SOCIETY FOR EXPERIMENTAL BIOLOGY

Journal of Experimental Botany, Vol. 74, No. 7 pp. 2295–2310, 2023
https://doi.org/10.1093/jxb/erac462 Advance Access Publication 23 November 2022

REVIEW PAPER

Functions and mechanisms of RNA helicases in plants

Xindi Li[1], Changhao Li[1], Jiaying Zhu[1], Songxiao Zhong[1], Hongliang Zhu[2] and Xiuren Zhang[1,3,*]

[1] Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843, USA
[2] College of Food Science and Nutritional Engineering, China Agricultural University, 100083 Beijing, China
[3] Department of Biology, College of Science, Texas A&M University, College Station, TX 77843, USA

* Correspondence: xiuren.zhang@tamu.edu

Received 29 August 2022; Editorial decision 14 November 2022; Accepted 21 November 2022

Editor: Pablo Manavella, Instituto de Agrobiotecnología del Litoral, Argentina

Abstract

RNA helicases (RHs) are a family of ubiquitous enzymes that alter RNA structures and remodel ribonucleoprotein complexes typically using energy from the hydrolysis of ATP. RHs are involved in various aspects of RNA processing and metabolism, exemplified by transcriptional regulation, pre-mRNA splicing, miRNA biogenesis, liquid–liquid phase separation, and rRNA biogenesis, among other molecular processes. Through these mechanisms, RHs contribute to vegetative and reproductive growth, as well as abiotic and biotic stress responses throughout the life cycle in plants. In this review, we systematically characterize RH-featured domains and signature motifs in Arabidopsis. We also summarize the functions and mechanisms of RHs in various biological processes in plants with a focus on DEAD-box and DEAH-box RNA helicases, aiming to present the latest understanding of RHs in plant biology.

Keywords: Abiotic stress, biotic stress, DEAD-box, DEAH-box, development, liquid–liquid phase separation, miRNA, pre-mRNA splicing, RNA helicase, RNA processing, transcription.

Introduction

Living organisms produce numerous species of RNA, such as mRNA, rRNA, tRNA, non-coding RNA (ncRNA), and so on. RNA transcripts form complicated structures that are believed to serve as a new layer of genetic information in different biological processes (Zhu *et al.*, 2021). Moreover, the dynamics of biological processes require that RNA–DNA, RNA–RNA, and RNA–protein must associate and dissociate from each other transiently and efficiently. Similar to protein folding, which requires protein chaperones, RNA molecules are prone to requiring RNA helicases (RHs) for conformational and compositional changes during various processes. Conventionally, RHs are a group of catalytic enzymes that can bind and hydrolyse NTP to unwind double-stranded RNAs. However, the term RNA helicase might not be accurate because increasing evidence shows that RHs are involved in disrupting or reconstituting RNA–protein interactions (Linder and Jankowsky, 2011; Gilman *et al.*, 2017; De Bortoli *et al.*, 2021; Donsbach and Klostermeier, 2021). Furthermore, RHs can ambiguously target DNA substrates and vice versa, and DNA helicases (DHs) and RHs are often grouped and discussed together.

According to amino acid sequence homology, oligomeric state, and substrate preferences, DHs and RHs identified in living organisms can be classified into six superfamilies (SF1, SF2, SF3, SF4, SF5, and SF6) (Singleton *et al.*, 2007). SF1 and SF2 members typically function as monomers, whereas the SF3–SF6 families display ring-shaped hexametric toroid structures (Patel and Picha, 2000; Lohman *et al.*, 2008;

Pyle, 2008; Dillingham, 2011) (Fig. 1A). The largest two superfamilies, SF1 and SF2, are further divided into various subgroups. For instance, SF1 members are sorted into three subgroups (Upf1-like, Pif-1-like, and UvrD-like/Rep), while SF2 members are grouped into nine different subfamilies based on their structural and mechanistic features. The nine subfamilies are known as DEAD-box, DEAH/RHA-box, Superkiller 2 (Ski2)-like, NS3/NPH-II, RecG-like, RecQ-like, Swi2/Snf2, XPD/Rad3/DinG, T1R, and XPF/Hef/ERCC4/RIG-I (Fairman-Williams *et al.*, 2010; Dillingham, 2011; Jankowsky *et al.*, 2011; Raney *et al.*, 2013). In eukaryotes, the majority of RHs belong to SF2 while some are SF1 members (Tuteja, 2018). Typically, six families of SF1 and SF2 possess RH activity (labeled in blue in Fig. 1A) while the other members have DH activity. A remarkable example is chromatin remodeling factor 2 (CHR2), an ATPase subunit in the Swi2/Snf2 complex, which also acts as an RH in altering the secondary structures of primary substrates of microRNAs (pri-miRNAs) to inhibit miRNA production, in addition to its well-known function in chromatin remodeling (Wang *et al.*, 2018).

In the past decade, RHs have been analysed and identified in several plants species including Arabidopsis, rice (*Oryza sativa*), maize (*Zea mays*), soybean (*Glycine max*), tomato (*Solanum lycopersicum*), cotton (*Gossypium raimondii*), wheat (*Triticum aestivum*), longan (*Dimocarpus longan*), canola (*Brassica napus*), barrel clover/barrel medic (*Medicago truncatula*), and chickpea (*Cicer arietinum*) (Umate *et al.*, 2010; Xu *et al.*, 2013a, b; Chen *et al.*, 2014; Tuteja, 2018; Zhang *et al.*, 2018; Cheng *et al.*, 2021; Ru *et al.*, 2021; Xu *et al*, 2021; Y. L. Wang *et al.*,

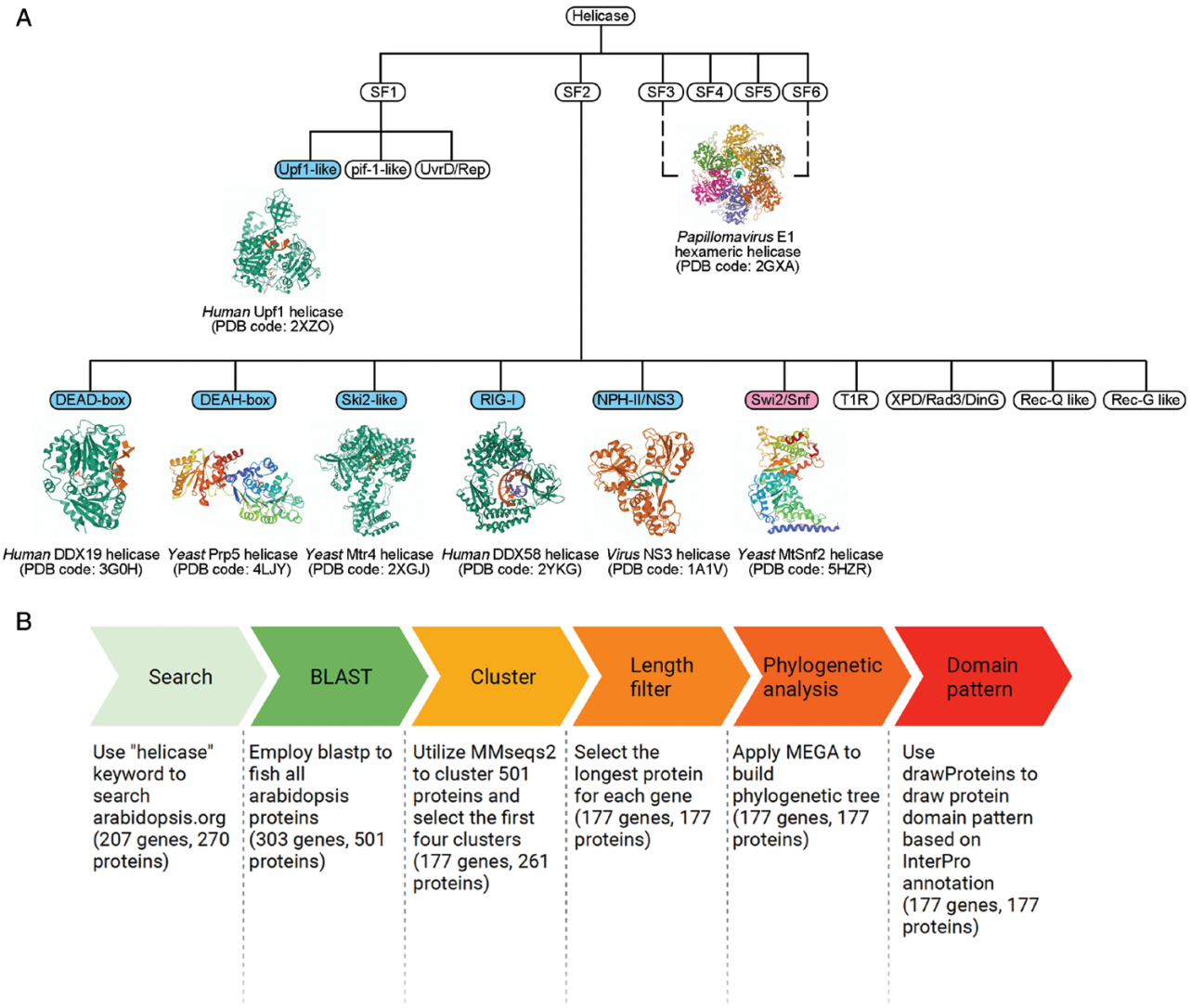

Fig. 1. Helicase classifications and RHs in Arabidopsis. (A) Classifications for DNA and RNA helicases. The blue regions show the canonical families with RH activity and the pink region shows the newly identified one. The structures of individual RHs in different families were retrieved from Protein Data Bank (http://www.pdb.org). (B) Pipeline for screening of RH candidates.

Table 1. Number of RHs in different plant species

Species	RH subfamily					Total	Reference
	DEAD-box	DEAH-box	Ski2-like	Swif2/Snf2	Others		
Arabidopsis (updated)	56	17	9	41	54	177	Tuteja (2018), Umate *et al.* (2010)
Oryza sativa	79	41	79	—	—	199	Tuteja (2018), Umate *et al.* (2010)
Zea mays	51	34	65	—	—	150	Xu *et al.* (2013b)
Glycine max	101	55	92	—	—	248	Xu *et al.* (2013b)
Solanum lycopersicum	42	36	52	—	27	157	Xu *et al.* (2013a)
Gossypium raimondii	51	52	58	—	—	161	Chen *et al.* (2014)
Triticum aestivum	141	—	—	—	—	141	Ru *et al.* (2021)
Dimocarpus longan	58	—	—	—	—	58	Xu *et al.* (2021)
Brassica napus	134	53	84	—	—	271	Zhang *et al.* (2018)
Medicago truncatula	52	38	80			170	Cheng *et al.* (2021)
Cicer arietinum	50	33	67			150	Yadav *et al.* (2022)

2022b; Yadav *et al.*, 2022 (Table 1; Supplementary Table S1). Recently, we performed an extensive computational analysis of the whole Arabidopsis genome (Fig. 1B) and identified all potential RH-like candidates. Briefly, we searched the 'helicase' keyword in the gene database and protein domain database of TAIR (https://www.arabidopsis.org) and recovered all annotated helicase genes or proteins with helicase domains. All these proteins were further employed as seed sequences to retrieve additional potential helicase-like proteins by blastp (Camacho *et al.*, 2009). Then, we used MMseqs2 (Steinegger and Soding, 2017) to cluster all blast-out protein sequences. The four most abundant clusters, which contain 177 genes encoding 261 proteins, were considered as the final helicase candidates. MEGA11 (Kumar *et al.*, 2018) was applied to draw the phylogenetic tree using the largest isoform of proteins (177 proteins) (Fig. 2A). The phylogenetic trees show that the 177 candidates are classified into different categories, which contain 56 DEAD-box RHs, 17 DEAH-box RHs, 9 Ski2-like RHs, and 41 Swi2/Snf2 RHs, among other RHs. The computational analysis also identified 16 proteins as potential RHs. In this review, we first summarize biochemical features of RHs and then focus on biological functions and action modes of RHs in plants, aiming to appreciate the increasingly important roles of RHs in plant biology.

Structural domains and signature motifs of RNA helicases

Detailed biochemical features of RHs have been summarized in recent reviews (Tuteja, 2018; De Bortoli *et al.*, 2021; Donsbach and Klostermeier, 2021). Generally speaking, SF1 and SF2 RHs in human and yeast share a helicase core with a highly similar structure, consisting of two Rec A domains (also name helicase 1 domain and helicase 2 domain) flanked by variable auxiliary N- and C-terminal extensions. There are 14 characteristic motifs located in the helicase core, which are sequentially termed motifs Q, I, Ia, Ib, Ic, II, III, IIIa, IV, Iva, V, Va, Vb, and VI (Cordin and Beggs, 2013; Jackson *et al.*, 2014; Jarmoskaite and Russell, 2014; Tuteja, 2018).

To characterize structural domains and signature motifs of RHs in Arabidopsis, we assessed the basic characteristics of plant RHs. The Bioconductor package drawProteins (Brennan, 2018) was applied to draw domain patterns based on the annotations from the InterPro database (Hunter *et al.*, 2009), and the motifs were identified by MEME (Bailey *et al.*, 2009). Similar to its counterparts in human and yeast, the helicase core of RHs in Arabidopsis contains two typical helicase domains (Fig. 2B). However, there are fewer conserved motifs located in the helicase core, suggesting increased diversity of RHs in plants compared with human and yeast. Most DEAD-box members in plants harbor the helicase core but lack additional domains in the C-terminal regions compared with DEAH-box and Ski2-like RHs. By contrast, almost all DEAH-box RHs are characterized by the oligonucleotide/oligosaccharide-binding fold (OB-fold) domain. The significance of the OB-fold domain in plant RHs remains to be explored, despite the domain being essential for activation of the RH PRP43 in yeast (Mouffok *et al.*, 2021).

Notably, several members of the DEAD-box and DEAH-box family RHs have a zinc finger domain, such as AT5G26742 (RH3) and AT4G01020. In contrast, Ski2-like RHs have multiple additional extensions in the C-terminal regions, including the exosome function-related Arch domain (Olsen and Johnson, 2021) and some unknown C-terminal domains. Remarkably, AT1G20960 (Brr2a), AT2G42270 (Brr2b), and AT5G61140 (Brr2c) are three unique RHs in Arabidopsis that encode proteins with extraordinary molecular mass (over 2000 amino acids), consisting of two repeat helicase cores. Additionally, the Swi2/Snf2 family has a zinc finger domain inserted in the middle of the helicase core to link helicase domain 1 and helicase domain 2. Although similar and conserved structural domains and motifs are characterized for the RHs from the same family, RHs beyond these four families show manifold functional domains and fewer conserved motifs (Fig. 2B).

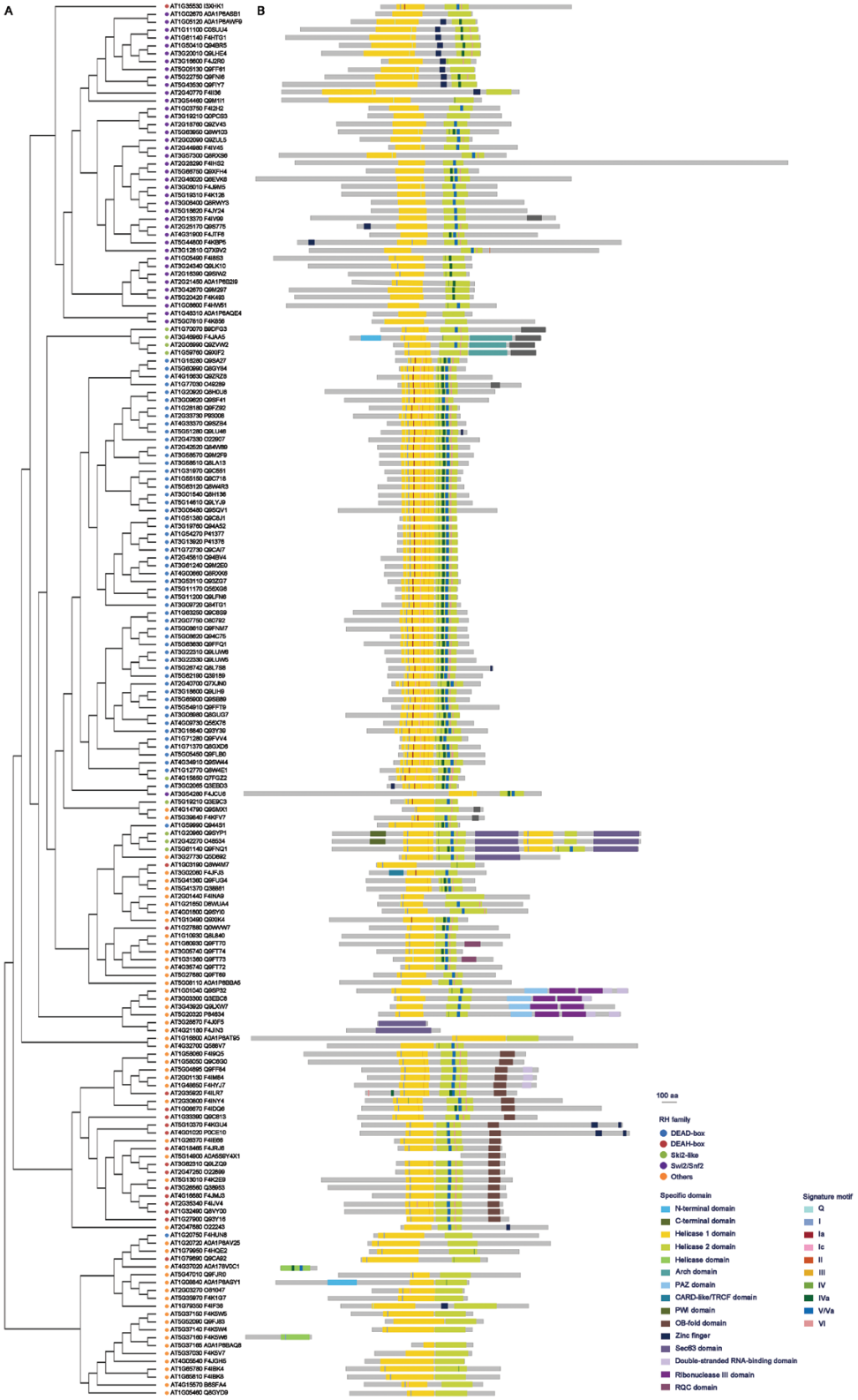

Fig. 2. Phylogenetic tree, domains, and motifs of different RH families in Arabidopsis. (A) Phylogenetic tree of RHs in Arabidopsis. Different families are labeled with distinct colors: DEAD-box (blue), DEAH-box (red), Ski2-like (green), Swi2/Snf2 (purple), and others (orange). The phylogenetic tree of 177 amino acid sequences was inferred using the Maximum Parsimony method in MEGA11. The most parsimonious tree with a length of 77 977 is shown. The consistency index was 0.519345 (0.479863), the retention index was 0.502416 (0.502416), and the composite index was 0.260928 (0.241091) for all sites and parsimony-informative sites (in parentheses). (B) Specific domains of all RHs in Arabidopsis are marked by distinct colors and conserved motifs are labeled in the helicase core. The schematic representations of the sequence features for RHs here are all proportional. The length for 100 amino acids is indicated by the scale bar.

Table 2. RHs function in abiotic and biotic stresses in Arabidopsis and other plants

Species	RHs	Stress	Reference
Abiotic stress			
Arabidopsis	RH17	Salt stress	Nguyen *et al.* (2018), Seok *et al.* (2020)
	RH31	Salt stress	Liu *et al.* (2021)
	SHI2	Salt stress and cold stress	Wang *et al.* (2020)
	STRS1 and STRS2	Salt stress, osmotic stress, and heat stress	Kant *et al.* (2007), Khan *et al.* (2014)
	LOS4	Cold stress and heat stress	Gong *et al.* (2005)
	RH50	Cold stress	Paieri *et al.* (2018)
Rice	OsTCD33	Cold stress	Xiaomei *et al.* (2020)
	OsRH42	Cold stress	Lu *et al.* (2020)
	OsTOGR1	Heat stress	Wang *et al.* (2016), Wang *et al.* (2020)
Wheat	TaDEAD-box57-3B	Salt stress, cold stress, and drought stress	Ru *et al.* (2021)
Physcomitrella patens	PpeIF4A	Salt stress	Tyagi *et al.* (2020)
Chrysanthemum	CmRH56	Drought stress	Zhang *et al.* (2022)
Canola	BnRH24	Cadmium stress	Zhang *et al.* (2018)
Biotic stress			
Arabidopsis	SMN2	*Pseudomonas syringae* pv. tomato DC3000	Takagi *et al.* (2020)
	RH6, RH8, and RH12	Turnip mosaic virus	Li *et al.* (2021)
	RH11 and RH37	*Botrytis cinerea*	He *et al.* (2021)
	PINP1	*Phytophthora sojae*	Gui *et al.* (2022)

Molecular roles of RHs in plants

In human and yeast, RHs in the SF2 family are known to participate in various processes of RNA metabolism such as transcription, pre-mRNA splicing, mRNA export, and mRNA degradation (Awasthi *et al.*, 2018; Giraud *et al.*, 2018; Khemici and Linder, 2018; De Bortoli *et al.*, 2021). Emerging evidence implicates RHs as RNA chaperones and ribonucleoprotein (RNP) remodelers (Jarmoskaite and Russell, 2014; Gilman *et al.*, 2017; Fu, 2020; Tauber *et al.*, 2020; Weis and Hondele, 2022). In contrast, the molecular functions of RHs in plants are less understood, despite a few reports that plant RHs are engaged in rRNA biogenesis and the nonsense-mediated mRNA decay (NMD) pathway (Liu and Imai, 2018; Sulkowska *et al.*, 2020).

RNA helicases contribute to transcription regulation and genome integrity

While studying the genetic regulation of megasporogenesis, Schmidt *et al.* (2011) performed transcriptome analysis of megaspore mother cells (MMCs) in Arabidopsis and found significant transcript enrichment of DEAD/DEAH-box helicases. This observation implies important roles for RHs in plant germline development. These authors characterized an ATP-dependent RNA helicase, MEM, which is specifically enriched in the MMC. Mutation in the *MEM* gene leads to defects in megasporogenesis and megagametogenesis and arrest at early embryonic stages. Notably, in *mem* mutants compared with wild-type plants, *LIKE HETEROCHROMATIN PROTEIN1/TERMINAL FLOWER2* (*LHP1/TFL2*), which encodes a protein that binds to the H3K27me3 group as a repressive epigenetic mark (Exner *et al.*, 2009), shows a distinct distribution. Furthermore, the distribution of the chromatin structure marker H2B is also changed in the gametophytic nuclei of the mutant. These findings imply that the RH regulates reproductive growth through fine-tuning epigenetic modifications. Similarly, the DEAD-box PpeIF4A (Pp3c6_1080V3.1), which has a canonical function in protein synthesis, has been newly shown to interact with LHP1-Interacting Factor2-Like1 (LIF2L1) in *Physcomitrella patens.* PpeLF4A is a positive regulator of salinity tolerance because deletion of the gene makes the plants less tolerant to salt stress (Tyagi *et al.*, 2020). Thus, PpeIF4A might coordinate with LIF2L1 to regulate the transcription of stress-responsive genes to confer plant adaption to salt stress (Table 2). This notwithstanding, the underlying mechanisms of how MEM and PpelF4A regulate transcription remain unclear.

Direct involvement of RHs in epigenetic silencing is exemplified by two RHs, STRESS RESPONSE SUPPRESSOR1 (STRS1) and STRS2. Through a functional genomic screening, they were identified as negative regulators for salt, osmotic, and heat stresses (Kant *et al.*, 2007). The STRS proteins exhibit RNA-dependent ATPase and RNA-unwinding activities (Khan *et al.*, 2014). Notably, the RHs are typically localized in the nucleolus, nucleoplasm, and chromocenters. However, STRS1 is mislocalized in the *hd2c* mutant, which is defective in histone deacetylase activity, whereas STRS2 shows mislocalization in several mutants of the RNA-directed DNA methylation (RdDM) pathway. Furthermore, heterochromatic RdDM target loci exhibit reduced DNA methylation and consequently increased expression in *strs* mutants. Thus, the STRS proteins contribute to epigenetic silencing, and through this function suppress the expression of stress-induced genes

and act as attenuators of stress responses in Arabidopsis (Khan *et al.*, 2014) (Table 2).

RHs exemplified by Rho-like DNA: RNA helicase (RHON1) can maintain genome integrity by releasing the R-loop triggered by transcription–replication head-on conflict (HO-TRC) (Yang *et al.*, 2020). The R-loop is a special chromosome structure containing a DNA–RNA hybrid and a displaced single-strand DNA, and is widely present in the nuclear and chloroplast genomes of Arabidopsis (Xu *et al.*, 2017; Yang *et al.*, 2017). Yang *et al.* (2017) reported that the accumulation of HO-TRC-formed R-loops in chloroplasts could result in chloroplast genome instability. The authors screened a loss-of-function mutant, *atrnh1c*, in which R-loop accumulation is detected. The *atrnh1c* mutant also exhibits yellowish leaves, small plant size, and low photosystem II efficiency. Later, they found that the developmental defects could be rescued by overexpression of *RHON1* (Yang *et al.*, 2020). RHON1 shares sequence similarity with RNase H1-like proteins, which are known to be associated with R-loop resolution in eukaryotes. Indeed, RHON1 possesses helicase activity and can resolve RNA from DNA–RNA hybrids. Additionally, the Comet and S9.6 immunostaining assays show that genome damage is reduced, and the R-loop is accumulated in the *rhon1* mutant under rifampicin treatment. The results imply that RHON1 is required for releasing RNA from HO-TRC to maintain chloroplast genome stability.

RNA helicases in pre-mRNA splicing

Removal of intronic regions converts pre-mRNA into mature mRNA to finally encode proteins, and this process is conducted through the spliceosome (Wilkinson *et al.*, 2020). The spliceosome consists of five small nuclear ribonucleoproteins (snRNPs: U1, U2, U4, U5, and U6) that undergo a series of dynamic transitions from a pre-assembled complex. In yeast, the compositional rearrangement of snRNPs is driven by eight SF2 RH members: DEAD-box PRP5, DEAD-box Sub2, Ski2-like Brr2a, DEAH-box PRP2, DEAH-box PRP16, DEAH-box PRP22, and DEAH-box PRP43 (De Bortoli *et al.*, 2021). Notably, alternative splicing events contribute to the diversity and complexity of spliced transcripts, and include exon skipping, intron retention (IR), mutually exclusive exons, alternative 5′ splice sites, and alternative 3′ splice sites. In plants, alternative splicing events are present in more than half of the coding genes (Reddy *et al.*, 2013).

In yeast, Brr2 activates spliceosomes by releasing U4 small nuclear RNA (snRNA) from U4/U5/U6 tri-snRNPs. In Arabidopsis, genetic screening of early flowering mutant recovers a hypomorphic allele of *Brr2a*. RNA-seq analysis indicates that only a small set of introns is mis-spliced in the *brr2a* mutant, and among the most sensitive transcripts is *FLC*. Thus, the defects in *FLC* splicing and greatly reduced *FLC* transcript levels are responsible for the early flowering phenotype in the *brr2a* mutant (Mahrez *et al.*, 2016). By analogy, the Arabidopsis homolog of DEAH-box PRP22, Root-initiation defective1 (RID1), interacts with AtGFA1, a component of U5 snRNP, to control the splicing of several essential pre-mRNAs. Through maintaining proper splicing of reproductive development-related transcripts, RID1 contributes to female gametophyte development (Zhu *et al.*, 2016).

Numerous RHs regulate alternative splicing events of involved transcripts to fine-tune plant responses to different biotic and abiotic stresses (Nidumukkala *et al.*, 2019; Pandey *et al.*, 2019, 2020). DEAD-box SHI2 and OsRH42, two plant homologs of yeast PRP5, were reported to promote gene expression through fulfilling proper splicing of cold response-related pre-mRNA under cold stress (Lu *et al.*, 2020; Wang *et al.*, 2020). Briefly, Wang *et al.* (2020) observed that IR isoforms of several cold-inducible transcripts including *COR15A*, *COR6.6/KIN2*, and *EDR10* are accumulated in the *shi2* mutant at low temperatures. This molecular phenotype implies that SHI2 might act as a component of the spliceosome and promote proper mRNA splicing of cold-response genes to confer plant resistance against cold stress. SHI2 also is associated with salinity stress (Table 2). *shi2* is more hyposensitive than the wild-type under salt stress, despite the transcripts of many salt-induced genes accumulating in the mutant. Further mechanistic studies suggest that SHI2 might repress the expression of salt-responsive genes by governing mRNA capping and polyadenylation site selection (Wang *et al.*, 2020). Lu *et al.* (2020) reported that OsRH42 directly binds with U2 snRNA and localizes in the splicing speckles. *OsRH42*-knockdown lines contain increased IR cases of cold-induced transcripts and show a reduced survival rate under cold stress. Surprisingly, *OsRH42*-overexpressed lines also display a disrupted splicing profile of the cold response-related transcripts at low temperatures, causing weaker cold tolerance. This finding means that the homeostasis of *OsRH42* is critical to governing the splicing of cold response transcripts to gain adaption to cold stress in rice (Lu *et al.*, 2020) (Table 2).

RNA helicases participate in miRNA production

miRNAs are a group of small non-coding RNA that regulate numerous developmental processes and plant responses to abiotic and biotic stresses (Li *et al.*, 2017). miRNAs are processed from primary miRNAs (pri-miRNAs) through microprocessor that consists of Dicer-like protein 1 (DCL1), HYPONASTIC LEAVES 1 (HYL1), and SERRATE (SE). Since precise processing and homeostasis of accumulating miRNAs are critical for their proper functionality in biology, the biogenesis and metabolism of miRNAs are fine-tuned through multiple regulatory layers. Multiple genetic pathways to control miRNA homeostasis converge on the multifunctional protein SE in Arabidopsis (Wang *et al.*, 2018; Li *et al.*, 2020; L. Wang *et al.*, 2022a). SE recruits Swi2/Snf2 ATPase subunit CHR2 to remodel the secondary structure of pri-miRNAs to inhibit miRNA production (Wang *et al.*, 2018).

A recent study of zygote division implicated another RH in miRNA production. Briefly, Hou *et al.* (2021) conducted a genetic screening of ethyl methane sulfonate mutagenesis and recovered a mutant in DEAD-box *RH27* with clear developmental defect. Whereas the strong mutant allele *rh27-1* displays a zygote-lethal phenotype, the weak mutant allele *rh27-2* has minor defects in embryogenesis and severely compromised stem cell homeostasis in shoot apical meristem and root apical meristem. Notably, the expression of several genes related to cell homeostasis increases alongside the down-regulation of their regulatory miRNAs in *rh27* mutants. Mechanistically, RH27 binds to pri-miRNAs and interacts with the key microprocessor components HYL1 and SE and an auxiliary component named DAWDLE (DDL). Thus, RH27 appears to affect zygote division and cell homeostasis through fine-tuning miRNA production.

Recently, Li *et al.* (2021) conducted a proteomics analysis of HYL1 protein to decode new components of microprocessors. RH6, RH8, and RH12 were uncovered as the new component of the HYL1-centered D-bodies in the nucleus. The term D-bodies was coined because the core components of the microprocessor, DCL1, HYL1, and possibly SE protein, are initially detected in discrete nuclear speckles (Fang and Spector, 2007). Supporting this proposal is a recent discovery that SE possesses a liquid-droplet feature and can drive D-body assembly and promote pri-miRNA processing (Xie *et al.*, 2021). Li *et al.* (2021) found that the three helicases also interact with and promote the phase separation of SE and drive the formation of D-bodies. In line with these observations, the knockdown mutants of the three helicases display reduced miRNA production (Li *et al.*, 2021). Intriguingly, the accumulation of these helicases in the nuclei decreases upon turnip mosaic virus infections (Table 2), which couples with the decrease of D-bodies. Instead, RH6, RH8, and RH12 appear to be hijacked or translocated by viral proteins to the perinuclear globular structure and on the chloroplast periphery, coupling with the formation of virus bodies where virus replication actively takes place (Li *et al.*, 2021). Thus, three helicases seem to concurrently regulate the assembly and disassembly of two macromolecular complexes in both nuclei and cytoplasm to adapt to physiological changes in the host–virus interaction. However, in a paradigm-shifting model, pri-miRNA processing can take place co-transcriptionally in plants, and D-bodies might not necessarily be the sole birthplace for miRNAs; rather, they might function as the reservoir for inactive microprocessor components, or partially participate in the miRNA production (Gonzalo, 2022; Zhu *et al.*, 2022). Thus, RH6/RH8/RH12 regulation of D-bodies might have new but yet to be understood roles in regulating plant defense against turnip mosaic virus.

PSR1-interacting protein 1 (PINP1) is another RH that contributes to miRNA production. *Phytophthora*-encoded Suppressor of RNA Silencing 1 (PSR1) is essential for the pathogen infection of the host. Qiao *et al.* (2015), found that PSR1 directly targets an evolutionarily conserved nuclear DEAH-box RH, PINP1. This protein, also known as PRP16, is a core pre-mRNA splicing factor. Silencing of *PINP1* impairs the assembly of miRNA-processing complexes in the nucleus, leading to developmental defects of plants and hypersusceptibility of the host to *Phytophthora* infection. In a follow-up study, Gui *et al.* (2022) showed that PINP1 could unwind RNA duplexes and bind to pri-miRNAs and general RNAs. The silencing of *PINP1* also results in genome-wide alternative splicing events, indicative of its dual functions in miRNA biogenesis and pre-mRNA processing. Interestingly, the genes showing alternative splicing in the *PINP1*-silencing line are closely related to a defense response and to small RNA (sRNA) biogenesis (Table 2). Although it is still unclear whether the RNA helicase activity of PINP1/PRP6 contributes to the defense response, these results indicate that RHs can regulate plant immunity through concordantly participating in two key RNA metabolic processes, pre-mRNA splicing and RNA silencing. Interestingly, PINP1 is reminiscent of DEAD-box RH DDX17 and SDE3 in animals and plants, which are involved in the sRNA biogenesis pathway and antiviral immunity (Garcia *et al.*, 2012; Moy *et al.*, 2014). Also, PINP1 mechanistically resembles the RH SMALL1 (SMA1), a homolog of the DEAD box pre-mRNA splicing factor PRP28 (Li *et al.*, 2018). SMA1 enhances the accumulation of DCL1 protein by promoting the splicing of the *DCL1* pre-mRNAs. Furthermore, SMA1 interacts with the DCL1 complex and positively influences pri-miRNA processing. Thus, SMA1 seems to have dual roles in promoting miRNA biogenesis in Arabidopsis (Li *et al.*, 2018).

RHs are not only regulators of miRNA production, but also can be the targets of miRNAs. In canola, BnRH24 is a negative regulator in the phytoremediation of the heavy metal cadmium (Cd) (Zhang *et al.*, 2018). High throughput degradome analysis revealed that *BnRH24* mRNA is a target of miR158. A Cd treatment assay in *B. napus* showed that expression of mature miR158 is induced whereas the *BnRH24* transcript is repressed. The inverse relationship of miR158 and *BnRH24* levels suggests Cd stress would trigger the repression of *BnRH24* through miR158-mediated cleavage of its target. Interestingly, transgenic Arabidopsis with ectopic expression of *BnRH24* displays defective root elongation, reduced mass production, and decreased chlorophyll content. Furthermore, the transgenic plants also have accumulated oxidative products and thiobarbituric acid reactive substances, indicative of weak stress tolerance under Cd-treated conditions. Taken together, these results suggest that the controlling of *BnRH24* by Cd-induced miR158 is crucial for Cd tolerance (Table 2).

RNA helicases and RNA trafficking

Besides being engaged in pre-mRNA splicing and sRNA production, RHs are also implicated in RNA trafficking. Earlier, Gong *et al.* (2005) discovered that a DEAD-box RNA helicase,

CRYOPHYTE/LOS4, is essential for the export of mRNA from nucleus to cytoplasm, and that through this the RH regulates plant development and stress responses in Arabidopsis (Table 2). The DEAD-box protein U2AF65-associated protein (UAP56), an RNA helicase in yeast and metazoans, is critically involved in mRNA splicing and export. Similarly, Arabidopsis UAP56 directly interacts with the mRNA export factors ALY2 and MOS11, suggestive of its critical role in mRNA export from plant cell nuclei (Kammel *et al.*, 2013).

RHs also contribute to the long-distance trafficking of RNA. Upon being infected by a pathogen, plants use extracellular vesicles (EVs) to deliver sRNAs into the pathogen and silence the virulence genes through a mechanism called 'cross-kingdom RNAi' (Cai *et al.*, 2018; Castillo-Gonzalez and Zhang, 2018). He *et al.* (2021) found that AGO1, RH11, and RH37 selectively bind to EV-enriched sRNAs, indicating their role in the critical loading of sRNA into EVs. Consistent with this, *rh11;rh37* shows increased susceptibility to *Botrytis cinerea* (Table 2). Thus, RHs can regulate plant immune response by controlling sRNA movement.

RNA helicases in liquid–liquid phase separation in P-bodies and stress granules

Liquid–liquid phase separation (LLPS), also called biomolecular condensation, is a feature of numerous membrane-less organelles in eukaryotic cells (Banani *et al.*, 2017; Shin and Brangwynne, 2017; Alberti, 2019; Xie *et al.*, 2021). LLPS allows the compartmentalization and organization of RNP macromolecules and specific cellular activities (Banani *et al.*, 2017; Shin and Brangwynne, 2017; Alberti, 2019). It is driven by multivalent protein–protein or protein–RNA interactions that often involve intrinsically disordered regions (IDRs) and/or low-complexity sequences in proteins. Among the membrane-less organelles are cytosolic P-bodies and related stress granules (SGs). P-bodies and SGs participate in translational control in cells (Corbet and Parker, 2019; Youn *et al.*, 2019). In P-bodies, mRNAs are devoid of translation initiation factors and thus are translationally stalled. These mRNAs can be further de-capped and degraded. Similarly, upon stress, mRNAs bind to the translational machinery and other proteins coalesce to form SGs, and global translation is paused. Both the assembly and disassembly of P-bodies or SGs are dynamic, and mRNAs in the organelles can be rescued in response to changes in physiological conditions.

It has been reported that the formation and turnover of P-bodies and SGs are driven by the DEAD-box RHs DHH1 and DED1 through LLPS in yeast (Mugler *et al.*, 2016; Hondele *et al.*, 2019). Similarly, Arabidopsis homologs of the yeast DHH1/DDX6, namely RH6, RH8, and RH12, which regulate the formation of D-bodies and virus bodies mentioned above, also contribute to the assembly and subcellular dynamics configurations of the P-bodies and SGs *in vivo* (Chantarachot *et al.*, 2020). In response to severe deficiency of RH6, RH8, and RH12 functions, the expression of defense and other stress-responsive mRNAs is up-regulated despite growth under standard conditions, with simultaneous repression of mRNAs required for general growth. It is noted that the three RHs facilitate the turnover of specific short-lived de-capping substrates that are enriched for stress and defense responses. They also act to restrict the precocious accumulation and ribosome association of stress-responsive mRNAs that are engaged in autoimmunity and growth inhibition under non-stress conditions. Otherwise, the stress-related mRNAs are stabilized and preferentially loaded into ribosomes in the *rh6;rh8;rh12* mutant, conferring auto-immunity. In summary, the three helicases act as redundant mRNA decay factors required for normal growth and development, and the decay of stress-responsive mRNAs under non-stress conditions is required for the maintenance of the growth/defense balance in plants (Chantarachot *et al.*, 2020). Altogether, RH6, RH8, and RH12 not only participate in plant defense against virus infection through regulating the formation and dynamics of D-bodies and virus bodies, but also regulates plant growth and development by controlling LLPS of two RNP complexes, the P-bodies and SGs.

Like RH6, RH8, and RH12, other RHs could also fine-tune the expression of stress-responsive genes by regulating SG dynamics (Jang *et al.*, 2020; Maruri-Lopez *et al.*, 2021). DEAD-box RH31 is identified as a component of SGs because the protein is co-localized with the SG marker RBP47. It has been found that the *RH31* knockout mutant is hypersensitive, whereas the overexpression line is hyposensitive to salt treatment. Liu *et al.* (2021) noticed that salinity stress could result in translocation of the RH31 from the nucleus to the cytoplasm, and the accumulated RH31 forms the SGs. Conversely, these condensates disappear when RH31–GFP seedlings are treated with cycloheximide, which blocks SG formation by disturbing mRNP homeostasis. Thus, RH31 is proposed to confer salt tolerance on Arabidopsis by participating in the formation of SGs, despite the fact that the deregulated mRNA targets remain to be identified (Liu *et al.*, 2021) (Table 2).

RNA helicases involved in RNA decay in exosomes

RHs can be constitutive components of RNA exosomes that account for the processing, surveillance, and turnover of both nuclear and cytoplasmic RNA (Lange *et al.*, 2014). Two well-known RHs in the exosome are HUA enhancer 2 (HEN2) and mRNA transport 4 (MTR4, see below). HEN2 is accumulated in the nucleoplasm and participates in the degradation of a large number of polyadenylated nuclear exosome substrates such as small nucleolar RNA and miRNA precursors, incompletely spliced mRNAs, and spurious transcripts produced from pseudogenes and intergenic regions (Lange *et al.*, 2014). In line with this observation is the recent discovery that increased accumulation of pri-miRNAs is detected in *se-2;hen2-2* double mutants compared with the single *se-2* mutant (Bajczyk *et al.*, 2020). Interestingly, HEN2 has been

recently reported to regulate pathogen resistance and innate immunity by governing the integrity of defense-related transcripts (Takagi *et al.*, 2020) (Table 2).

SUPERKILLER (SKI) complexes degrade aberrant cytoplasmic RNAs that are derived from RNA silencing processes or from the NMD surveillance system (Linder and Owttrim, 2009). The RH SKI2 is a critical component of the SKI complex and contributes to the decay of 5′ cleavage fragments of miRNA targets in Arabidopsis (Branscheid *et al.*, 2015). By contrast, the SF1 member RH Up Frameshift1 (UPF1) is engaged in the NMD pathway and targets the transcripts with premature termination codons (Arciga-Reyes *et al.*, 2006). Of note, *UPF1* loss-of-function mutants display a range of defective-growth phenotypes, including jagged leaves, fused flowers, larger-size seeds, seedling lethality, and stunted growth (Arciga-Reyes *et al.*, 2006; Yoine *et al.*, 2006). Interestingly, UPF1 has been recently reported to play important roles in alternative splicing and translation regulation (Raxwal *et al.*, 2020).

RNA helicases involved in the biogenesis of nuclear rRNA and ribosome

Many RHs contribute to ribosome biogenesis, and through this function regulate plant reproductive growth. One example is DEAD-box RH36/SLOWWALKER3 (SWA3), which is essential for female gametogenesis (Huang *et al.*, 2010; Liu *et al.*, 2010). The *swa3* mutant generated by the *Ds* transposon insertion system shows postponed megagametogenesis development at four- or eight-nucleate stages and abnormal polarity of synergids. Sequence analysis indicates that SWA3 is a homolog of Dbp8 in yeast, which is essential for the biogenesis of yeast 18S rRNA by interacting with the protein Esf2 (Liu *et al.*, 2010). At the same time, Huang *et al.* (2010) also reported an allele of *rh36-1* and found that female gametogenesis is delayed in the mutant. Furthermore, asynchronous development of the female gametophytes was found within a single pistil. Notably, the pleiotropic phenotype is correlated to defective processing of rRNA such as 18S pre-rRNA. DEAD-box RH29 was identified through genetic screening and characterized to function in development and maturation of gametophytes (Chen *et al.*, 2020). RH29 is highly and specifically expressed in gametophytic cells and is required for functional maturation of male and female gametophytes. It is of note that RH29 shares a high amino acid sequence identity with Dbp10p in yeast, which is involved in ribosome biogenesis, suggesting that RH29 may modulate male/female gametophyte development through regulating protein synthesis.

The DEAD-box helicase eIF4A-1 is best known for being involved in translation initiation, and its loss leads to a defect in ovule development (Bush *et al.*, 2015). RH17 was also found to function in ovule development (Stein *et al.*, 2021). It has been shown that the *rh17* mutant displays supernumerary reproductive cell lineages in the female flower tissues (ovules), causing the formation of two embryos per seed from time to time. Furthermore, seed coat and putative endosperm development are frequently initiated autonomously in the mutant. These observations indicate that RH17 is involved in the repression of reproductive fate and of elements of seed development in the absence of fertilization. Additional transcriptome analysis implies that RH17 regulates the process by controlling the genetic pathways of ribosome biosynthesis, stress response, hormones, and seed coat development (Table 2).

Similarly, several RHs also regulate vegetative growth by controlling rRNA biogenesis in Arabidopsis. Lange *et al.* (2011) reported that *mtr4* has phenotypes of aberrant vein patterning and pointed true leaves, reminiscent of ribosome biogenesis mutants. Indeed, some mature rRNA by-products are accumulated, while mature 5.8S rRNA is down-regulated in *mtr4*. In a subsequent study, Lange *et al.* (2014) found that MTR4 is a component of nuclear exosomes and plays a major role in the degradation of rRNA precursors and rRNA maturation by-products. Thus, MTR4 controls vegetative growth by regulating the rRNA biogenesis pathway.

Like MTR4, RH7 and RH57 also regulate plant growth and development by participating in rRNA biogenesis, whereas the knockout mutants of DEAD-box *RH7* (*rh7-2* and *rh7-3*) show delayed seed germination, narrow points on the first leaves, shoot roots, aberrant floral development, and reduced plant status (Huang *et al.*, 2016). The deficiency of RH57 leads to enhanced sensitivity to glucose and abscisic acid (Hsu *et al.*, 2014). Taken together, RH36/SWA3, RH29, RH17, eIF4A-1, MTR4, RH7, and RH57 are all engaged in different aspects of rRNA biogenesis or translation regulation. The coincidental findings highlight the significant regulatory roles of RHs in the reproductive and vegetative processes at the protein synthesis level in Arabidopsis.

RHs related to rRNA biogenesis also play important roles in the growth and development of other plants. A recent study in *Medicago truncatula* revealed that silencing of *DEAD-box MtRH10* represses the development of roots accompanied by a smaller number of roots and reduced primary root length. It has been noted that all ribosome-associated genes of roots decreased in silenced plants, suggesting that MtRH10 positively regulates root development by involving the ribosome biogenesis pathway (Camborde *et al.*, 2022). In rice, DEAD-box Thermotolerant Growth Required1 (OsTOGR1) has been also identified to improve thermotolerance by positively regulating mature rRNA production. OsTOGR1 is a pre-rRNA chaperone as a partner of the small subunit (SSU) complex. The *togr1-1* mutant exhibits a stunted pre-rRNA maturation process with highly abundant intermediates accumulating during pre-rRNA processing at high temperatures, indicating that OsTOGR1 promotes rRNA maturation under heat stress (Wang *et al.*, 2016) (Table 2). Later, OsTOGR1 was also found to enhance resistance to heat stress when it was exogenously expressed in Chinese cabbage (*Brassica rapa* L. ssp. *Chinensis*). The study showed that transgenic cabbage seedlings grow and develop better at high temperatures as evidenced by

longer hypocotyl, higher chlorophyll content, and better germination (Yarra and Xue, 2020).

RNA helicases function in organelles

RHs can be trafficked into different organelles to fulfill their regulatory functions. The null mutation of three chloroplast-localized DEAD-box members, *RH3*, *Increased Size Exclusion limit2* (*ISE2*), and *RH22*, are embryo lethal or defective, but their regulatory mechanisms of embryo development are distinct (Asakura *et al.*, 2012; Hou *et al.*, 2021). It was found that RH3 regulates the splicing of several genes with group II introns (Asakura *et al.*, 2012). Group II introns are a large class of self-catalytic ribozymes that can fold into a conserved secondary structure of six domains (DI–DVI) extending from a central hub. The conserved secondary structure of group II introns and defective splicing in *rh3* strongly suggest that RH3 might participate in the folding or unfolding of RNA secondary structure of the targeted transcripts. Through this regulation, RH3 contributes to embryogenesis, chloroplast development, and photosynthesis. Similarly, ISE2 is required for the splicing of group II introns from chloroplast transcripts. In addition, ISE2 is also engaged in other aspects of chloroplast RNA metabolism because the loss of *ISE2* can cause defects in C-to-U RNA editing and defective processing of chloroplast ribosomal RNAs. These results suggest that ISE2 is different from the canonical Ski2-like RNA helicases that typically function in degrading RNA in cytoplasm, despite the high sequence similarity between ISE2 and the Ski2-helicases (Carlotto *et al.*, 2016; Bobik *et al.*, 2017).

Chi *et al.* (2012) found that the DEAD-box *RH22* knockdown mutant has virescent seedlings. Importantly, the mutant also contains accumulated precursors of 23S and 4.5S rRNA. Chi *et al.* experimentally validated that RH22 interacts with 50S ribosomal subunits like RPL24, suggesting RH22 takes part in chloroplast ribosome assembly to regulate plant development. Like RH22, DEAD-box RH50 has been identified as a chloroplast rRNA maturation factor that is required for the maturation of the 23S and 4.5S rRNAs (Paieri *et al.*, 2018). In that study, RNA-immunoprecipitation showed that the transcripts from the 23S–4.5S intergenic region accumulate in *rh50-1*. Furthermore, the abundance of intermediate transcripts located in 23S–4.5S intergenic regions increases significantly, while the levels of mature 23S and 4.5S rRNAs decrease in the cold-sensitive mutant *rh50-1*. Since RH50 is characterized as an RH, it is speculated that the enzyme might act in unwinding paired rRNA of the 23S–4.5S intergenic region to facilitate rRNA cleavage processing during biogenesis. Due to its critical role in rRNA maturation in the chloroplast, deficiency of the gene confers cold sensitivity in plants (Table 2).

RHs have been reported to regulate the functions of the chloroplast in other plants. In tomato, a T-DNA insertion mutant in *SlDEAD39*, named *restored cell structure by salinity* (*res*), displays obvious growth inhibition with damaged cell structure (Garcia-Abellan *et al.*, 2015; Capel *et al.*, 2020). In the *res* mutant, immature fragments of unprocessed or partially processed 23S are abnormally enriched; in contrast, the fully processed mature fragments are barely detectable. The results imply that SlDEAD39 is engaged in the proper processing of premature 23S rRNA. Indeed, the authors further determined that the defective cleavage site in *res* is located on the hidden break site B, one of two critical sites to process premature 23S rRNA into the mature fragments. Together, these studies indicate that DEAD-box SlDEAD39 acts in tomato vegetative development by regulation of 23S rRNA maturation (Capel *et al.*, 2020).

RNA helicases function in metabolism

As discussed in the above section, plant RHs play diverse roles in all aspects of RNA metabolism as catalytic enzymes by acting on different substrates (Fig. 3). RHs are also engaged in various metabolic pathways but with yet unknown targets. In chrysanthemum, a DEAD-box member, CmRH56, regulates negatively rhizome outgrowth under water deficiency (Zhang *et al.*, 2022). CmRH56 is expressed specifically in the rhizome shoot apex, and lack of CmRH56 causes fewer rhizomes under drought conditions. In contrast, *CmRH56*-overexpressed plants show increased survival through induced rhizomes under water deficiency. Mechanistically, CmRH56 might contribute to rhizomes growth with drought stress through inhibiting gibberellin biosynthesis.

In rice, DEAD-box chloroplast-located OsTCD33 regulates resistance to cold stress by controlling the chlorophyll biosynthesis pathway (Xiaomei *et al.*, 2020). TaDEAD-box57-3B is associated with cold stress in rice (Ru *et al.*, 2021). TaDEAD-box57-3B is proposed to contribute to drought, salt, and cold stress by regulating membrane lipid peroxidation (Maruri-Lopez *et al.*, 2021). In *Physcomitrium patens*, Perroud *et al.* (2021) found that deletions of individual DEAD-box PpRH1 and PpRH2 cause visible defective development in protonemata and gametophores. The double mutant displays significantly increased starch granules, indicative of changes in photosynthetic activity.

Conclusion and future perspectives

Accumulating evidence has revealed the significance of plant RHs in all aspects of RNA metabolism, including transcription, pre-mRNA splicing, miRNA production, RNP dynamics, RNA export, RNA stability, and protein translation. Through these mechanisms, RHs participate in almost every biological process in plants (Fig. 4). From the perspective of biological functions, there are lots of exciting studies ahead. One critical question is whether all or most of the RHs modulate molecular and biological processes by altering RNA secondary structure. Despite the important roles of RHs in biology, the significance of helicase activity is still unclear. For example, a recent study by

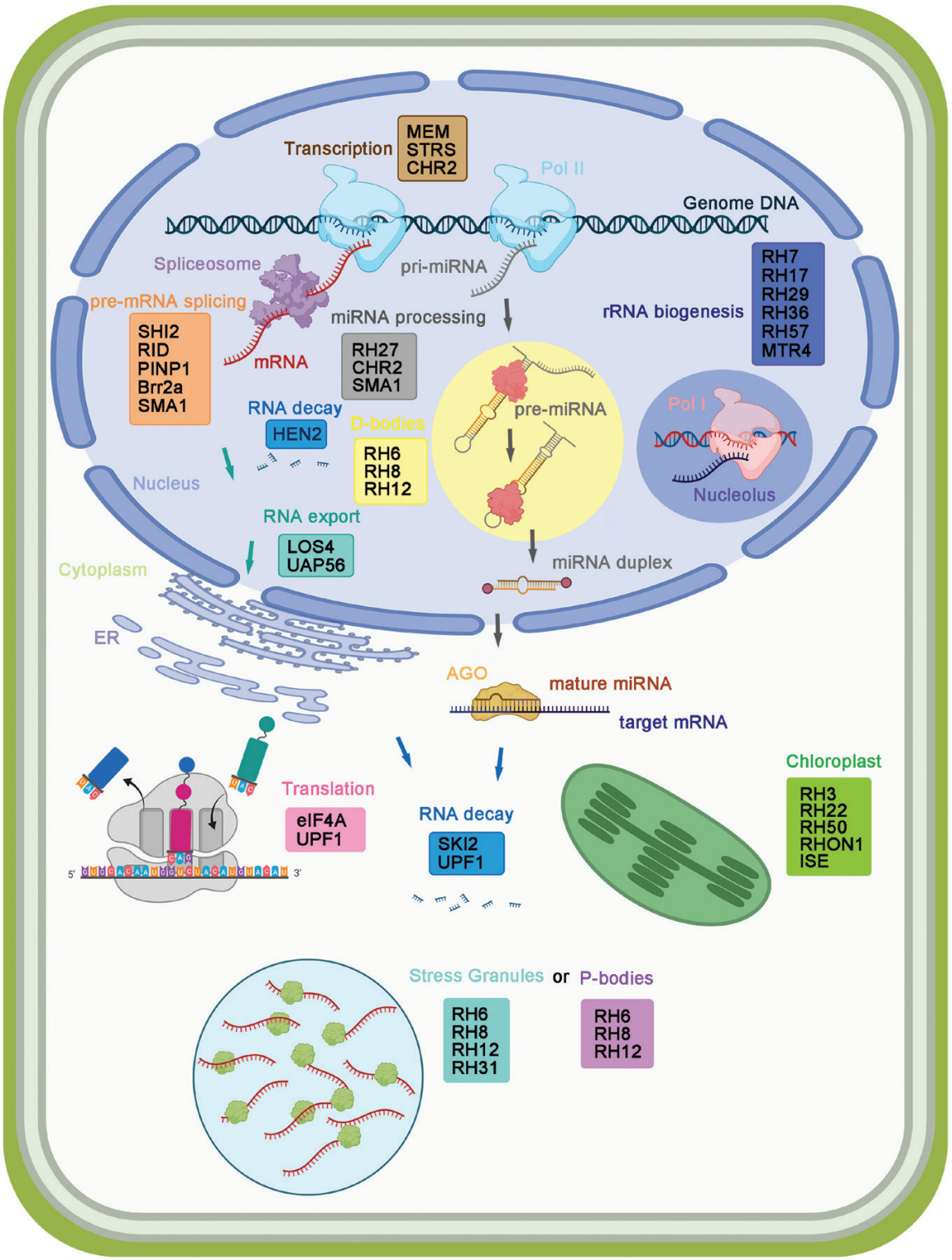

Fig. 3. RHs are engaged in multiple molecular processes in Arabidopsis. Individual RHs involved in diverse kinds of RNA metabolism are labeled with distinct colors and include those involved in transcription, pre-mRNA splicing, formation of LLPS (D-bodies, P-bodies, and stress granules), miRNA processing, mRNA export, RNA degradation, protein translation, and organelle-related functions.

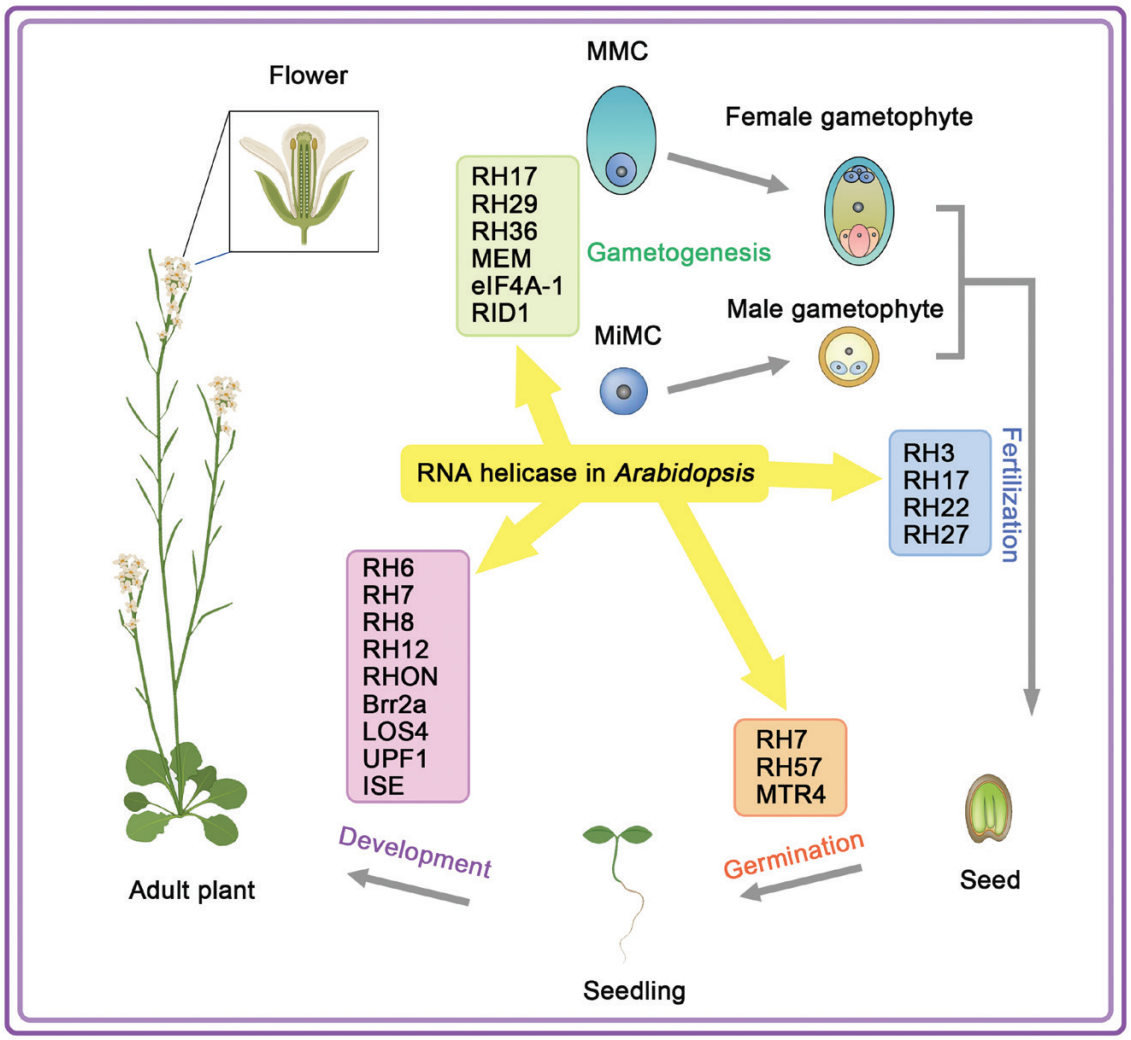

Fig. 4. RHs take participate in the whole life cycle of Arabidopsis plants. RHs are involved in every stage of plant growth and development in Arabidopsis, including gametogenesis (green), fertilization (blue), germination (orange), and development (purple).

Chantarachot *et al.* (2020) suggested that plant RH6, RH8, and RH12 facilitate the decay of short-lived RNAs, and it is worth exploring whether the helicase activity of the RHs is required for this process and what the structural features are of short-live RNAs in the *rh6;rh8;rh12* mutant. What is more, most current studies have focused on the roles of RHs in RNA and RNA–proteins, while only limited studies have demonstrated that RHs regulate RNA–DNA hybrids, in terms of R-loops, which have increasingly been shown to have important roles in eukaryotes. Furthermore, it has not been reported whether plant RHs function independently from their unwinding activity. Last but not the least, understanding the functions of RHs entails the decoding of RH-bound substrates. Excitingly, the advancement of cutting-edge next-generation sequencing-based methods provides a gateway to this goal. RNA-immunoprecipitation combined with sequencing (RIP-seq) or crosslinking, and immunoprecipitation combined with sequencing (CLIP-seq) (Zambelli and Pavesi, 2015; Hafner *et al.*, 2021) would be powerful approaches. The advantages of these approaches are that they could capture the transient interaction of RHs with their substrates. In addition, spatial–temporal techniques can be explored to study functions and mechanisms of RHs. Single-molecule techniques, such as single-molecule RNA-seq (smRNA-seq) (Bayega *et al.*, 2018), individual nucleotide CLIP-seq (iCLIP-seq) (Huppertz *et al.*, 2014), and single-molecule fluorescence resonance energy transfer (SM-FRET) (Lerner *et al.*, 2018), would facilitate the study of RHs. For example, smRNA-seq provides more detailed information on RH targets, including regulation of isoform production and different preferences for different RNA isoforms. iCLIP would allow precisely pinpointing of the binding sites of RHs at individual nucleotide resolution. SM-FRET facilitates the understanding of interacting cofactors and specific targets. In summary, molecular mechanisms of RHs in biological processes have been characterized well in mammals and yeast, while mechanistic studies in plant RHs are still in their infancy.

Supplementary data

The following supplementary data are available at *JXB* online.

Table S1. All RH candidates in Arabidopsis.

Acknowledgements

We apologize to colleagues whose research was not cited here due to the limitation of space.

Conflict of interest

The authors have no conflicts to declare.

Funding

The work in the Zhang laboratory was supported by grants from National Institutes of Health (NIH) (GM127742 and GM132401) to XZ. XL was partially supported by China Council Scholarship.

Data availability

The Uniprot accession numbers and gene locus of all potential RH candidates supporting blastp data are available within the paper.

References

Alberti S, Gladfelter A, Mittag T. 2019. Considerations and challenges in studying liquid-liquid phase separation and biomolecular condensates. Cell **176**, 419–434.

Arciga-Reyes L, Wootton L, Kieffer M, Davies B. 2006. UPF1 is required for nonsense-mediated mRNA decay (NMD) and RNAi in Arabidopsis. The Plant Journal **47**, 480–489.

Asakura Y, Galarneau E, Watkins KP, Barkan A, van Wijk KJ. 2012. Chloroplast RH3 DEAD Box RNA helicases in maize and Arabidopsis function in splicing of specific group II introns and affect chloroplast ribosome biogenesis. Plant Physiology **159**, 961–974.

Awasthi S, Verma M, Mahesh A, Khan MIK, Govindaraju G, Rajavelu A, Chavali PL, Chavali S, Dhayalan A. 2018. DDX49 is an RNA helicase that affects translation by regulating mRNA export and the levels of pre-ribosomal RNA. Nucleic Acids Research **46**, 6304–6317.

Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren JY, Li WW, Noble WS. 2009. MEME SUITE: tools for motif discovery and searching. Nucleic Acids Research **37**, W202–W208.

Bajczyk M, Lange H, Bielewicz D, Szewc L, Bhat SS, Dolata J, Kuhn L, Szweykowska-Kulinska Z, Gagliardi D, Jarmolowski A. 2020. SERRATE interacts with the nuclear exosome targeting (NEXT) complex to degrade primary miRNA precursors in Arabidopsis. Nucleic Acids Research **48**, 6839–6854.

Banani SF, Lee HO, Hyman AA, Rosen MK. 2017. Biomolecular condensates: organizers of cellular biochemistry. Nature Reviews. Molecular Cell Biology **18**, 285–298.

Bayega A, Fahiminiya S, Oikonomopoulos S, Ragoussis J. 2018. Current and future methods for mRNA analysis: a drive toward single molecule sequencing. Gene Expression Analysis **1783**, 209–241.

Bobik K, McCray TN, Ernest B, Fernandez JC, Howell KA, Lane T, Staton M, Burch-Smith TM. 2017. The chloroplast RNA helicase ISE2 is required for multiple chloroplast RNA processing steps in *Arabidopsis thaliana*. The Plant Journal **91**, 114–131.

Branscheid A, Marchais A, Schott G, Lange H, Gagliardi D, Andersen SU, Voinnet O, Brodersen P. 2015. SKI2 mediates degradation of RISC 5′-cleavage fragments and prevents secondary siRNA production from miRNA targets in *Arabidopsis*. Nucleic Acids Research **43**, 10975–10988.

Brennan P. 2018. drawProteins: a Bioconductor/R package for reproducible and programmatic generation of protein schematics. F1000Research **7**, 1105.

Bush MS, Crowe N, Zheng T, Doonan JH. 2015. The RNA helicase, eIF4A-1, is required for ovule development and cell size homeostasis in Arabidopsis. The Plant Journal **84**, 989–1004.

Cai Q, Qiao LL, Wang M, He BY, Lin FM, Palmquist J, Huang SND, Jin HL. 2018. Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. Science **360**, 1126–1129.

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. BMC Bioinformatics **10**, 421.

Camborde L, Kiselev A, Pel MJC, Le Ru A, Jauneau A, Pouzet C, Dumas B, Gaulin E. 2022. An oomycete effector targets a plant RNA helicase involved in root development and defense. New Phytologist **233**, 2232–2248.

Capel C, Albaladejo I, Egea I, *et al*. 2020. The *res* (*restored cell structure by salinity*) tomato mutant reveals the role of the DEAD-box RNA helicase SlDEAD39 in plant development and salt response. Plant, Cell & Environment **43**, 1722–1739.

Carlotto N, Wirth S, Furman N, Ferreyra Solari N, Ariel F, Crespi M, Kobayashi K. 2016. The chloroplastic DEVH-box RNA helicase INCREASED SIZE EXCLUSION LIMIT 2 involved in plasmodesmata regulation is required for group II intron splicing. Plant, Cell & Environment **39**, 165–173.

Castillo-Gonzalez C, Zhang X. 2018. The Trojan horse of the plant kingdom. Cell Host and Microbe **24**, 1–3.

Chantarachot T, Sorenson RS, Hummel M, *et al*. 2020. DHH1/DDX6-like RNA helicases maintain ephemeral half-lives of stress-response mRNAs. Nature Plants **6**, 675.

Chen D, Wang Y, Zhang W, Li N, Dai B, Xie F, Sun Y, Sun M, Peng X. 2020. Gametophyte-specific DEAD-box RNA helicase 29 is required for functional maturation of male and female gametophytes in Arabidopsis. Journal of Experimental Botany **71**, 4083–4092.

Chen J, Zhang Y, Liu J, Xia M, Wang W, Shen F. 2014. Genome-wide analysis of the RNA helicase gene family in *Gossypium raimondii*. International Journal of Molecular Sciences **15**, 4635–4656.

Cheng J, Zhou SS, Yang K, Yu HY, Chen RR, Zeng LM, Li H, Wang YH, Song JB. 2021. Identification of RNA helicases in *Medicago truncatula* and their expression patterns under abiotic stress. Physiology and Molecular Biology of Plants **27**, 2283–2296.

Chi W, He B, Mao J, Li Q, Ma J, Ji D, Zou M, Zhang L. 2012. The function of RH22, a DEAD RNA helicase, in the biogenesis of the 50S ribosomal subunits of Arabidopsis chloroplasts. Plant Physiology **158**, 693–707.

Corbet GA, Parker R. 2019. RNP granule formation: lessons from P-bodies and stress granules. Cold Spring Harbor Symposia on Quantitative Biology **84**, 203–215.

Cordin O, Beggs JD. 2013. RNA helicases in splicing. RNA Biology **10**, 83–95.

De Bortoli F, Espinosa S, Zhao R. 2021. DEAH-Box RNA helicases in pre-mRNA splicing. Trends in Biochemical Sciences **46**, 225–238.

Dillingham MS. 2011. Superfamily I helicases as modular components of DNA-processing machines. Biochemical Society Transactions **39**, 413–423.

Donsbach P, Klostermeier D. 2021. Regulation of RNA helicase activity: principles and examples. Biological Chemistry **402**, 529–559.

Exner V, Aichinger E, Shu H, Wildhaber T, Alfarano P, Caflisch A, Gruissem W, Kohler C, Hennig L. 2009. The chromodomain of LIKE HETEROCHROMATIN PROTEIN 1 is essential for H3K27me3 binding and function during *Arabidopsis* development. PLoS One **4**, e5335.

Fairman-Williams ME, Guenther UP, Jankowsky E. 2010. SF1 and SF2 helicases: family matters. Current Opinion in Structural Biology **20**, 313–324.

Fang Y, Spector DL. 2007. Identification of nuclear dicing bodies containing proteins for microRNA biogenesis in living *Arabidopsis* plants. Current Biology **17**, 818–823.

Fu XD. 2020. RNA helicases regulate RNA condensates. Cell Research **30**, 281–282.

Garcia D, Garcia S, Pontier D, Marchais A, Renou JP, Lagrange T, Voinnet O. 2012. Ago hook and RNA helicase motifs underpin dual roles for SDE3 in antiviral defense and silencing of nonconserved intergenic regions. Molecular Cell **48**, 109–120.

Garcia-Abellan JO, Fernandez-Garcia N, Lopez-Berenguer C, *et al*. 2015. The tomato res mutant which accumulates JA in roots in non-stressed conditions restores cell structure alterations under salinity. Physiologia Plantarum **155**, 296–314.

Gilman B, Tijerina P, Russell R. 2017. Distinct RNA-unwinding mechanisms of DEAD-box and DEAH-box RNA helicase proteins in remodeling structured RNAs and RNPs. Biochemical Society Transactions **45**, 1313–1321.

Giraud G, Terrone S, Bourgeois CF. 2018. Functions of DEAD box RNA helicases DDX5 and DDX17 in chromatin organization and transcriptional regulation. BMB Reports **51**, 613–622.

Gong ZZ, Dong CH, Lee H, Zhu JH, Xiong LM, Gong DM, Stevenson B, Zhu JK. 2005. A DEAD box RNA helicase is essential for mRNA export and important for development and stress responses in Arabidopsis. The Plant Cell **17**, 256–267.

Gonzalo L, Tossolini I, Gulanicz T, *et al*. 2022. R-loops at microRNA encoding loci promote co-transcriptional processing of pri-miRNAs in plants. Nature Plants **8**, 402–418.

Gui XM, Zhang P, Wang D, Ding Z, Wu X, Shi JX, Shen QH, Xu YZ, Ma WB, Qiao YL. 2022. *Phytophthora* effector PSR1 hijacks the host pre-mRNA splicing machinery to modulate small RNA biogenesis and plant immunity. The Plant Cell **34**, 3443–3459.

Hafner M, Katsantoni M, Köster T, Marks J, Mukherjee J, Staiger D, Ule J, Zavolan M. 2021. CLIP and complementary methods. Nature Reviews. Methods Primers **1**, 20.

He BY, Cai Q, Qiao LL, Huang CY, Wang SM, Miao WL, Ha T, Wang YS, Jin HL. 2021. RNA-binding proteins contribute to small RNA loading in plant extracellular vesicles. Nature Plants **7**, 342.

Hondele M, Sachdev R, Heinrich S, Wang J, Vallotton P, Fontoura BMA, Weis K. 2019. DEAD-box ATPases are global regulators of phase-separated organelles. Nature **573**, 144–148.

Hou XL, Chen WQ, Hou Y, Gong HQ, Sun J, Wang Z, Zhao H, Cao X, Song XF, Liu CM. 2021. DEAD-BOX RNA HELICASE 27 regulates microRNA biogenesis, zygote division, and stem cell homeostasis. The Plant Cell **33**, 66–84.

Hsu YF, Chen YC, Hsiao YC, Wang BJ, Lin SY, Cheng WH, Jauh GY, Harada JJ, Wang CS. 2014. AtRH57, a DEAD-box RNA helicase, is involved in feedback inhibition of glucose-mediated abscisic acid accumulation during seedling development and additively affects pre-ribosomal RNA processing with high glucose. The Plant Journal **77**, 119–135.

Huang CK, Huang LF, Huang JJ, Wu SJ, Yeh CH, Lu CA. 2010. A DEAD-box protein, AtRH36, is essential for female gametophyte development and is involved in rRNA biogenesis in *Arabidopsis*. Plant and Cell Physiology **51**, 694–706.

Huang CK, Shen YL, Huang LF, Wu SJ, Yeh CH, Lu CA. 2016. The DEAD-Box RNA helicase AtRH7/PRH75 participates in pre-rRNA processing, plant development and cold tolerance in Arabidopsis. Plant and Cell Physiology **57**, 174–191.

Hunter S, Apweiler R, Attwood TK, *et al*. 2009. InterPro: the integrative protein signature database. Nucleic Acids Research **37**, D211–D215.

Huppertz I, Attig J, D'Ambrogio A, Easton LE, Sibley CR, Sugimoto Y, Tajnik M, Konig J, Ule J. 2014. iCLIP: protein-RNA interactions at nucleotide resolution. Methods **65**, 274–287.

Jackson RN, Lavin M, Carter J, Wiedenheft B. 2014. Fitting CRISPR-associated Cas3 into the helicase family tree. Current Opinion in Structural Biology **24**, 106–114.

Jang GJ, Jang JC, Wu SH. 2020. Dynamics and functions of stress granules and processing bodies in plants. Plants **9**, 1122.

Jankowsky A, Guenther UP, Jankowsky E. 2011. The RNA helicase database. Nucleic Acids Research **39**, D338–D341.

Jarmoskaite I, Russell R. 2014. RNA helicase proteins as chaperones and remodelers. Annual Review of Biochemistry **83**, 697–725.

Kammel C, Thomaier M, Sorensen BB, Schubert T, Langst G, Grasser M, Grasser KD. 2013. *Arabidopsis* DEAD-Box RNA helicase UAP56 interacts with both RNA and DNA as well as with mRNA export factors. PLoS One **8**, e60644.

Kant P, Kant S, Gordon M, Shaked R, Barak S. 2007. STRESS RESPONSE SUPPRESSOR1 and STRESS RESPONSE SUPPRESSOR2, two DEAD-box RNA helicases that attenuate Arabidopsis responses to multiple abiotic stresses. Plant Physiology **145**, 814–830.

Khan A, Garbelli A, Grossi S, *et al*. 2014. The Arabidopsis STRESS RESPONSE SUPPRESSOR DEAD-box RNA helicases are nucleolar- and chromocenter-localized proteins that undergo stress-mediated relocalization and are involved in epigenetic gene silencing. The Plant Journal **79**, 28–43.

Khemici V, Linder P. 2018. RNA helicases in RNA decay. Biochemical Society Transactions **46**, 163–172.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution **35**, 1547–1549.

Lange H, Sement FM, Gagliardi D. 2011. MTR4, a putative RNA helicase and exosome co-factor, is required for proper rRNA biogenesis and development in *Arabidopsis thaliana*. The Plant Journal **68**, 51–63.

Lange H, Zuber H, Sement FM, *et al*. 2014. The RNA helicases AtMTR4 and HEN2 target specific subsets of nuclear transcripts for degradation by the nuclear exosome in *Arabidopsis thaliana*. PLoS Genetics **10**, e1004564.

Lerner E, Cordes T, Ingargiola A, Alhadid Y, Chung S, Michalet X, Weiss S. 2018. Toward dynamic structural biology: Two decades of single-molecule Förster resonance energy transfer. Science **359**, 288.

Li Q, Liu NK, Liu Q, *et al*. 2021. DEAD-box helicases modulate dicing body formation in *Arabidopsis*. Science Advances **7**, eabc6266.

Li SJ, Castillo-Gonzalez C, Yu B, Zhang XR. 2017. The functions of plant small RNAs in development and in stress responses. The Plant Journal **90**, 654–670.

Li SJ, Xu R, Li AX, *et al*. 2018. SMA1, a homolog of the splicing factor Prp28, has a multifaceted role in miRNA biogenesis in Arabidopsis. Nucleic Acids Research **46**, 9148–9159.

Li YJ, Sun D, Ma ZY, *et al*. 2020. Degradation of SERRATE via ubiquitin-independent 20S proteasome to survey RNA metabolism. Nature Plants **6**, 970.

Linder P, Jankowsky E. 2011. From unwinding to clamping – the DEAD box RNA helicase family. Nature Reviews. Molecular Cell Biology **12**, 505–516.

Linder P, Owttrim GW. 2009. Plant RNA helicases: linking aberrant and silencing RNA. Trends in Plant Science **14**, 344–352.

Liu M, Shi DQ, Yuan L, Liu J, Yang WC. 2010. *SLOW WALKER3*, encoding a putative DEAD-box RNA helicase, is essential for female gametogenesis in *Arabidopsis*. Journal of Integrative Plant Biology **52**, 817–828.

Liu YA, Liu SJ, Shi HY, Ma JY, Jing M, Han YZ. 2021. The TSN1 binding protein RH31 is a component of stress granules and participates in regulation of salt-stress tolerance in *Arabidopsis*. Frontiers in Plant Science **12**, 804356.

Liu YL, Imai R. 2018. Function of plant DExD/H-Box RNA helicases associated with ribosomal RNA biogenesis. Frontiers in Plant Science **9**, 125.

Lohman TM, Tomko EJ, Wu CG. 2008. Non-hexameric DNA helicases and translocases: mechanisms and regulation. Nature Reviews. Molecular Cell Biology **9**, 391–401.

Lu CA, Huang CK, Huang WS, Huang TS, Liu HY, Chen YF. 2020. DEAD-Box RNA Helicase 42 plays a critical role in pre-mRNA splicing under cold stress. Plant Physiology **182**, 255–271.

Mahrez W, Shin J, Munoz-Viana R, Figueiredo DD, Trejo-Arellano MS, Exner V, Siretskiy A, Gruissem W, Kohler C, Hennig L. 2016. BRR2a affects flowering time via FLC splicing. PLoS Genetics **12**, e1005924.

Maruri-Lopez I, Figueroa NE, Hernandez-Sanchez IE, Chodasiewicz M. 2021. Plant stress granules: trends and beyond. Frontiers in Plant Science **12**, 722643.

Mouffok S, Capeyrou R, Belhabich-Baumas K, Joret C, Henras AK, Humbert O, Henry Y. 2021. The G-patch activators Pfa1 and PINX1 exhibit different modes of interaction with the Prp43 RNA helicase. RNA Biology **18**, 510–522.

Moy RH, Cole BS, Yasunaga A, Gold B, Shankarling G, Varble A, Molleston JM, TenOever BR, Lynch KW, Cherry S. 2014. Stem-loop recognition by DDX17 facilitates miRNA processing and antiviral defense. Cell **158**, 764–777.

Mugler CF, Hondele M, Heinrich S, Sachdev R, Vallotton P, Koek AY, Chan LY, Weis K. 2016. ATPase activity of the DEAD-box protein Dhh1 controls processing body formation. eLife **5**, e18746.

Nguyen LV, Seok HY, Woo DH, Lee SY, Moon YH. 2018. Overexpression of the DEAD-box RNA helicase gene *AtRH17* confers tolerance to salt stress in *Arabidopsis*. International Journal of Molecular Sciences **19**, 3777.

Nidumukkala S, Tayi L, Chittela RK, Vudem DR, Khareedu VR. 2019. DEAD box helicases as promising molecular tools for engineering abiotic stress tolerance in plants. Critical Reviews in Biotechnology **39**, 395–407.

Olsen KJ, Johnson SJ. 2021. Mtr4 RNA helicase structures and interactions. Biological Chemistry **402**, 605–616.

Paieri F, Tadini L, Manavski N, Kleine T, Ferrari R, Morandini P, Pesaresi P, Meurer J, Leister D. 2018. The DEAD-box RNA helicase RH50 is a 23S-4.5S rRNA maturation factor that functionally overlaps with the plastid signaling factor GUN1. Plant Physiology **176**, 634–648.

Pandey S, Muthamilarasan M, Sharma N, Chaudhry V, Dulani P, Shweta S, Jha S, Mathur S, Prasad M. 2019. Characterization of DEAD-box family of RNA helicases in tomato provides insights into their roles in biotic and abiotic stresses. Environmental and Experimental Botany **158**, 107–116.

Pandey S, Prasad A, Sharma N, Prasad M. 2020. Linking the plant stress responses with RNA helicases. Plant Science **299**, 110607.

Patel SS, Picha KM. 2000. Structure and function of hexameric helicases. Annual Review of Biochemistry **69**, 651–697.

Perroud PF, Demko V, Ako AE, Khanal R, Bokor B, Pavlovic A, Jasik J, Johansen W. 2021. The nuclear GUCT domain-containing DEAD-box RNA helicases govern gametophytic and sporophytic development in *Physcomitrium patens*. Plant Molecular Biology **107**, 307–325.

Pyle AM. 2008. Translocation and unwinding mechanisms of RNA and DNA helicases. Annual Review of Biophysics **37**, 317–336.

Qiao Y, Shi J, Zhai Y, Hou Y, Ma W. 2015. *Phytophthora* effector targets a novel component of small RNA pathway in plants to promote infection. Proceedings of the National Academy of Sciences, USA **112**, 5850–5855.

Raney KD, Byrd AK, Aarattuthodiyil S. 2013. Structure and mechanisms of SF1 DNA helicases. Advances in Experimental Medicine and Biology **767**, 17–46.

Raxwal VK, Simpson CG, Gloggnitzer J, Entinze JC, Guo WB, Zhang RX, Brown JWS, Riha K. 2020. Nonsense-mediated RNA decay factor UPF1 is critical for posttranscriptional and translational gene regulation in Arabidopsis. The Plant Cell **32**, 2725–2741.

Reddy AS, Marquez Y, Kalyna M, Barta A. 2013. Complexity of the alternative splicing landscape in plants. The Plant Cell **25**, 3657–3683.

Ru JN, Hou ZH, Zheng L, *et al*. 2021. Genome-wide analysis of DEAD-box RNA helicase family in wheat (*Triticum aestivum*) and functional identification of TaDEAD-box57 in abiotic stress responses. Frontiers in Plant Science **12**, 797276.

Schmidt A, Wuest SE, Vijverberg K, Baroux C, Kleen D, Grossniklaus U. 2011. Transcriptome analysis of the *Arabidopsis* megaspore mother cell uncovers the importance of RNA helicases for plant germline development. PLoS Biology **9**, e1001155.

Seok HY, Nguyen LV, Nguyen DV, Lee SY, Moon YH. 2020. Investigation of a novel salt stress-responsive pathway mediated by Arabidopsis DEAD-Box RNA helicase gene *AtRH17* using RNA-Seq analysis. International Journal of Molecular Sciences **21**, 1595.

Shin Y, Brangwynne CP. 2017. Liquid phase condensation in cell physiology and disease. Science **357**, eaaf4382.

Singleton MR, Dillingham MS, Wigley DB. 2007. Structure and mechanism of helicases and nucleic acid translocases. Annual Review of Biochemistry **76**, 23–50.

Stein RE, Nauerth BH, Binmoller L, Zuhl L, Loreth A, Reinert M, Ibberson D, Schmidt A. 2021. RH17 restricts reproductive fate and represses autonomous seed coat development in sexual *Arabidopsis*. Development **148**, dev198739.

Steinegger M, Soding J. 2017. MMseqs2 enables sensitive protein sequence searching for the analysis of massive data sets. Nature Biotechnology **35**, 1026–1028.

Sulkowska A, Auber A, Sikorski PJ, Silhavy D, Auth M, Sitkiewicz E, Jean V, Merret R, Bousquet-Antonelli C, Kufel J. 2020. RNA helicases from the DEA(D/H)-box family contribute to plant NMD efficiency. Plant and Cell Physiology **61**, 144–157.

Takagi M, Iwamoto N, Kubo Y, *et al*. 2020. *Arabidopsis SMN2/HEN2*, encoding DEAD-Box RNA helicase, governs proper expression of the resistance gene *SMN1/RPS6* and is involved in dwarf, autoimmune phenotypes of *mekk1* and *mpk4* mutants. Plant and Cell Physiology **61**, 1507–1516.

Tauber D, Tauber G, Khong A, Van Treeck B, Pelletier J, Parker R. 2020. Modulation of RNA condensation by the DEAD-box protein eIF4A. Cell **180**, 411.

Tuteja R. 2018. Helicases from all domains of life. Academic Press.

Tyagi V, Parihar V, Malik G, Kalra V, Kapoor S, Kapoor M. 2020. The DEAD-box RNA helicase eIF4A regulates plant development and interacts with the hnRNP LIF2L1 in *Physcomitrella patens*. Molecular Genetics and Genomics **295**, 373–389.

Umate P, Tuteja R, Tuteja N. 2010. Genome-wide analysis of helicase gene family from rice and *Arabidopsis*: a comparison with yeast and human. Plant Molecular Biology **73**, 449–465.

Wang B, Chai H, Zhong Y, Shen Y, Yang W, Chen J, Xin Z, Shi H. 2020. The DEAD-box RNA helicase SHI2 functions in repression of salt-inducible genes and regulation of cold-inducible gene splicing. Journal of Experimental Botany **71**, 1598–1613.

Wang X, Kong R, Zhang T, Gao Y, Xu J, Piao Z, Lee G, Lin D, Dong Y. 2020. A DEAD-box RNA helicase TCD33 that confers chloroplast development in rice at seedling stage under cold stress. Journal of Plant Physiology **248**, 153138.

Wang D, Qin BX, Li X, Tang D, Zhang Y, Cheng ZK, Xue YB. 2016. Nucleolar DEAD-box RNA helicase TOGR1 regulates thermotolerant growth as a pre-rRNA chaperone in rice. PLoS Genetics **12**, e1005844.

Wang L, Yan XX, Li YJ, *et al*. 2022a. PRP4KA phosphorylates SERRATE for degradation via 20S proteasome to fine-tune miRNA production in *Arabidopsis*. Science Advances **8**, eabm8435.

Wang YL, Liao JW, Wu J, Huang HM, Yuan ZX, Yang W, Wu XY, Li X. 2022b. Genome-wide identification and characterization of the soybean DEAD-box gene family and expression response to rhizobia. International Journal of Molecular Sciences **23**, 1120.

Wang Z, Ma Z, Castillo-Gonzalez C, Sun D, Li Y, Yu B, Zhao B, Li P, Zhang X. 2018. SWI2/SNF2 ATPase CHR2 remodels pri-miRNAs via Serrate to impede miRNA production. Nature **557**, 516–521.

Weis K, Hondele M. 2022. The role of DEAD-box ATPases in gene expression and the regulation of RNA-Protein condensates. Annual Review of Biochemistry **91**, 197–219.

Wilkinson ME, Charenton C, Nagai K. 2020. RNA Splicing by the spliceosome. Annual Review of Biochemistry **89**, 359–388.

Xiaomei W, Rongrong K, Ting Z, Yuanyuan G, Jianlong X, Zhongze P, Gangseob L, Dongzhi L, Yanjun D. 2020. A DEAD-box RNA helicase TCD33 that confers chloroplast development in rice at seedling stage under cold stress. Journal of Plant Physiology **248**, 153138.

Xie D, Chen M, Niu J, Wang L, Li Y, Fang X, Li P, Qi Y. 2021. Phase separation of SERRATE drives dicing body assembly and promotes miRNA processing in *Arabidopsis*. Nature Cell Biology **23**, 32–39.

Xu RR, Zhang SH, Lu LT, Cao H, Zheng CC. 2013a. A genome-wide analysis of the RNA helicase gene family in *Solanum lycopersicum*. Gene **513**, 128–140.

Xu RR, Zhang SZ, Huang JG, Zheng CC. 2013b. Genome-wide comparative in silico analysis of the RNA helicase gene family in *Zea mays* and

Glycine max: a comparison with *Arabidopsis* and *Oryza sativa*. PLoS One **8**, e78982.

Xu W, Xu H, Li K, Fan YX, Liu Y, Yang XR, Sun QW. 2017. The R-loop is a common chromatin feature of the *Arabidopsis* genome. Nature Plants **3**, 704–714.

Xu XP, Chen XH, Shen X, Chen RZ, Zhu C, Zhang ZH, Chen YK, Lin WZ, Xu XH, Lin YL. 2021. Genome-wide identification and characterization of DEAD-box helicase family associated with early somatic embryogenesis in *Dimocarpus longan* Lour. Journal of Plant Physiology **258**, 153364.

Yadav S, Yadava YK, Kohli D, Meena S, Kalwan G, Bharadwaj C, Gaikwad K, Arora A, Jain PK. 2022. Genome-wide identification, *in silico* characterization and expression analysis of the RNA helicase gene family in chickpea (*C. arietinum* L.). Scientific Reports **12**, 9778.

Yang Z, Hou QC, Cheng LL, Xu W, Hong YT, Li S, Sun QW. 2017. RNase H1 cooperates with DNA gyrases to restrict R-Loops and maintain genome integrity in *Arabidopsis* chloroplasts. The Plant Cell **29**, 2478–2497.

Yang Z, Li MM, Sun QW. 2020. RHON1 co-transcriptionally resolves R-Loops for *Arabidopsis* chloroplast genome maintenance. Cell Reports **30**, 243.

Yarra R, Xue Y. 2020. Ectopic expression of nucleolar DEAD-Box RNA helicase OsTOGR1 confers improved heat stress tolerance in transgenic Chinese cabbage. Plant Cell Reports **39**, 1803–1814.

Yoine M, Nishii T, Nakamura K. 2006. *Arabidopsis* UPF1 RNA helicase for nonsense-mediated mRNA decay is involved in seed size control and is essential for growth. Plant and Cell Physiology **47**, 572–580.

Youn JY, Dyakov BJ, Zhang J, Knight JD, Vernon RM, Forman-Kay JD, Gingras AC. 2019. Properties of stress granule and P-body proteomes. Molecular Cell **76**, 286–294.

Zambelli F, Pavesi G. 2015. RIP-Seq data analysis to determine RNA-protein associations. Methods in Molecular Biology **1269**, 293–303.

Zhang L, Xu Y, Liu X, *et al*. 2022. The chrysanthemum DEAD-box RNA helicase CmRH56 regulates rhizome outgrowth in response to drought stress. Journal of Experimental Botany **73**, 5671–5681.

Zhang XD, Sun JY, You YY, Song JB, Yang ZM. 2018. Identification of Cd-responsive RNA helicase genes and expression of a putative BnRH24 mediated by miR158 in canola (*Brassica napus*). Ecotoxicology and Environmental Safety **157**, 159–168.

Zhu DZ, Zhao XF, Liu CZ, Ma FF, Wang F, Gao XQ, Zhang XS. 2016. Interaction between RNA helicase ROOT INITIATION DEFECTIVE 1 and GAMETOPHYTIC FACTOR 1 is involved in female gametophyte development in Arabidopsis. Journal of Experimental Botany **67**, 5757–5768.

Zhu J, Yan X, Zhang X. 2022. The R-loop influences miRNA birth place. Nature Plants **8**, 320–321.

Zhu JY, Li CH, Peng X, Zhang XR. 2021. RNA architecture influences plant biology. Journal of Experimental Botany **72**, 4144–4160.