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Posttranscriptional control of plant development

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Abstract

Genetic studies have provided increasing evidence that proteins involved in all aspects of RNA metabolism, such as RNA processing, transport, stability, and translation, are required for plant development and for plants' responses to the environment. Such proteins act in floral transition, floral patterning, and signaling by abscisic acid, low temperature and circadian rhythms. Although some of these proteins belong to core RNA metabolic machineries, others may have more specialized cellular functions. Despite the limited knowledge of the underlying molecular mechanisms, posttranscriptional regulation is known to play a key role in the control of plant development.

Introduction

The generation of functional proteins from genes is a complex process that involves multiple stages, which can be categorized on the basis of the substrates being DNA, RNA, or protein. In this review, we narrowly define posttranscriptional processes as events in gene expression that involve RNA, such as RNA processing, RNA export, RNA decay, and translation.

While a protein-coding gene is being transcribed by RNA polymerase II (RNA pol II) into a pre-mRNA, several RNA-processing machineries are recruited by the carboxy-terminal domain of the largest subunit of RNA pol II to the emerging pre-mRNA for its processing [1,2]. A cap structure is added to the nascent transcript and bound by the heterodimeric cap-binding complex (CBC). Intronic sequences are removed through splicing reactions, and polyadenylation signals in the elongating transcript are recognized by the cleavage and polyadenylation complex, which cleaves the RNA and adds a poly A tail to the 3' end of the transcript. These processing events do not occur in isolation but are instead interlinked and affect downstream events such as RNA export and translation. The CBC-bound cap stimulates splicing, polyadenylation, and RNA export [3]. Splicing can both enhance polyadenylation at the terminal exon and inhibit polyadenylation at sites within introns [4,5]. Successful splicing also helps to recruit an export factor onto the mRNA to facilitate its nuclear export [6]. Once outside the nucleus, the CBC is replaced by eIF4E, which together with polyA-binding proteins stimulates the initiation of translation [7]. In addition, RNAs are subject to degradation by ribonucleases in both nuclear and cytoplasmic compartments [8,9].

All aspects of RNA metabolism are accompanied by the activities of a myriad of RNA-binding proteins. Most RNA-binding proteins contain one or more conserved domains, such as the RNA-recognition motif (RRM), the K-homology (KH) motif, RGG (Arg-Gly-Gly) boxes, and double-stranded RNA-binding domains (dsRBDs) [10,11]. A survey of the *Arabidopsis* genome for RNA-binding proteins revealed 196 RRM- and 26 KH-containing proteins [12]. Another survey of the *Arabidopsis* genome identified 262 splicing-related proteins (<http://www.plantgdb.org/AtGDB/prj/SRGD/ASRP-home.php>). Although most of these proteins haven't been characterized experimentally, forward and reverse genetic approaches are beginning to reveal a requirement for proteins that have roles in RNA metabolism in plant development (Table 1).

Developmental transitions

Genetic analyses have uncovered several genes that encode RNA-binding proteins in the autonomous floral-promotion pathway. This pathway accelerates floral transition by negatively regulating the expression of *FLOWERING LOCUS C (FLC)*, a central repressor of flowering [13–15]. The characterization of *FCA* provided an elegant example of posttranscriptional regulation in gene expression and in plant development. *FCA* gives rise to four transcripts (α , β , δ and γ) due to the alternative splicing of introns 3 and 13, and to polyadenylation within intron 3. The β form, which is generated by polyadenylation within intron 3, is the most abundant form, but only the γ form can generate the functional FCA protein containing two RRM motifs and a WW domain [16,17•]. *FCA* negatively regulates its own expression by promoting the usage of the polyadenylation site within intron 3, thereby suppressing the formation of the functional γ form [18•]. This regulation is responsible for the temporal and spatial accumulation of functional FCA [17•,18•].

FY, another member of the autonomous pathway, interacts physically with FCA and shares high sequence similarity to a yeast polyadenylation factor, Pfs2p [19•]. FCA–FY interaction is required for the regulated 3' end processing of *FCA* pre-mRNA [19•]. Another autonomous pathway gene, *FPA*, also encodes a potential RNA-binding protein, which has three RRM motifs [20]. Although loss-of-function mutations in *FCA*, *FY*, and *FPA* lead to increased accumulation of *FLC* RNA [21], the molecular mechanism through which *FLC* is directly or indirectly regulated by these RNA-binding proteins remains a mystery, and undoubtedly an active area for future research.

Genetic characterization of vegetative phase change in *Arabidopsis* revealed two nucleocytoplasmic transport receptors, HASTY (HST), an ortholog of mammalian exportin-5, and PAUSED (PSD), an ortholog of mammalian exportin-t [22•,23•]. Exportin-5 may mediate the export of dsRBD proteins [24] and dsRNAs that have structural similarities to microRNA precursors [25•]. Exportin-t mediates tRNA export [26]. *hst-6* [22•], *psd-13* [23•] and *psd-6* [27•], potential null mutations, do not result in lethality. Even the *hst-6 psd-13* double mutant is viable [23•].

Floral patterning

As a class-C gene in flower development, *AGAMOUS* (*AG*) specifies the identities of stamens and carpels [28]. Recessive mutations in *HUA1* and *HUA2* enhance the floral homeotic phenotype of the weak *ag-4* mutant [29]. The *hua1-1 hua2-1* double mutant shows occasional stamen→petal and partial carpel→sepal transformation, weak phenotypes that indicate the partial loss of class-C function in the flower [30,31]. Another genetic screen in the *hua1-1 hua2-1* background resulted in the isolation of recessive mutations in *HUA ENHANCER1* (*HEN1*), *HEN2*, *HEN4*, and *PSD* (*HEN5*), which greatly enhance the homeotic phenotypes of the *hua1-1 hua2-1* double mutant [27•,30,31,32••].

The molecular nature of the *HUA* and *HEN* genes suggests that these genes have a role in RNA metabolism. *HUA2* encodes a novel protein [29] with a regulation of nuclear pre-mRNA (RPR) domain that was first recognized in several metazoan nuclear RNA-processing proteins [33,34]. The RPR domain mediates the interaction of these metazoan proteins with the largest subunit of the carboxy-terminal domain of RNA pol II [35]. *HUA1* encodes an RNA-binding protein that has six CCCH-type zinc fingers [36]. *HEN1* encodes a novel protein with two putative dsRBDs [30]. *HEN2* encodes a DEXH RNA helicase [31] that is highly similar to yeast Dob1p (Dependent on eIF4B) [37], whose activity is required for the nuclear exosome, an exonuclease complex that acts in ribosomal RNA biogenesis and in nuclear pre-mRNA degradation [38]. *HEN4* contains KH domains [32••]. *PSD* (*HEN5*) is the *Arabidopsis* ortholog of mammalian exportin-t [23•,27•].

Single mutations in *HEN1* and *PSD* result in pleiotropic phenotypes [23•,27•,30], indicating that these genes play general roles in gene expression. Indeed, *HEN1* appears to act in microRNA biogenesis [39], and *PSD* is a tRNA nuclear export receptor [23•]. *AG* expression is reduced in *hua1 hua2 hen1* or *hua1 hua2 psd* flowers [27•,30], probably forming the basis of the floral phenotypes of these triple mutants, but *AG* is probably just one of many genes whose expression is affected by *hen1* or *psd* mutations. Single mutations in *HUA1*, *HUA2*, *HEN2*, and *HEN4*, on the other hand, cause few phenotypes [29,31,32••], indicating that these mutations have little effect on gene expression in general.

HUA1, *HUA2*, *HEN2*, and *HEN4* appear to modulate *AG* expression through splicing or polyadenylation [32••]. In *hua1 hua2 hen2* or *hua1 hen2 hen4* mutants, the abundance of *AG* mRNA is reduced relative to that of wildtype plants and, concomitantly, two larger RNA species accumulate to higher levels. These two larger transcripts appear to result from alternative polyadenylation events within the large second intron. It is not known, however, whether the primary defect is premature polyadenylation or inefficient splicing of the second intron, which results in the utilization of cryptic polyadenylation signals in the second intron. Thus, *HUA1*, *HUA2*, *HEN2*, and *HEN4* promote the production of *AG* mRNA by facilitating efficient splicing or by preventing alternative polyadenylation. These genes do not appear to be required for the processing of pre-mRNAs from other floral homeotic genes.

It is interesting to note the parallels between the autonomous floral promotion pathway and the *AG* homeotic pathway. Both pathways employ proteins in RNA metabolism to control

the expression of two MADS-box genes with large introns. *FLC* and *AG* may not be direct targets of the RNA-binding proteins, but current evidence suggests that a similar molecular mechanism may be involved, that is, the regulation of gene expression by alternative RNA processing.

Signaling

Analyses of abscisic acid (ABA) signaling revealed four proteins that have roles in RNA metabolism: ABA-HYPERSENSITIVE1 (ABH1), SUPERSENSITIVE TO ABA AND DROUGHT1 (SAD1), HYPONASTIC LEAVES1 (HYL1), and ABA-ACTIVATED PROTEIN KINASE (AAPK)-INTERACTING PROTEIN1 (AKIP1). As the first three proteins have been the subjects of a previous review [40], we describe them only briefly. *abh1*, *sad1*, and *hyl1* mutants all show hypersensitivity to ABA during seed germination, but *hyl1* and *sad1* mutants appear to have more pleiotropic phenotypes [41–43]. The HYL1 protein contains two dsRBDs [41]. Both ABH1 and SAD1 appear to be the only *Arabidopsis* orthologs of subunits of basic RNA-processing machineries in yeast and metazoans [42,43]. Intriguingly, expression studies indicate that only a small set of mRNAs is affected by mutations in these genes, even by a null mutation in *abh1* [42,43]. How mRNAs are specifically targeted by the machineries that involve ABH1 and SAD1 remains a mystery.

Studies of AKIP1, a substrate of the AAPK, demonstrate that the phosphorylation of an RNA-binding protein can alter its target specificity. AKIP is a nuclear protein that shares sequence similarities with heterogeneous ribonucleoprotein (hnRNP)A/B. ABA-induced phosphorylation of AKIP1 results in a rapid concentration of AKIP in nuclear speckles, and also renders AKIP1 able to bind dehydrin mRNA [44••].

Temperature and circadian signaling also involve proteins that have posttranscriptional roles. LOW EXPRESSION OF OSMOTICALLY RESPONSIVE GENES1 (LOS1), a translation elongation factor2-like protein [45••], and LOS4, a DEAD-box RNA helicase [46•], are required for low-temperature-induced gene expression. Transcripts of two glycine-rich RNA-binding proteins, AtGRP7 and AtGRP8, show circadian oscillations. AtGRP7 regulates the rhythmic expression of itself and of *AtGRP8* through the alternative splicing of its own pre-mRNA and that of *AtGRP8* [47,48•].

Developmental roles of RNA-binding proteins explored using reverse genetics

Several plant RNA-binding proteins that were identified on the basis of homology to their metazoan counterparts have been studied. Examples include AtSRp30, a serine/arginine-rich (SR) protein [49], poly(A)-binding proteins [50,51], the U1-70K protein and its interacting SR proteins [52,53], and an exonuclease AtRrp41p [54] from *Arabidopsis*. Several *Nicotiana glauca* proteins, such as UBPI, a novel hnRNP-like protein [55], the UBPI-interacting proteins UBA1 and UBA2 [56], and RBP45 and RBP47, two oligouridylate-specific hnRNP-like proteins [57], have also been characterized. Although most studies have focused on the expression patterns of genes that encode RNA-binding proteins or on the biochemical activities of the proteins, the developmental roles of some RNA-binding

proteins have been explored. For example, the overexpression of AtSRp30 in *Arabidopsis* leads to alternative RNA processing and delays developmental transitions [49].

MicroRNAs and plant development

microRNAs have also emerged as posttranscriptional regulators in plant development, but we do not discuss this topic here as it has been reviewed recently [58,59].

Conclusions

Among the proteins with RNA-metabolic roles that have been identified through forward genetic screens, some have clear orthologs that are components of core RNA-processing machineries in other species. Others do not have clear orthologs in other species and may function as subsidiary proteins that interface with core RNA-processing machineries, either providing substrate specificity or determining the type of processes that occur on the RNA substrates. These RNA-binding proteins likely act on some, but not all, RNAs. Consistent with this, loss-of-function mutations in these genes have mild or ‘specific’ developmental consequences. Examples of this type of gene are *FCA*, *HUA1*, *HUA2*, and *HEN4*. A seemingly surprising finding from the studies of RNA-metabolic genes carried out to date is that reduction-of-function or loss-of-function mutations in genes that appear to be subunits of core RNA-processing machineries do not result in lethality. On the contrary, only mild or even ‘specific’ defects result from such mutations and, in some cases, the expression of only a small number of genes is affected. Examples of such genes are *FY*, *ABH1*, *PSD*, *HST*, and *SADI*. One explanation for this is that hypomorphic rather than null mutations in these genes were being studied. The *fy* alleles, for example, may not be null alleles [19•]. Another possible explanation is redundancy, either redundant RNA-processing machineries or homologous genes that have similar activities might exist. The fact that apparent null mutants of *ABH1* and *PSD* are viable, despite both *ABH1* and *PSD* being single copy genes, is not as surprising as it may seem as the yeast orthologs of these genes are also dispensable for viability [60,61]. These findings indicate the existence of redundant RNA-metabolic machineries.

The requirement for RNA-binding proteins in plant development has clearly been established and boils down to the requirement for these proteins for the expression of certain genes. Do these proteins really ‘control’ plant development? Perhaps one criterion in establishing an RNA-binding protein as a regulator of plant development is that the protein regulates the expression of its target genes, resulting in temporally or spatially distinct patterns of gene expression during development or in response to environmental stimuli. Many proteins simply play a permissive role in the expression of their target genes. Some of these proteins may also be considered as developmental regulators as long as they meet a second criterion, that is, that they affect only the expression of a small set of genes, one of which is a key developmental regulator.

The regulation of gene expression at posttranscriptional levels can modulate the accumulation of functional proteins or lead to the production of different protein isoforms in response to different developmental and environmental cues. Although genetic studies in

plants have revealed many RNA-binding proteins that have potential roles in the posttranscriptional regulation of plant development, future challenges lie in understanding the molecular mechanisms of their developmental functions. This understanding will largely depend on the elucidation of the biochemical actions of these proteins, and on the identification of their targets in relevant developmental and environmental contexts.

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Abbreviations

AAPK	ABA-activated protein kinase
ABA	abscisic acid
ABH1	ABA-HYPERSENSITIVE1
AG	<i>AGAMOUS</i>
AKIP1	AAPK-interacting protein1
AtGRP	<i>Arabidopsis thaliana</i> glycine-rich protein
CBC	cap-binding complex
dsRBD	double-stranded RNA-binding domain
FLC	<i>FLOWERING LOCUS C</i>
HEN1	<i>HUA ENHANCER1</i>
hnRNP	heterogeneous ribonucleoprotein
HST	HASTY
HYL1	HYPONASTIC LEAVES1
KH	K-homology
LOS1	LOW EXPRESSION OF OSMOTICALLY RESPONSIVE GENES1
Pfs2p	polyadenylation factor1 subunit2p
PSD	PAUSED
RNA pol II	RNA polymerase II
RPR	regulation of nuclear pre-mRNA
RRM	RNA-recognition motif
SAD1	SUPERSENSITIVE TO ABA AND DROUGHT1

SR

serine/arginine-rich

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Table 1

RNA metabolic proteins in developmental and signaling pathways.

Motif	Metazoan/ yeast homolog	Cellular function	Possible targets ^a	Subcellular localization	Reference(s)
Floral transition					
FCA	WW, RRM	Pre-mRNA processing	FCA	Nuclear	[16,17•,18•]
			FLC?		
FY	PPLP, WD	Polyadenylation	FCA		[19•]
			FLC?		
FPA	RRM		FLC?		[20]
Vegetative phase change					
HST	HEAT repeats	Nucleocytoplasmic transport		Periphery of the nucleus	[22•]
PSD	RAN-binding	Nucleocytoplasmic transport			[23•,27•]
Floral patterning					
HUA1	Zinc finger	Pre-mRNA processing	AG	Nuclear	[29,36]
HUA2	PWWP, RPR	Pre-mRNA processing	AG		[29,32•]
HEN1	dsRBD	miRNA and siRNA metabolism	AG		[30,39]
HEN2	DEXH-box helicase	RNA processing or degradation	AG		[31,32•]
HEN4	KH	Pre-mRNA processing	AG	Nuclear	[32•]
PSD	RAN-binding	Nucleocytoplasmic transport	AG		[27•]
Signaling					
ABH1	CBP80	mRNA Cap binding	AtPP2C etc.	Mainly nuclear	[42]
AKIP1	hnRNP A/B		Dehydrin	Nuclear	[44•]
HYL1	dsRBD				[41]
SAD1	Sm domain	RNA metabolism	AtPP2C etc.		[43]
LOS1	eEF-2	RNA metabolism	CBF		[45•]
LOS4	DEAD-box RNA helicase	Protein synthesis	CBF	Nuclear and cytoplasmic	[46•]
AtGRP7	Glycine-rich, RRM	Alternative splicing	AtGRP7	Nuclear	[48•]
			AtGRP8		

^a Some may not be direct targets. '?' denotes a suspected target.

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Abbreviations: AIPP2C, *A. thaliana* protein phosphatase 2C; CBF, C-repeat element binding factor; CBP80, cap-binding protein80; eEF-2, eukaryotic elongation factor-2; HEAT, Huntingtin, Elongation factor3, PP2A, TOR1; miRNA, microRNA; siRNA, small interfering RNA.