The Dynamic Kaleidoscope of RNA Biology in Plants[1]

The kaleidoscope of RNA biology within plants is explored in this Focus Issue of eight *Update* reviews, one letter, and 22 research articles. The contributions are sorted by RNA type, location of synthesis, and activity. But the picture is not simple: the relationship between DNA and RNA is kaleidoscopic, with mirrors, reflections, and repeated patterns, easily shifted and altered by composition. RNAs have dynamic structures, undergo a multitude of modifications, and serve diverse functions during development and environmental responses. They act, following base modification and processing, at varied or specific locations, involving complex processes controlled by a colorful palette of RNA-binding proteins and other RNAs. RNAs can deliver a message to cells beyond their site of synthesis, even transcending organismal and kingdom boundaries. As when patterns change as a kaleidoscope is turned, RNAs can be shape shifted through isoform production, processing, covalent modifications, and decay processes. The recognition of an epitranscriptome–specific adenosines converted to methyl-6-adenosine (m^6A) and other posttranscriptional modifications not encoded by DNA–has stimulated researchers to probe the cause and effect of these RNA variations. This collection of articles illustrates vibrant activity and illuminates emerging opportunities for the application of RNA biology in biotechnology and agriculture.

MIRRORS AND REFLECTION: CHROMATIN, TRANSCRIPTION, AND COTRANSCRIPTIONAL PROCESSES

Most articles in this Focus Issue consider RNAs encoded in the cell nucleus. The production of gene transcripts involves epigenetic regulation of chromatin conformation that influences the accessibility of transcription factors to their binding sites for the activation of transcription as well as the regulation of cotranscriptional processes. The activation of transcription of a protein-coding gene by RNA Polymerase II (RNAPII) is essential for the production of a nascent transcript. After polymerization of the first eight to 16 nucleotides, these precursor mRNAs (pre-mRNAs) are cotranscriptionally modified by the addition of a 5′-m^7Gppp cap to the first nucleotide of the transcript (Bentley, 2005). This modification enhances the processivity of transcriptional elongation, cotranscriptional intron splicing, and subsequent cleavage and addition of the 3′-polyadenylate tail that is not encoded by the DNA template. Fully processed mRNAs are exported to the cytoplasm, where they undergo a pioneer round of translation that recognizes premature stop codons and other aberrancies (Matsui et al., 2019). Those that pass this quality control continue to be translated, but those that fail are degraded (Chantarachot and Bailey-Serres, 2018).

CHROMATIN AND HISTONES

The regulation of chromatin organization through the modification of histones controls many developmental and environmental responses. The *Update* by Ueda and Seki (2020) summarizes progress in deciphering the roles and interrelationships between the histone modifications and variations that influence drought, salinity, heat, and cold stress responses of plants. These include acetylation, methylation, phosphorylation, and ubiquitination of specific residues of core histones, primarily Histone 3 (H3). Specific histone modifications are associated with gene activation or repression. This issue includes a description of dynamics in a histone deacetylase (HDAC) in cucumber (*Cucumis sativus*) associated with cell proliferation during fruit development (Zhang et al., 2020). HDACs remove acetylation on residues of H3 tails, increasing chromatin compaction and dampening transcriptional activity. A short cucumber fruit determined by a deficiency in *SHORT FRUIT1* (*SF1*), a homolog of Arabidopsis (*Arabidopsis thaliana*) *HISTONE DEACETYLASE COMPLEX1* (*HDAC1*), displays reduced cell division during fruit development. Binding of SF1 near the transcription start site of specific genes correlates with reduction of H3 Lys-9 acetylation (H3K9ac) and H3 Lys-14 acetylation (H3K14ac), as observed for HDACs across eukaryotes (Liu et al., 2014). SF1 targets include genes involved in cell proliferation with H3K9ac and H3K14ac marks positioned within the gene body. This raises the possibility that position-dependent SF1 association with nucleosomes may positively modulate the transcription of certain genes.

Regions of chromatin can vary in nucleosome density and compaction. Plants encode many chromatin remodelers, such as the Polycomb Repressive Complexes (PRCs)

[1]This work was supported by the U.S. National Science Foundation (MCB-1716913, IOS-1856749, and IOS-1936492) and the U.S. Department of Agriculture-National Institute for Food and Agriculture (2017-67013-26194 and 2019-67013-29313) to J.B.-S., by the NSFC (31871234), the Program for Guangdong Introducing Innovative and Entrepreneurial Teams (2016ZT06S172), and the Shenzhen Sci-Tech Fund (KYTDPT20181011104005) to J.Z., and by the Grants-in-Aid for Scientific Research (KAKENHI) in Innovative Areas of the Japan Society for the Promotion of Science of the Ministry of Education, Culture, Sports, and Technology of Japan (18H04791 and 18H04705) and RIKEN to M.S.

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www.plantphysiol.org/cgi/doi/10.1104/pp.19.01558

that have histone methyltransferase activity that places the H3 Lys-27 methylation mark associated with silencing of transcription (Ojolo et al., 2018). Kumar et al. (2020) describe two PRC components of potato (*Solanum tuberosum*), *StBMI1-1* and *StMSI1*, that regulate tuber development by controlling the expression of *microRNA156* (*miR156*) and hormonal responses in a photoperiod-dependent manner. They propose that cross talk between histone modifiers regulates *miRNA156* to alter aerial tuber formation under short-day conditions.

Histone Modifications and 3′-End Processing

Most reports on histone modifications focus on transcriptional regulation, but they can affect the cotranscriptional processes of intron splicing and 3′-cleavage and polyadenylation site selection. The Arabidopsis histone methyltransferase SET DOMAIN GROUP8 (SDG8) mediates genome-wide changes in H3 Lys-36 methylation (H3K36me3) within the body of genes that regulate carbon and light responses (Li et al., 2015). It was hypothesized that SDG8-mediated placement of H3K36me3 affects transcriptional elongation or RNA processing. Following up on this, Li et al. (2020) demonstrate that H3K36me3 placement is influenced by nitrogen availability and regulates the production of transcript isoforms, including the full-length isoform of *CCT101* (a CONSTANS, CO-like, and TOC1 motif protein). Nitrogen depletion or the loss of SDG8 activity through mutation lowers H3K36me3 on nucleosomes within the *CCT101* gene body and is associated with the production of a truncated RNA isoform, demonstrating that chromatin marks influence 3′-end processing.

Histone modification, transcription, and transcript isoform production can form a complex and circular network, as elegantly demonstrated by the floral repressor *FLOWERING LOCUS C* (*FLC*) of Arabidopsis (Wu et al., 2020). *FLC* controls the autonomous pathway of flowering. Central to *FLC* regulation is the production of transcript isoforms defined by the site of 3′-poly(A) addition on *COOLAIR*, a long noncoding RNA (lncRNA) produced by antisense transcription of *FLC*. A short *COOLAIR* transcript forms when 3′-cleavage and polyadenylation occurs proximal to the transcription start site of the lncRNA. Proximal poly(A) site selection requires components of the cleavage and polyadenylation machinery, including the nucleus-localized RNA-binding protein FCA that is necessary for 3′-end processing. The *Update* by Wu et al. (2020) is an excellent primer on the factors involved in *FLC* regulation that have been identified in screens for *suppressors of overexpressed FCA*. These mutants uncover machinery necessary for polyadenylation, splicing, transcriptional elongation, and histone modification. It turns out that production of the short *COOLAIR* transcript influences regional histone modifications on *FLC*. These limit the level of production of a full-length *FLC* mRNA that encodes a functional MADS box transcription factor. New data resolve the distribution and dynamics of histone modifications promoted by active 3′-proximal polyadenylation of *COOLAIR*. Key is the recruitment of the H3K4me2 demethylase encoded by *FLOWERING LOCUS D* (*FLD*) to the *FLC* gene body to promote the removal of H3K4me2. This is associated with a loss of H3K4me3 and elevation of H3K27me3 across the complete *FLC* gene body, promoting silencing. A challenge is to understand how 3′-end processing of *COOLAIR* transcripts invokes changes in histone modifications, including the establishment of H3K27me3. This is likely mediated by the PRC2 component CURLYLEAF and at least one factor required for 3′-proximal polyadenylation of *COOLAIR*. The *Update* considers the possible roles of transcriptional elongation rate and noncoding RNA production in this interplay. *FLC* regulation demonstrates that chromatin states associated with transcriptional activity can be reinforced by cotranscriptional RNA processing events to determine environmentally controlled developmental processes.

FLC illustrates a connection between chromatin and polyadenylation site selection. Alternative polyadenylation site selection is observed for over 60% of Arabidopsis and is associated with developmental and environmental regulation. Both pre-mRNA sequence determinants and proteins guide cleavage and polyadenylation. There is a growing number of examples where the cause and consequence of alternative polyadenylation have been analyzed. Variations in site selection that result in distinct 3′untranslated regions (UTRs) can result in presence versus absence of a specific cis-sequence or structure that is targeted by an RNA-binding protein or miRNA (Hunt, 2019). These 3′ UTR features can influence mRNA stability, translation, and trafficking. A database reported in this issue is a resource for the identification, visualization, and expression data of alternative polyadenylation sites for six species (rice [*Oryza sativa*, *japonica* and *indica* accessions], Arabidopsis, *Medicago truncatula*, red clover [*Trifolium pratense*], bamboo [*Phyllostachys edulis*], and *Chlamydomonas reinhardtii*; Zhu et al., 2020).

Splicing

The selection of alternative splice sites on pre-mRNAs contributes to the diversity of the proteome (Reddy et al., 2013; Cao and Ma, 2019; Chaudhary et al., 2019). Numerous factors regulate alternative splicing, determining temporal and spatial production of multiple transcript isoforms from the majority of protein-coding genes. The regulated retention of an intron often results in the presence of a premature termination codon on an mRNA that triggers nonsense-mediated decay during the pioneer round of translation (Shaul, 2015; Ohtani and Wachter, 2019). Selection of alternative 5′ or 3′ splice donor sites can lead to the synthesis of proteins targeted to specific subcellular locations or with alternate biochemical properties. Splicing is influenced by epigenetic modifications, chromatin state, and the rate of

RNAPII elongation but is generally controlled by splicing factors, including Ser-Arg proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs; Chaudhary et al., 2019). These guide the spliceosome in splice site recognition. Environmental cues influence alternative splicing. Lin et al. (2020) show in this issue that PHYTOCHROME4 (PpPHY4), a sensor of red and far-red light and regulator of photosynthetic capacity, interacts with a splicing regulator classified as an hnRNP in the moss *Physcomitrella patens* to increase its abundance. This PHY4 and PphnRNP-F1 interaction cooperatively regulates 70% of the intron retention events promoted by red light. Complementary analyses are used to show that the trans-acting splice regulator PphnRNP-F1 binds to a repetitive exonic cis-regulatory motif just 5′ of the retained intron. Thus, a light sensor can influence alternative splicing mediated by F1-hnRNP, one of the many types of RNA-binding proteins that constitute hnRNP complexes. Splicing is also regulated by proteins that influence RNA structure. Another research article identifies a member of a large family of RNA helicase (RH) proteins needed to maintain splicing homeostasis under cold temperatures in rice (Lu et al., 2020). The abundance of this RH needs to be appropriately regulated, as both reduction or elevation of *OsRH42* transcript accumulation perturbs splicing homeostasis under cold stress conditions.

RNA splicing events can form rare circular RNAs (circRNAs) by a noncanonical backsplicing event of pre-RNAs including lncRNAs (Chaudhary et al., 2019). Complementary pairing between a circRNA and DNA can form an R-loop (RNA:DNA hybrid) that influences splicing. In this issue, a genome-wide survey of circRNA was performed on accessions of rice differing in resistance or susceptibility to the rice blast *Magnaporthe oryzae*, with the aim of identifying circRNAs associated with immunity (Fan et al., 2020). The study provides evidence of genotypic and conditional variation in circRNA accumulation and identifies a circRNA that appears to contribute to innate immunity through an undefined mechanism.

Synthetic Gene Regulation by Riboswitches

Riboswitches are small cis-regulatory RNA elements that undergo conformational change in response to ligand binding (Sherwood and Henkin, 2016). Riboswitch-ligand interaction can be used to activate self-cleavage of a transcript leading to degradation. Shanidze et al. (2020) mobilize the theophylline-dependent aptazyme (*aTheoAz*) derived from the hammerhead ribozyme of the water-borne parasite *Schistosoma mansoni* in Arabidopsis. Insertion of *aTheoAz* in the 3′ UTR of a nucleus-encoded GFP reporter gene enabled a dose-dependent down-regulation of transcript accumulation by theophylline. This synthetic riboswitch proved effective for the conditional complementation of seedling-lethal mutants of *ONE HELIX PROTEIN1* (*OHP1*), encoding an integral thylakoid protein that is required for functional photosystems (Beck et al., 2017). An *OHP1* transgene equipped with the *aTheoAz* riboswitch rescued photosynthetic function in an *ohp1* mutant when grown in the absence of theophylline. These transgenics displayed reduced growth and photosynthetic activity when grown in the presence of the drug. This synthetic riboswitch may be useful for the tunable control of transcript accumulation in a biotechnological context.

FRAGMENTATION AND NEW ASSOCIATION: SMALL RNAS

Plants produce a cadre of small regulatory RNAs (sRNAs) by multiple pathways that act in various ways to alter the regulation of genes or transcripts through base complementarity. The miRNA class of sRNAs determines posttranscriptional gene regulation (Rogers and Chen, 2013). These are synthesized by RNAPII and processed by Dicer-like (DCL) endonucleases to produce a miRNA/miRNA* duplex, which is further modified before assembly into an Argonaute (AGO) protein. This RNA-protein complex guides the post-transcriptional regulation of complementary transcripts, influencing transcript abundance through endonucleolytic cleavage and in some cases limiting translation (Rogers and Chen, 2013; Hudzik et al., 2020). The small-interfering RNA (siRNA) class of sRNAs can be powerful transcriptional gene silencers of viruses, inappropriately expressed transgenes, and transposable elements, through RNA-directed DNA methylation pathway amplification (Borges and Martienssen, 2015). siRNAs are generated by cleavage of double-stranded RNAs (dsRNAs) by specific DCLs. They can cause gene silencing in a non-cell-autonomous manner due to their movement through plasmodesmata to adjacent cells and more distantly through the phloem. Dalakouras et al. (2020) provide an *Update* on siRNA production and consider their direct application to manipulate gene activity and provide protection from biotic stressors in the field. Nakano et al. (2020) describe an updated MPSS database that provides sequence-based information on miRNAs and evidence of their cleavage activity. The revisions include new tools and deeper coverage for a greater number of plant species and fungi. Other articles in this issue highlight miRNAs, sRNAs, and sRNA transfer between organisms as described below.

miRNAs and sRNAs

miRNAs are the topic of two articles related to environmental responses. A study of Arabidopsis determined through mutant analyses that AGO1 and AGO4 are involved in the regulation of hypoxia-induced gene expression and submergence survival (Loreti et al., 2020). *ago1* mutants are more susceptible to submergence. An

AGO4-dependent RNA-directed DNA methylation pathway that promotes localized heterochromatin was found to influence the expression of *HOMOLOG OF RPW8 4* (*HR4*). The regional methylation of *HR4* was reduced in *ago4-1* plants as well as in those that constitutively express a stable version of RELATED TO AP2 12, a transcriptional regulator of genes associated with anaerobic metabolism. This is the first evidence that the AGO1 and AGO4 pathways contribute to submergence survival. Another report identifies a natural variant of *miR397* in Arabidopsis that influences circadian period and flowering time, describing a feedback loop that includes direct regulation of *miR397* by the circadian clock regulator CIRCADIAN CLOCK ASSOCIATED1 (Feng et al., 2020).

Plants produce other sRNA types with roles in development, such as the phased secondary small-interfering RNAs (phasiRNAs) produced during male microsporogenesis (Zhai et al., 2015). A study in Arabidopsis found that certain meiocyte-specific sRNAs are involved in double-strand break repair (Huang et al., 2019). A comparison of the sRNAomes of meiocytes of soybean (*Glycine max*) and cucumber with those of Arabidopsis uncovered conservation in miRNAs and phasiRNAs in all three species (Huang et al., 2020). *miR390* and *miR167* are highly abundant in the meiocytes of all three species, whereas soybean has species-specific meiocyte phasiRNAs. These findings beg further exploration.

The use of hybrid seed for the production of maize (*Zea mays*) and other crops significantly bolsters seed production due to the relatively poorly understood phenomenon of hybrid vigor (Springer and Stupar, 2007). To test the hypothesis that sRNAs contribute to heterosis, sRNA variation was surveyed in a collection of tissues from a large sampling of inbreds and hybrids of representative heterotic groups (Crisp et al., 2020). These researchers found that sRNA expression patterns are influenced by development and genotype. Although this deep examination found no clear evidence that sRNAs contribute to heterotic phenotypes, this study provides valuable information on 21-, 22-, and 24-nucleotide sRNA populations mapped across the nuclear genome of eight inbreds and 12 hybrids of maize.

sRNA Transfer between Organisms

The topic of transspecies transfer of small regulatory RNAs is the focus of a valuable *Update* (Hudzik et al., 2020). Gene silencing by siRNAs is an essential component of plant defense from viruses, invasive fungi, and oomycetes. This review details the evidence that plant miRNAs and siRNAs are mobilized into necrotic and hemibiotrophic pathogens as a shotgun strategy of defense, as the delivery of dsRNA and sRNA from the plant to a pathogen can provide protection. Indeed, transgenic plants that produce dsRNAs that are complementary to pathogen transcripts show increased resistance. This host-induced gene silencing, mediated by the mobilization of plant sRNAs into an invading eukaryotic pathogen, provides a promising strategy for protection against biotrophic/hemibiotrophic filamentous fungi and oomycetes, nematodes, and parasitic plants. Intriguingly, sRNA movement also occurs in the opposite direction, from pathogens and parasites into plants, to control plant gene activity. Work in this area of sRNA biology may provide new strategies for plant protection that hopefully can extend to prokaryotic pathogens, including those vectored by insects.

SHAPESHIFTING: RNA MODIFICATIONS AND THE EPITRANSCRIPTOME

The production of functional structural and regulatory RNAs requires the synthesis of nucleotides (Witte and Herde, 2020), gene transcription, and myriad posttranscriptional processes. Advances in mass spectrometry and next-generation sequencing methods have piqued awareness of covalent modifications of RNAs, beyond the addition of the m^7G-5′-cap to pre-mRNAs, specific modifications of pre-rRNAs that facilitate processing and ribosome biogenesis, and the targeted editing of cytosine to uracil (C-to-U) of plastid and mitochondrial transcripts. The most widely studied epitranscriptomic modification is m^6A.

m^6A Modification

An *Update* contributed by Arribas-Hernández and Brodersen (2020) considers the m^6A modification present on many plant transcripts and compares the proteins involved in targeted modification and the impact on posttranscriptional processes, by comparing knowledge from plants with that of other eukaryotes. This review is complemented by two research articles on the m^6A epitranscriptome of maize, one considering the relevance of the modification in mRNA translation (Luo et al., 2020) and the other evaluating coevolutionary patterns of genes with m^6A modification in the two subgenomes of maize that arose through an ancient genome duplication (Miao et al., 2020).

C-to-U Editing in Organelles

Many of the transcripts synthesized within plastids and mitochondria are modified posttranscriptionally by RNA editing. This is typically C-to-U editing, a process guided by a class of nucleus-encoded and modular RNA sequence-specific RNA-binding proteins, the pentatricopeptide repeat proteins (PPRs; Small et al., 2019). Edited sites vary across species and genera, with each edit requiring one or more PPR to facilitate the process. Species of *Selaginella*, seedless vascular plants, display a large number of highly variable edited sites within their plastid transcripts, which not surprisingly is accompanied by a pronounced expansion of PPR gene families (Smith, 2020). Edits in

these organellar RNAs can be required to form a start codon, generate synonymous and nonsynonymous mutations, determine appropriate processing, and in rare cases create a stop codon.

LOCATION AND RELOCATION: RNA MOVEMENT

Visualization

It is challenging to monitor spatial and temporal dynamics in the subcellular location of one or more RNAs. Fluorescence in situ hybridization can pinpoint loci within chromosomes at high resolution but is rarely applied to monitor gene transcripts. Having shown by the use of imaging that transcripts encoding certain cell cycle regulators are sequestered within the nucleus of Arabidopsis (Yang et al., 2017), this team further refined their methods for high-resolution confocal imaging of specific RNAs within sectioned tissues (Yang et al., 2020). The technique begins with hybridization of digoxigenin- or fluorescein-labeled antisense probes to target RNAs in prepared tissue sections. Samples are processed by incubation with digoxigenin- or fluorescein-labeled antibodies conjugated to horseradish peroxidase. This allows tyramide signal amplification at the site where an RNA is located through peroxide-catalyzed covalent binding to fluorochrome-labeled tyramides. The authors demonstrate tyramide signal amplification fluorescence in situ hybridization on sectioned shoot apical meristems, resolving *WUSCHEL* mRNA in its niche and the temporal accumulation of *HISTONE H4* (*HIS4*) mRNA, which is limited to the S-phase of the cell cycle. They also demonstrate covisualization of distinct transcripts within primordia as well as transcripts enriched in the nucleus or cytoplasm, including an lncRNA. By counterstaining with diamidino-2-phenylindole, they resolve the sequential accumulation of *HIS4* and *CYCLIN-DEPENDENT PROTEIN KINASE1;2* (*CYCB1;2*) during the progression of the cell cycle in the shoot meristem. RNA visualization methods that can resolve single RNA molecules and RNA-protein complexes, are a new frontier in plant biology. This particular method is timely given that genomic technologies that monitor RNAPII activity and nascent RNA synthesis uncover modulation of transcript termination and nuclear export (Hetzel et al., 2016; Palovaara et al., 2017; Reynoso et al., 2018; Zhu et al., 2018; Lee and Bailey-Serres, 2019; Reynoso et al., 2019).

Movement

hnRNP assembly on pre-mRNA transcripts commences during transcription, guides processing, and can ultimately contribute to the regulation of nuclear export, pioneer translation, translation, turnover, and mRNA localization. Another *Update* follows the transport and localization of mRNA within the cell cytoplasm, highlighting the importance of cis-regulatory zipcode sequences and varied RNA-binding proteins that orchestrate mRNA localization and other processes (Tian et al., 2020). These include the non-sequence-specific K homology motif, zinc finger, cold shock domain, RGG (Arg-Gly-Gly) box, and RNA helicase (DEAD/DEAH box) families as well as the sequence-specific PPR and Puf RNA-binding repeat families of plants (Dedow and Bailey-Serres, 2019; Prall et al., 2019). In rice, seed storage protein transcripts are trafficked to specific subdomains of the endoplasmic reticulum during endosperm maturation. The *Update* summarizes the resolution of key trans-acting proteins that bind to zipcodes and function in the cytoskeleton-mediated transport of mRNAs to their site of translation. The review also considers mRNA targeting to the surface of chloroplasts and mitochondria, thought to enrich the localized synthesis of specific outer membrane-associated and imported proteins. Technologies that utilize proximity-based labeling of RNAs within a subcellular context (Wang et al., 2019) could complement visualization studies and enhance understanding of transcript targeting. Finally, symplastic and long-distance transport of mRNAs via the phloem is considered. The best characterized long-distance transported mRNAs are involved in plant development.

COMPLEX ASSOCIATIONS: POSTTRANSCRIPTIONAL PROCESSES

mRNA Turnover

The essential role of an mRNA is to provide a template for the synthesis of a defined polypeptide by the ribosome. But other fates are closely linked to mRNA translation, including mRNA decay and sequestration (Chantarachot and Bailey-Serres, 2018). mRNA turnover proceeds via several pathways, one of which involves the removal of the 5′-m^7G-cap structure by a decapping complex. This complex includes the decapping enzyme DCP2, cofactors, and regulators that modulate decapping and link the process to translation. One of the regulators is the scaffold protein VARICOSE (VCS), known to interact with several decapping and decay components. In this issue, Kawa et al. (2020) dissect the role of abscisic acid-independent activation of SNF1-RELATED PROTEIN KINASE2 (SnRK2) family members in the phosphorylation of specific residues of VCS and VARICOSE RELATED (VCR) of Arabidopsis. They report a combination of redundancy and specificity in phosphorylation of VCS and VCR residues by SnRK2.5, SnRK2.6, and SnRK2.10. This activation is rapid and likely tissue specific within roots. Although the outcome of these phosphorylation events remains unclear, mutants in several components of the 5′ to 3′ decay pathway are shown to contribute to root system architecture and development in seedlings exposed to salt stress.

The process of mRNA decay takes place within cellular subdomains called protein bodies (PBs) that are marked by the presence of mRNAs and decapping complex proteins. mRNAs can be translationally

inactive and sequestered from the translational apparatus in stress granules (SGs). Microscopically visible foci of PB and SG components increase in size in response to varied stress conditions. This condensation involves disordered proteins that form multivalent or intermolecular protein-protein and protein-RNA interactions that promote aggregation, a process that can be rapidly reversed (Chantarachot and Bailey-Serres, 2018). The liquid-liquid phase separation described for PBs was also recently noted for complexes involved in the regulation of *FLC* expression in the nucleus (Wu et al., 2020).

Translation

Cytosolic mRNA translation is also highly regulated, reflecting the complexity of the translational mechanism (Browning and Bailey-Serres, 2015) as well as the diversity of nucleotide composition, primary sequence, and structural qualities that constitute regulatory elements of mRNAs (Roy and von Arnim, 2013; Merchante et al., 2017). Many recent studies consider the dynamics of mRNA translation, including five articles in this issue. The first appriases stored RNAs and their mobilization into active translation complexes during the early germination stages of Arabidopsis (Bai et al., 2020). Using biochemical fractionation methods, this report evaluates the ribosomes of dry seeds, demonstrating an abundance of monoribosomes (monosomes) that are loaded with mRNA. By profiling these transcripts and those associated with the rapidly formed polysomes of germinating seed, they confirm that close to 20% of the preloaded monosomes are primed for translational activity in early germination. These transcripts are enriched in a GA-rich 5′ UTR motif. An accompanying proteomic study confirms that the monosome complexes of dry seed include RNA-binding proteins associated with PBs and SGs as well as a number of abundant seed storage proteins. The complexity of the monosome proteome raises new questions. Are there distinctions in the proteins associated with the subset of monosomes that are primed for translation upon germination? Do PB- or SG-associated ribosome complexes constitute a similar or distinct type of repressed mRNPs?

m^6A Modification, Translation, and Other Processes

The second article surveys global m^6A-modified RNAs by immunoprecipitation and the relationship between this mark and translation by polysome profiling in two maize inbreds (Luo et al., 2020). In nonplants, m^6A is associated with the promotion or repression of translation. This depends upon the transcript, the position and context of the modification, and specific protein readers (Arribas-Hernández and Brodersen, 2020). The maize study recognized a consensus motif associated with m^6A sites that is enriched in 3′ UTRs and could be associated with the selection of the site of 3′-polyadenylation. It also reports a negative association between translational status (the proportion of an individual gene transcript associated with polysomes) and the presence of m^6A modification. The comparison between inbreds identified common as well as noncommon m^6A sites, which tended to be associated with alternative splicing. There is considerable room for future analyses of the functions of m^6A, as only a small proportion of the transcriptome has a particular m^6A modification. Adding to the intrigue are the findings of Miao et al. (2020) related to gene evolution and m^6A modification. They report that transcripts produced from the two subgenomes of maize differ, with higher m^6A in transcripts of the singleton and duplicated genes of the subgenome that has more actively expressed genes.

Upstream Open Reading Frame Control of Translation

The translation of many mRNAs is conditionally modulated by the presence of one or more upstream open reading frame (uORF) in the 5′ leader of an mRNA that precedes the main open reading frame (mORF; von Arnim et al., 2014). uORFs generally repress the translation of the mORF, but this regulation can be conditionally or developmentally reversed (van der Horst et al., 2020). Gene transcript isoforms can also vary in the presence versus absence of uORFs due to alternative splicing. A new example of this is provided by Dong et al. (2020). In their study of Arabidopsis, they note that the gene encoding a repressor of light responses, *PHYTOCHROME-INTERACTING FACTOR3* (*PIF3*), is regulated by alternative splicing and uORF-mediated translational repression. More specifically, a mutant with unusually high levels of photoactivated phytochrome B accumulates a *PIF3* transcript with a 5′ retained intron. This isoform possesses a 5′ leader with multiple uORFs, including a 129-nucleotide uORF that represses mORF translation in a reporter assay. This intron retention event is enriched in *PIF3* transcripts produced in the light in plants grown in a short-day diurnal cycle. By contrast, the fully spliced *PIF3* isoform is produced during the dark period and is associated with PIF3's positive role in elongation growth. Similarly, rice *NITROGEN LIMITATION ADAPTATION1* (*OsNLA1*), important in the degradation of several phosphate transporters, produces a transcript isoform by alternative splicing with an inhibitory 5′ uORF under phosphate-replete conditions (Yang et al., 2020). In this case, the uORF is proposed to fine-tune phosphate-stimulated production of NLA1.

The *Update* by van der Horst et al. (2020) tenders the hypothesis that the ribosome can act as a metabolite multisensor in uORF-mediated translational control. Support is provided with a survey of sequence conserved-peptide uORFs (CPuORFs) that dampen translation when a specific metabolite is present (i.e. Suc, polyamines, thermospermine, phosphocholine,

ascorbate, galactinol, and boron). The stalling of ribosomes along these CPuORFs or at their stop codons is confirmed in ribosome-footprinting studies and is dependent upon the encoded peptide and metabolic status (Yamashita et al., 2017; van der Horst et al., 2019). Ribosome stalling also can occur in a metabolite-specific manner during mORF translation, as *S*-adenosyl-L-Met promotes ribosome stalling on *CYSTATHIONINE GAMMA SYNTHASE1* (*CGS1*) mRNA, triggering its degradation. This mechanism limits the synthesis of CGS that catalyzes the first committed step in Met biosynthesis. The ribosome metabolite-sensor hypothesis is bolstered by three-dimensional single-complex cryoelectron microscopy of bacterial ribosomes that has revealed interactions between nascent polypeptides and a specific metabolite within the ribosome exit channel that stall translational elongation. To test the relationship between a nascent conserved peptide and the ribosome as a metabolite sensor in plants, it may be feasible to purify ribosomes translating a specific CPuORF to view peptide-metabolite interactions within the exit channel by high-resolution cryoelectron microscopy. It is exciting to anticipate single mRNA-ribosome resolution of translational control in plants.

Within Plastids and Mitochondria

Gene regulation in chloroplasts and mitochondria is extensively determined by posttranscriptional processes. These include C-to-U editing as well as extensive editing of polycistronic mRNAs. The processing mechanisms are surprisingly complex and guided by many PPRs and other proteins, often in a transcript-specific manner. Two research articles provide examples along these lines in plastids. The first describes a single plastid gene that requires a specific member of the mitochondria-type transcription terminator factor gene family for proper transcription termination in Arabidopsis (Xiong et al., 2020). The second article examines chloroplast transcript levels and their association with ribosomes in tobacco (*Nicotiana tabacum*) in response to high light (Schuster et al., 2020). These authors show that *psbA*, encoding the highly labile D1 reaction center protein of PSII, is the principal plastid transcript that is translationally up-regulated. It had been anticipated that the shift from low light to high light would result in broader changes in translation. This points to highly tuned modulation of activity of specific RNAs in plastids.

CLOSING REMARKS

A kaleidoscope as analogy to describe RNA biology captures the complexity and dynamics of the form, function, and fates associated with these molecules in plants. This Focus Issue will expand online as new articles are published in *Plant Physiology* over the next 2 years. We anticipate continued discovery in the realm of plant RNA biology, including findings with applications in synthetic biology and the improvement of the health, yields, and nutritional value of crops.

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ACKNOWLEDGMENTS

We thank all of the authors, reviewers, and editors, especially Sarah M. Assmann, who contributed to this Focus Issue. We thank Thanin Chantarachot and Sarah M. Assmann for comments on this editorial.

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