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REVIEW PAPER

Plant long non-coding RNAs: biologically relevant and mechanistically intriguing

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Abstract

Long non-coding RNAs (lncRNAs) are a group of RNAs greater than 200 nucleotides in length exhibiting low or no coding potential that are involved in diverse biological functions through their molecular interaction with proteins, DNA, or other RNAs. With the emergence of advanced high-throughput RNA sequencing technologies, tens of thousands of novel long non-coding RNAs have been identified in plant transcriptomes in the last decade. More importantly, functional studies revealed that several lncRNAs play key regulatory roles in plant development and stress responses. In this review, we focus on summarizing recent progress uncovering regulatory roles and mechanisms of lncRNAs during the plant life cycle, and briefly discuss the possible biotechnological applications of lncRNAs for plant breeding.

Keywords: Alternative splicing, chromatin loop, gene translation, histone modification, long noncoding RNA, protein–protein interactions, protein relocalization, R-loop, target mimic, transcription factor.

Introduction

The central dogma of molecular biology pinpoints RNA as a key actor in the transfer of genetic information from DNA to proteins (Crick, 1970). Nevertheless, the advent of novel sequencing technologies has revealed that eukaryotic genomes are pervasively transcribed although a large number of RNA molecules do not encode proteins. These so-called non-coding transcripts were initially considered as transcriptional noise because of their unknown function. Long non-coding RNA (lncRNA) is a class of non-coding RNAs longer than 200 nt that are associated with biological functions (Wierzbicki *et al.*, 2021). During the last couple of decades, an increasing number of lncRNAs have been identified in the plant kingdom, but only a small fraction have been functionally characterized (Ariel *et al.*, 2015; Lucero *et al.*, 2021). Among them, several lncRNAs have been linked to diverse aspects of plant development and the response to environmental changes. In this review, we summarize recent progress in lncRNA-mediated regulation in plants, and discuss potential applications in plant breeding.

LncRNAs modulating the behavior of transcriptional regulators

Transcription factors (TFs) play essential roles in diverse developmental processes and stress responses in plants (Ramachandran *et al.*, 1994; Singh *et al.*, 2002; Yang *et al.*, 2012). They are able to bind specific DNA sequences through their DNA binding domains and interact with different proteins of transcriptional complexes that initiate gene expression (Yamasaki *et al.*, 2013; Inukai *et al.*, 2017). In addition to regulating lncRNA transcription, TFs can regulate gene expression through direct interaction with lncRNAs (Fig. 1A). The *VERNALIZATION1* (*VRN1*) gene encodes an APETALA1 (AP1)-like MADS-box TF, expressed at a low level in the vegetative stage of winter wheat (Yan *et al.*, 2004; Trevaskis, 2010). During the early period of vernalization in winter wheat, the lncRNA *VAS*, which is derived from the *TaVRN1* gene, was specifically induced. *VAS* could physically associate with a bZIP transcription factor, TaRF2b, and assist it to bind and activate *TaVRN1* together with TaRF2a, ultimately accelerating flowering (S. Xu *et al.*, 2021). Interestingly, there is a chromatin loop structure including *VAS* and the TaRF2b binding sequence motif, together with the cohesion factor PDS5A identified among *VAS*-associated proteins, implying that *VAS* might also participate in the regulation of local chromatin structural dynamics. In Arabidopsis, the lncRNA *AUXIN-REGULATED PROMOTER LOOP* (*APOLO*) could regulate the plant responses to cold through cooperation with a TF. It directly interacted with WRKY42 and jointly formed a novel ribonucleoprotein complex to shape the epigenetic environment of *ROOT HAIR DEFECTIVE 6* (*RHD6*) and activate its transcription, which modulated the transcriptional root hair program inducing cell expansion in response to cold by stimulating the expression of *ROOT HAIR DEFECTIVE*

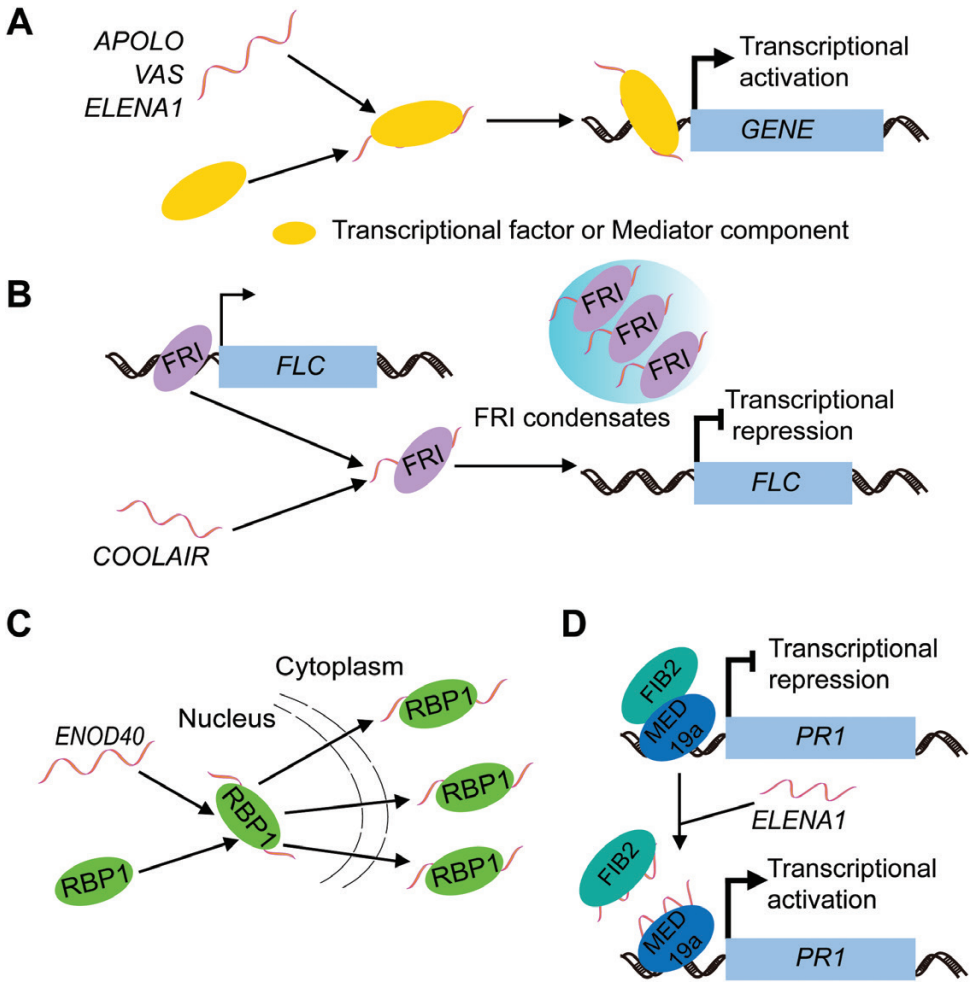

Fig. 1. LncRNA regulates the action of transcriptional regulators. (A) LncRNA interacts directly with a TF or mediator subunit to activate expression of a target gene, e.g. *APOLO*, *VAS*, and *ELENA1*. (B) *COOLAIR* associated with the transcriptional activator FRIGIDA (FRI) to repress *FLC* transcription. The interaction between FRI and *COOLAIR* results in the accumulation of FRI nuclear condensates that sequester FRI away from the *FLC* promoter. (C) Nucleus-localized RBP binds to lncRNA and then transports it to the cytoplasm, e.g. *MtENOD40*. (D) LncRNA interacts directly with proteins and inhibits their interaction, e.g. *ELENA1*.

SIX-LIKE 2 (*RSL2*) and *RSL4* (Moison *et al.*, 2021). The *APOLO*–WRKY42 ribonucleoprotein complex was also able to bind and positively mediate the expression of several cell wall EXTENSIN (EXT)-encoding genes including a key regulator of root hair growth, *EXT3*, triggering root hair cell elongation (Pacheco *et al.*, 2021). Besides their direct interaction with TFs, lncRNAs may also interact with Mediator, which is a general interactor with TF activation domains. In Arabidopsis, the flg22- and elf18-induced lncRNA *ELF18-INDUCED LONG-NONCODING RNA1* (*ELENA1*) directly interacted with Mediator subunit 19a (MED19a) and affected enrichment of MED19A on the *PATHOGENESIS-RELATED GENE1* (*PR1*) promoter, thereby positively regulating Arabidopsis resistance to *Pseudomonas syringae* pv. *tomato* strain DC3000 by modulating *PR1* expression (Seo *et al.*, 2017). In addition to activating target gene expression, lncRNAs are able to trigger gene repression. FRIGIDA (FRI) acted as a scaffold protein interacting with FRL1, FES1, SUF4, and FLX to form a transcription activator complex, resulting in the activation of *FLOWERING LOCUS C* (*FLC*) transcription (Choi *et al.*, 2011). A specific isoform of *COOLAIR*, a group of antisense lncRNAs, could interact with FRI to promote cold-induced nuclear condensate formation, which sequestered FRI away from the *FLC* promoter and repressed its transcription (Zhu *et al.*, 2021) (Fig. 1B). Collectively, lncRNA can interact with TFs or associated proteins to form a ribonucleoprotein complex to coordinate transcriptional regulatory networks in plants.

Nucleo-cytoplasmic partitioning of regulatory proteins is crucial in diverse plant biological processes (Merkle, 2003), and many proteins have been found to be involved in nucleo-cytoplasmic partitioning, such as importin α (Chang *et al.*, 2012), importin β (Zhao *et al.*, 2007), and ring finger proteins (Lim *et al.*, 2013). LncRNAs have also been reported to mediate protein relocalization in plants (Fig. 1C). *ENOD40* is a lncRNA expressed at an early stage in root nodule organogenesis in legumes, and in soybean *ENOD40* also encodes two peptides of 12 and 24 amino acid residues that could bind to sucrose synthase (Röhrig *et al.*, 2002). Interestingly, in *Medicago truncatula*, RNA Binding Protein 1 (RBP1) localized to nuclear speckles in plant cells but was exported into the cytoplasm during nodule development in *ENOD40*-expressing cells. A yeast three-hybrid experiment and an *in vivo* assay showed that MtRBP1 interacted with the *ENOD40* RNA, indicating that *ENOD40* mediated cytoplasmic relocalization of nucleus-localized MtRBP1 (Campalans *et al.*, 2004).

Protein–protein interactions occur in the plant cell to establish the macromolecular complexes and networks accountable for the regulation of gene expression. Recent findings indicate that lncRNAs regulate plant biological processes by affecting protein–protein interactions (Fig. 1D). In addition to MED19a, it was proposed that *ELENA1* directly interacts with FIBRILLARIN 2 (FIB2) in the nucleoplasm and nucleolus, and that it could dissociate the FIB2–MED19a complex and release FIB2 from the *PR1* promoter to activate *PR1* expression (Seo *et al.*, 2019). In rice, *MISSEN* is a maternally expressed lncRNA that competitively inhibited the interaction between tubulin and a helicase family protein, HeFP, which negatively regulated endosperm development (Zhou *et al.*, 2021).

In addition to the mechanisms of lncRNA-mediated regulation discussed above, lncRNAs are able to regulate gene expression through transcription. Thus the non-coding antisense RNA *asDOG1* strongly suppressed *Delay of Germination 1* (*DOG1*) expression during seed maturation *in cis* (Fedak *et al.*, 2016); the transcription of a cryptic antisense *CBF1* lncRNA (*asCBF1*) generated by RNA polymerase II (RNAPII) read-through transcription of a lncRNA, *SVALKA*, resulted in RNAPII collision to limit the expression of full-length *CBF1* (Kindgren *et al.*, 2018); and up-regulation of *COOLAIR* was associated with *FLC* transcriptional shutdown (Zhao *et al.*, 2021).

LncRNA guiding histone modifications

Histone post-translational modifications affect gene transcription by modulating the chromatin status to facilitate or block the binding of various proteins to chromatin. This phenomenon includes a range of chemical groups that can be added to different amino acids of histones under the action of specific modifying enzymes *in vivo*, including methylation, acetylation, phosphorylation, ubiquitination, and sumoylation, among others (Hsieh and Fischer, 2005). Emerging evidence shows that lncRNAs participate in diverse biological processes in plants by modulating histone modifications to influence gene expression (Fonouni-Farde *et al.*, 2021) (Fig. 2). In Arabidopsis, lncRNA *COLDAIR*, transcribed in the sense direction from the first intron of *FLC*, was the first characterized plant lncRNA closely participating in chromatin modification (Heo and Sung, 2011). It was suggested that *COLDAIR* could interact with the Polycomb Repressive Complex 2 (PRC2) component CLF and guide PRC2 to *FLC* chromatin, leading to H3K27me3 deposition and *FLC* gene silencing during exposure to prolonged low temperature (Heo and Sung, 2011; Kim *et al.*, 2017). Similarly, *AG-incRNA4* is transcribed from the second intron of *AGAMOUS* (*AG*) and was found to associate with CLF and recruit PRC2 to the *AG* locus, which increased H3K27me3 levels and inhibited *AG* transcription in Arabidopsis leaf tissues (Wu *et al.*, 2018). The lncRNA *salicylic acid biogenesis controller 1* (*SABC1*) fine-tuned salicylic acid biosynthesis through recruiting PRC2 to its neighboring gene, *NAC3*, encoding a NAC TF, to reduce its transcription via H3K27me3 deposition, which balanced plant immunity and growth (N. Liu *et al.*, 2022). On the other hand, activation of histone marks can also be modulated by lncRNAs. The natural antisense transcript (NAT) lncRNA produced from the *MADS AFFECTING FLOWERING4* (*MAF4*) locus, *MAS*, is induced by cold and was linked to *MAF4* transcriptional activation. *MAS* directly bound to WDR5a, a core component

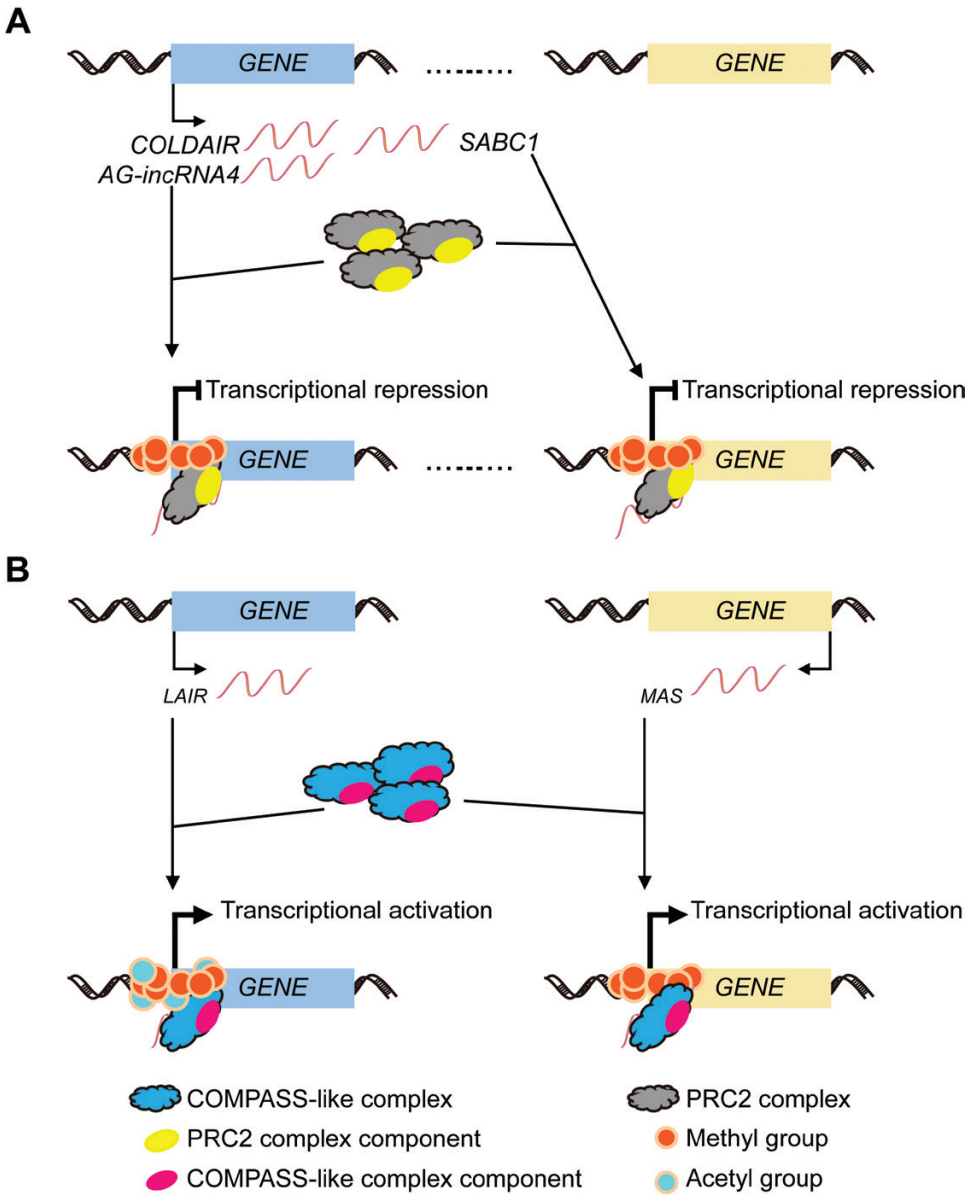

Fig. 2. LncRNA modulates histone modification by recruiting chromatin-modifying complexes to regulate gene expression. LncRNA can interact directly with a component of PRC2 (A), e.g. *COLDAIR*, *AG-incRNA4*, and *SABC1*, or COMPASS-LIKE (B), e.g. *MAS* and *LAIR*, for repressing or activating gene expression, respectively.

of the COMPASS-LIKE complex, and then recruited it to the *MAF4* locus, resulting in enhanced H3K4me3 to promote *MAF4* transcription and repress Arabidopsis precocious flowering (Zhao *et al.*, 2018). Similarly, the lncRNA *LRK Antisense Intergenic RNA* (*LAIR*) is transcribed from the antisense strand of the neighboring gene *LRK* (leucine-rich repeat receptor kinase) in rice, and it regulates rice plant growth and increases grain yield. RNA-binding proteins OsMOF and OsWDR5, which participate in H3K4me3 and H4K16ac histone modification complexes, were found to recognize *LAIR*, and both the lncRNA and the two epigenetic modification proteins could target the *LRK1* genomic region. Overexpressing *LAIR* resulted in an increase of both H3K4me3 and H4K16ac levels at the *LRK1* chromatin region and activation of transcription of *LRK1* (Wang *et al.*, 2018).

Chromatin loop formation mediated by lncRNA

Genes are encompassed in dynamic chromatin loop structures that juxtapose regulatory elements to activate or repress transcription. Growing evidence suggests that lncRNAs could recruit chromatin-modifying complexes to shape local

3D chromatin architecture and alter target gene activity in plants (Rodriguez-Granados *et al.*, 2016; Kim and Sung, 2021) (Fig. 3). In Arabidopsis, auxin induced RDD (ROS1, DML2, and DML3)-mediated DNA demethylation on the lncRNA *APOLO* locus, opening the chromatin loop harboring the promoter region of *APOLO*'s neighboring gene, *PINOID* (*PID*), which triggered RNA polymerase (Pol) II divergent transcription of both *PID* and *APOLO* loci. After that, Pol V was recruited to the *APOLO* locus, prompting siRNA-mediated DNA methylation. The Polycomb Repressive Complex 1 (PRC1) component LIKE HETEROCHROMATIC PROTEIN 1 (LHP1) could directly bind to the *APOLO* Pol II transcript to restore loop formation, and then Pol IV/Pol V-dependent DNA methylation assisted in the stabilization of this loop (Ariel *et al.*, 2014). In addition, *APOLO* was involved in chromatin loop formation not only at its neighboring *PID* locus, but also at a plethora of distal loci via sequence complementarity and DNA–RNA duplex (R-loops) formation (Ariel *et al.*, 2020) (Fig. 3A, B). *COLDWRAP* is a vernalization-induced lncRNA that is transcribed from the proximal promoter of the Arabidopsis floral repressor gene *FLC*. During vernalization, *COLDWRAP*, together with another lncRNA derived from the first intron of *FLC*, *COLDAIR*, directly interacts with a component of PRC2, CLF. It was suggested that *COLDWRAP* and *COLDAIR* participate in the formation of an intragenic chromatin loop between the promoter and 3′ end of the first intron of *FLC*, which is involved in establishing stable Polycomb-mediated silencing of *FLC* (Kim and Sung, 2017). An additional chromatin loop exists between the 5′ and 3′ flanking regions of the *FLC* locus (Fig. 3C) that is disrupted during vernalization, and it is hypothesized that the disruption of this loop might facilitate *COOLAIR* expression by revealing *cis*-elements that contribute to the cold induction of antisense transcription (Crevillen *et al.*, 2013; Zhu *et al.*, 2015). In Arabidopsis response to abscisic acid (ABA), the lncRNA *MARneral Silencing* (*MARS*), encoded in the marneral cluster, decoyed LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) away from the cluster and promoted the formation of a chromatin loop bringing together the *MARNERAL SYNTHASE 1* (*MRN1*) proximal promoter and an enhancer element enriched in ABA-related TF binding sites, which led to an ABA-mediated transcriptional activation (Roulé *et al.*, 2022).

Regulatory R-loops formation mediated by lncRNAs

R-loops are three-stranded structures that include a DNA–RNA hybrid and a displaced single-stranded DNA that play diverse roles in genome organization and gene regulation in plants (Xu *et al.*, 2017). It has been shown that plant lncRNAs are also able to participate in R-loop formation (Fig. 3B, D).

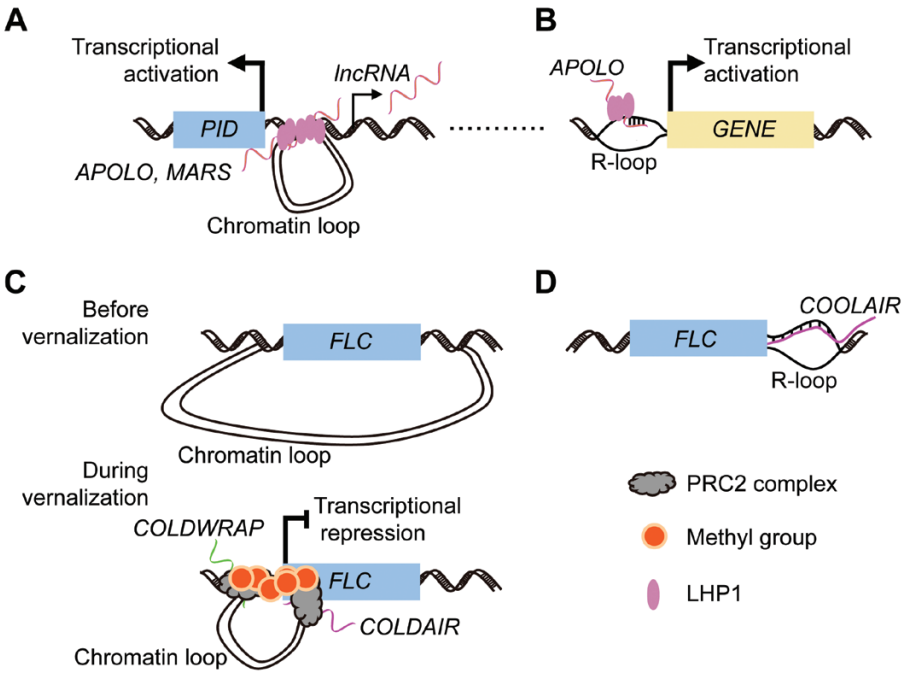

Fig. 3. LncRNA regulates gene expression through establishing chromatin loops and forming R-loops. LncRNA not only is involved in the chromatin loop formation between lncRNA and its target (A), e.g. *APOLO* and *MARS*, but also regulates the distal gene expression through forming an R-loop (B), e.g. *APOLO*. LncRNA not only is involved in the intragenic chromatin loop formation (C), e.g. *COLDWRAP* and *COLDAIR*, but also regulates a *cis* target expression through forming an R-loop (D), e.g. *COOLAIR*.

The *APOLO* lncRNA can also recognize distant independent loci, including a subset of auxin-responsive genes, by short sequence complementarity and the formation of R-loops (Ariel *et al.*, 2020) (Fig. 3B). The invasion of *APOLO* into the target DNA double strand decoyed the plant PRC1 component LHP1 and modulated local chromatin 3D conformation, resulting in coordinating transcription of topologically non-associated auxin-responsive genes during lateral root development in Arabidopsis. An R-loop was also generated by the *COOLAIR* transcript at the 3′-end of *FLC* (Sun *et al.*, 2013) (Fig. 3D), and stabilization of this *COOLAIR*-induced R-loop could enable an RNA binding protein, FCA, with its direct partner, FY/WDR33, and other 3′-end processing factors, to polyadenylate the nascent *COOLAIR* transcript, which cleared the R-loop and recruited the chromatin modifiers to silence the *FLC* locus (C. Xu *et al.*, 2021). Interestingly, a circular RNA derived from exon 6 of the *SEPALLATA3* (*SEP3*) gene can regulate *SEP3* pre-mRNA splicing through binding to this gene locus and forming an R-loop, thereby leading to abnormal flower development (Conn *et al.*, 2017). Moreover, a recent finding showed that co-transcriptional pri-miRNA processing is promoted by R-loops established near the transcription start site regions of *MIRNA*s (Gonzalo *et al.*, 2022). Therefore, it will be exciting to find more evidence that plant lncRNAs are involved in regulatory R-loop-mediated co- and post-transcriptional processes in future.

LncRNAs regulating alternative splicing

Although there are a large number of studies that consider gene expression regulation directed by lncRNAs at the transcriptional level, there is also increasing evidence that lncRNAs may regulate expression at the post-transcriptional level, such as alternative splicing (AS). In eukaryotes, non-consecutive exons are separated into multiple segments by introns in the gene. These genes are transcribed into pre-mRNA, and then undergo a few more processes including RNA splicing to become mature mRNA. RNA splicing events are mainly classified into two modes, constitutive splicing and AS. Constitutive splicing is the process of intron removal and exon ligation in the order in which they are present in a gene, while AS implies that multiple transcripts can be derived from a single gene by intron retention of exon skipping, diversifying the resulting transcriptome and proteome (Nilsen and Graveley, 2010). It has been well known that AS plays a fundamental role in plant growth, development, and responses to external cues (Staiger and Brown, 2013). LncRNAs have also been shown to play a crucial role in the regulation of AS in plants (Romero-Barrios *et al.*, 2018) (Fig. 4). In Arabidopsis, the lncRNA *Alternative Splicing Competitor* (*ASCO*) was found to modulate AS through interacting with two nuclear speckle RNA-binding proteins (NSRs), AtNSRa and AtNSRb (Bardou *et al.*, 2014). Both *ASCO* overexpressing lines and *nsra/b* double mutants exhibited an altered ability to form lateral roots in response to auxin. Analysis of AS events between WT and *nsra/b* mutant plants with or without auxin treatment revealed that the splicing of a large number of auxin-related genes was disturbed in *nsra/b* mutants, and some of them behaved accordingly in *ASCO* overexpressing lines. An *in vitro* assay showed that *ASCO* could act as a competitor with other target mRNAs for binding to NSRs, implying that *ASCO* could regulate AS by affecting the affinity of NSRs for their targets during auxin response in roots (Bardou *et al.*, 2014). Besides AS regulation mediated by *ASCO*–NSR interaction in the auxin response, a great number of deregulated and differentially spliced genes related to biotic stress and flagellin response were identified in both *ASCO* knockdown and overexpressing plants. *ASCO* was also able to bind to two core components of the spliceosome, PRP8a and SmD1b, which recognized subsets of AS-regulated flg22-regulatory genes. Moreover, *ASCO* overexpression competed for PRP8a binding to particular mRNA targets, which impaired the recognition of specific flagellin-related transcripts by PRP8a (Rigo *et al.*, 2020).

LncRNAs regulating gene translation

mRNA is translated into protein by ribosomes (Crick, 1970). Strikingly, thousands of evolutionarily conserved small open reading frames (smORFs, <100 codons) have been found in lncRNAs in plants, and ribosome-footprinting studies proved that lncRNAs produce detectable peptides in Arabidopsis, demonstrating that they are a reservoir of conserved and differentially regulated small peptide-coding genes (Bazin *et al.*, 2017; Fesenko *et al.*, 2021). In addition, lncRNAs can be functional as positive or negative regulators to enhance or inhibit gene expression via influencing the polysome association of transcripts (Fig. 5). *cis*-NAT$_{PHO1;2}$ is a *cis*-natural antisense transcript of *OsPHO1;2* that is the functional ortholog of *AtPHO1* and involved in phosphate loading into the xylem in rice (Secco *et al.*, 2010). Although the *OsPHO1;2* transcript levels remain stable under P_i deficiency, expression of both

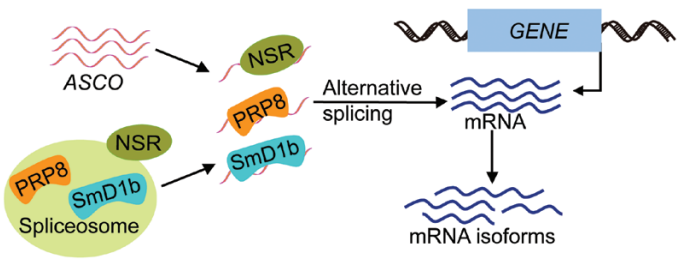

Fig. 4. Regulatory lncRNA mediates gene function by affecting alternative splicing. LncRNA competitively binds to nuclear speckle RNA-binding proteins (NSRs) or components of the spliceosome to influence alternative splicing, e.g. *ASCO*.

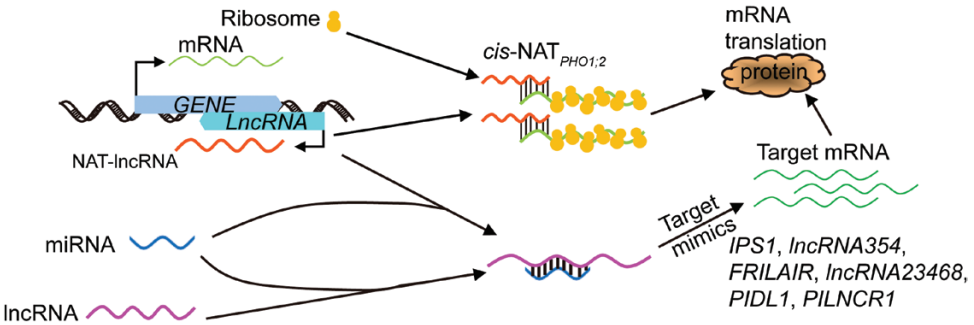

Fig. 5. LncRNA regulates translation of protein-coding genes. LncRNA enhances the association of mRNA with polysomes, thereby promoting its target gene translation, e.g. *cis*-NAT$_{PHO1;2}$. LncRNA can act as the miRNA target mimic for inhibiting its interaction with authentic targets, e.g. *IPS1*, *lncRNA354*, *FRILAIR*, *lncRNA23468*, *PIDL1*, and *PILNCR1*.

cis-NAT$_{PHO1;2}$ and the PHO1;2 protein increased under the same nutrient deficiency. Either downregulating or constitutively overexpressing *cis*-NAT$_{PHO1;2}$ had no effect on *PHO1;2* mRNA level, while there was a strong decrease and increase of PHO1;2 protein level in *cis*-NAT$_{PHO1;2}$ RNAi and overexpressing lines, respectively. Raising *cis*-NAT$_{PHO1;2}$ expression leads to a shift of both the sense *PHO1;2* and the antisense NAT toward the translationally active polysomes, thereby promoting *PHO1;2* translation and affecting phosphate homeostasis and plant fitness (Jabnoune *et al.*, 2013). Structural analyses showed that a high GC content region in *PHO1;2* produces a structure inhibiting the binding of the 60S subunit to the 40S. In the presence of *cis*-NAT$_{PHO1;2}$, a localized sense–antisense inter-molecular interaction results in an alteration of this inhibitory structure, leading to increased formation of the 80S complex in *PHO1;2* and enhancing its translation (Reis *et al.*, 2021). Similarly, *cis*-NATs have been found to regulate cognate sense mRNA translation in Arabidopsis (Bazin *et al.*, 2017; Deforges *et al.*, 2019), such as *cis*-NAT$_{CuAO1}$ and *cis*-NATAT$_{3G26240}$, which can repress and enhance translation of their cognate mRNAs, respectively (Deforges *et al.*, 2019). In cassava, an intergenic lncRNA, *cold-responsive intergenic lncRNA 1* (*CRIR1*), whose expression was significantly induced by cold stress, interacted directly with a cold shock domain-containing protein, COLD SHOCK PROTEIN 5 (MeCSP5), and increased its translational yield to cope with cold stress (Li *et al.*, 2022).

LncRNAs titrating miRNAs

It is well known that miRNA can direct RNA-induced silencing complex (RISC) to cleave a target mRNA or arrest its translation by perfectly or imperfectly pairing with the miRNA recognition elements in the target genes (Rogers and Chen, 2013). Increasing evidence suggests that in plants lncRNAs harboring highly similar miRNA recognition elements as miRNA targets are able to act as miRNA target mimics to inhibit miRNA activity through competitively binding to miRNAs and blocking their interaction with authentic targets (Franco-Zorrilla *et al.*, 2007; Wu *et al.*, 2013; Liu *et al.*, 2015) (Fig. 5). In Arabidopsis, a phosphate starvation-induced lncRNA, *INDUCED BY PHOSPHATE STARVATION 1* (*IPS1*), had a 23-nt motif complementary to miR399 and functioned as the target mimic of miR399, sequestering miRNA399 away from its bona fide target, *PHO2*, thereby enhancing *PHO2* expression and modulating P_i content in shoot (Franco-Zorrilla *et al.*, 2007). A cotton *lncRNA354* worked as an endogenous target mimic for miR160b, which inhibited miR160b-mediated degradation of *GhARF17/18* and modulated plant response to salt stress (Zhang *et al.*, 2021). A novel strawberry lncRNA, *FRILAIR*, was identified that harbored a miR397 binding site and served as a noncanonical target mimic of miR397, thereby modulating the expression of *LAC11a*, which is the miR397 target, and affecting strawberry fruit ripening (Tang *et al.*, 2021). Endogenous target mimicry directed by lncRNA has been discovered in diverse plant species, such as the *lncRNA23468*–miR482b module in tomato (Jiang *et al.*, 2019), the *PIDL1*–miR399 module in *Medicago truncatula* (Wang *et al.*, 2017) and the *PILNCR1*–miR399 module in maize (Du *et al.*, 2018).

Conclusions

Understanding the diversity of roles carried out by lncRNAs in a wide range of aspects of the plant life cycle will be key to studying plant lncRNA, and novel genetic tools such as the CRISPR–Cas systems that can be applied for targeted genome editing, triggering repression, or activation of gene expression are able to speed up this process. In addition, several notes of caution should be considered before applying precise genome editing tools to study biological functions of the targeted genes. For producing lncRNA loss of function mutants, it would be more appropriate to mutate lncRNA regions that do not overlap with other functionally relevant genomic regions (Summanwar *et al.*, 2020). However, we still cannot fully exclude the potential side effects induced by precision genome editing albeit with careful design, as mentioned

above. For example, a promoter deletion of *COOLAIR* not only represses its transcription (Luo *et al.*, 2019; Zhao *et al.*, 2021), but also generates a novel convergent antisense transcript (*CAS*) originating from the first intron of *FLC* (Zhao *et al.*, 2021). Moreover, the generation of novel *COOLAIR–CAS* transcripts from intragenic regions in *FLC* has occurred at each attempt to remove *COOLAIR* (Zhao *et al.*, 2021). Therefore, it will be more reliable to further confirm lncRNA function by applying complemented lines, and this strategy is suitable for lncRNA working together with other factors such as RNA binding protein. A better choice for studying lncRNA functions mediated by its transcriptional activity is to repress lncRNA expression through RNA interference-mediated post-transcriptional silencing, such as by using artificial miRNA. Additionally, although overexpression is vulnerable to technical artifacts, it also remains a vital tool, because altered phenotypes for lncRNAs are seen only upon overexpression in contrast to knock down/knock out in many cases (Wierzbicki *et al.*, 2021). In total, to ensure the appropriate attribution of a phenotype to the desired lncRNA with precise genome editing tools such as the CRISPR–Cas system, things to take into account before manipulating lncRNA include the lncRNA locus, which should be carefully studied for neighboring or overlapping genes at the guide design stage. The expression output of neighboring or overlapping genes should also be monitored in parallel to the lncRNA, and phenotypes—if not mediated in *cis*—should be reproducible with RNAi-mediated approaches or rescued by exogenous expression of lncRNA (Goyal *et al.*, 2017). Once the biological functions of lncRNAs are uncovered, their molecular basis will be the next challenge to attempt. Identifying molecular partners in the interplay with lncRNAs is of paramount importance, and the rising number of available biochemical methods will be not only helpful for this but also for unveiling the molecular mechanism behind the biology of lncRNAs.

With global population currently 8 billion and predicted to rise to 9.7–10 billion by 2050, there is an urgent need to produce high-yielding crops that are able to cope with a range of environmental stressors (Gupta *et al.*, 2020). Traditional plant breeding techniques are not able to solve the emerging challenges because they are time-consuming and labor-intensive. With the rapid development of biological techniques in recent decades, genetic engineering has been successfully applied to breeding strategies for accelerating improvement of desired traits of plants, so-called molecular breeding. Nevertheless, breeders have already realized that current genetic resources mainly from protein-coding genes are not enough, and increasing numbers of regulatory genes are required. Now, regulatory noncoding RNAs mediating plant growth and stress responses from transcription to translation are emerging into the spotlight as target material for genetic engineering. LncRNAs, as a class of regulatory noncoding RNAs, are increasingly being related to the regulation of plant life. When functions and mechanisms of a growing number of lncRNAs are characterized, it will be possible for specific lncRNA loci to be genetically edited for plant improvement. So far, a series of lncRNAs have been shown to regulate crop reproductive growth, such as overexpression of lncRNA *LAIR* increasing rice grain yield (Wang *et al.*, 2018), expression of lncRNA *Ef-cd* reducing rice maturity duration without yield penalty (Fang *et al.*, 2019), and lncRNA *PMS1T* regulating photoperiod-sensitive male sterility in rice (Fan *et al.*, 2016). Besides their potential to boost crop production, lncRNAs can also be used for other aspects of plant breeding such as fruit quality improvement. For example, overexpressing the lncRNA *FRILAIR* could increase the content of the main soluble sugars, sucrose, glucose, and fructose (Tang *et al.*, 2021). Additionally, lncRNAs are an important asset for improving plant stress resistance, such as overexpression of *lncRNA08489* enhancing the resistance of tomato plants to *Phytophthora infestans* (W. Liu *et al.*, 2022) and knock down of *lncRNA973* reducing salt stress tolerance in cotton (Zhang *et al.*, 2019). Interestingly, a recent study successfully demonstrated the importance of *COOLAIR*-mediated *FLC* transcriptional silencing in natural conditions (Zhao *et al.*, 2021), suggesting that lncRNAs whose functions have been studied in laboratory conditions can form a pool of potential tools for plant improvement. Double-stranded RNAs are increasingly used as exogenous biomolecules for crop protection (Dalakouras *et al.*, 2020), but the use of lncRNAs modulating transcriptional or post-transcriptional regulation of gene expression remains mostly unexplored. Therefore, it can be expected that lncRNAs will become a valuable genetic resource for plant breeding and versatile exogenous tools in the near future.

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Author contributions

DW conceived the review topic and structure; DW, FA, and JY wrote the manuscript.

Conflict of interest

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