Catalytic activities, molecular connections, and biological functions of plant RNA exosome complexes

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Abstract

RNA exosome complexes provide the main 3'–5' exoribonuclease activities in eukaryotic cells and contribute to the maturation and degradation of virtually all types of RNA. RNA exosomes consist of a conserved core complex that associates with exoribonucleases and with multimeric cofactors that recruit the enzyme to its RNA targets. Despite an overall high level of structural and functional conservation, the enzymatic activities and compositions of exosome complexes and their cofactor modules differ among eukaryotes. This review highlights unique features of plant exosome complexes, such as the phosphorolytic activity of the core complex, and discusses the exosome cofactors that operate in plants and are dedicated to the maturation of ribosomal RNA, the elimination of spurious, misprocessed, and superfluous transcripts, or the removal of mRNAs cleaved by the RNA-induced silencing complex and other mRNAs prone to undergo silencing.

Introduction

Transcription produces primary transcripts that require multiple processing steps to become functional RNAs. These processing steps include the elimination of 5', 3' or internal sequences, as well as nucleotide additions and modifications. The transcription, processing, and assembly of RNAs and their association with proteins into functional ribonucleoproteins (RNPs) are prone to error and are constantly monitored by nuclear RNA surveillance mechanisms that ensure the rapid degradation of truncated, misprocessed, and misassembled transcripts (Bresson and Tollervey, 2018; Schmid and Jensen, 2018; Peck et al., 2019). RNA degradation also contributes to RNA processing by trimming 3' or 5'-extremities and eliminates the processing and transcription by-products and other nonfunctional RNAs produced by pervasive transcription (Arraiano et al., 2013; Jensen et al., 2013; Tomecki et al., 2017). The efficient elimination of nonfunctional RNAs in the nucleus is important for resolving RNA/DNA hybrids, avoiding the sequestration of RNA processing factors, and preventing the export and nonproductive assembly of faulty mRNAs with ribosomes (Kilchert et al., 2016; Ogami et al., 2018). In the cytosol, translation-coupled RNA degradation prohibits the production of truncated proteins, enables the recycling of stalled ribosomes, and prevents endogenous mRNAs from becoming targets of posttranscriptional gene silencing (PTGS; Labno et al., 2016; Hung and Slotkin, 2021). Both nuclear and cytosolic RNA degradation pathways also ensure the timely turnover of RNA and thereby define (together with transcriptional control) cellular RNA homeostasis. Finally, RNA degradation is one of the mechanisms that enable rapid responses to developmental signals, environmental changes, and pathogen infection (Prall et al., 2019).

RNA exosome complexes catalyze the 3'–5' exoribonucleolytic degradation of almost all types of RNA in both nuclear and cytoplasmic compartments (Chekanova et al., 2007; Gudipati et al., 2012; Schneider et al., 2012). RNA
exosomes consist of a conserved core complex composed of nine subunits, Exo9, which associates in a compartment-specific manner with ribonucleases and other cofactors such as RNA helicases, RNA binding proteins, poly(A) polymerases, and proteins that mediate protein–protein interactions (Figure 1 and Table 1). These cofactors often assemble into higher-order complexes that assist in RNA recognition and unwinding or link exosome-mediated RNA degradation to other cellular processes, such as ribosome biogenesis or transcription, splicing, nuclear export, and translation. The basic principle that Exo9 associates with ribonucleases and various additional cofactors to form functionally specialized exosome complexes is conserved in all eukaryotes. However, exosome activity, as well as the compositions and functions of exosome cofactors, has diverged in fungi, animals, and plants. Several recent reviews discuss the activity, interactions, and functions of the exosome and its cofactors in yeasts and animals, including humans (Puno et al., 2019; Schmid and Jensen, 2019; Tudek et al., 2019; Fasken et al., 2020; Fraga de Andrade et al., 2020; Garland and Jensen, 2020; Nair et al., 2020; Weick and Lima, 2021)

This review summarizes the recent progress in understanding RNA exosome complexes and their functions in the model plant Arabidopsis thaliana (Arabidopsis). We discuss the importance of exosome-mediated RNA degradation for developmental processes and for protecting endogenous mRNAs against spurious silencing.

The exosome core complex

Plant RNA exosomes have a unique phosphorolytic activity

Exo9 adopts a barrel-like shape with a central channel. Three heterodimers of RNPase PH-like domain proteins (RRP41–RRP45, MTR3–RRP42, and RRP46–RRP43) form a ring, and three proteins with S1 and/or KH RNA-binding domains (RRP4, RRP40, and CSL4) form a cap-like structure on top of the ring (Figure 2; Liu et al., 2006). Exo9 is structurally and evolutionarily related to bacterial polynucleotide phosphorylases (PNPases) and archaeal exosomes (Tsanova and van Hoof, 2010; Januszyk and Lima, 2014). Archaeal exosomes, consisting of three RRP41–RRP42 heterodimers, harbor three catalytic sites located at the interfaces of each dimer inside the channel (Figure 2A; Böttner et al., 2005; Liu et al., 2006). By contrast, Arabidopsis Exo9 can degrade RNA substrates (Sikorska et al., 2017). Tagged Exo9 purified from Arabidopsis uses inorganic phosphate to cleave phosphodiester bonds and therefore degrades RNA into nucleosides diphosphates (Figure 2B).

Recombinant RRP46 of rice (Oryza sativa), which forms homodimers independently of Exo9, was also proposed to have a phosphorolytic RNase activity, in addition to a hydrolytic DNase activity (Yang et al., 2010). OsRRP46’s phosphorolytic RNase activity remains intriguing, as OsRRP46 contains only the acidic residue necessary for the cleavage of the phosphodiester bond but lacks the phosphate

Figure 1 The RNA exosome and its cofactors. Exosome complexes consist of a conserved core complex (Exo9) that associates with two classes of exoribonucleases and with RNA helicases of the MTR4/SKI2 family. These RNA helicases help with the unwinding and threading of RNA through the central channel of the core complex. RNA helicases also serve as a binding platform for other cofactors including poly(A) polymerases, RNA binding proteins, and proteins that mediate protein interactions. The composition of exosomes and cofactor complexes depends on their intracellular localization and is in part conserved and in part variable in different eukaryotes.
| Localization | Name in Arabidopsis | AGI | Human Homolog | S. pombe Homolog | Comment |
|--------------|---------------------|-----|---------------|------------------|---------|
| Nuclear and cytosolic | CSL4 | At5g38890 | EXOSC1 | Csd4 | Exo9 subunit, S1 RNA binding domain |
| | RRP4 | At1g03360 | EXOSC2 | Rrp4 | Exo9 subunit, S1 and KH RNA binding domains |
| | RRP40 | At2g25355 | EXOSC3 | Rrp40 | Exo9 subunit, S1 and KH RNA binding domains |
| | RRP41 | At3g61620 | EXOSC4 | Rrp41 | Exo9 subunit, RNase PH fold, confers phosphorolytic activity to plant Exo9 |
| | RRP45A | At3g12990 | EXOSC9 | Rrp45 | Exo9 subunit, RNase PH fold, encoded by duplicated genes in Arabidopsis and close relatives |
| | RRP45B (CER7) | At3g60500 | | | |
| | RRP46 | At2g07110 | EXOSC5 | Rrp46 | Exo9 subunit, RNase PH fold |
| | RRP43 | At1g60080 | EXOSC8 | Rrp43 | Exo9 subunit, RNase PH fold |
| | MTR3 (RRP41L) | At4g27490 | EXOSC6 | Mtr3 | Exo9 subunit, RNase PH fold |
| | RRP42 | At3g07750 | EXOSC7 | Rrp42 | Exo9 subunit, RNase PH fold |
| | DIS3 (RRP44) | At2g17510 | DI3 | Dis3 | Exoribonuclease (RNase II family), endonuclease activity conferred by PIN domain, binds Exo9 |
| Nuclear | RRP6L2 | At5g35910 | EXOSC10 | Rrp6 | Exoribonuclease (RNase D family), predominantly nucleolar |
| | RRP47 | At5g25080 | C1D | Cu1 | RRP6 cofactor |
| | MPP6 | At5g9460 | MPP6 | Mpp6 | Yeast and human homologs stabilize the interaction of Exo9 with certain cofactors |
| | MTR4 | At1g59760 | MTR4 (SKIV2L2) | Mtr4 | RNA helicase, predominantly nucleolar, associates with Exo9 and preribosomes |
| | TRL | At5g53770 | PAPD5 (TRF4-2) | Cid4 | Nucleotidyl transferase, predominantly nucleolar |
| | MEE44 | At4g00060 | | | Nucleotidyl transferase, TRF4 homolog, not yet investigated |
| | HEN2 | At2g06990 | | Mti1 | RNA helicase, predominantly nucleoplasmic, associated with EXO9 and NEXT, colocalizes with SOP1 in nuclear speckles |
| | RBM7 | At4g10110 | RBM7 | No homolog | RNA binding protein (RRM RNA binding domain), nucleoplasmic and NEXT subunit |
| | ZCCHC8A, ZCCHC8B | At5g38600 | ZCCHC8 | No homolog | Zn-finger proteins, nucleoplasmic, NEXT subunits, interact with SE |
| | | At1g67210 | | | |
| | CBP20 | At5g44200 | NCBP2 | Cbc2 | Subunit of the CBC, RRM RNA binding domain |
| | CBP80 | At2g13540 | NCBP1 | Cbc1 | Subunit of the CBC |
| | SE | At2g27100 | ARS2 | Pir2 | Multifunctional Zn-finger protein required for nuclear RNA sorting |
| | SOP1 | At1g21580 | ZC3H38 | Red5 | Zn-finger protein, co-localized with HEN2 in nucleoplasmic speckles, and putative PAXT component |
| | RBM26/27 | At3g27700 | RBM26, RBM27 | Rmn1 | RNA binding protein, putative PAXT component |
| | PABN1 | At5g51120 | PABPN1 | Pab2 | Nuclear poly(A) binding protein, putative PAXT component |
| | Not identified | No clear homologs | ZCCHC7 (AIR1) | Air1 | Zn-knuckle protein, part of the TRAMP complex in yeast and human |
| | Not identified | No clear homologs | ZC3H18 | | Zn-finger protein, subunit of PAXT in human |
| | Not identified | No clear homologs | ZFC3H1 | Red1 | Zn-finger protein, subunit of PAXT in human |
| Cytosolic | SK12 | At3g46960 | Sk2VL | Sk2 | RNA helicase, subunit of the SKI complex |
| | SK13 | At1g76630 | TTC37 | Sk3 | Tetra tricopeptide repeat protein, subunit of the SKI complex |
| | SK18 (VIP3) | At4g29830 | WDR61 | Sk8 | WD40 repeat-containing protein, subunit of the SKI complex, has a second function as a subunit of the nuclear PAF complex |
| | SK7 | At5g10630 | HS51LV3 | Sk7 | Binds to Exo9 and the SKI complex, has a GTPase domain of unknown function |
| | RST1 | At3g27670 | FOCAD | No homolog | ARM repeats, associated with EXO9 and RIPR |
| | RIPR | At5g44150 | Not conserved | | No known domains, associated with RST1, SK7, and the Ski complex |
coordination motif found in RNase PH, PNPase, and archaeal or plant RRP41 proteins. Whether the OsRRP46 subunit contributes to the catalytic activity of rice Exo9 is unknown. However, the lysine and glutamine residues that are required for RNA binding by OsRRP46 based on structural data (Yang et al., 2010) are not conserved beyond monocots. It is therefore impossible to predict a possible conservation of RRP46 catalytic activity in plants based on sequence alignments. The possibility that Exo9 subunits form alternative complexes separately from the RNA exosome has also not been investigated in other plants.

In Arabidopsis, the catalytic activity of Exo9 is not essential for viability. Nevertheless, the amino acid motifs required for phosphate coordination, magnesium coordination, and RNA binding are strictly conserved in the RRP41 proteins of land plants, mosses, and green algae (Figure 2D). This observation is a strong indication for a deep conservation of a phosphorolytically active Exo9 in the green lineage (Sikorska et al., 2017). Interestingly, the genomes of some red algae and early-branching eukaryotes including certain Amoebozoa, the human pathogen Nagleria fowleri (Heterobolosa), and Capsaspora owczarzaki (Ophistokonta), a unicellular relative of metazoans, also encode RRP41 proteins with intact active sites. This observation suggests that Exo9 complexes with phosphorolytic activity may also exist outside the plant lineage. If the Exo9 activity in these species is experimentally proven, this would indicate that the primordial phosphorolytic activity borne by prokaryotic enzymes has been lost multiple times during evolution. Why it has been lost in yeast and animals but preserved in plants is one of the intriguing questions in the field.

Three catalytic activities of plant exosomes cooperate during rRNA processing

Yeast and human exosome complexes owe their ribonucleolytic activity to the association of Exo9 with two hydrolytic ribonucleases (Figure 3A). DIS3 (aka RRP44) is a processive RNase II-type enzyme that binds to the bottom of both nuclear and cytosolic Exo9 and has both exo and endoribonucleolytic activities (Chlebowski et al., 2011; Figure 3A).
Figure 3 Cooperation of enzymatic activities within exosome complexes. A, Catalytic activities of eukaryotic exosomes. Plant Exo9 has phosphorolytic activity, while yeast and human Exo9 are catalytically inert. RRP6L2 and RRP6 proteins are distributive, hydrolytic exoribonucleases. In yeast and human, RRP6 binds to the tops of nuclear exosome complexes. DIS3/RRP44 proteins bind to the bottom of Exo9 and confer processive, hydrolytic exoribonuclease activity as well as endonucleolytic activity. DIS3L is a related exoribonuclease that binds to cytoplasmic exosomes in human and animals. B, Simplified diagram illustrating the contributions of the three catalytic activities of Exo9, RRP44, and RRP6L2 to the processing and degradation of rRNA in Arabidopsis. The functions of two nucleolar exosome cofactors, the RNA helicase MTR4 and the poly(A) polymerase TRL are also indicated. C, Rosette phenotypes of rrp41 mutant plants expressing either a catalytically active or inactive version of RRP41 in the WT and mutant backgrounds. rrp6l2 and mtr4 are null mutants, and RRP44KD are knockdown mutants.
Animals have a second paralog, DIS3L, which lacks the endoribonucleolytic site and binds only to cytosolic Exo9. ScRRP6/HsEXOSC10 is a distributive enzyme of the RNase D-type that binds to the tops of nuclear exosome complexes. RRP6 proteins have also exosome-independent functions in the 3’-processing of certain snRNAs and 18S ribosomal RNA (rRNA) precursors (Callahan and Butler, 2008; Preti et al., 2013; Sloan et al., 2013; Taftorbe et al., 2013). The genomes of most plant species encode one DIS3/RRP44, which is essential in Arabidopsis and hereafter named RRP44, and two or three RRP6-like proteins named RRP6L1–3 (Chekanova et al., 2007; Lange et al., 2008; Zhang et al., 2010; Kumakura et al., 2013). To date, only RRP6L2 is a proven exosome co-factor in Arabidopsis. RRP6L1 is dispensable for exosome-mediated RNA degradation (Zhang et al., 2014), and the functions of RRP6L3 have not yet been elucidated. RRP6L2 is enriched in nucleoli and contributes together with RRP44 and Exo9 to the maturation or degradation of rRNAs (Figure 3B; Lange et al., 2008; Sikorska et al., 2015; Sikorska et al., 2017). RRP6L2 also has an exosome-independent function in trimming specific 18S precursors, similar to its human counterpart EXOSC10 (Preti et al., 2013; Sloan et al., 2013; Taftorbe et al., 2013; Sikorski et al., 2015).

The structures and catalytic properties of yeast and human exosome complexes containing DIS3/RRP44 and RRP6 have been intensively studied (Liu et al., 2007; Januszyk et al., 2011; Wasmuth and Lima, 2012; Makino and Conti, 2013; Fromm et al., 2017; Axhem et al., 2020). Plant exosome complexes have not yet been reconstituted, and the complexes that have thus far been purified from Arabidopsis contain (besides Exo9) only trace amounts of RRP44 and no RRP6-like protein (Chekanova et al., 2007; Lange et al., 2014, 2019). However, some insight into the teamwork of RRP44, RRP6L2, and Exo9s activity was provided by the analysis of rRNA processing intermediates in single, double, and triple mutant plants (Sikorska et al., 2017). The processing of rRNA precursors and the elimination of rRNA processing by-products is one of the conserved and quantitatively most important functions of eukaryotic exosomes (Figure 3B; Henras et al., 2015; Sáez-Vásquez and Delseny, 2019). Briefly, 18S, 5.8S, and 25S/5S rRNAs are produced from a polycistronic 35S (yeast) or 45S (plants) precursor. This requires the removal of external and internal transcribed spacers (ETS/ITS) by endoribonucleolytic cleavages and exoribonucleolytic degradation by both 5’–3’ and 3’–5’exoribonucleases (Tomecki et al., 2017). The phosphoro-lytic activity of AtExo9 degrades specific fragments that are generated during the elimination of the 5’-ETS and cooperates with RRP6L2 and RRP44 for the 3’-trimming of 5.8S rRNA precursors (Sikorska et al., 2017). The cooperation of the three catalytic activities is further evidenced by plant phenotypes. While loss of RRP6L2 or AtExo9’s catalytic activity produces no obvious phenotype, double mutants lacking RRP6L2 and AtExo9s activity show developmental defects characteristic of mild ribosome biogenesis mutants, notably disturbed vein patterns in cotyledons (Byrne, 2009; Sikorska et al., 2017), and smaller rosette leaves (Figure 3C). Downregulation of RRP44 in leaves only slightly affected leaf and rosette size (Kumakura et al., 2013), but deleting RRP6L2 and/or mutating AtExo9’s activity in an RRP44 knockdown background led to curled leaves and much smaller rosettes (Figure 3C; Sikorska et al., 2017). Whether these phenotypes are only due to impaired ribosome biogenesis is not yet clear. However, the additive character of these plant phenotypes highlights the cooperation of RRP44, RRP6L2, and Exo9’s activity in vivo.

Nuclear exosomes

Two RNA helicases associate with nuclear exosome complexes in plants

Both nuclear and cytosolic exosome complexes bind RNA helicases of the MTR4/SKI2 family, which unwind RNA substrates and help thread them into the exosome barrel (Johnson and Jackson, 2013). MTR4/SKI2 RNA helicases possess a characteristic arch domain with a KOW motif that is recognized by various proteins harboring arch-interacting motifs (AIMs; Jackson et al., 2010; Weir et al., 2010; Halbach et al., 2012). MTR4/SKI2 helicases themselves, or their interacting factors, can serve as scaffolds for the assembly of complex cofactors that assist in RNA recognition and degradation and/or recruit the exosome to transcription and processing sites or ribosomes (Weick and Lima, 2021).

The central cofactor of nuclear exosomes is the RNA helicase MTR4. Saccharomyces cerevisiae and humans possess a single essential MTR4 protein that is required for all nuclear exosome functions (Schmid and Jensen, 2019). By contrast, fission yeast (Schizosaccharomyces pombe) and all members of the green lineage from unicellular green algae to flowering plants have two functionally specialized MTR4 homologs, named MTR4 and MTR4-like 1 (MTL1) in S. pombe (Lee et al., 2013), and MTR4 and HUA ENHANCER2 (HEN2) in plants (Western et al., 2002; Lange et al., 2014). Both S. pombe MTR4 and MTL1 are essential, but plant mutants lacking either of the nuclear proteins MTR4 or HEN2 have only mild, though distinct, developmental defects under normal growth conditions. However, double mutants are rarely obtained and if so, seedlings do not develop true leaves and die early (Lange et al., 2014). This suggests first, that MTR4 and HEN2 can partially replace each other, and second, that at least one nuclear RNA helicase (MTR4 or HEN2) is required for postgerminative viability.

How MTR4 or HEN2 bind to plant exosome complexes has not yet been investigated. SpRRP6 is required to recruit exosome complexes to MTL1-containing foci (Shichino et al., 2020), and ScMTR4 is recruited to a composite surface formed by RRP6 and RRP47/Lrp1(C1D in humans), a small accessory protein that binds to the N-terminal PMC2NT domain of ScRRP6 (Schuch et al., 2014; Figure 4A). Together, these data suggest that RRP6 is necessary for the association of MTR4 and Exo9. However, hSMT4 and RRP6 both bind to the EXOSC2 (RRP44) subunit of the core
complex, and the binding of hsMTR4 induces a conformational change that displaces the catalytic center of EXOSC10 (RRP6) and C1D from the channel entry site (Weick et al., 2018). The small RNA binding protein MPP6 also bridges MTR4 to the ScRRP40/HsEXOSC3 subunit (Wasmuth et al., 2017; Falk et al., 2017a). Comparable structural data for plant exosomes are currently lacking. A fraction of affinity-purified Arabidopsis exosome complexes contain MTR4 or its nucleoplasmic homolog HEN2 (Figure 4B), and conversely, tagged versions of MTR4 or HEN2 pull down Exo9.
(Lange et al., 2014). The interaction of RRP6L2 with RRP47 is conserved in Arabidopsis (Sikorski et al., 2015), and a putative homolog of MPP6 is encoded by At5g59460. Yet, none of these proteins was detected by mass spectrometry analyses using MTR4, HEN2, or three different subunits of AtExo9 as bait (Chekanova et al., 2007; Lange et al., 2014, 2019). The small size of AtMPP6 and RRP47 may not favor their detection by mass spectrometry, and the absence of RRP6L2 in exosome purifications from Arabidopsis could be linked to sample preparation and/or reflect protein instability or transient associations with Exo9. However, the data available to date would also be in line with the idea that binding of MTR4 or HEN2 to plant Exo9 does not require the presence of an RRP6-like protein.

**AtMTR4 recruits the exosome to preribosomal particles**

Arabidopsis MTR4 is predominantly found in nucleoli (Lange et al., 2011). Plants lacking MTR4 share morphological features with other ribosome biogenesis mutants, such as upward curled rosette leaves (Figure 3C), distorted vein patterns, fused cotyledons, and pointed first leaves (Lange et al., 2011; Weis et al., 2015; Ohbayashi et al., 2017; Sikorska et al., 2017). Furthermore, MTR4 co-purifies with a number of predicted ribosomal biogenesis factors (Figure 4C; Lange et al., 2014) whose yeast homologs are associated with both early and late 60S preribosomal particles (Kressler et al., 2017). In line with these findings, mtr4 plants accumulate the 5.8S rRNA precursor 7S and the full-length P–P′ fragment of the 5′-ETS, while levels of nonribosomal nuclear RNAs are hardly affected (Lange et al., 2011). Taken together, these data indicate that the predominant function of plant MTR4 is to assist in the processing of rRNA and the degradation of rRNA maturation by-products.

A fraction of baker’s yeast MTR4 molecules are also tethered to preribosomes (Figure 4D; Thoms et al., 2015; Schuller et al., 2018). Utp18, an early-acting factor required for removing the 5′-ETS, recruits MTR4 to 90S preribosomal particles (Thoms et al., 2015). Nop53 is a late-acting factor that recruits MTR4 to pre-60S ribosomal particles to trim the 3′-ends of 5.8S rRNA precursors. Both Utp18 and Nop53 possess an AIM that binds to the KOW motif in MTR4’s arch domain (Thoms et al., 2015; Fromm et al., 2017; Falk et al., 2017b; Schuller et al., 2018). This AIM is conserved in plant UTP18 and NOP53 homologs (Thoms et al., 2015), but their direct binding to Arabidopsis MTR4 has not yet been demonstrated. Of note, an insertion of approximately 10 amino acids is present in the KOW domains of all plant MTR4 proteins but absent in its HEN2 orthologs (Lange et al., 2014). Whether and how these extra amino acids affect the structure, binding properties, and biological functions of plant MTR4 proteins would be an interesting question to address.

In fungi and animals, a portion of MTR4 is incorporated into TRAMP complexes (Figure 4D). Besides MTR4, TRAMP contains a Zn-knuckle protein (Sc/SpAir1/2, HsZCCHC7) and a terminal nucleotidyl transferase that adds nontemplated A-tails to exosome substrates (ScTrf4/S, SpCid14, and HsPADS/TRF4-2) (LaCava et al., 2005; Vanacova et al., 2005; Wyers et al., 2005; Win et al., 2006; Lubas et al., 2011; Figure 4D). Humans have one TRAMP complex that resides in nucleoli (Lubas et al., 2011; Sudo et al., 2016). Baker’s yeast has three distinct TRAMP complexes (Trf4/Air1, Trf4/Air2, and Trf5/Air1) that have both common and specific targets (San Paolo et al., 2009; Delan-Forino et al., 2020). In both yeast and human, TRAMP complexes are dispensable for 5.8S rRNA processing but contribute to the degradation of a variety of other nuclear exosome substrates (Sudo et al., 2016; Delan-Forino et al., 2020). The existence of a TRAMP-like complex has not yet been reported in plants. The Arabidopsis genome encodes two putative homologs of TRF4/S named MEE44 (At4g00060) and TRF4/S-LIKE (TRL; At5g53770). The function of MEE44 has not yet been studied. TRL adds nontemplated adenosines to the 3′-ends of several rRNA degradation intermediates (Sikorski et al., 2015), but the full set of its substrates is yet to explore. Although a physical interaction between TRL or MEE44 with MTR4 or HEN2 remains a possibility, it has not been experimentally supported so far. Indeed, neither TRL nor MEE44 was detected by mass spectrometry among potential interactants co-purifying with MTR4 and HEN2.

**HEN2 is the main exosome cofactor involved in nuclear RNA surveillance**

While Arabidopsis MTR4 is mostly involved in rRNA processing/degradation, its paralog HEN2 targets predominantly non-rRNAs for degradation by nuclear exosome complexes (Figure 5; Lange et al., 2014; Kindgren et al., 2018, 2020; Bajczyk et al., 2020; Gao et al., 2020; Thieffry et al., 2020; Thomas et al., 2020). HEN2 substrates include long noncoding RNAs (lncRNAs), precursors and processing intermediates of small nuclear RNAs (snRNAs), small nuclear RNAs (snRNAs), and primary microRNAs (miRNAs), as well as by-products produced by miRNA processing. Importantly, loss of HEN2 or the downregulation of Exo9 core subunits does not affect the steady-state levels of mature snORNs, snRNAs, or miRNAs and therefore, HEN2 and the exosome are not required for their production (Chekanova et al., 2007; Lange et al., 2014; Bajczyk et al., 2020; Gao et al., 2020).

A pioneering study using tiling arrays revealed that the plant exosome degrades a vast array of cryptic nuclear transcripts, including antisense transcripts, transcripts generated from nongenic regions, and transcripts corresponding to the 5′-regions of protein-coding genes (Chekanova et al., 2007). Since then, studies employing diverse protocols based on high-throughput sequencing have precisely characterized several types of short-lived nuclear transcripts and confirmed the essential role of the RNA exosome and HEN2 in their degradation. One group of cryptic nuclear RNAs that is rapidly eliminated by HEN2 and the nuclear exosome is the short promoter–proximal RNAs (spRNAs). spRNAs...
are produced from nascent transcripts by cleavage and polyadenylation shortly after initiation at positions of promoter–proximal RNA polymerase II (RNAPII) stalling (Thomas et al., 2020). HEN2 and the RNA exosome also degrade several types of antisense RNAs produced at or near protein-coding genes. Antisense RNAs initiated within 500-bp upstream of the transcription start sites of protein-coding genes are called divergent noncoding transcripts or promoter upstream transcripts (PROMPTs; Kindgren et al., 2020; Thieffry et al., 2020). PROMPTs are widespread in mammals but are detected at only a small percentage of Arabidopsis genes (Kindgren et al., 2020; Thieffry et al., 2020).

Other short-living antisense transcripts degraded by the RNA exosome initiate within 5′-regions of protein-coding genes (convergent antisense [CAS]) or within 3′-untranslated regions (poly(A)-site antisense [PAS]) (Kindgren et al., 2020; Thieffry et al., 2020). In addition, hen2 mutants accumulate transcripts with 3′-extensions downstream of the annotated cleavage and polyadenylation sites that correspond to 3′-read-through transcripts (Lange et al., 2014; Bajczyk et al., 2020; Parker et al., 2020). Finally, HEN2 promotes the degradation of unspliced, mis-spliced, or inefficiently spliced mRNAs (Lange et al., 2014; Hématy et al., 2016). Taken together, these data show that HEN2 is the major nuclear cofactor for the exosome-mediated elimination of spurious, defective, nonfunctional, and superfluous transcripts, including precursors of noncoding RNAs and pre-miRNAs. Plants lacking HEN2 have a disturbed phyllotaxis, slightly larger flowers, and occasionally increased numbers of flower organs (Western et al., 2002; Lange et al., 2014). Understanding how the failure to degrade various nuclear transcripts is related to the morphological phenotypes of hen2 mutants is certainly an interesting future research direction that will elucidate the role of nuclear RNA surveillance at the organismic level.

HEN2 assembles into the nuclear exosome targeting complex

In addition to its association with Exo9, HEN2 is a component of the nuclear exosome targeting (NEXT) complex. In human cells where it was identified, the NEXT complex is composed of the nucleoplasmic fractions of hMTR4, ZCCHC8, and RBM7 (Lubas et al., 2011). Arabidopsis NEXT complexes contain HEN2, RBM7, ZCCHC8A, and ZCCHC8B (Figure 6). ZCCHC8A and ZCCHC8B are closely related and
The nature and polyadenylation status of the RNA substrates of the NEXT complex and the (hypothetical) PAXT connection have not been defined. The nature and polyadenylation status of the RNA substrates of the NEXT complex following the posttranscriptional addition of adenines. (Bajczyk et al., 2020), the substrates of plant NEXT complexes are currently unknown. Human NEXT is required for RNA fate (Giacometti et al., 2017; Schulze et al., 2018; O'Sullivan and Howard, 2017). Some of these interactions are mutually exclusive. Known interactants of Arabidopsis SE include the miRNA processing factors DCL1 and HYAPONASTIC LEAVES1 (HYL1), subunits of RNAII including CPL1 and its partner protein HOS5 (RCF3), splicing factors such as subunits of the U1snRNP, components of the THO–TREX complex involved in mRNA export, and NEXT (Chen et al., 2015; Knop et al., 2017; Speth et al., 2018; Bajczyk et al., 2020). The emerging picture is that the CBC–SE/ARS complex provides a dynamic platform for the assembly of functionally distinct RNPs and thereby functions as a central nuclear sorting hub for RNA fate (Giacometti et al., 2017; Schulze et al., 2018; Garland and Jensen, 2020). In line with the model that SE recruits the NEXT complex to promote RNA degradation (Lubas et al., 2011; Andersen et al., 2013), plants with impaired SE function accumulate many known targets of HEN2 and the nuclear exosome, including precursors of miRNAs and snoRNAs, snRNAs, IncRNAs, and short transcripts from protein-coding genes (Bajczyk et al., 2020). These short transcripts often comprise the first intron, suggesting they are produced by premature termination and do not undergo splicing.

Mutating Arabidopsis RBM7 or ZCCHC8A/B has no obvious effects on plant growth and development, and with the exception of miRNA precursors and maturation by-products (Bajczyk et al., 2020), the substrates of plant NEXT complexes are currently unknown. Human NEXT is required for the degradation of a subset of PROMPTs, enhancer RNAs, several IncRNAs, and 3′-extended snoRNAs or snRNAs, and iCLIP experiments confirmed that these transcripts are bound by HsRBM7 (Lubas et al., 2011, 2015; Andersen et al., 2013). Interestingly, RBM7 binding sites were enriched close to the transcription start sites of genes producing PROMPTs, enhancer RNAs, or short RNAs generated by promoter–proximal transcription termination. Furthermore, RBM7 binding sites were particularly enriched within the first 1,000 nt of pre-mRNAs. These data indicate that RBM7 is loaded onto newly synthesized RNA to enable immediate NEXT- and exosome-mediated degradation as soon as a 3′-extremity becomes unprotected (Lubas et al., 2015).

**NEXT connects the exosome to SERRATE and the CBC**

In addition to exploiting the RNA binding properties of RBM7, the NEXT complex can find newly transcribed exosome targets through its interaction with SERRATE (SE; ARS2/Pir2 in animals and S. pombe) and the nuclear cap-binding complex (CBC; Andersen et al., 2013; Lange et al., 2014; Lubas et al., 2015, 2020). The nuclear CBC consists of two subunits, CBC20 and CBC80, which bind to the m7G cap of nascent RNAII transcripts (Gonatopoulos-Pournatzis and Cowling, 2014). SE/ARS2 is a nuclear Zn-finger protein that interacts with the CBC, and at the same time, with various factors involved in transcription, transcription termination, 3′-end maturation, RNA processing, export, and degradation (O’Sullivan and Howard, 2017). Some of these interactions are mutually exclusive. Known interactants of Arabidopsis SE include the miRNA processing factors DCL1 and HYAPONASTIC LEAVES1 (HYL1), subunits of RNAII including CPL1 and its partner protein HOS5 (RCF3), splicing factors such as subunits of the U1snRNP, components of the THO–TREX complex involved in mRNA export, and NEXT (Chen et al., 2015; Knop et al., 2017; Speth et al., 2018; Bajczyk et al., 2020). The emerging picture is that the CBC–SE/ARS complex provides a dynamic platform for the assembly of functionally distinct RNPs and thereby functions as a central nuclear sorting hub for RNA fate (Giacometti et al., 2017; Schulze et al., 2018; Garland and Jensen, 2020). In line with the model that SE recruits the NEXT complex to promote RNA degradation (Lubas et al., 2011; Andersen et al., 2013), plants with impaired SE function accumulate many known targets of HEN2 and the nuclear exosome, including precursors of miRNAs and snoRNAs, snRNAs, IncRNAs, and short transcripts from protein-coding genes (Bajczyk et al., 2020). These short transcripts often comprise the first intron, suggesting they are produced by premature termination and do not undergo splicing.

Human CBC/ARS2 associates with NEXT via an interaction between MTR4 and SE (Fan et al., 2017; Melko et al., 2020). An additional bridge between SE and NEXT is formed by the Zn-finger protein ZC3H18 (Andersen et al., 2013; Giacometti et al., 2017; Winczura et al., 2018). By contrast, Arabidopsis HEN2 does not physically interact with SE, and sequence homologs of ZC3H18 seem to be absent from
plant genomes. Instead, plant CBC–SE contacts the NEXT complex via a direct interaction between SE and the ZCCHC8 subunit (Figure 5; Bajczyk et al., 2020).

A PAXT connection in plants?
Human MTR4 can assemble into NEXT, but alternatively and probably less frequently, with the large Zn-finger protein ZFC3H1 (Meola et al., 2016; Ogami et al., 2017). MTR4’s interactions with NEXT components or ZFC3H1 are mutually exclusive. The MTR4–ZFC3H1 dimer can contact different partners, among them ZC3H18 and CBC-ARS, the Zn-finger protein ZC3H3, the RNA binding proteins RBM26 and/or RBM27, and, in an RNA-dependent manner, the nuclear poly(A) binding protein PABN1 (Meola et al., 2016; Silla et al., 2018, 2020; Wu et al., 2020a). The interaction network of the core module MTR4–ZFC3H1 with PABN and the ZC3H3–RBM26/27 and ZC3H18–CBC–ARS subcomplexes is called the poly(A) exosome targeting (PAXT) connection. PAXT targets include IncRNAs, spliced transcripts deriving from genes hosting snoRNA genes in their introns, and truncated transcripts produced from protein-coding genes with intrinsic polyadenylation sites (Meola et al., 2016; Ogami et al., 2017). The current notion is that NEXT primarily targets transcripts with heterogeneous 3’-ends directly after their release from the transcription machinery, while PAXT promotes the degradation of transcripts with defined 3’-ends generated by canonical cleavage and polyadenylation. However, when NEXT function is impaired, its substrates can be polyadenylated and become targets of PAXT (Wu et al., 2020a; Figure 6).

Both MTR4 and ZFC3H1 are also crucial for retaining poly(A)+ exosome targets in nuclear foci upon the downregulation of exosome activity (Ogami et al., 2017; Silla et al., 2018). Moreover, MTR4 and ZFC3H1 compete with the export factor THO4/ALYREF for the binding to CBC-ARS2. This competition constitutes a crucial barrier that prevents the export of nuclear exosome targets to the cytosol (Fan et al., 2017; Silla et al., 2018). The importance of this function is underlined by the global reduction of polysomes and translation in cells depleted of MTR4 or ZFC3H1, likely due to the heavy loading of short open reading frame-containing, prematurely terminated transcripts or PROMPTs onto ribosomes (Ogami et al., 2017). A modular interaction network similar to PAXT also operates in fission yeast: Mtl1, one of the two MTR4 homologs in S. pombe, forms a complex named Mtl1-Red1-Core (MTREC) or nuclear RNA silencing with the ZFC3H1 homolog Red1 (Lee et al., 2013; Egan et al., 2014; Zhou et al., 2015). MTREC can associate with various other partners, among them the canonical poly(A) polymerase Pia1, and CBC-Ars2 and Red5-Rrn1-Pab2 (the orthologs of ZC3H3-RBM26/27-PABN1; Zhou et al., 2015).

A possible Arabidopsis homolog of the Zn-finger protein ZFC3H1/Red1 may be encoded by At3g27700, although its overall sequence conservation is rather poor, except for a short stretch containing the Zn-finger motif. Similarly, the sequence conservation between RBM27 and its closest Arabidopsis homolog, encoded by At3g27700, is restricted to the Zn-finger motif and the RNA recognition domain. Yet, a genetic screen for mutations that suppress the phenotype of pas2-1 mutants identified a putative Arabidopsis ortholog of ZC3H3/Red5 named SUPPRESSOR OF PAS2 (SOP1, encoded by At1g21580; Hématy et al., 2016). In the pas2-1 mutant, a point mutation in a splicing donor site severely affects the production of the PAS2-1 mRNA, encoding an essential factor for very long-chain fatty acid biosynthesis. Compared to pas-2 single mutants, pas-2 sop1 plants accumulate higher levels of PAS2-1 pre-mRNA, and the failure to degrade this pre-mRNA likely compensates for its inefficient splicing and allows the translation of some functional PAS2 protein (Hématy et al., 2016). sop1 mutants also accumulate some other nonspliced mRNAs and other types of HEN2 targets including 3’-extended mRNAs, IncRNAs, and some primary (pri)-miRNAs (Hématy et al., 2016; Gao et al., 2020). By contrast, loss of SOP1 does not affect levels of snoRNA precursors or other small noncoding RNAs, which accumulate in hen2 mutants and are likely targets of NEXT. Hence, SOP1 is required for the degradation of a subset of HEN2 targets, but the features that define plant transcripts as substrates of SOP1 (or NEXT) still need to be defined.

In S. pombe and in mammalian cells depleted of exosome subunits, the SOP1 homologs ZC3H3/Red5 as well as ZFC3H1/Red1 and poly(A)+ RNA accumulate in nuclear foci (Hurt et al., 2009; Yamanaka et al., 2010; Sugiyama et al., 2013; Silla et al., 2018). Arabidopsis SOP1 colocalizes with ectopically expressed HEN2 in nucleoplasmic speckles (Hématy et al., 2016). These speckles are not observed with GFP-tagged version of the NEXT subunits RBM7 and ZCCHC8, both of which show a rather diffuse nucleoplasmic localization in both plants and human cells (Silla et al., 2018; Bajczyk et al., 2020). In Arabidopsis, nucleoplasmic speckles were recently also observed with fluorescent versions of FLOWERING CONTROL LOCUS A (FCA) (Fang et al., 2019). FCA is an RNA binding protein best known for its role in the autonomous pathway of flowering time regulation (Wu et al., 2020b). However, FCA generally represses the production of 3’-read-through transcripts by promoting cleavage and polyadenylation at promoter–proximal sites (Sommez et al., 2011; Duc et al., 2013). In line with its function in poly(A) site selection, FCA co-purifies with the polymerase and nuclelease modules of the RNA 3’-end processing machinery (Fang et al., 2019). In addition, FCA affinity-purification captured HEN2, SOP1, PABN1, the putative RBM26/27 homolog At3g27700, and SE (Fang et al., 2019). These data support the speculation that HEN2 and SOP1 are recruited to sites of RNA 3’-end processing to trigger the degradation of read-through transcripts, perhaps in a PAXT-like context.

Unraveling the interacting network of factors that recruit plant nuclear exosome complexes to specific types of transcripts may not only provide mechanistic insights, but might also elucidate whether and how RNA degradation is
integrated with regulatory processes at the transcriptional and chromatin organization levels.

**Nuclear RNA surveillance competes with miRNA processing**

Recent genetic and molecular evidence for the competition between pri-miRNA processing and nuclear RNA surveillance machineries came from the analysis of plants with compromised functions of HEN2 and either SE or HYL1 (Bajczyk et al., 2020; Gao et al., 2020). SE and HYL1 are multifunctional proteins that, together with DCL1, are core members of the miRNA processing machinery. In addition to its nuclear function in miRNA biogenesis, HYL1 promotes miRNA-mediated translational repression in the cytoplasm (Yang et al., 2021). Besides miRNA processing, SE modulates several transcriptional and posttranscriptional processes, such as constitutive and alternative splicing of pre-mRNAs, the transcription of intronless genes, and the expression of transposons. SE is an essential gene (Lobbes et al., 2006), but hypomorphic mutants such as se-2 are viable. Both se-2 and the knockout mutant hyl1-2 display a pleiotropic phenotype with hyponastic and serrated rosette leaves, a characteristic feature of miRNA biogenesis mutants (Grigg et al., 2005; Yang et al., 2006; Laubinger et al., 2008). Interestingly, the loss of HEN2 partially rescued the developmental defects of se-2 and hyl1-2 mutants (Bajczyk et al., 2020; Gao et al., 2020). SOP1 loss of function also partially suppressed the morphological phenotype of hyl1-2 mutants (Gao et al., 2020). In se-2/hen2, hyl1-2/ hen2, and hyl1-2/sop1 plants, primary miRNAs accumulate, and both mature miRNAs and miRNA-target mRNAs are restored to near-normal levels, explaining the partial suppression of the se-2 and hyl1-2 mutant phenotype by mutations in HEN2 or SOP1. These observations suggest that the impaired degradation of miRNA precursors mediated by HEN2 allows for slow miRNA processing despite the absence of an optimal microprocessor complex. The rescue of the pas2-1 splice site mutant by loss of SOP1, HEN2, or a point mutation in the exosome core subunit RRP4 suggests a similar relationship between pre-mRNA degradation and splicing (Hématy et al., 2016). These data emphasize the finding that precursor degradation competes with RNA processing, and they fit well with the current hypothesis that nuclear RNA surveillance constantly degrades all RNAs that are not protected by efficient assembly within processing or transport RNPs (Bresson and Tollervey, 2018; Tudek et al., 2018).

The plant exosome is genetically, physically, and functionally linked to polyadenylation and 3′-end processing

HEN2 was originally identified in a genetic screen for mutations that enhance the morphological defects of hua1 hua2 mutant plants (Western et al., 2002; Cheng et al., 2003). HUA1 and HUA2 (HUA means “flower” in Chinese) were previously selected in genetic screens aimed at identifying regulators of AGAMOUS (AG) expression (Chen and Meyerowitz, 1999). AG is one of the homeotic transcription factors that specify floral organ identity in Arabidopsis (Thomson et al., 2017). AG expression is mainly controlled by transcriptional regulators that bind to DNA sequences located in the large second intron of the AG locus. However, genetic screens for regulators of AG expression identified a number of factors required for the production of AG mRNA, namely HUA1, HUA2, HEN4, and HEN2 (Chen and Meyerowitz, 1999; Western et al., 2002; Cheng et al., 2003). HUA2 and three related proteins named HUA2-like 1–3 are plant-specific proteins that have a PWWP domain and a proline-rich domain, both of which are proposed to mediate protein–protein interactions, and an RNAPIII CTD interaction domain (Jali et al., 2014). HUA1 and HEN4 are Zn-finger and KH-domain RNA binding proteins, respectively (Li et al., 2001; Cheng et al., 2003). Loss of either HUA1, HUA2, or HEN4 resulted in the accumulation of truncated AG transcripts composed of the two first exons, a large portion of the second intron, and a poly(A) tail (Western et al., 2002; Cheng et al., 2003). The same transcript was detected (at lower levels) in hyn2 mutants. Yet, mutating HEN2 in hua1, hua2, hyn4 single or double mutant backgrounds increased the accumulation of misprocessed AG transcripts, decreased the levels of correctly processed AG mRNA, and strongly enhanced the homeotic conversions of flower organs (Western et al., 2002; Cheng et al., 2003). Two newer studies showed that HUA1 and HEN4 form a complex with two additional KH-domain proteins, FLOWERING LOCUS WITH KH (FLK) DOMAINS and PEPPER (PEP; Rodríguez-Cazorla et al., 2015, 2018). This complex and HUA2, collectively dubbed “HUA–PEP activity,” are required for the production of AG mRNA and some other large intron-containing mRNAs including SHATTERPROOF1 (SHP1), SHP2, and SEEDSTICK, encoding homeotic transcription factors that redundantly confer ovule identity (Rodríguez-Cazorla et al., 2015, 2018). The current hypothesis is that HUA–PEP associates with nascent AG transcripts to block a cryptic cleavage and polyadenylation site located in the second intron (Cheng et al., 2003; Rodríguez-Cazorla et al., 2015). HEN2’s function in this context is likely to promote the rapid degradation of misprocessed mRNAs in case of their accidental production. Yet, the identification of HEN2 as an enhancer of the hua1 hua2 phenotype (Western et al., 2002) suggests that the accumulation of misprocessed mRNAs exerts negative feedback on HUA–PEP’s function or on the production of mRNAs that rely on it. A possible scenario is that the degradation of misprocessed RNAs is required to prevent the sequestration of HUA–PEP components on such molecules. Another possibility is that the accumulation of truncated HUA–PEP targets interferes with the transcriptional control of gene expression at these loci.

HUA1, HEN4, and the RNA helicase HEN2 are localized to nuclear speckles and can be co-purified with FCA and other RNA 3′-end processing factors (Cheng et al., 2003; Lange et al., 2014; Fang et al., 2019). HUA1, HEN4, FLK, PEP, and HEN2 were also captured with tag-purified FLOWERING
CONTROL PROTEIN A (FPA), another RNA binding protein that functions largely redundantly with FCA in poly(A) site selection and 3′-end processing (Sonmez et al., 2011; Duc et al., 2013; Parker et al., 2021). At least a portion of FPA colocalizes with FCA (Fang et al., 2019). Yet, while the interactome of FCA contained putative PAXT subunits, FPA co-purified with the NEXT subunit ZCCHC8A (Fang et al., 2019; Parker et al., 2021). These data suggest that a modular interaction network might establish multiple connections between RNA degradation and 3′-end processing in plants, similar to what is observed in human cells (Wu et al., 2020a).

FPA-dependent alternative polyadenylation affects a large number of loci encoding nucleotide-binding and leucine-rich repeat (NLR) proteins, the major class of R-genomes in plants (Parker et al., 2021). NLR proteins are intracellular immune receptors that detect the presence of pathogens and activate the innate immune response (van Wersch et al., 2020). One FPA-dependent NLR locus is RESPONSE TO PSEUDOMONAS6 (RPS6; Parker et al., 2021). In noninfected plants, one type of transcript derived from RPS6 is constitutively degraded by HEN2-mediated RNA surveillance (Takagi et al., 2020; Parker et al., 2021). Another type of transcript is constitutively degraded by nonsense mediated-decay (NMD; Gloggnitzer et al., 2014; Parker et al., 2021). Interestingly, the strong autoimmune phenotype that is observed in NMD mutants or upon constant activation of the signaling cascade that is activated by RPS6 can be rescued by mutating HEN2 (Gloggnitzer et al., 2014; Takagi et al., 2020). If and how the rescue of this phenotype is indeed linked to the over-accumulation of the HEN2-targeted RPS6 transcripts has not yet been investigated. Still, the take-home message that can be extracted from the already existing genetic, proteomic, and transcriptomic data is that exosome-mediated RNA degradation is not only a clean-up mechanism for defective or superfluous RNAs, but it is also embedded in the regulation of gene expression steering specific developmental steps and stress responses.

Exosome-mediated RNA degradation and heterochromatin formation

Physical connections and diverse functional relationships between exosome-mediated RNA degradation and chromatin remodeling by both RNAi-dependent and RNAi-independent pathways have mostly been characterized in S. pombe and recently also in mice (Vasiljeva et al., 2008; Bühler, 2009; Guttman et al., 2011; Shah et al., 2014; Martiensen and Moazed, 2015; Brönnner et al., 2017; Singh et al., 2018; Garland et al., 2019; Shipkova et al., 2020). Whether such relationships also exist in plants is currently unclear. Downregulating the exosome increases the steady-state levels of spurious transcripts generated from heterochromatic regions such as repeats and transposons but has no effect on the production of the small RNAs that direct DNA methylation and histone modification at those loci (Shin et al., 2013). siRNA-independent pathways that target chromatin remodelers to individual protein-coding genes in response to developmental and environmental stimuli often depend on lncRNAs, and many of these lncRNAs are indeed targets of HEN2 and the nuclear exosome. Yet, whether the accumulation of such lncRNAs alters the chromatin status at these loci has not yet been investigated.

Cytoplasmic exosomes

The cytoplasmic exosome cooperates with the Ski complex in translation-dependent RNA quality control

The default pathway for cytoplasmic bulk mRNA turnover begins with the shortening of poly(A) tails by deadenylases, followed by decapping and exonucleolytic degradation by 5′–3′-exoribonucleases, mainly XRN1 in yeast and animals and XRN4 in plants (Nagarajan et al., 2013; Mugridge et al., 2018). The 3′–5′-degradation can be performed by the cytoplasmic exosome together with the Ski complex (Schaeffer et al., 2011) and, in most eukaryotes except S. cerevisiae, by DIS3L2 (SOV in Arabidopsis), an exoribonuclease related to DIS3 that operates independently of the exosome (Zhang et al., 2010; Lubas et al., 2013; Malecki et al., 2013). Due to a high level of redundancy, individual RNA degradation mutants do not display important changes in steady-state mRNA levels (Labno et al., 2016; Sorenson et al., 2018). Yet, the exosome has a well investigated role in so-called RNA quality control (RQC) pathways that make a considerable contribution to the physiological regulation of mRNA levels. A hallmark of these pathways is the stalling or collision of ribosomes at specific features (D’Orazio and Green, 2021; Morris et al., 2021). No-go decay (NGD) removes mRNAs with rare codons or strong secondary structures (Harigaya and Parker, 2010). NMD recognizes mRNAs with translation termination codons upstream of an exon–exon junction, or with long 3′-UTRs (Lykke-Andersen and Jensen, 2015). In plants, NMD restricts viral infections and degrades a substantial proportion of the transcript variants produced by alternative splicing, often in response to endogenous or exogenous stimuli (Garcia et al., 2014; Ohtani and Wachter, 2019). Nonstop decay (NSD) degrades mRNA lacking translation termination signals (Klaue and van Hooft, 2012). An important function of plant NSD is to eliminate the 5′-fragments of miRNA-targeted mRNAs following cleavage by the RNA-induced silencing complex (RISC; Szańczezy-Kardoss et al., 2018). The 3′-ends of NSD substrates are directly accessible for 3′–5′-degradation. NGD and NMD trigger either rapid deadenylation and decapping or endonucleolytic cleavage. The resulting 3′-fragment is degraded by XRN4, while the 5′-fragment is eliminated by cytoplasmic exosome complexes. Yet, it is important to keep in mind that the biological functions of the plant cytoplasmic exosome were mostly assessed in the Arabidopsis Columbia-0 accession, which lacks a fully functional homolog of the cytoplasmic 3′–5′-exoribonuclease SOV (SUPRESSOR OF VARICOSE) (Zhang et al., 2010). The extent to which SOV contributes to NMD, NSD, or NGD has not yet been investigated.
The central cofactor of cytoplasmic exosomes is the Ski complex composed of the RNA helicase SKI2 (SKIV2L in animals), the tetratricopeptide repeat protein SKI3 (TTC37), and two copies of the WD40-repeat protein SKI8 (WDR61, also named VIP3 in plants) (Schmidt et al., 2016; Figure 7). Both yeast and animal SKI2/SKIV2L bind closely to the mRNA entry site of elongation complexes (Schmidt et al., 2016; Tuck et al., 2020). Crosslinking and analysis of c-DNAs (CRAC) mapping of SKIV2Ls binding sites revealed that SKIV2L binds to all mRNA regions associated with ribosomes, such as 5′-UTRs, upstream open reading frames (uORFs), and coding sequences, and is enriched at proline or other codon combinations that trigger ribosome stalling (Tuck et al., 2020). Cryo-EM data suggest that the binding of the yeast Ski complex to ribosomes induces a conformational change that replaces structural elements that otherwise inhibit the ATPase activity of SKI2 and enable the formation of an opening through which the 3′-end of the mRNA can be channeled to the exosome (Schmidt et al., 2016). A recent in vitro study using reconstituted human Ski complexes demonstrated that ATP hydrolysis drives the nucleotide-by-nucleotide extraction of mRNAs from 80S ribosomes, which facilitates mRNA degradation by exosome complexes (Zinoviev et al., 2020). Moreover, the presence of Ski enables the dissociation of ribosomes by ABCE1, PELOTA, and the translational GTPase HBS1, which together initiate the recycling of stalled elongation complexes in the context of NSD and NGD pathways (Zinoviev et al., 2020). The emerging picture is that a principal and conserved function of the Ski complex is the constant monitoring of translating ribosomes to extract the RNA and initiate ribosome recycling in case there are problems. In yeast, a subpopulation of Ski complexes is not bound to ribosomes but instead associates with the yeast-specific protein Ska1p for the degradation of lncRNAs, poorly translated mRNAs, and mRNAs with long 3′-UTRs (Zhang et al., 2019). Whether such alternative Ski complexes that assist in the degradation of non-translating RNAs also exist in other species is currently unknown.

**RST1 and RIPR may link plant Ski complexes to Exo9**

In vivo, Ski and exosome complexes are bridged by the SKI7 protein (Araki et al., 2001). SKI7 possesses two N-terminal domains that are necessary and sufficient for its role in RNA degradation and mediate the binding of SKI7 to the Ski and exosome complexes (Wang et al., 2005; Kowalinski et al., 2015; Horikawa et al., 2016). Yeast SKI7 also contains a guanosine triphosphate (GTP) binding domain that resembles the translational GTPase domain of the ribosome dissociation factor HBS1. However, SKI7 does not bind directly to ribosomes, and the molecular function of this domain is still under discussion (Schmidt et al., 2016). In many organisms including humans and plants, the mRNAs encoding SKI7 and HBS1 are produced from a single locus by alternative splicing (Kalisiak et al., 2017; Marshall et al., 2018). Arabidopsis SKI7 and HBS1 mRNAs differ only by the presence of an extra exon encoding the exosome-binding domain of SKI7 (Brunkard and Baker, 2018; Marshall et al., 2018). HsSKI7 is shorter than HsHBS1 and lacks the GTP binding domain that is present in yeast SKI7 (Kalisiak et al., 2017; Marshall et al., 2018). In both animals and plants, SKI7 copurifies with affinity-tagged exosome and Ski complexes (Zhang et al., 2015; Kalisiak et al., 2017; Lange et al., 2019).

In addition to SKI7, the Arabidopsis exosome copurifies with RESURRECTION 1 (RST1), a large ARM repeat protein,
and RST1 INTERACTING PROTEIN (RIPR), a protein without known domains and that has no sequence homologs outside plants (Lange et al., 2019). Both proteins are exclusively cytoplasmic. The reverse IP experiments using RST1 and RIPR as baits confirmed their association with the exosome complex and indicated that they may link Exo9 with the Ski complex (Lange et al., 2019). A recent study showed that RST1 and RIPR were required for the degradation of NGD and NSD model substrates that were transiently expressed in Nicotiana benthamiana leaves (Auth et al., 2021). Yet, the molecular functions of RST1 and RIPR remain to be identified. The mammalian homolog of RST1, named FOCAD, also interacts with the Ski complex, and with the metazoan-specific RGG/RG domain RNA binding protein AVEN (Thandapani et al., 2015; Tuck et al., 2020). CRAC data suggest that AVEN, similar to SKI2, binds to the mRNA entry sites of ribosomes, and to 5′-UTRs, uORFs, and coding regions (Tuck et al., 2020). Knockout of AVEN increased both ribosome stalling and Ski binding at regions enriched in GC-rich sequences and in regions predicted to form paired nucleotides or G-quadruplexes (Thandapani et al., 2015; Tuck et al., 2020). The current model is that AVEN binding counteracts ribosome stalling and Ski recruitment at structured RNA regions, thereby preventing the unwanted degradation of mRNAs (Tuck et al., 2020). The coordinated functions of AVEN and the Ski complex regulate the expression of many mRNAs, among them uORF-containing mRNAs and replication-dependent histone mRNAs during the cell cycle (Tuck et al., 2020). Unexpectedly, knockout of AVEN also increased SKI2 binding on ribosome-bound, structured noncoding RNAs with small ORFs that originated from loci upstream or antisense to protein-coding genes or from intergenic regions (Tuck et al., 2020). Whether AVEN engages with such transcripts to enable the translation of small peptides, or whether the actions of AVEN and the Ski complex on those transcripts is another layer of RNA surveillance to eliminate nonfunctional RNAs that escape nuclear RNA degradation, is not yet understood.

The role of FOCAD in this scenario is not yet clear, and whether plant RST1 and/or RIPR are involved in a similar mechanism needs to be investigated. Interestingly, Arabidopsis mutants bearing a T-DNA insertion in the gene encoding both HBS1 and SKI7 accumulate a vast number of nongenic transcripts, suggesting that noncoding transcripts escaping nuclear RNA surveillance (Szádeczky-Kardoss et al., 2018) are indeed degraded by the cytoplasmic exosome in plants. Nonetheless, whether their degradation is coupled to ribosome association remains to be studied.

**RNA degradation by the cytoplasmic exosome prevents the posttranscriptional silencing of endogenous genes and transgenes**

Several lines of evidence indicate that RNA degradation by the cytoplasmic RNA exosome limits the posttranscriptional silencing of endogenous genes and transgenes. For instance, both exosome subunits and cytoplasmic cofactors surfaced in genetic screens involving epicuticular wax biosynthesis in Arabidopsis (Daszkowska-Golec, 2020). Epicuticular waxes are a layer of very long-chain hydrocarbons that cover the outer surfaces of land plants and protect them from water loss and pathogen attack. Genetic strategies to identify mutations causing glossy green stems due to the absence of epicuticular waxes identified the Exo9 core subunit CER7 (RP45B) and all three subunits of the Ski complex (Hooker et al., 2007; Zhao and Kunst, 2016). The glossy stem phenotype is shared by plants lacking RST1 and RIPR (Chen et al., 2005; Lange et al., 2019; Yang et al., 2020). Key to understanding this phenomenon was the identification of factors involved in PTGS, including SGS3, RDR6, and AGO1 as suppressors of the cer7 mutation (Lam et al., 2012, 2015). These pioneering studies revealed that the wax-deficient phenotype is caused by the accumulation of 21 nt siRNAs that trigger silencing of CER3 mRNA, encoding a wax biosynthetic enzyme (Lam et al., 2012, 2015; Figure 7). Importantly, these genetic screens were performed using the Landsberg erecta accession (Chen et al., 2005; Hooker et al., 2007; Rowland et al., 2007; Lam et al., 2012, 2015; Zhao and Kunst, 2016; Yang et al., 2020), which actually possesses a fully functional SOV. Therefore, preventing the silencing of CER3 is a nonredundant function of the cytoplasmic exosome. Surprisingly, and as yet unexplained, mutations in the three SKI complex subunits also suppress the wax phenotype of cer7 mutants, although the SKI complex is a co-factor of the exosome (Zhao and Kunst, 2016). The genome-wide identification of siRNAs confirmed that the exosome, Ski, RST1, and RIPR are required to prevent the unwanted silencing of endogenous genes (Brunscheid et al., 2015; Lam et al., 2015; Lange et al., 2019). The siRNAs that accumulate in RNA degradation mutants are named RNA-Quality-Control-dependent siRNAs (rqc-siRNAs) or coding-transcript-derived siRNAs (ct-siRNA; Moreno et al., 2013; Zhang et al., 2015).

Several independent forward and reverse genetic strategies identified RST1, SKI2, or SKI3 as suppressors of transgene silencing (Brunscheid et al., 2015; Yu et al., 2015; Lange et al., 2019; Li et al., 2019). A recent genetic screen implicated RST1 in vacuole trafficking (Zhao et al., 2019), and another recent study reported that the loss of SKI2 increases autophagosome numbers and impairs the RNA-uptake ability of vacuoles (Floyd et al., 2021). Further investigation will reveal whether the lack of exosome-related RNA degradation factors indirectly perturbs these processes by promoting PTGS of key genes involved in vacuolar RNA uptake or trafficking or because RST1 and SKI2 have moonlighting functions.

Not surprisingly, XRN4 and decapping factors also suppress PTGS (Gazzani et al., 2004; Gy et al., 2007; Gregory et al., 2008; Martínez de Alba et al., 2015). Plants that are simultaneously impaired in both 3′–5′- and 5′–3′-degradation pathways show dramatically increased production of ct-siRNAs (Zhang et al., 2015). This finding highlights the notion that 3′–5′- and 5′–3′-degradation pathways play largely redundant roles in the degradation of mRNAs, and
that this redundancy is pivotal for preventing the production of siRNAs from endogenous mRNAs. The high level of redundancy and the fast conversion of improperly degraded mRNA into siRNAs also explain why almost no accumulation of full-length mRNAs or RISC-cleaved fragments is observed in individual degradation mutants. The importance of bidirectional RNA degradation for avoiding silencing of endogenous mRNAs is highlighted by the fact that the seedling lethality of xrn4 skl2 or decapping mutants is rescued by mutating components of the PTGS machinery (Martinez de Alba et al., 2015; Zhang et al., 2015).

Many of the endogenous mRNAs prone to producing ct-siRNAs in RNA degradation mutants are targets of miRNA-guided RISC cleavage (Zhang et al., 2015; Lange et al., 2019). This observation fits well with the documented roles of SKI2/exosome complexes and XRN4 in degrading 5’ and 3’-RISC-cleaved fragments (Souret et al., 2004; Branscheid et al., 2015). Yet, some of the mRNAs that undergo silencing in RNA degradation mutants, including CER3, are not known targets of miRNAs. Whether such mRNAs trigger ribosome stalling and/or are co-translationally cleaved by other mechanisms independent from the RISC has not yet been determined, but such processes were recently shown to trigger siRNA biogenesis from transposon RNAs (Kim et al., 2021).

In addition, the lack of a poly(A) tail activates siRNA biogenesis by promoting RDR6 recruitment (Luo and Chen, 2007; Baeg et al., 2017; Iwakawa et al., 2021). Because deadenylated mRNAs are substrates of both the RNA exosome and the decapping machinery, the swift elimination of deadenylated mRNAs is likely key to avoid unwanted siRNA biogenesis from mRNAs. The global picture emerging from all these studies is that most mRNAs can likely trigger gene silencing if not properly degraded.

**Perspectives**

The recent advances discussed here revealed a hitherto unappreciated diversity of catalytic activities, cofactors, and biological functions associated with RNA exosome complexes in Arabidopsis compared to yeasts and humans. To date, RNA substrates of the RNA exosome and its co-factors have mostly been identified by transcriptomics approaches in mutant backgrounds. Alternative strategies such as CLIP or HyperTRIBE have been implemented in Arabidopsis to identify RNA substrates that interact with proteins involved in RNA metabolism (Meyer et al., 2017; Köster and Staiger, 2021; Arribas-Hernández et al., 2021a, 2021b). Provided that these techniques are sensitive enough to detect exosome substrates in a wild-type background, they should be decisive for uncovering the modes of action of nuclear and cytoplasmic cofactors of the RNA exosome in order to fully appreciate all of its functions in plants.

Yet, various exciting questions remain to answer. Many groups have reported that exosome-mediated RNA degradation suppresses PTGS, but understanding the molecular scenarios underlying this crucial function of the RNA exosome is definitely one of the key issues to be solved. The incidents that lead to the production of rqc-siRNA are likely of rather diverse nature. One of these could be the initial slicing guided by a small RNA (miRNA or siRNA), and we must now decipher the chain of molecular events that eventually trigger rqc-siRNA biogenesis while other RISC-induced cleavages do not. A recent study proposed that the exosome limits siRNA amplification by reducing the dwell time of RISC on miRNA-targeted mRNA (Vigh et al., 2021). Endoribonucleolytic cleavages not mediated by the RISC, translation issues, or excessive shortening of the poly(A) tail are also among the many possible ways to generate RNA species that need to be rapidly cleared by the RNA exosome to prevent rqc-siRNA biogenesis. Despite the sophisticated sequencing protocols now being developed, the characterization of those RNAs remains challenging due to their transient nature and their rapid conversion into siRNAs. Time-resolved studies will be crucial for fully understanding these processes.

Another important question that needs to be clarified is whether and how exosome-dependent RNA degradation modulates heterochromatin formation in plants. Also, cross-talk between RNA degradation and transcription is emerging in other eukaryotes but is as yet unexplored in plants. Would exosome-mediated RNA degradation be integrated in this crosstalk? Phylogenetic analysis suggests that the unique Exo9 phosphorolytic activity that has been demonstrated in Arabidopsis may have been retained throughout the green lineage. What is the biological function of Exo9's phosphorolytic activity? We envisage two hypotheses that are not mutually exclusive. One idea is that the phosphorolytic site of RRP41, which is positioned inside the channel of Exo9, allows an RNA substrate to be further trimmed compared to RRP44 positioned at the bottom of Exo9. Could such a trimming function compensate for an unstable interaction of plant Exo9 with RRP6L2? The genome-wide identification of RNAs that are trimmed by Exo9 would require an extraordinary sequencing resolution and depth. The second hypothesis to explain the conservation of a phosphorolytic site is grounded on a key feature of this type of activity: its reversibility. It is likely that Exo9's phosphorolytic activity can synthesize untemplated RNA extensions in vivo, as previously reported for other PNPases such as the archaean exosome or bacterial and chloroplastic PNPases. Yet, does the plant RNA exosome indeed tail RNA substrates, under which biological conditions, and for what purpose?

Another underdeveloped area of research concerns the impact of tailing by terminal nucleotidyl transferases on the function of plant RNA exosomes. Besides the adenylation of nuclear exosome substrates, uridylation is emerging as an important regulator of RNA fate. Per se, RNA uridylation can have multiple outcomes, depending on the nature of the RNA substrate and/or the interaction network of the respective terminal uridine transferases (Scheer et al., 2016; De Almeida et al., 2018; Zigâcková and Vanačová, 2018). Uridylation of RISC-cleaved fragments and noncoding RNAs with unprotected 3’-ends is predominantly catalyzed by
HESO1 and proposed to facilitate 3′→5′-degradation (Zhao et al., 2012; Ren et al., 2014; Zuber et al., 2018). Yet, experimental evidence for the stimulation of exosome or SOV activity by U-tails is still lacking in plants, and this point will certainly need to be clarified. Understanding how adenylation or uridylation of RNA substrates regulate the RNA exosome functions entails the use of dedicated sequencing technologies to accurately monitor nucleotide extensions. Several such protocols based on Illumina sequencing are currently being used, and direct RNA sequencing by Oxford Nanopore Technologies also stands among the promising techniques to analyze the 3′-extensions. Several such protocols based on Illumina will certainly need to be clarified. Understanding how adenylation by U-tails is still lacking in plants, and this point is of interest.

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