



Composition and function of P bodies in *Arabidopsis thaliana*

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mRNA accumulation is tightly regulated by diverse molecular pathways. The identification and characterization of enzymes and regulatory proteins involved in controlling the fate of mRNA offers the possibility to broaden our understanding of posttranscriptional gene regulation. Processing bodies (P bodies, PB) are cytoplasmic protein complexes involved in degradation and translational arrest of mRNA. Composition and dynamics of these subcellular structures have been studied in animal systems, yeasts and in the model plant *Arabidopsis*. Their assembly implies the aggregation of specific factors related to decapping, deadenylation, and exoribonucleases that operate synchronously to regulate certain mRNA targets during development and adaptation to stress. Although the general function of PB along with the flow of genetic information is understood, several questions still remain open. This review summarizes data on the composition, potential molecular roles, and biological significance of PB and potentially related proteins in *Arabidopsis*.

Keywords: P bodies, decapping, deadenylation, mRNA decay, post-transcriptional gene regulation, RNA-protein complex, stress

INTRODUCTION

The diversity of eukaryotic mRNA decay pathways illustrates the importance of nullifying transcripts as a mechanism to ensure proper course of gene expression and prevent the accumulation of aberrant mRNA (Houseley and Tollervey, 2009; Christie et al., 2011). These molecular mechanisms that abrogate accumulation of transcripts and proteins depend on the formation of RNA-protein complexes (RNPs). Under specific conditions or developmental cues, eukaryotic cells sort proteins, and mRNA in microscopically detectable complexes, commonly called cytoplasmic foci, categorized depending on the protein composition. Immunodetection of foci of the mice 5'-exoribonuclease XRN1 indicated that some pathways of RNA degradation might occur in protein speckles (Bashkirov et al., 1997). The 5'-exoribonuclease activity depends on the removal of 5' cap structure 7-methyl-Guanosine-diphosphate (m7GDP). Interestingly, subsequent localization studies in yeast demonstrated that decapping-associated proteins are also localized in cytoplasmic foci, which were called Processing bodies, P bodies (PB; Sheth and Parker, 2003) or GW bodies in mammals (Eystathioy et al., 2003). Further microscopical and biochemical studies ended up proposing the composition of PB, which is conserved in eukaryotic organisms (Parker and Sheth, 2007). Similar structures have been identified in the protists *Trypanosoma cruzi* and *Entamoeba histolytica* (Holetz et al., 2007; López-Rosas et al., 2012). Electronic microscopy observations in human cells revealed that PB are clustered strands of 10–15 nm of diameter (Souquere et al., 2009).

Formation of PB depends on the availability of free cytoplasmic mRNA or mRNA not associated with polysomes (Teixeira et al., 2005). This correlation has been reported in *Arabidopsis* by trapping mRNA into polysomes by short treatments with

cycloheximide (CHX; Weber et al., 2008). However, endogenous signaling events influence the formation of PB in *Saccharomyces cerevisiae* (Kilchert et al., 2010; Ramachandran et al., 2011).

The mRNAs aggregated into PB are translationally repressed and potentially degraded, even their re-incorporation in ribosomes is plausible. Decapping, deadenylation and 5'-exonucleolytic decay are biochemically related enzymatic modifications catalyzed in PB. Besides their involvement in RNA decay, these proteins may secure the translational arrest. Accumulation of related decay factors in PB implicates spatially ordered mechanisms of post-transcriptional regulation. As the activity of such factors in modifying mRNA is most likely irreversible, other mechanisms, possibly occurring outside PB and before the transcript is loaded into them, are necessary to accomplish a selective and controlled delivery, degradation, or translational arrest. It is crucial for the living cell because the knockout of some of those components causes lethality or severely abnormal phenotypes in *Arabidopsis*.

The intrinsic dynamics of PB in controlling the mRNA fate could lead a functional link between PB and other mRNA-containing cytoplasmic foci, for example, stress granules (sg) and heat shock granules (HSG). In human cells, SG are composed by proteins related to initiation of translation such as eukaryotic initiation factors (eIF3, eIF4E, eIFG), ribosomal subunits, and poly(a)-binding protein1 (PABP-1; Anderson and Kedersha, 2008). Assembly of SG is triggered by environmental stress and requires mRNA associated with 48S complexes, thus, assembly can be disrupted by CHX. The HSG described in *Arabidopsis* and tomato are formed upon prolonged heat treatment, they contain heat shock proteins and polyadenylated mRNA (Nover et al., 1989; Weber et al., 2008). The physical proximity between SG/HSG and PB has been evidenced in human cells and

Arabidopsis (Kedersha et al., 2005; Weber et al., 2008). Such proximity might be necessary for the proper sorting of mRNAs after release from polysomes or to facilitate the exchange of mRNAs and proteins in a confined subcellular space. As SG formation is triggered by stress, they might protect transcripts during the lapse of stress and re-initiate translation once conditions get more favorable. If the translation of some transcripts aggregated into SG is not required, the proximity between foci might facilitate transport and decay into PB. Although some proteins related to RNA metabolism can associate with SG (Anderson and Kedersha, 2008), modification of mRNA targets into SG should be demonstrated. In contrast, the battery of proteins involved in RNA decay contained in PB favors a scenario where target mRNAs are translationally repressed and degraded, either they are released from polysomes, SG, or directly delivered after nuclear export.

COMPOSITION OF P BODIES

Subunits of decapping complex, the 5'-exoribonuclease, and deadenylases are prominent markers of PB in eukaryotic organisms. Such core decay factors are distinguishable from other accessory proteins because they have the potential to modify the structure of mRNA. RNPs accessing into PB may be decorated with proteins that can interact with decay factors to mediate their activity within PB. Those proteins are distributed in different subcellular locations, including PB. Their frequency of association with PB might be synchronized with endogenous or exogenous signals that control the fate of specific mRNAs as a mechanism to fulfill requirements of the cell. The microRNA (miRNA)-dependent endonuclease argonaute1 (AGO1), components of nonsense-mediated mRNA decay (NMD) and RNA-binding proteins such as tandem zinc finger proteins occasionally localize in PB, as so far, their functional connection to PB is hypothetical in *Arabidopsis*. The **Figure 1** illustrates reported and putative PB components in *Arabidopsis*.

DECAPPING COMPLEX

The cleavage of the 5' cap compromises mRNA to 5' to 3' exonucleolytic decay, apparently in an irreversible way, hence, decapping activity is tightly regulated (Li and Kiledjian, 2010). In yeast and animals, decapping complex consists on several subunits such as DCP1, DHH1p, EDC3, SCD6, PAT1, and LSM1-7 which mediate the activity of the catalytic subunit DCP2 (Kulkarni et al., 2010; Zheng et al., 2011). The decapping complex in *Arabidopsis* is formed by the catalytic subunit DCP2, and the subunits DCP1, DCP5, and varicose (VCS; Xu et al., 2006), although other proteins can potentially affect decapping activity.

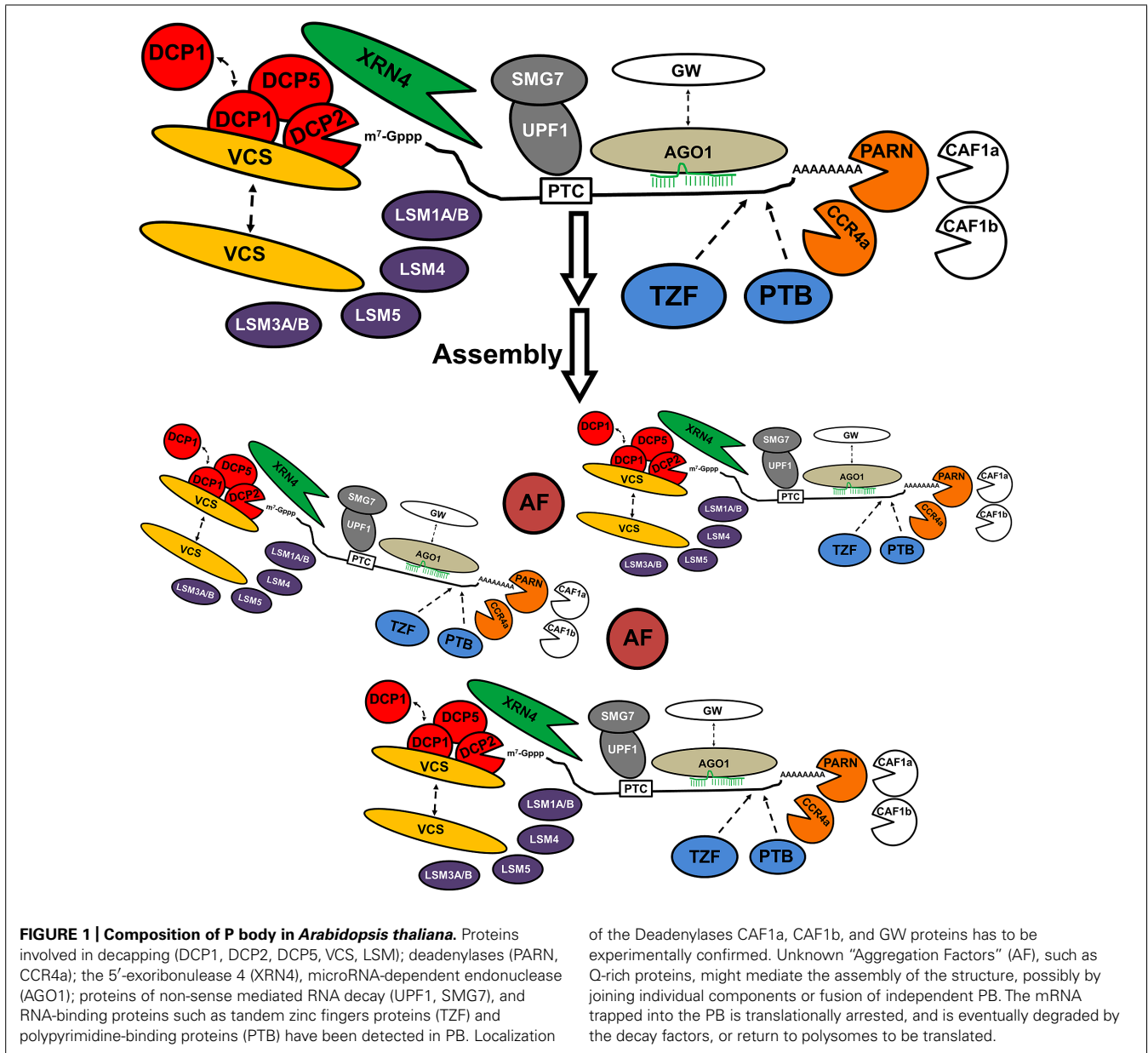
The *Arabidopsis* DCP2 harbors a conserved nudix hydrolase domain essential for the decapping activity, and physically interacts with DCP5, and VCS (Xu et al., 2006). In the same report, Xu et al. (2006) demonstrated that DCP1 stimulates decapping activity *in vivo*. DCP1 contains an EVH1-like domain potentially involved in protein-protein interactions (She et al., 2004). DCP1 interacts with itself, and experimental evidences in *Nicotiana benthamiana* demonstrated DCP1 does not interact with DCP2 (Iwasaki et al., 2007), indicating that additional components are required to link the function of DCP1 to the catalysis of

DCP2. DCP1 and DCP2 may be physically close each other due to their interaction with VCS. Interaction between VCS, DCP1, and DCP2 was demonstrated *in vivo* while yeast two hybrid experiments revealed interaction between VCS and DCP2 and VCS with itself (Xu et al., 2006; Goeres et al., 2007). VCS contains two protein-protein interaction WD40 motifs, although they are not necessary to the interaction with DCP1 and DCP2. Deletion of WD40 repeats in *Ge-1*, the human homolog of VCS, did not compromised the localization into PB, then, WD40 repeats probably function in the recruitment of another mediators of decapping (Yu et al., 2005).

The *Arabidopsis* DCP5 is required for decapping and repression of translation *in vivo* (Xu and Chua, 2009). PB visualized by expressing DCP1-CFP are smaller in knockdown *dcp5-1* compared to Col-0. This comparison indicates DCP5-mediated assembly of PB is necessary for proper decapping. Impaired translational repression or decapping in *dcp5-1* might cause the release of some mRNA from PB, thus, smaller size of PB in *dcp5-1* might be a consequence of their decreased capacity to retain mRNA. Interaction between DCP5 and the RNA chaperone cold shock domain protein 3 AtCSP3 was reported (Kim et al., 2013) and possibly indicates that unwinding secondary structures of RNA might be necessary to facilitate decapping or inhibition of translation. The yeast DEAD box helicase DHH1p and homolog proteins in animals promote decapping and inhibit translation (Kulkarni et al., 2010). *Arabidopsis* lacks a homolog of DHH1p, but other proteins might replace its function through analogous processes.

Pat1 (protein-associated with topoisomerases) is conserved in different organisms, involved in activation of decapping and inhibition of translation (Coller and Parker, 2005; Scheller et al., 2007). In concordance with the formation of PB and inhibition of translation, nutrient deprivation causes nuclear accumulation of tRNAs in yeast. The yeast *dhh1Δ* and *pat1Δ* mutants failed to accumulate tRNAs into nucleus during starvation, but *xrn1Δ* showed normal tRNA nuclear accumulation (Hurto and Hopper, 2011). These evidences suggest that the formation of PB is accompanied by the nuclear accumulation of tRNAs in a DHH1p-Pat1-dependent manner as a mechanism to ensure inhibition of translation. The *Arabidopsis* genes *At1g79090*, *At3g22270*, and *At4g14990* are annotated as *Pat1* (<http://www.arabidopsis.org>), experimental evidences are required to confirm their localization and molecular function.

Sm-like proteins (LSM) interact each other to form complexes to activate decapping and modulate splicing in yeast (Tharun et al., 2000). The *Arabidopsis* LSM1-7 proteins form a cytoplasmic heptameric complex. LSM1A-GFP, LSM1B-GFP, LSM3A-GFP, LSM3B-GFP, and LSM4-GFP localize to cytoplasmic foci in root tips of stably transformed plants (Perea-Resa et al., 2012). Formation of these foci is sensitive to CHX, and the co-localization with RFP-DCP1 confirmed their physical association with PB. Heat stress facilitates the detection of LSM1-4-GFP containing cytoplasmic foci, it tempts to consider that LSM proteins are simultaneously incorporated in distinct cytoplasmic foci. Given that cytoplasmic foci localization of GFP-DCP2 and GFP-VCS is depleted in *lsm1alsm1b*, both proteins might participate in the assembly of PB or, at least influence the localization of VCS and DCP2.



Decapping activity is prevented by the translation initiation factor eIF4E, a 5' cap-binding protein (von der Haar et al., 2004). Its localization in PB and SG is documented in humans and yeast (Andrei et al., 2005; Brengues and Parker, 2007), but the homolog of *Arabidopsis* was only detected in SG (Weber et al., 2008).

5'-EXORIBONUCLEASE

Decapped mRNA is prone to 5' to 3' decay. The *Arabidopsis* XRN4 complements the yeast *xrn1Δ* mutant (Olmedo et al., 2006). XRN4 co-localizes to DCP1 in *Arabidopsis*, furthermore, PB visualized with DCP1 antiserum were quite observable in the knockout *xrn4* line compared to wild type plants (Weber et al., 2008). This interesting comparison suggests the loss of 5'-exoribonuclease activity in *xrn4* enhances the assembly of PB by over accumulation

of potentially uncapped mRNA. XRN4 might prevent accumulation of uncapped intermediates potentially involved in short interfering (siRNA) biogenesis (Gregory et al., 2008), and also participates in the degradation of selected 3' end products from miRNA-mediated decay (Rymarquis et al., 2011).

DEADENYLATION

Deadenylases and their regulatory proteins play an essential role both in 5' to 3' RNA decay and formation of PB (Zheng et al., 2008). Polyadenylated mRNA is aggregated in SG and HSG of tomato cells, thus, deadenylation might be necessary to sort mRNAs into different foci. Shortening of poly-A might exclude poly-A binding proteins and proteins associated with translation to decrease the efficiency of translation and produce mRNAs accessible to PB components. The deadenylation is catalyzed

by the poly-A-specific ribonuclease AtPARN and the carbon catabolite repressor 4-CCR4 associated factor 1-CAF1 complex (CCR4-CAF1). Both AtPARN and CCR4a co-localize to DCP1 in *Agro*-infiltrated tobacco leaves (Moreno et al., 2013). The activity of CCR4-CAF1 complex is regulated by the NOT1-5 proteins in yeast and humans, nevertheless their function has not been confirmed in *Arabidopsis* (Zheng et al., 2011; Abbasi et al., 2013).

ARGONAUTE1

ARGONAUTE (AGO) proteins have miRNA-dependent RNA slicer activity and are part of the RNA interference effector complex (RISC). Interaction between AGO proteins and the PB-associated GW182 (TNRC6A) is critical to gene silencing in humans (Lazzaretti et al., 2009). Early immunofluorescence studies of GW182 (TNRC6A) in mammalian cells revealed co-localization with proteins of decapping in foci, were initially known as GW-bodies (Eystathiou et al., 2003). Human AGO1 and AGO2 co-localize to DCP1 (Liu et al., 2005). GW182 and its isoforms contain a GW motif and a Q-rich region which contributes to the localization into PB, but is not required for silencing (Eulalio et al., 2007). Q or N-rich regions in other PB-related proteins might provoke a general aggregation of other components (Reijns et al., 2008). GW-containing proteins have been identified *Arabidopsis* by a computational approach. However, experimental evidence is required to confirm the potential association with AGO proteins (Karlowski et al., 2010; Gu et al., 2012). *Arabidopsis* AGO1 is associated with microRNAs, endogenous, and transgene derived siRNAs (Baumberger and Baulcombe, 2005), its potential localization in PB is documented (Pomeranz et al., 2010a). The accumulation of 3'-end products of miRNA-mediated decay in the *Arabidopsis xrn4* mutant suggests that AGO slicer activity acts upstream XRN4, possibly associated with PB (Rymarquis et al., 2011). Moreover, the increase of potential targets of miRNA in *dcp1*, *dcp2*, and *vcs* suggests a functional link between decapping and AGO1 activity (Motomura et al., 2012).

Localization of AGO1 varies under different conditions. *GFP-AGO1* driven by the native *AGO1* promoter is detectable in cytosol, nuclear envelope and the proximity to Golgi apparatus (Derrien et al., 2012). The viral suppressor P0 triggers the localization of GFP1-AGO1 into vesicles as a necessary early step in its degradation by autophagy.

The translational repression activity of AGO1 has been uncovered for specific miRNA targets. For example, the superoxide dismutase *CSD2* mRNA is targeted by the miR398 to repress translation under "low Cu⁺⁺" conditions, this mechanism requires VCS (Brodersen et al., 2008). Both miRNA and AGO1 are detectable in polysomes to repress translation of the targets independent of the AGO1 slicer activity (Lanet et al., 2009). These results proposed that AGO1 and VCS sequester mRNA into PB to prevent their translation. Nevertheless, the translational repression activity of AGO1 rather requires the integral Endoplasmic reticulum (ER) protein altered meristem program1 (ATM1) and possibly its paralog LAMP1 (Li et al., 2013). Increased amounts of miRNA target transcripts such as *AGO1*, *PHB*, and *CSD2* in membrane bound polysomes of the double mutant *atm1lamp1*

suggests that ATM1 and LAMP1 displace miRNA target transcripts from translation in membrane bound polysomes. The specificity in the inhibition might depend in the miRNA-dependent ability of AGO1 to recognize unique mRNAs. Although VCS participates in translational arrest, the evidences illustrate that the AGO1-dependent translational inhibition is related to ER and not to PB.

NONSENSE-MEDIATED mRNA DECAy

The NMD refers a surveillance system that detects and eliminates mRNAs containing premature termination codons (PTC) and also controls expression of wild type genes with long 3'-UTR, mRNAs harboring 3'-UTR-located introns, and upstream open reading frames (uORF)-containing genes, defined as small ORFs encoded in the 5'-UTR of major ORFs (mORF; Hayden and Jorgensen, 2007; Kerényi et al., 2008). The up-frameshift proteins UPF1, UPF2, and UPF3 are essential factors of NMD. In yeast, UPF1-3 are involved in recognition and targeting PTC-containing mRNA to PB (Sheth and Parker, 2006; Nyikó et al., 2009). UPF1-3 are cytoplasmic, but they co-localize to DCP2 in the *dcp1Δ* strain, theoretically by accumulation of non-degraded aberrant mRNAs or other substrates of NMD.

Arabidopsis UPF1 is also cytoplasmic, and its incorporation in PB depends on 14-3-3-like protein SMG7 (Méraï et al., 2012). A RNP formed by the NMD target, the presumably phosphorylated UPF1, UPF2, and UPF3 could be transported to PB by SMG7 to promote the degradation of the target. As several components of NMD -such as *SMG5* and *SMG6*- are absent in *Arabidopsis*, the localization of UPF1 and SMG7 in PB would indicate that 5' to 3' decay could have a predominant role in NMD. *XRN4*-silenced tobacco leaves accumulated PTC-containing mRNA at equal levels than the control leaves (Méraï et al., 2012). This results contradicts the model in yeast, where XRN1 is involved in NMD. So far, the role of decapping complex in plant NMD is not demonstrated, and evidences of 3' to 5' decay in PB are lacking. If decapping is dispensable for plant NMD, XRN4 would be dispensable as well. Plant XRN4-independent events of NMD and the role of decapping in this pathway have to be clarified in the future.

TANDEM ZINC FINGER PROTEINS AND OTHER RNA-BINDING PROTEINS

RNPs shuttle between cytoplasm-polysomes-PB and other cytoplasmic foci as a dynamic mechanism to regulate the fate of mRNAs. Microscopical observations and analysis of polysomal profiles revealed movement of mRNA to PB triggered under glucose deprivation in yeast; and selected mRNAs are newly detectable in polysomal fractions after readdition of glucose (Bregues et al., 2005). The mRNAs harbor diverse *cis*-elements potentially recognized by RNA-binding proteins, siRNAs or microRNAs that regulate their maturation, stability, translation rate, as well as localization elements or Zipcodes, involved in the delivery of transcripts to specific subcellular compartments (Ahmed et al., 2011). Specificity in the reactions catalyzed in PB might be conferred by RNA-binding proteins involved in the recognition of targets prior to their delivery in PB.

The human tandem CCCH-type zinc finger proteins such as TTP, are detectable in PB. TTP exists in complex with the proteins

DCP1, DCP2, and XRN1 (Lykke-Andersen and Wagner, 2005; Franks and Lykke-Andersen, 2007). The RNA-binding activity of TTP and similar proteins is conferred by the CCCH-type zinc finger motifs. TTP binds to the class II AU-rich sequence 5'-UUUUUUUU-3' (class II ARE) located in the 3'-UTR of specific mRNAs. TTP enhances decapping of ARE-containing RNA *in vitro*, which suggests that targets of TTP are prone to decapping after their delivery into PB (Fenger-Grøn et al., 2005). Beyond its role in ARE-containing mRNA decay, TTP has been postulated as a nucleation factor of PB since microscopic detection of GFP-HsDCP1 increases by overexpression (OE) of *TTP* (Franks and Lykke-Andersen, 2007).

The *Arabidopsis* CCCH-containing Tandem Zinc Finger protein family consists of 11 members. Transient expression in protoplasts revealed all of them localize to cytoplasmic foci, and the co-localization with PB and SG markers has been demonstrated for specific members (Pomeranz et al., 2010a,b; Bogamuwa and Jang, 2013; Maldonado-Bonilla et al., 2014).

Localization of TZF proteins is highly dependent on the experimental conditions that influence the physiology and requirements of the cell. Nuclear, cytoplasmic, and nucleocytoplasmic distribution was reported to SZF1/TZF11, SZF2/TZF10, OXS2/TZF7, TZF2, and TZF3, by using either transient expression in onion epidermal cells or stable expression in transgenic plants (Sun et al., 2007; Blanvillain et al., 2011; Lee et al., 2012). Those reports ruled out any localization in cytoplasmic foci or PB.

In planta detection of TZF1 in putative PB depends on Methyl Jasmonate (MeJA) treatment or wounding (Pomeranz et al., 2010a) unlike TZF4, TZF5, and TZF6, whose localization in putative PB is detectable in intact plants (Bogamuwa and Jang, 2013). TZF1 accumulates into the nucleus by inhibiting XPO1-mediated nuclear export with Leptomycin B (LMB; Pomeranz et al., 2010a), evidencing a nucleocytoplasmic shuttling. Once in cytoplasm, TZF1 has potential to be incorporated in PB or SG, though the signals, factors or functional domains of TZF1 that promote assembly in PB are still unknown. The localization of TZF9 is also affected by LMB in *Arabidopsis* protoplasts, however, further experiments has to be performed in order to confirm the nucleocytoplasmic shuttling (Maldonado-Bonilla et al., 2014). The nucleocytoplasmic shuttling of TZF proteins might indicate they have different functions both in nucleus and cytoplasm, or perhaps, that the proteins recognize the potential mRNA target during or after transcription. A hypothetical RNP formed in the nucleus might determine the fate of the mRNA once exported in the cytoplasm.

In vitro evidences indicate that TZF proteins bind to RNA, DNA, or even possess ribonuclease activity (Pomeranz et al., 2010a; Blanvillain et al., 2011; Lee et al., 2012; Maldonado-Bonilla et al., 2014). Directs mRNA targets of *Arabidopsis* TZF proteins have not been reported so far, and critical amino acid substitutions in the zinc finger motifs might entail their inability to recognize the class II ARE sequence. However, the arginine-rich region preceding CCCH zinc fingers also contributes in the RNA-binding activity (Qu et al., 2014).

Arabidopsis polypyrimidine tract-binding proteins PTB1, PTB2, and PTB3 are involved in alternative splicing—a nucleus-localized process—, however they are also detectable in PB (Stauffer

et al., 2010). As alternative splicing can produce PTC-containing mRNAs (Wang and Brendel, 2006), these proteins might traffic to PB as part of the mechanism of degradation of substrates of NMD.

The human poly-C binding protein 2 (PCBP2) shuttles between nucleus and cytoplasm, and is detectable in PB and SG. Different RNA-binding KH domains are critical to confer its localization in both cytoplasmic foci (Fujimura et al., 2008). This changing localization may be necessary to join different protein complexes to regulate translation or transcript stability. *Arabidopsis* possesses 26 KH-containing proteins, the arrangement and architecture of KH domains of PEPPER (At4g26000) and At1g14170 are reminiscent to PCBP2 (Lorkovič and Barta, 2002; Ripoll et al., 2006). Subcellular localization studies on these proteins might complement their characterization.

The subcellular dynamics of RNA-binding proteins mentioned above might be related to direct or indirect interaction with cytoskeletal proteins. Myosin Va co-localizes to PB markers, co-precipitates with the 5' cap-binding protein eIF4E, and is required to the assembly of PB in HeLa cells (Lindsay and McCaffrey, 2011). Although eIF4E is not accumulated in PB in plants, direct interaction between myosins and DCP1 has been recently reported in *Arabidopsis* (Steffens et al., 2014). This evidence might motivate to further investigate movement of PB and RNP along actin filaments or microtubules.

BIOLOGICAL RELEVANCE OF PB COMPONENTS IN DEVELOPMENT AND RESPONSE TO STRESS

Analysis of the phenotype of mutant lines has uncovered the importance of PB machinery for plant development and responses to stress. The **Table 1** summarizes *Arabidopsis* PB-related genes and the phenotypes of the corresponding knockout and OE lines.

Post-embryonic lethality of *Arabidopsis dcp1* and *dcp2* mutants, and severe phenotypic defects in *vcs* and *dcp5* mutants such as abnormal patterning of vascular tissue, defects in the shoot apical meristem, chlorosis, slow germination, and temperature sensitivity illustrate functional decapping components are essential for optimal plant development (Xu et al., 2006; Goeres et al., 2007; Iwasaki et al., 2007; Xu and Chua, 2009). Enhanced decapping activity is also necessary to adaptation to abiotic stress. *Arabidopsis sad1(lsm5)* is hypersensitive to ABA and drought (Xiong et al., 2001). *Arabidopsis* DCP1 is phosphorylated by the stress-related MAP kinase MPK6 in response to dehydration. Comparison of capped to uncapped transcripts ratios, and global transcript profiling in different mutants and transgenic lines unveiled that MPK6-mediated phosphorylation of DCP1 increases decapping activity in concert with DCP5 (Xu and Chua, 2012). As MAP kinase activity is triggered by other signals, it is worth to investigate whether decapping activity is modulated *via* phosphorylation of DCP1 in response to other adverse conditions.

The function of LSM proteins is crucial for plant growth (Perea-Resca et al., 2012). In *Arabidopsis*, some transcripts with prolonged half-life in *xrn4* mutant are also stabilized in *lsm1alsm1b* and *lsm5* mutants. Since capping is increased in some of the stabilized transcripts, LSM1A, LSM1B, and LSM5 might stimulate decapping (Golisz et al., 2013). The stabilization of mRNAs such as *WRKY40*, *WRKY33*, and *ACS6* in *lsm1alsm1b* and *lsm5* could be considered

Table 1 | Knock-out and over expression (OE) of P body-related genes alters development and adaptation to stress (view text for details).

Gene (AGI code)	Function	Mutant/OE line	Phenotype	Reference
Decapping				
<i>DCP1</i> (At1g08837)	Regulatory subunit	<i>dcp1-1, dcp1-2</i>	Post-embryonic lethal	Xu et al. (2006), Iwasaki et al. (2007)
<i>DCP2/TDT</i> (At5g13570)	Catalytic subunit	<i>dcp2-1, tdt-1</i>	Post-embryonic lethal, severe morphological defects	Xu et al. (2006), Goeres et al. (2007)
<i>DCP5</i> (At1g26110)	Regulatory subunit	<i>dcp5-1</i>	Pale and weak cotyledons, abnormal rosette leaves, and vascular patterning	Xu and Chua (2009)
<i>VCS</i> (At3g13300)	Subunit of decapping complex, scaffold	<i>vcs-1, vcs-7</i>	Abnormal leaves, vascular defects, temperature-sensitive	Goeres et al. (2007), Xu and Chua (2009)
<i>LSM1a</i> (At1g19120)	Promoter of decapping, assembly of PB	<i>lsm1a/lsm1b</i> (double knockout)	Growth delay, abnormal leaves, lack of apical dominance	Perea-Resa et al. (2012), Golisz et al. (2013)
<i>LSM1b</i> (At3g14080)	Promoter of decapping, assembly of PB	<i>lsm1a/lsm1b</i> (double knockout)	Growth delay, abnormal leaves, lack of apical dominance	Golisz et al. (2013)
<i>LSM5/SAD1</i> (At5g48870)	Promoter of decapping and splicing	<i>sad1</i>	Enhanced ABA-sensitivity	Xiong et al. (2001)
5'-Exoribonuclease				
<i>XRN4/EIN5</i> (At1g54490)	5'-exoribonuclease	<i>ein5-1, ein5-6, xrn4-3</i>	Ethylene-insensitive	Olmedo et al. (2006), Potuschak et al. (2006)
Deadenylation				
<i>PARN</i> (At1g55870)	Poly(A)-ribonuclease	<i>parn-1, parn-2</i>	Embryogenesis arrest	Reverdatto et al. (2004)
<i>CAF1a</i> (At3g44260)	3'-5' Deadenylase	<i>Atcaf1a-1</i>	Enhanced susceptibility to <i>Pseudomonas syringae</i> . Altered response to ROS and NaCl	Liang et al. (2009), Walley et al. (2010)
<i>CAF1b</i> (At5g22250)	3'-5' Deadenylase	35S::CAF1a <i>Atcaf1b-2</i>	Increased response to <i>P. syringae</i> Altered response to ROS and NaCl	Walley et al. (2010) Walley et al. (2010)
RNA-induced silencing				
<i>AGO1</i> (At1g48410)	microRNA-dependent endonuclease. Translational repressor.	<i>ago1-24, ago1-25, ago1-26</i> <i>ago1-27</i>	Dark green, and serrated leaves. Self-sterility Hyper susceptible to cucumber mosaic virus	Morel et al. (2002)
Nonsense-mediated mRNA decay				
<i>UPF1</i> (At5g47010)	Component of NMD	<i>upf1-3</i> <i>upf1-5</i>	Post-embryonic lethal Dwarf, curly leaves. Increased resistance to <i>P. syringae</i>	Arciga-Reyes et al. (2006) Jeong et al. (2011)
<i>SMG7</i> (At5g19400)	Component of NMD, 14-3-3-like	<i>smg7-1, smg7-2, smg7-3</i>	Dwarf, constitutive defense responses, infertility	Riehs-Kearnan et al. (2012)

(Continued)

Table 1 | Continued

Tandem zinc finger proteins	
<i>TZF1</i> (At2g25900)	RNA/DNA binding 35S:TZF1 Enhanced sensitivity to ABA and tolerance to abiotic stress Lin et al. (2011)
<i>TZF2/OZF1</i> (At2g19810)	Ribonuclease activity (<i>in vitro</i>) 35S:OZF1 Enhanced tolerance to oxidative stress Huang et al. (2011)
<i>TZF3</i> (At4g29190)	Ribonuclease activity (<i>in vitro</i>) 35S:TZF2 Enhanced sensitivity to ABA and decreased response to JA Lee et al. (2012)
<i>TZF4</i> (At1g03790)	? 35S:TZF3 Enhanced sensitivity to ABA and decreased response to JA Lee et al. (2012)
<i>TZF5</i> (At5g44260)	? som-1, som-2, som-3, tzf4 35S:TZF4 Early germination, irrespective of light regimen Kim et al. (2008), Bogamuwa and Jang (2013)
<i>TZF6/PEI1</i> (At5g07500)	? tzf5 35S:TZF5 Delayed germination, dark green and smaller size Bogamuwa and Jang (2013)
	35S:antisensePEI1 Delayed germination, dark green leaves, and smaller plant size Li and Thomas (1998), Bogamuwa and Jang (2013)
	tzf6 Early germination
	35S:TZF6 Delayed germination, dark green leaves, and smaller plant size
<i>TZF7/OXS2</i> (At2g41900)	Transcription factor oxs2-1, oxs2-2, oxs2-4, oxs2-6 Early flowering, sensitive to diamide Blauvillain et al. (2011)
	35S:OXS2 (cytoplasmic version) Delayed flowering
<i>TZF9</i> (At5g58620)	RNA-binding activity (<i>in vitro</i>) tzf9 Attenuated response to pathogen-associated molecular patterns Maldonado-Bonilla et al. (2014)
<i>TZF10</i> (At2g40140)	? tzf10/szf2 Abnormal development of vascular tissue Gandotra et al. (2013)
	35S:TZF10 Increased susceptibility to <i>Botrytis cinerea</i> AbuQamar et al. (2006), Sun et al. (2007)
<i>TZF11</i> (At3g55980)	? tzf11/szf1 enhanced sensitivity to ABA and NaCl Sun et al. (2007)
	35S:TZF11 Tolerant to NaCl Sun et al. (2007)
	Enhanced sensitivity to ABA Sun et al. (2007)
	Tolerant to NaCl Sun et al. (2007)

as evidence that modulation of decapping activity takes part during execution of plant defense to pathogens or response to stress (Golisz et al., 2013).

Disruption of *XRN4/EIN5* in *Arabidopsis* affects the response to ethylene. Reduction in the accumulation of the transcription factor EIN3 in *ein5* mutant is consequence of the increased levels of F-box proteins EBF1 and EBF2 which target EIN3 to degradation in an Ubiquitin-dependent manner (Olmedo et al., 2006; Potuschak et al., 2006).

Knockout of the deadenylase *AtPARN*, caused embryogenesis arrest, indicating this enzyme might be indispensable to deadenylate bulk mRNA during embryo development (Reverdatto et al., 2004). Selective deadenylation mediated by CCR4-CAF1 proteins is necessary to regulate responses to stress. Knockout mutant lines *atac1a* and *atac1b* display enhanced susceptibility to *Pseudomonas syringae* and altered response to abiotic stress (Liang et al., 2009; Walley et al., 2010). AtCAF1A and AtCAF1B are necessary to respond to *P. syringae*, possibly by deadenylating jasmonic acid-responsive mRNAs or negative regulators of plant defense. The higher sensitivity to reactive oxygen species (ROS) of *atac1a* and *atac1b* could be helpful to investigate the potential link between AtCAF1A/B, the response to ROS and the defense against *P. syringae*.

Severe vegetative and reproductive defects in *ago1* mutants confirms the importance of AGO1 for plant development. Hypomorphic *ago1* plants permitted higher accumulation of Cucumber Mosaic Virus (CMV) and displayed enhanced symptoms, which evidences the role of AGO1 in posttranscriptional gene silencing, virus resistance and degradation of exogenous RNAs (Morel et al., 2002).

POTENTIAL FUNCTIONAL LINK BETWEEN NMD PATHWAY AND PB

NMD pathway is essential for seed development. Homozygous *upf1-2* and *smg7* are lethal (Yoine et al., 2006; Riehs-Kearnan et al., 2012). Weak mutant allele of *UPF1* (*upf1-5*) and *upf3* showed anomalies in vegetative growth and flowering (Arciga-Reyes et al., 2006). Elevated defense-related gene expression and salicylic acid (SA) accumulation of *upf3* may reflect the reduction in the growth of *P. syringae* (Jeong et al., 2011). A selected group of WRKYs is up-regulated in *upf1-5* and *upf3*, suggesting UPF1/3 are involved in the decay of WRKY transcripts. Coincidentally, CHX promotes accumulation of those WRKY mRNAs in wild type plants. The effect of CHX might imply the arrest of mRNAs in polysomes that impedes the recognition of UPF1 as an early step in NMD. XRN4 is dispensable for decay of PTC-containing transcripts (Mérail et al., 2012). Hence, targets of NMD could be transiently delivered within PB by UPF1 and SMG7 as a mechanism to inhibit translation, and, in some point, the target might be released from PB and degraded by other nucleolytic pathways. The link between UPF1 and the transport of mRNA targets to PB should be investigated in the future.

Conserved uORF homology groups have been identified in *Arabidopsis* and rice by following a *in silico* approach (Hayden and Jorgensen, 2007). Some genes encoding transcription factors, kinases, or enzymes of polyamine metabolism harbor uORF that potentially induces ribosome stalling or reduces stability of the mORF. If the translation or accumulation of uORF perturbs

the translation of the mORF, the latter one gets prone to be recognized by components of the NMD pathway. Depletion of *GFP* expression driven by uORF in *Agro*-infiltrated *Nicotiana benthamiana* strengthen this potential mechanism, although not all the uORF-containing transcripts would be substrates of NMD (Nyikó et al., 2009). It is worth to investigate whether the decay of mORF depends on decapping, deadenylation, and 5'-exoribonuclease activity.

IS THE FUNCTION TZF PROTEINS ASSOCIATED WITH PB?

Knockout and OE of *TZF* genes has revealed their involvement in ABA signaling and response to stress (AbuQamar et al., 2006; Sun et al., 2007; Blanvillain et al., 2011; Huang et al., 2011; Lin et al., 2011; Lee et al., 2012). The changing localization and *in vitro* RNA-binding and ribonuclease activity of TZF proteins suggests that their occasional association with PB might be biologically relevant. However, there are not evidences that indicate the phenotype of such mutants and OE lines is a consequence of mis-regulation of transcripts aggregated in PB.

The human CCH protein TTP promotes mRNA decay by interaction with DCP2. Simultaneous presence of the target and TTP in PB has been demonstrated (Fenger-Grøn et al., 2005; Franks and Lykke-Andersen, 2007). The rice TZF1-GFP is detectable in PB upon salt stress, the recombinant protein binds to the 3'-UTR of selected stress related genes which are down-regulated in the OE line (Jan et al., 2013), indicating that OsTZF1 might lead the decay of these mRNA targets. Additional experiments have to be designed to investigate whether OsTZF1 destabilizes targets by stimulation of decapping. If this hypothesis is validated, similar experiments could be performed in *Arabidopsis* as starting point to demonstrate the functional link between TZF proteins and the decapping activity localized in PB.

CONCLUDING REMARKS AND PERSPECTIVES

The aggregation of related decay factor in PB enables an organized set of mechanisms to prevent translation and catalyze the decay of specific transcripts. The core of plant PB is consensually known and different shuttling proteins might be involved in delivering mRNA targets and promoting degradation or storage via their interactions with core components, which is critical in regulating plant development and response to stress.

Current availability of genomes and transcriptome data encourages us to use the well-known information of *Arabidopsis* to *in silico* identify genes encoding components of PB as a first approach to study these foci in other plant models. The *in silico* analysis also allows the identification of *cis*-elements in potential mRNA targets.

Several methodologies to investigate RNA-protein interactions, isolation of polysomal mRNA and high resolution cell imaging used in yeast and animal systems have to be set up in model plants. Such technical package is necessary to further molecular characterization of proteins associated to PB, as well as the identification mRNA targets and RNA sequences recognized by RNA binding proteins. By overcoming technical challenges, we will broaden our understanding about the dynamics, plasticity, and mechanisms related to the PB function.

Finally, as knockout or overexpression of some PB-related proteins alters the response to environmental stresses, we can use those genes to design novel strategies of post-transcriptional gene regulation to improve crop protection and production.

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REFERENCES

- Abbasi, N., Park, Y.-I., and Choi, S.-B. (2013). RNA deadenylation and decay in plants. *J. Plant Biol.* 56, 198–207. doi: 10.1007/s12374-013-0201-8
- AbuQamar, S., Chen, X., Dhawan, R., Bluhm, B., Salmeron, J., Lam, S., et al. (2006). Expression profiling and mutant analysis reveals complex regulatory networks involved in *Arabidopsis* response to Botrytis infection. *Plant J.* 48, 28–44. doi: 10.1111/j.1365-313X.2006.02849.x
- Ahmed, F., Benedito, V. A., and Zhao, P. X. (2011). Mining functional elements in messenger RNAs: overview, challenges, and perspectives. *Front. Plant Sci.* 2:84. doi: 10.3389/fpls.2011.00084
- Anderson, P., and Kedersha, N. (2008). Stress granules: the Tao of RNA triage. *Trends Biochem. Sci.* 33, 141–150. doi: 10.1016/j.tibs.2007.12.003
- Andrei, M. A., Ingelfinger, D., Heintzmann, R., Achsel, T., Rivera-Pomar, R., and Lührmann, R. (2005). A role for eIF4E and eIF4E-transporter in targeting mRNPs to mammalian processing bodies. *RNA* 11, 717–727. doi: 10.1261/rna.2340405
- Arciga-Reyes, L., Wootton, L., Kieffer, M., and Davies, B. (2006). UPF1 is required for nonsense-mediated mRNA decay (NMD) and RNAi in *Arabidopsis*. *Plant J.* 47, 480–489. doi: 10.1111/j.1365-313X.2006.02802.x
- Bashkurov, V. I., Scherthan, H., Solinger, J. A., Buerstedde, J. M., and Heyer, W. D. (1997). A mouse cytoplasmic exoribonuclease (mXRN1p) with preference for G4 tetraplex substrates. *J. Cell Biol.* 136, 761–773. doi: 10.1083/jcb.136.4.761
- Baumberger, N., and Baulcombe, D. C. (2005). *Arabidopsis* ARGONAUTE1 is an RNA slicer that selectively recruits microRNAs and short interfering RNAs. *Proc. Natl. Acad. Sci. U.S.A.* 102, 11928–11933. doi: 10.1073/pnas.0505461102
- Blanvillain, R., Wei, S., Wei, P., Kim, J. H., and Ow, D. W. (2011). Stress tolerance to stress escape in plants: role of the OXS2 zinc-finger transcription factor family. *EMBO J.* 30, 3812–3822. doi: 10.1038/emboj.2011.270
- Bogamuwa, S., and Jang, J.-C. (2013). The *Arabidopsis* tandem CCCH zinc finger proteins AtTZF4, 5 and 6 are involved in light-, abscisic acid- and gibberellic acid-mediated regulation of seed germination. *Plant. Cell Environ.* 36, 1507–1519. doi: 10.1111/pce.12084
- Bregues, M., and Parker, R. (2007). Accumulation of polyadenylated mRNA, Pab1p, eIF4F and eIF4G with P-bodies in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 18, 2592–2602. doi: 10.1091/mbc.E06
- Bregues, M., Teixeira, D., and Parker, R. (2005). Movement of eukaryotic mRNAs between polysomes and cytoplasmic processing bodies. *Science* 310, 486–489. doi: 10.1126/science.1115791
- Brodersen, P., Sakvarelidze-Achard, L., Bruun-Rasmussen, M., Dunoyer, P., Yamamoto, Y. Y., Sieburth, L., et al. (2008). Widespread translational inhibition by plant miRNAs and siRNAs. *Science* 320, 1185–1190. doi: 10.1126/science.1159151
- Christie, M., Brosnan, C. A., Rothnagel, J. A., and Carroll, B. J. (2011). RNA decay and RNA silencing in plants: competition or collaboration? *Front. Plant Sci.* 2:99. doi: 10.3389/fpls.2011.00099
- Coller, J., and Parker, R. (2005). General translational repression by activators of mRNA decapping. *Cell* 122, 875–886. doi: 10.1016/j.cell.2005.07.012
- Derrien, B., Baumberger, N., Schepetilnikov, M., Viotti, C., De Cillia, J., Ziegler-Graff, V., et al. (2012). Degradation of the antiviral component ARGONAUTE1 by the autophagy pathway. *Proc. Natl. Acad. Sci. U.S.A.* 109, 15942–15946. doi: 10.1073/pnas.1209487109
- Eulalio, A., Behm-Ansmant, I., Schweizer, D., and Izaurralde, E. (2007). P-body formation is a consequence, not the cause, of RNA-mediated gene silencing. *Mol. Cell Biol.* 27, 3970–3981. doi: 10.1128/MCB.00128-07
- Eystathiou, T., Jakymiw, A., and Chan, E. K. L. (2003). The GW182 protein colocalizes with mRNA degradation associated proteins hDcp1 and hLSm4 in cytoplasmic GW bodies. *RNA* 9, 1171–1173. doi: 10.1261/rna.5810203
- Fenger-Grøn, M., Fillman, C., Norrild, B., and Lykke-Andersen, J. (2005). Multiple processing body factors and the ARE binding protein TTP activate mRNA decapping. *Mol. Cell* 20, 905–915. doi: 10.1016/j.molcel.2005.10.031
- Franks, T. M., and Lykke-Andersen, J. (2007). TTP and BRF proteins nucleate processing body formation to silence mRNAs with AU-rich elements. *Genes Dev.* 21, 719–735. doi: 10.1101/gad.1494707
- Fujimura, K., Kano, F., Murata, M., and Fujimura, K. E. N. (2008). Identification of PCBP2, a facilitator of IRES-mediated translation, as a novel constituent of stress granules and processing bodies. *RNA* 14, 425–431. doi: 10.1261/rna.780708
- Gandotra, N., Coughlan, S. J., and Nelson, T. (2013). The *Arabidopsis* leaf provascular cell transcriptome is enriched in genes with roles in vein patterning. *Plant J.* 74, 48–58. doi: 10.1111/tj.12100
- Goeres, D. C., Van Norman, J. M., Zhang, W., Fauver, N. A., Spencer, M., and Sieburth, L. E. (2007). Components of the *Arabidopsis* mRNA decapping complex are required for early seedling development. *Plant Cell* 19, 1549–1564. doi: 10.1105/tpc.106.047621
- Goliz, A., Sikorski, P. J., Kruszka, K., and Kufel, J. (2013). *Arabidopsis thaliana* LSM proteins function in mRNA splicing and degradation. *Nucleic Acids Res.* 41, 6232–6249. doi: 10.1093/nar/gkt296
- Gregory, B. D., O'Malley, R. C., Lister, R., Urlich, M. A., Tonti-Filippini, J., Chen, H., et al. (2008). A link between RNA metabolism and silencing affecting *Arabidopsis* development. *Dev. Cell* 14, 854–866. doi: 10.1016/j.devcel.2008.04.005
- Gu, X.-L., Wang, H., Huang, H., and Cui, X.-F. (2012). SPT6L encoding a putative WG/GW-repeat protein regulates apical-basal polarity of embryo in *Arabidopsis*. *Mol. Plant* 5, 249–259. doi: 10.1093/mp/sss073
- von der Haar, T., Gross, J. D., Wagner, G., and McCarthy, J. E. G. (2004). The mRNA cap-binding protein eIF4E in post-transcriptional gene expression. *Nat. Struct. Mol. Biol.* 503–511. doi: 10.1038/nsmb779
- Hayden, C. A., and Jorgensen, R. A. (2007). Identification of novel conserved peptide uORF homology groups in *Arabidopsis* and rice reveals ancient eukaryotic origin of select groups and preferential association with transcription factor-encoding genes. *BMC Biol.* 5:32. doi: 10.1186/1741-7007-5-32
- Hoeltz, F. B., Correa, A., Avila, A. R., Nakamura, C. V., Krieger, M. A., and Goldenberg, S. (2007). Evidence of P-body-like structures in *Trypanosoma cruzi*. *Biochem. Biophys. Res. Commun.* 356, 1062–1067. doi: 10.1016/j.bbrc.2007.03.104
- Houseley, J., and Tollervey, D. (2009). The many pathways of RNA degradation. *Cell* 136, 763–776. doi: 10.1016/j.cell.2009.01.019
- Huang, P., Chung, M.-S., Ju, H.-W., Na, H.-S., Lee, D. J., Cheong, H.-S., et al. (2011). Physiological characterization of the *Arabidopsis thaliana* oxidation-related zinc finger 1, a plasma membrane protein involved in oxidative stress. *J. Plant Res.* 124, 699–705. doi: 10.1007/s10265-010-0397-3
- Hurto, R. L., and Hopper, A. K. (2011). P-body components, Dhh1 and Pat1, are involved in tRNA nuclear-cytoplasmic dynamics. *RNA* 17, 912–924. doi: 10.1261/rna.2558511
- Iwasaki, S., Takeda, A., Motose, H., and Watanabe, Y. (2007). Characterization of *Arabidopsis* decapping proteins AtDCP1 and AtDCP2, which are essential for post-embryonic development. *FEBS Lett.* 581, 2455–2459. doi: 10.1016/j.febslet.2007.04.051
- Jan, A., Maruyama, K., Todaka, D., Kidokoro, S., Abo, M., Yoshimura, E., et al. (2013). OsTZF1, a CCCH-tandem zinc finger protein, confers delayed senescence and stress tolerance in rice by regulating stress-related genes. *Plant Physiol.* 161, 1202–1216. doi: 10.1104/pp.112.205385
- Jeong, H.-J., Kim, Y. J., Kim, S. H., Kim, Y.-H., Lee, I.-J., Kim, Y. K., et al. (2011). Nonsense-mediated mRNA decay factors, UPF1 and UPF3, contribute to plant defense. *Plant Cell Physiol.* 52, 2147–2156. doi: 10.1093/pcp/pcr144
- Karłowski, W. M., Zielezinski, A., Carrère, J., Pontier, D., Lagrange, T., and Cooke, R. (2010). Genome-wide computational identification of WG/GW Argonaute-binding proteins in *Arabidopsis*. *Nucleic Acids Res.* 38, 4231–4245. doi: 10.1093/nar/gkq162
- Kedersha, N., Stoecklin, G., Ayodele, M., Yacono, P., Lykke-Andersen, J., Fritzler, M. J., et al. (2005). Stress granules and processing bodies are dynamically linked

- sites of mRNP remodeling. *J. Cell Biol.* 169, 871–884. doi: 10.1083/jcb.2005.02088
- Kerényi, Z., Mérai, Z., Hiripi, L., Benkovics, A., Gyula, P., Lacomme, C., et al. (2008). Inter-kingdom conservation of mechanism of nonsense-mediated mRNA decay. *EMBO J.* 27, 1585–1595. doi: 10.1038/emboj.2008.88
- Kilchert, C., Weidner, J., Prescianotto-baschong, C., and Spang, A. (2010). Defects in the Secretory Pathway and high Ca²⁺ induce multiple P-bodies. *Mol. Biol. Cell* 21, 2624–2638. doi: 10.1091/mbc.E10
- Kim, D. H., Yamaguchi, S., Lim, S., Oh, E., Park, J., Hanada, A., et al. (2008). SOMNUS, a CCCH-type zinc finger protein in *Arabidopsis*, negatively regulates light-dependent seed germination downstream of PIL5. *Plant Cell* 20, 1260–1277. doi: 10.1105/tpc.108.058859
- Kim, M.-H., Sonoda, Y., Sasaki, K., Kaminaka, H., and Imai, R. (2013). Interactome analysis reveals versatile functions of *Arabidopsis* COLD SHOCK DOMAIN PROTEIN 3 in RNA processing within the nucleus and cytoplasm. *Cell Stress Chaperones* 18, 517–525. doi: 10.1007/s12192-012-0398-3
- Kulkarni, M., Ozgur, S., and Stoecklin, G. (2010). On track with P-bodies. *Biochem. Soc. Trans.* 38, 242–251. doi: 10.1042/BST0380242
- Lanet, E., Delannoy, E., Sormani, R., Floris, M., Brodersen, P., Crété, P., et al. (2009). Biochemical evidence for translational repression by *Arabidopsis* microRNAs. *Plant Cell* 21, 1762–1768. doi: 10.1105/tpc.108.063412
- Lazzaretti, D., Tournier, I., and Izaurralde, E. (2009). The C-terminal domains of human TNRC6A, TNRC6B, and TNRC6C silence bound transcripts independently of argonaute proteins. *RNA* 15, 1059–1066. doi: 10.1261/rna.1606309
- Lee, S., Jung, H. J., Kang, H., and Kim, S. Y. (2012). *Arabidopsis* zinc finger proteins AtC3H49/AtTZF3 and AtC3H20/AtTZF2 are involved in ABA and JA responses. *Plant Cell Physiol.* 53, 673–686. doi: 10.1093/pcp/pcs023
- Li, S., Liu, L., Zhuang, X., Yu, Y., Liu, X., Cui, X., et al. (2013). microRNAs inhibit the translation of target mRNAs on the endoplasmic reticulum in *Arabidopsis*. *Cell* 153, 562–574. doi: 10.1016/j.cell.2013.04.005.microRNAs
- Li, Y., and Kiledjian, M. (2010). Regulation of mRNA decapping. *Wiley Interdiscip. Rev. RNA* 1, 253–265. doi: 10.1002/wrna.15
- Li, Z., and Thomas, T. L. (1998). PEI1, an embryo-specific zinc finger protein gene required for heart-stage embryo formation in *Arabidopsis*. *Plant Cell* 10, 383–398. doi: 10.1105/tpc.10.3.383
- Liang, W., Li, C., Liu, F., Jiang, H., Li, S., Sun, J., et al. (2009). The *Arabidopsis* homologs of CCR4-associated factor 1 show mRNA deadenylation activity and play a role in plant defence responses. *Cell Res.* 19, 307–316. doi: 10.1038/cr.2008.317
- Lin, P.-C., Pomeranz, M. C., Jikumaru, Y., Kang, S. G., Hah, C., Fujioka, S., et al. (2011). The *Arabidopsis* tandem zinc finger protein AtTZF1 affects ABA- and GA-mediated growth, stress and gene expression responses. *Plant J.* 65, 253–268. doi: 10.1111/j.1365-313X.2010.04419.x
- Lindsay, A. J., and McCaffrey, M. W. (2011). Myosin Va is required for P body but not stress granule formation. *J. Biol. Chem.* 286, 11519–11528. doi: 10.1074/jbc.M110.182808
- Liu, J., Valencia-Sanchez, M. A., Hannon, G. J., and Parker, R. (2005). MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. *Nat. Cell Biol.* 7, 719–723. doi: 10.1038/ncb1274
- López-Rosas, I., Orozco, E., Marchat, L. A., García-Rivera, G., Guillen, N., Weber, C., et al. (2012). mRNA decay proteins are targeted to poly(A)+ RNA and dsRNA-containing cytoplasmic foci that resemble P-bodies in *Entamoeba histolytica*. *PLoS ONE* 7:e45966. doi: 10.1371/journal.pone.0045966
- Lorković, Z. J., and Barta, A. (2002). Genome analysis: RNA recognition motif (RRM) and K homology (KH) domain RNA-binding proteins from the flowering plant *Arabidopsis thaliana*. *Nucleic Acids Res.* 30, 623–635. doi: 10.1093/nar/30.3.623
- Lykke-Andersen, J., and Wagner, E. (2005). Recruitment and activation of mRNA decay enzymes by two ARE-mediated decay activation domains in the proteins TTP and BRF-1. *Genes Dev.* 19, 351–361. doi: 10.1101/gad.1282305
- Maldonado-Bonilla, L. D., Eschen-Lippold, L., Gago-Zachert, S., Tabassum, N., Bauer, N., Scheel, D., et al. (2014). The *Arabidopsis* tandem zinc finger 9 protein binds RNA and mediates pathogen-associated molecular pattern-triggered immune responses. *Plant Cell Physiol.* 55, 412–425. doi: 10.1093/pcp/pt175
- Mérai, Z., Benkovics, A. H., Nyikó, T., Debreczeny, M., Hiripi, L., Kerényi, Z., et al. (2012). The late steps of plant nonsense-mediated mRNA decay. *Plant J.* 73, 50–62. doi: 10.1111/tpj.12015
- Morel, J., Godon, C., Mourrain, P., Béclin, C., Boutet, S., Feuerbach, F., et al. (2002). Fertile Hypomorphic ARGONAUTE (ago1) Mutants Impaired in Post-Transcriptional Gene Silencing and Virus Resistance. *Plant Cell* 14, 629–639. doi: 10.1105/tpc.010358
- Moreno, A. B., Martínez de Alba, A. E., Bardou, F., Crespi, M. D., Vaucheret, H., Maizel, A., et al. (2013). Cytoplasmic and nuclear quality control and turnover of single-stranded RNA modulate post-transcriptional gene silencing in plants. *Nucleic Acids Res.* 41, 4699–4708. doi: 10.1093/nar/gkt152
- Motomura, K., Le, Q. T. N., Kumakura, N., Fukaya, T., Takeda, A., and Watanabe, Y. (2012). The role of decapping proteins in the miRNA accumulation in *Arabidopsis thaliana*. *RNA Biol.* 9, 644–652. doi: 10.4161/rna.19877
- Nover, L., Scharf, K., and Neumann, D. (1989). Cytoplasmic heat shock granules are formed from precursor particles and are associated with a specific set of mRNAs. *Mol. Cell. Biol.* 9, 1298–1308. doi: 10.1128/MC9.3.1298
- Nyikó, T., Sonkoly, B., Mérai, Z., Benkovics, A. H., and Silhavy, D. (2009). Plant upstream ORFs can trigger nonsense-mediated mRNA decay in a size-dependent manner. *Plant Mol. Biol.* 71, 367–378. doi: 10.1007/s11103-009-9528-4
- Olmedo, G., Guo, H., Gregory, B. D., Nourizadeh, S. D., Aguilar-Henonin, L., Li, H., et al. (2006). Ethylene-insensitive5 encodes a 5' → 3' exoribonuclease required for regulation of the EIN3-targeting F-box proteins EBF1/2. *Proc. Natl. Acad. Sci. U.S.A.* 103, 13286–13293. doi: 10.1073/pnas.0605528103
- Parker, R., and Sheth, U. (2007). P bodies and the control of mRNA translation and degradation. *Mol. Cell* 25, 635–646. doi: 10.1016/j.molcel.2007.02.011
- Perea-Resca, C., Hernández-Verdeja, T., López-Cobollo, R., del Mar Castellano, M., and Salinas, J. (2012). LSM proteins provide accurate splicing and decay of selected transcripts to ensure normal *Arabidopsis* development. *Plant Cell* 24, 4930–4947. doi: 10.1105/tpc.112.103697
- Pomeranz, M. C., Hah, C., Lin, P.-C., Kang, S. G., Finer, J. J., Blackshear, P. J., et al. (2010a). The *Arabidopsis* tandem zinc finger protein AtTZF1 traffics between the nucleus and cytoplasmic foci and binds both DNA and RNA. *Plant Physiol.* 152, 151–165. doi: 10.1104/pp.109.145656
- Pomeranz, M., Lin, P., Finer, J., and Jang, J. (2010b). AtTZF gene family localizes to cytoplasmic foci. *Plant Signal. Behav.* 5, 190–192. doi: 10.1104/pp.109.145656.n
- Potuschak, T., Vansiri, A., Binder, B. M., Lechner, E., Vierstra, R. D., and Genschik, P. (2006). The exoribonuclease XRN4 is a component of the ethylene response pathway in *Arabidopsis*. *Plant Cell* 18, 3047–3057. doi: 10.1105/tpc.106.046508
- Qu, J., Kang, S. G., Wang, W., Musier-Forsyth, K., and Jang, J. C. (2014). *Arabidopsis thaliana* tandem zinc finger 1 (AtTZF1) protein in RNA binding and decay. *Plant J.* 78, 452–467. doi: 10.1111/tpj.12485
- Ramachandran, V., Shah, K. H., and Herman, P. K. (2011). The cAMP-dependent protein kinase signaling pathway is a key regulator of P body foci formation. *Mol. Cell* 43, 973–981. doi: 10.1016/j.molcel.2011.06.032
- Reijns, M. A., Alexander, R. D., Spiller, M. P., and Beggs, J. D. (2008). A role for Q/N-rich aggregation-prone regions in P-body localization. *J. Cell Sci.* 121, 2463–2472. doi: 10.1242/jcs.024976
- Reverdatto, S. V., Dutko, J. A., Chekanova, J. A., and Hamilton, D. A. (2004). mRNA deadenylation by PARN is essential for embryogenesis in higher plants. *RNA* 10, 1200–1214. doi: 10.1261/rna.7540204.mRNAs
- Riehs-Kearnan, N., Gloggnitzer, J., Dekrout, B., Jonak, C., and Riha, K. (2012). Aberrant growth and lethality of *Arabidopsis* deficient in nonsense-mediated RNA decay factors is caused by autoimmune-like response. *Nucleic Acids Res.* 40, 5615–5624. doi: 10.1093/nar/gks195
- Ripoll, J. J., Ferrándiz, C., Martínez-Laborda, A., and Vera, A. (2006). PEPPEP, a novel K-homology domain gene, regulates vegetative and gynoecium development in *Arabidopsis*. *Dev. Biol.* 289, 346–359. doi: 10.1016/j.ydbio.2005.10.037
- Rymarquis, L. A., Souret, F. F., and Green, P. J. (2011). Evidence that XRN4, an *Arabidopsis* homolog of exoribonuclease XRN1, preferentially impacts transcripts with certain sequences or in particular functional categories. *RNA* 17, 501–511. doi: 10.1261/rna.2467911
- Scheller, N., Resa-Infante, P., de la Luna, S., Galao, R. P., Albrecht, M., Kaestner, L., et al. (2007). Identification of PatL1, a human homolog to

- yeast P body component Pat1. *Biochim. Biophys. Acta* 1773, 1786–1792. doi: 10.1016/j.bbamcr.2007.08.009
- She, M., Decker, C. J., Sundramurthy, K., Liu, Y., Chen, N., Parker, R., et al. (2004). Crystal structure of Dcp1p and its functional implications in mRNA decapping. *Nat. Struct. Mol. Biol.* 11, 249–256. doi: 10.1038/nsmb730
- Sheth, U., and Parker, R. (2003). Decapping and decay of RNAs occur in cytoplasmic processing bodies. *Science* 300, 805–808. doi: 10.1126/science.1082320
- Sheth, U., and Parker, R. (2006). Targeting of aberrant mRNAs to cytoplasmic processing bodies. *Cell* 125, 1095–1109. doi: 10.1016/j.cell.2006.04.037
- Souquere, S., Mollet, S., Kress, M., Dautry, F., Pierron, G., and Weil, D. (2009). Unravelling the ultrastructure of stress granules and associated P-bodies in human cells. *J. Cell Sci.* 122, 3619–3626. doi: 10.1242/jcs.054437
- Stauffer, E., Westermann, A., Wagner, G., and Wachter, A. (2010). Polypyrimidine tract-binding protein homologues from *Arabidopsis* underlie regulatory circuits based on alternative splicing and downstream control. *Plant J.* 64, 243–255. doi: 10.1111/j.1365-313X.2010.04321.x
- Steffens, A., Jaegle, B., Tresch, A., Hülkamp, M., and Jakoby, M. (2014). Processing body movement in *Arabidopsis thaliana* depends on an interaction between myosins and DCP1. *Plant Physiol.* 164, 1879–1892. doi: 10.1104/pp.113.233031
- Sun, J., Jiang, H., Xu, Y., Li, H., Wu, X., Xie, Q., et al. (2007). The CCCH-type zinc finger proteins AtSZF1 and AtSZF2 regulate salt stress responses in *Arabidopsis*. *Plant Cell Physiol.* 48, 1148–1158. doi: 10.1093/pcp/pcm088
- Teixeira, D., Sheth, U., Valencia-Sanchez, M. A., Brengues, M., and Parker, R. (2005). Processing bodies require RNA for assembly and contain nontranslating mRNAs. *RNA* 11, 371–382. doi: 10.1261/rna.7258505
- Tharun, S., He, W., Mayes, A. E., Lennertz, P., Beggs, J. D., and Parker, R. (2000). Yeast Sm-like proteins function in mRNA decapping and decay. *Nature* 404, 515–518. doi: 10.1038/35006676
- Walley, J. W., Kelley, D. R., Nestorova, G., Hirschberg, D. L., and Dehesh, K. (2010). *Arabidopsis* deadenylases AtCAF1a and AtCAF1b play overlapping and distinct roles in mediating environmental stress responses. *Plant Physiol.* 152, 866–875. doi: 10.1104/pp.109.149005
- Wang, B.-B., and Brendel, V. (2006). Genomewide comparative analysis of alternative splicing in plants. *Proc. Natl. Acad. Sci. U.S.A.* 103, 7175–7180. doi: 10.1073/pnas.0602039103
- Weber, C., Nover, L., and Fauth, M. (2008). Plant stress granules and mRNA processing bodies are distinct from heat stress granules. *Plant J.* 56, 517–530. doi: 10.1111/j.1365-313X.2008.03623.x
- Xiong, L., Gong, Z., Rock, C. D., Subramanian, S., Guo, Y., Xu, W., et al. (2001). Modulation of abscisic acid signal transduction and biosynthesis by an Sm-like protein in *Arabidopsis*. *Dev. Cell* 1, 771–781. doi: 10.1016/S1534-5807(01)00087-9
- Xu, J., and Chua, N.-H. (2009). *Arabidopsis* decapping 5 is required for mRNA decapping, P-body formation, and translational repression during postembryonic development. *Plant Cell* 21, 3270–3279. doi: 10.1105/tpc.109.070078
- Xu, J., and Chua, N.-H. (2012). Dehydration stress activates *Arabidopsis* MPK6 to signal DCP1 phosphorylation. *EMBO J.* 31, 1975–1984. doi: 10.1038/emboj.2012.56
- Xu, J., Yang, J.-Y., Niu, Q.-W., and Chua, N.-H. (2006). *Arabidopsis* DCP2, DCP1, and VARICOSE form a decapping complex required for postembryonic development. *Plant Cell* 18, 3386–3398. doi: 10.1105/tpc.106.047605
- Yoine, M., Nishii, T., and Nakamura, K. (2006). *Arabidopsis* UPF1 RNA helicase for nonsense-mediated mRNA decay is involved in seed size control and is essential for growth. *Plant Cell Physiol.* 47, 572–580. doi: 10.1093/pcp/pcj035
- Yu, J. H., Yang, W., Gulick, T. O. D., and Bloch, K. D. (2005). Ge-1 is a central component of the mammalian cytoplasmic mRNA processing body. *RNA* 11, 1795–1802. doi: 10.1261/rna.2142405.cleavage
- Zheng, D., Chen, C.-Y. A., and Shyu, A.-B. (2011). Unraveling regulation and new components of human P-bodies through a protein interaction framework and experimental validation. *RNA* 17, 1619–1634. doi: 10.1261/rna.2789611
- Zheng, D., Ezzeddine, N., Chen, C.-Y. A., Zhu, W., He, X., and Shyu, A.-B. (2008). Deadenylation is prerequisite for P-body formation and mRNA decay in mammalian cells. *J. Cell Biol.* 182, 89–101. doi: 10.1083/jcb.200801196

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