNA under phosphate
starvation and thereby increases access of the large ribosomal subunit to the initiation codon. Translation of \textit{PHO1.2} increases and elevated levels of \textit{PHO1.2} transporter allow for efficient redistribution of inorganic phosphate (Pi). (E) A thermosensitive hairpin in the \textit{PIF7} 5' UTR adopts a more relaxed conformation at elevated temperatures, which acts as a translational enhancer. Increased levels of \textit{PIF7} subsequently promote temperature-induced hypocotyl elongation. (F) \textit{MiR156/157} bind to the 3' UTR of \textit{SPL3} and trigger the transcript's degradation via \textit{ARGONAUTE 1} (AGO1). \textit{MiR156/157} levels are reduced at elevated temperature, allowing \textit{SPL3} protein to accumulate and induce flowering. (G) An m^\text{6}A modification in the \textit{PIF4} 3' UTR under red light destabilises the transcript, thereby promoting photomorphogenesis. (H) Formation of an RG4 in the \textit{CORG1} 3' UTR at low temperature prevents degradation of the transcript and thereby attenuates growth in the cold. (H) Selection of a distal polyadenylation site in the \textit{HKT1} 3' UTR enhances translation under salt stress; increased production of the HKT1 transporter subsequently increases export of sodium ions and thereby promotes salt tolerance in \textit{Spartina alterniflora}. Arrows indicate positive regulation; blunt arrows indicate negative regulation; dotted arrows denote environmental effects. Ribosomes are depicted in bright blue.
Figure 1. Post-transcriptional mechanisms controlling gene expression in plants. After transcription by RNA polymerase II (RNA Pol II), a pre-mRNA undergoes processing, which includes attachment of the m7G cap structure at the 5’ end, cleavage and polyadenylation at the 3’ end as well as splicing and nucleotide modifications such as m5A (1). The resulting mature mRNA is then tightly packed into a ribonucleoprotein complex and exported into the cytoplasm through nuclear pore complexes (2). Some cytoplasmic mRNAs will immediately undergo translation (2), which is tightly regulated at the level of initiation (and includes recruitment of the pre-initiation complex, scanning of the 5’ UTR and assembly of the full ribosome at the AUG initiation codon). mRNAs can also be sequestered into biomolecular condensates, from which they are later re-released (4). Finally, mRNAs are subjected to degradation (5), which can be a result of mRNA quality control or specific RNA modifications; it can also occur cotranslationally or within biomolecular condensates. Solid arrows indicate movement of molecular components during translation; dotted arrows indicate the mRNA’s progression through consecutive stages of gene expression. Blue spheres represent proteins or (ribonucleo)protein complexes; numbers within the spheres indicate specific eukaryotic initiation factors (eIFs).
Figure 2. Regulatory features of 5’ and 3’ UTRs and their role in environmental responses. (A) UTRs act as hubs for post-transcriptional regulation of mRNA function and thereby ultimately determine the abundance of the encoded protein. Regulatory features of UTRs include binding sites for RBPs, miRNAs and IncRNAs, uORFs, RNA secondary structures such as hairpins and RNA G-quadruplexes (RG4s) as well as sites for splicing, nucleotide modification and polyadenylation, the latter determined with the help of far (FUE) and near upstream elements (NUE). (B–I) Examples of UTR-mediated regulatory processes that operate in response to environmental signals. (B) PNT1 recruits the active phyB photoreceptor under red light, inhibiting translation of POR1 mRNA and thereby regulating plant greening. (C) PhyB, together with the splicing factors RRC1 and SFPS, triggers intron retention in the PIF3 5’ UTR; the retained intron contains a uORF that downregulates PIF3 translation in the light and thereby promotes seedling de-etiolation. (D) Binding of the IncRNA cis-NATPHO1.2 triggers structural rearrangements in the 5’ UTR and coding region of the rice PHO1.2 mRNA under phosphate starvation and thereby increases access of the large ribosomal subunit to the initiation codon. Translation of PHO1.2 increases and elevated levels of PHO1.2 transporter allow for efficient redistribution of inorganic phosphate (Pi). (E) A thermosensitive hairpin in the PIF7 5’ UTR adopts a more relaxed conformation at elevated temperatures, which acts as a translational enhancer. Increased levels of PIF7 subsequently promote temperature-induced hypocotyl elongation. (F) MIR156/157 bind to the 3’ UTR of SPL3 and trigger the transcript’s degradation via ARGONAUTE 1 (AGO1). MIR156/157 levels are reduced at elevated temperature, allowing SPL3 protein to accumulate and induce flowering. (G) An m7G modification in the PIF4 3’ UTR under red light destabilises the transcript, thereby promoting photomorphogenesis. (H) Formation of an RG4 in the CORGI 3’ UTR at low temperature prevents degradation of the transcript and thereby attenuates growth in the cold. (H) Selection of a distal polyadenylation site in the HKT1 3’ UTR enhances translation under salt stress; increased production of the HKT1 transporter subsequently increases export of sodium ions and thereby promotes salt tolerance in Spartina alterniflora. Arrows indicate positive regulation; blunt arrows indicate negative regulation; dotted arrows denote environmental effects. Ribosomes are depicted in bright blue.