Trends in Plant Science

CellPress OPEN ACCESS

Review

LncRNAs: the art of being influential without protein

Lorena Ramírez Gonzales [1,6], Suze Blom [2,3,6], Rossana Henriques [4,5], Christian W.B. Bachem [1,*] and Richard G.H. Immink [2,3,*]

The plant long noncoding (lnc)RNA field is on the brink of transitioning from large-scale identification of lncRNAs to their functional characterization. Due to the cross-kingdom conservation of interaction types and molecular functions, there is much to be learned from mammalian lncRNA research. Here, we discuss the different molecular processes involving lncRNAs from the regulation of chromatin to splicing. Furthermore, we discuss the lncRNA interactome, which includes proteins, other RNAs, and DNA. We explore and discuss how mammalian lncRNA functionalities could be reflected in similar pathways in plants and hypothesize that several breakthroughs in mammalian research could lead to the discovery of novel plant lncRNA molecular functions. Expanding our knowledge of the biological role of lncRNAs and their multiple applications paves the way for future agricultural applications.

Highlights

We can learn from the mechanistic understanding of the functions of long noncoding (lnc)RNA by exploring research in the mammalian field.

LncRNAs are associated with a plethora of specific silencing and activating chromatin-remodeling events.

Plant lncRNAs in R-loops could affect local chromatin remodeling and transcription in response to environmental conditions, representing a dynamic adaptation mechanism.

LncRNAs can modulate alternative splicing events through competition with pre-mRNAs and by post-transcriptional regulation of the alternative splicing machinery.

The long-distance transport capability of lncRNAs makes them interesting candidates to investigate as novel hormone-like molecules.

Many of the characterized plant lncRNAs modulate genes associated with important agricultural traits, providing opportunities for the development of lncRNA-based biomarkers with breeding applications.

The emergence of lncRNA research in plant science

Within the plant noncoding (nc)RNA field, **lncRNAs** (see Glossary) are still relatively poorly understood regulators. These molecules are over 200 nucleotides long and are not commonly translated into proteins [1,2]. Since the widespread application of genome-wide sequencing, hundreds of thousands of lncRNAs have been found in dozens of plant species (reviewed in [3,4]). To keep track of these fast-expanding lists of plant lncRNAs, specific classification groups were created based either on their position and genome orientation (reviewed in [5]) or on their structure and known or predicted molecular functions (reviewed in [6,7]). However, this led to a 'numbers game', where the vast number of lncRNAs found is disproportional to the number of individual lncRNAs with an assigned biological significance or unique molecular functionality. Furthermore, these large-scale sequencing studies raise the question of whether all identified lncRNAs are biologically relevant or simply transcriptional noise.

Mammalian lncRNAs have been more widely studied and shown to be involved in a range of molecular processes, including **chromatin** organization, associated transcriptional regulation, or alternative mRNA splicing. To achieve this diversity in molecular functions, a broad range of lncRNA interaction types occur, such as lncRNA–protein interactions, lncRNA–DNA interactions, and lncRNA–RNA interactions [6,8].

For the relatively few plant lncRNAs that have been functionally characterized, similar molecular activities were identified that show a degree of cross-kingdom conservation (reviewed in [9]). Given this conservation, parallels between the mammalian and plant fields can be drawn and used to provide further insights into plant lncRNA function. In this review, we discuss striking examples of functionally characterized plant lncRNAs with different molecular activities. We have selected examples of lncRNAs involved in regulating chromatin and DNA methylation, lncRNAs in **R-loop** formation, lncRNA–RNA interactions, lncRNAs participating in splicing

[1]Plant Breeding, Wageningen University and Research, Wageningen, The Netherlands
[2]Laboratory of Molecular Biology, Wageningen University and Research, Wageningen, The Netherlands
[3]Bioscience, Wageningen University and Research, Wageningen, The Netherlands
[4]School of Biological, Earth, and Environmental Sciences, University College Cork, Cork, Ireland
[5]Environmental Research Institute, University College Cork, Cork, Ireland

regulation, lncRNAs as **natural antisense transcripts (NATs)**, and lncRNAs acting as potential mobile signals. We contextualize and extend these with examples of what is known in the animal field. We focus on lncRNAs the functions of which were experimentally validated *in vitro* or *in vivo*. Rather than providing a comprehensive overview of the lncRNA field, our aim is to sketch a roadmap for plant scientists on lncRNA research progress, taking advantage from knowledge collected in the mammalian field.

[6]These authors contributed equally to this work

*Correspondence:
Christian.Bachem@wur.nl
(C.W.B. Bachem) and
Richard.Immink@wur.nl
(R.G.H. Immink).

LncRNAs in epigenetics and chromatin interactions

The epigenomic landscape includes two main chemical modifications: chromatin marks located on histone tails, and DNA methylation, where the methyl groups are directly linked to cytosines in the DNA. Both types of modification require specific protein complexes, which are involved in altering and maintaining the epigenome [10]. These complexes are categorized into three classes: **epigenetic** 'writers', which add chromatin marks or DNA methylation; 'erasers', which remove chromatin marks or DNA methylation; and 'readers', which recognize these chemical modifications and convert them into specific biological processes. LncRNAs can interact with these chromatin modifiers and modulate both histone and DNA methylation, such as by guiding the modifier complexes to specific loci. Although the process of adding and removing epigenetic marks is conserved in plants and animals, the specific positioning of histone marks and the type of DNA methylation differ. While animals have only CG methylation of DNA, plants have CG, CHH, and CHG methylation. Nevertheless, DNA methylation appears to fulfil similar roles between the kingdoms [10,11], such as transcriptional silencing, transcriptional activation, or precursor mRNA (pre-mRNA) splicing (reviewed in [12]). Therefore, parallels between lncRNA–chromatin interactions in mammals and plants may still be drawn.

The relevance of drawing parallels between plants and mammals is exemplified in recent work comparing the plant lncRNA AUXIN-REGULATED PROMOTER LOOP (*APOLO*) and human lncRNA UHRF1 protein-associated transcript (*UPAT*) [13]. *UPAT* was found to interact with the epigenetic reader PHD and RING finger domain-containing protein (UHRF1) and to stabilize it, aiding its role in DNA methylation maintenance [14]. In plants, *APOLO* interacts with the VARIANT IN METHYLATION1 (VIM1) protein, which is a homolog of UHRF1 and also acts as DNA methylation reader, suggesting a conserved regulatory mechanism (reviewed in [15]).

Interestingly, *APOLO* interacts with VIM1 and also with LIKE HETEROCHROMATIN PROTEIN 1 (LHP1), a component of the epigenetic complex POLYCOMB REPRESSIVE COMPLEX 1 (PRC1) [13,16]. The functional similarities between *APOLO* and *UPAT* were further extended and confirmed by showing that *UPAT* is also able to interact with the plant proteins LHP1 and VIM1 *in vivo* [13]. Additionally, ChIP assays revealed a reduced presence of both LHP1 and VIM1 at the *YUCCA2* locus in *APOLO* or *UPAT* overexpression lines in arabidopsis (*Arabidopsis thaliana),* revealing a similar and conserved activity of plant and human lncRNAs. This is especially stunning considering the vast evolutionary distance between plants and animals and because the two lncRNAs differ completely at the sequence level [13]. This example highlights the value of drawing comparisons between plants and animals as a tool to improve our understanding of plant lncRNA functionality.

UPAT does not affect DNA methylation directly through its interaction with the DNA methylation reader UHRF1. Rather, it affects DNA methylation through regulation of DNA methyltransferase (DNMT)-1 protein levels, by mediating the degradation or inhibiting degradation of DNMT1 depending on the interaction partners of UHFR1 [17]. As *UPAT* inhibits degradation of UHRF1 [14], it indirectly regulates DNA methylation. In plants, *APOLO* interacts with VIM1, which is also considered a DNA methylation reader. Therefore, extrapolating from mammalian data, it

would be interesting to see whether *APOLO* also affects DNA methylation through promoting VIM1 interactions with DNA methylases.

Additionally, the direct interaction of lncRNAs with DNA methylation writers has also been characterized in mammals. For example, the mammalian Hox antisense intergenic RNA (*HOTAIR*) lncRNA is known to interact with DNA methylation writers from the DNMT family, including DNMT1, DNMT3a, and DNMT3b [18–20]. Interestingly, *HOTAIR* appears to affect both methylation and demethylation of DNA through different interactions, representing a dual mechanism not yet described in plants. On the one hand, *HOTAIR* recruits DNMT3a to catalyze DNA methylation at the promoter of specific target genes, contributing to their silencing [20]. On the other hand, *HOTAIR* was found to interact with DNMT1 to inhibit DNA methylation of a target promoter, allowing transcriptional activation to occur [19]. Furthermore, *HOTAIR* promotes *DNMT1*, *DNMT3a*, and *DNMT3b* transcript expression [21], suggesting its involvement in a strictly controlled regulatory network. It also affects the distribution of various histone marks through interactions with multiple writer complexes, suggesting a broader role in chromatin regulation by a single lncRNA (reviewed in [22]). The DNMT1 family is relatively conserved; in plants, one of its members is METHYL TRANSFERASE 1 (MET1) [23], which could also be under the control of a lncRNA.

Besides DNA methylation, both mammal and plant lncRNAs have been associated with histone protein modifications. Probably the best-studied example of a histone modification linked to lncRNAs is represented by trimethylation of histone 3 lysine 27 (H3K27me3), a chromatin mark associated with transcriptional silencing via the POLYCOMB REPRESSIVE COMPLEX 2 (PRC2) (reviewed in [7,24,25]). For example, CURLY LEAF (CLF), a component of PRC2 in plants, was found to be associated with *COLD ASSISTED INTRONIC NONCODING RNA* (*COLDAIR*) [26] and *AGAMOUS (AG)-incRNA4* lncRNAs [27]. These interactions result in H3K27me3 deposition and transcriptional silencing at *FLOWERING LOCUS C* (*FLC*), as part of the vernalization response, and at *AG* to restrict its expression domain to the floral reproductive organs, respectively.

In the mammalian field, lncRNAs were shown to associate with a wider range of histone protein modifications [28,29], suggesting an extensive relationship between lncRNA and chromatin-remodeling events. These findings could be used to broaden the scope of research on plant lncRNA and histone interactions. In this respect, the modulation by lncRNAs of H3K4me3 deposition via association with its writer complex (COMPASS) represents a nice example. This was initially described in mammals [30,31] but a similar pathway exists in plants. This is also the case for *MAF4 ANTISENSE RNA* (*MAS*), a NAT originating from the *MADS AFFECTING FLOWERING 4* (*MAF4*) locus (Figure 1). Arabidopsis *MAS* was found to interact with WDR5a, a component of the H3K4me3 writer COMPASS-like complex [32]. *MAS* guides this writer complex to *MAF4*, promoting H3K4me3 deposition and, consequently, activating its transcription *in cis*, suppressing precocious flowering.

Although functionally characterized plant lncRNAs associated with the regulation of histone mark deposition or removal are limited, the rice lncRNA *LRK ANTISENSE INTERGENIC RNA* (*LAIR*) was found to interact both with H3K4me3 and H4K16ac writers to affect these histone marks [33]. In peanut, the lncRNA *AhDONE40* was shown to interact with a predicted ortholog of an arabidopsis H3K36me3 writer, although its role in regulating H3K36me3 deposition remains unknown [34]. Furthermore, downregulation of the rice lncRNA *TWISTED LEAF* (*TL*) increased H3K36me2, H3K36me3, and H3K4me3 deposition *in cis*, but its molecular function in this process is yet to be demonstrated [35]. Although the exact contribution of plant lncRNAs to the regulation of histone marks remains to be fully explored, it would be interesting to see whether they could also modulate the accumulation of other chromatin marks already known to be affected by lncRNAs in mammals, such as H3K9me3 [36–38], H3K27ac, and H3K14ac [39].

Glossary

Alternative splicing (AS): process where a precursor RNA can be converted into more than one mRNA isoform through differential removal of intronic sequences by splicing factors.
Chromatin: structure of proteins and DNA that encapsulates the genome, comprises mainly histones, the chemical structure of which can affect the accessibility of the genomic DNA.
Epigenetics: effects on transcription or gene function that are not determined through coding DNA sequences. Epigenetics can involve either the addition of methyl groups to the DNA or changes to the chemistry of the chromatin.
Long non-coding RNAs (lncRNAs): RNA molecules longer than 200 nucleotides that do not encode proteins longer than 100 amino acids.
MiRNAs: small noncoding RNAs 21–24 nucleotides in length that can bind to complementary mRNAs to modulate them post transcriptionally.
Natural antisense transcripts (NATs): a type of lncRNA that is transcribed from the opposite DNA strand of another transcriptional unit (e.g., a protein-coding gene or lncRNA).
R-loops: structures where RNA binds to an exposed single-stranded piece of DNA, resulting in an RNA:DNA hybrid.
Splicing factors: proteins that are part of the splicing machinery involved in the removal of introns from pre-mRNAs.
Triple helix: structure where three strands of either DNA, RNA, or in combination, form three helices that bind to each other using hydrogen bonds. It occurs in the nucleus when a nucleotide sequence binds to the exterior of the double helix formed by the genomic DNA strands.

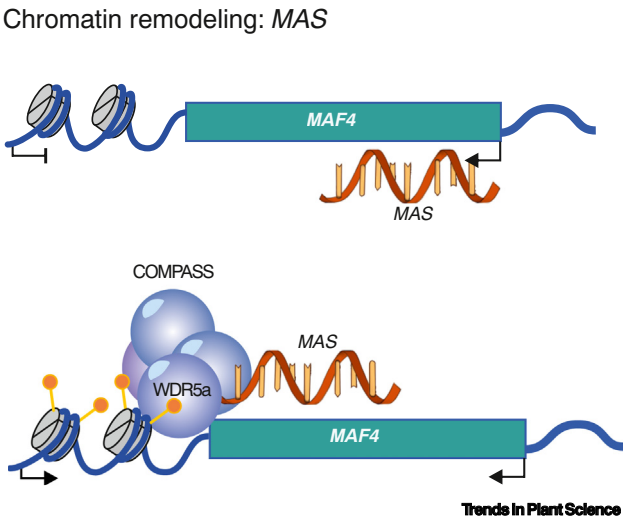

Figure 1. The NAT *MAS* regulates *MAF4* transcription *in cis* through chromatin remodeling. The lncRNA *MAS* is transcribed antisense from the *MAF4* locus. *MAS* interacts with the H3K4me3 writing complex COMPASS through interaction with its component WDR5a. Thus, *MAS* recruits COMPASS to deposit H3K4me3 at the transcriptional start site of *MAF4*. This results in transcriptional activation of this locus. Abbreviations: lncRNA, long noncoding RNA; *MAF4*, *MADS AFFECTING FLOWERING 4; MAS*, MAF4 ANTISENSE RNA; NAT, natural antisense transcript; WDR5a, WD40 REPEAT 5A.

LncRNAs involved in R-loop formation

When RNA interacts with a single-strand DNA molecule through a complementary sequence, an RNA–DNA hybrid is formed (an R-loop). Additionally, RNA can also interact with the exterior of the major groove in the DNA double helix, forming a **triple helix** RNA•DNA-DNA structure (Box 1). Other types of regulatory lncRNA–DNA structure have also been identified (reviewed in [40]).

R-loops are associated with an open chromatin landscape in both plants and animals, with low levels of CG DNA methylation and H3K27me3, and higher levels of H3K4me3 and H3K27ac [41–43]. R-loops often occur when the DNA strands are separated during transcription and can affect transcriptional elongation and the conformation of the surrounding chromatin (reviewed in [44]). Furthermore, R-loop enrichment in promoters and actively transcribed genes in arabidopsis strongly suggests a role for these dynamic structures in transcriptional regulation [41]. Notably, in mammals, antisense lncRNAs can themselves be generated by R-loops, which are formed when a nascent transcript invades the DNA double helix behind elongating RNA polymerase II (Pol II) [45]. However, our focus here is on lncRNA molecular functionality in R-loop formation and associated consequences rather than on lncRNA generation through R-loops.

In arabidopsis, *APOLO* forms R-loops *in trans* at target genes through interaction with a GAAGAA DNA motif by a six-base pair (bp) complementary sequence (UUCUUC) [46,47]. As mentioned earlier, *APOLO* can interact with LHP1, a component of the epigenetic programming complex PRC1 [47]. At high expression levels, *APOLO* could act as a decoy for LHP1, preventing its functioning [46,47].

In addition, the interaction of *APOLO* with VIM1 leads to the formation of an R-loop at the *YUC2* locus [13]. Interestingly, this R-loop forms at 23°C, while no R-loop and also no VIM1 binding at the *YUC2* locus occurs at higher temperatures (29°C) (Figure 2). This suggests that the formation of the *APOLO*-based R-loop is necessary to bring the epigenetic writer near the target locus and to alter the local chromatin at 23°C.

Moreover, circular RNAs (circRNAs) are also involved in R-loop formation [48,49]. CircRNAs are a class of ncRNA molecule generated by back splicing from intronic or exonic sequences,

Box 1. LncRNAs in triple helices

Certain lncRNAs with a complementary sequence length of at least 19 bases can interact with the exterior of nucleotides in the major groove of the double-stranded DNA (dsDNA) helix and establish specific hydrogen bonds (Hoogsteen bonds) [140] (Figure I). The structures created from these lncRNA interactions are called triple helices, also known as triplexes (reviewed in [141,142]). Hoogsteen bonds are commonly annotated by '•', (whereas Watson–Crick pairing is annotated with '-'). The different types of triple helix discovered thus far are RNA•DNA-DNA helices, RNA•RNA-RNA helices, and DNA•DNA-DNA helices.

Mammalian lncRNA-based triple helices are involved in multiple molecular processes both *in cis* and *in trans*, including transcriptional activation [143], repression [144], or the modification of chromatin [145]. Triple helixes appear to be enriched in promoter sequences [146,147]. However, characterized lncRNAs in triple-helical structures are limited due to the difficulty in studying these structures. In plants, the lncRNA triple-helix field is still in its infancy and, therefore, could benefit from methodologies and tools developed for mammalian systems. For instance, specific bioinformatic tools have been developed to find triple helix-forming elements in RNAs, including Triplexator [146] and the Triplex Domain Finder [147]. These tools test strings of DNA and RNA for complementary sequences, where minimum length and error margin can be defined. Using these tools, potential triple helix-forming elements have been found in a lncRNA in cotton [148] and in hundreds of lncRNAs in rice [149]. Many of the motifs found in rice have complementary sequences in neighboring genes, suggesting they form triple-helical structures *in cis* [149]. Furthermore, Triplexator was used to identify a potential triplex-forming motif in *COOLAIR* located in a region needed for its interaction with *FLC* locus chromatin. This triplex-forming motif in *COOLAIR* was shown by an *in vitro electrophoretic mobility shift assay (EMSA)* to bind to the complementary dsDNA sequence of *FLC* [129]. However, more work is needed to confirm that triple-helix structures are formed *in vivo*.

Unfortunately, triple-helical interaction confirmation *in vivo* is challenging. Chromatin Isolation by RNA Purification (ChIRP) in combination with bioinformatic tools, such as Triplexator or Triplex Domain Finder, could constitute a good indication that a lncRNA forms a triple helix at a specific DNA target site (e.g., as used in [145]); however, it by itself is not conclusive. Alternatively, a recently developed untargeted genome-wide approach may be implemented for the isolation of RNA•DNA-DNA helices [150]. Furthermore, the fluorescent dye Thiazole Orange (TO), an *in vivo* approach, specifically stains triple helices in living mammalian cells, making this an interesting tool to visualize triple-helix formation [151]. TO live cell assays have been performed successfully in tobacco BY2 cells, indicating that it can be used in plants [152]. X-ray crystallography and nuclear magnetic resonance (NMR) also have been applied successfully to confirm RNA•RNA-RNA triple helices (reviewed in [153]). However, thus far, these methods have yet to be applied in RNA•DNA-DNA studies. Nevertheless, they could be valuable to finally confirm the existence of RNA•DNA-DNA triple helices *in vivo,* both in mammals and plants.

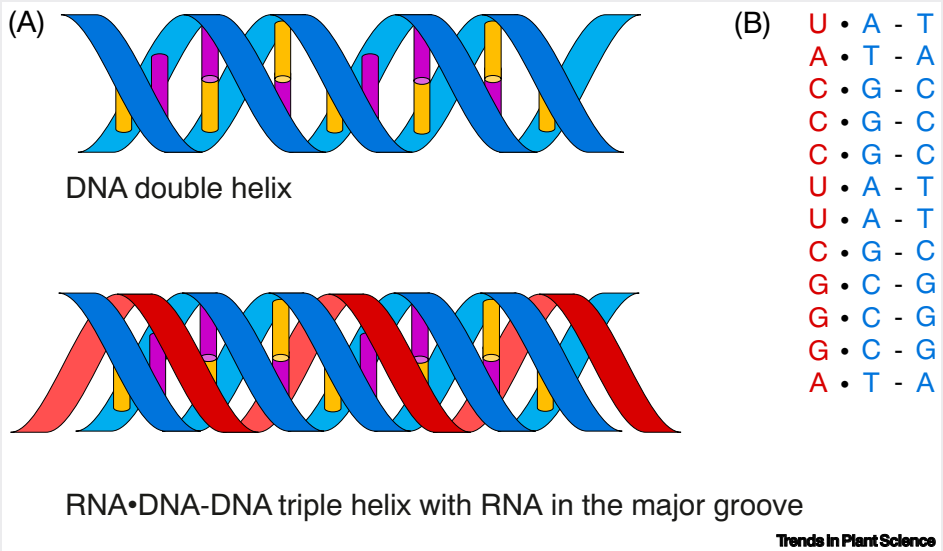

Figure I. Triple-helix formation through interaction between RNA and double-stranded (ds)DNA. (A) Simplified overview of an RNA•DNA-DNA triple-helix formation through RNA interaction (red) with the DNA double helix (blue). The invading RNA strand binds through Hoogsteen base pairing within the major groove of the DNA double helix. (B) Example of the annotation of a triple helix with RNA (red) and a DNA double helix (blue). Hoogsteen bonds are annotated by '•', whereas Watson–Crick pairing is annotated by '-'.

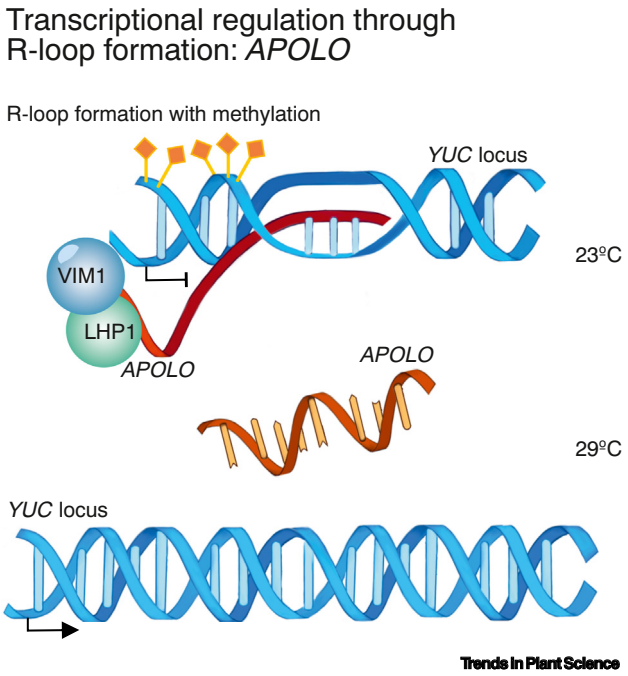

Figure 2. *APOLO* regulates *YUC2*expression through R-loop formation. At 23°C, *APOLO* is able to form an R-loop at the *YUC2* locus through a complementary sequence. *APOLO* can interact with the DNA methylation writer VIM1, which methylates the *YUC2* promoter, resulting in its transcriptional repression. Furthermore, APOLO interacts with the PRC1 protein LHP1. At 29°C, *APOLO* is unable to form an R-loop at the *YUC2* locus, allowing its transcription to occur. Abbreviations: *APOLO*, AUXIN-REGULATED PROMOTER LOOP; LHP1, LIKE HETEROCHROMATIN PROTEIN 1; PRC1, POLYCOMB REPRESSIVE COMPLEX 1; VIM1, VARIANT IN METHYLATION1; *YUC2*, YUCCA2.

presenting a covalently closed loop structure in which their 3′ and 5′ ends are joined together [50]. For example, in arabidopsis, exon six of *SEPALLATA3* (*SEP3*) gives rise to a circRNA that enhances the expression of the *SEP3.3* isoform lacking exon six [48]. This mechanism operates via R-loop formation through direct interaction of the *SEP3* circRNA with the genomic locus of its cognate, resulting in transcriptional pausing and, consequently, altered floral organ development.

Data from the mammalian field suggest that R-loops in an open chromatin landscape actively facilitate chromatin relaxation. For example, TCF21 antisense RNA inducing promoter demethylation (*TARID*) is a lncRNA forming an R-loop, which is recognized by ten-eleven translocation (TET) and growth arrest and DNA damage protein 45A (GADD45A). TET and GADD45A promote DNA demethylation at the *TCF21* locus and it was proposed that GADD45A acts as a reader of R-loops and, consequently, recruits the TET demethylase [51,52].

In conclusion, these studies show that lncRNA-based R-loops have a role in chromatin remodeling and transcriptional regulation. It would be interesting to determine whether lncRNA-based R-loops can also recruit other transcriptional or epigenetic regulators to loci of protein-coding or noncoding genes in plants.

LncRNA–RNA interactions

LncRNAs can interact with multiple types of RNA through complementary Watson–Crick base pair binding. In plants, lncRNAs interact with various **miRNAs** (reviewed in [8]). This specific interaction type, known as target mimicry, is proposed to result in competition between the mRNA and the lncRNA to bind miRNAs. MiRNAs are small RNAs of 21–24 nucleotides in length that are known to regulate target genes at the post-transcriptional level through interaction with mRNAs [53]. For instance, one of the first reports on this topic refers to the arabidopsis lncRNA *INDUCED BY PHOSPHATE STARVATION1* (*IPS1*), which partly complements and binds to miR399. However, due to a few mismatches in the sequence, this double-stranded RNA

(dsRNA) is not cleaved. Therefore, *IPS1* decoys miR399, leading to an increase in its mRNA target *PHO2* and a dynamically and precisely altered phosphate-starvation response [54]. Differences in miRNA regulation between mammals and plants have been extensively reviewed [55]. In plants, the most common mode of action involves miRNA-dependent target cleavage and degradation through the RNA-induced silencing complex (RISC). Although plant miRNAs and their targets are almost fully complementary [56–61], mismatches between the 10- and 11-bp position in the 5′ of the miRNA can occur and enhance target mimicry [58,62]. By contrast, miRNA regulation in mammals occurs commonly via translation inhibition of their mRNA targets [56,57,63–66]. Interestingly, only a short seed region (6–8 bp) usually matches the miRNA to its target [56], which needs to be loaded into Argonaute (AGO) protein to form the RISC. Although mammals have four AGO proteins, only one is active, indicating the lack of function of miRNAs via transcriptional inactivation [67,68]. Instead, miRNA regulation in mammals relies on other proteins that function during translation inhibition [69,70], although no precise molecular mechanism has yet been identified accounting for the preference of miRNAs to promote either mRNA translation or degradation.

MiRNA sponging can also occur via circRNA regulation. Mammalian circRNA can sponge miRNAs to modulate mRNA translation, affecting the protein levels of the miRNA target [71,72]. Although these circRNAs show low expression overall, they are relatively stable and, furthermore, appear to be highly abundant in a tissue-specific manner [73–75]. For instance, circRNA-Sirtuin1 (circ-Sirt1) binds to miR-132/212, increasing SIRT1 protein levels and affecting the inflammatory pathway [71]. In plants, a recent study described circRNA involvement in miRNA sponging, whereby *Os06circ02797* sponges OsMIR408, regulating salinity stress during seed germination. In agreement, the targets of OsMIR408 showed reduced expression in an *OS06circ02797Δ1* mutant [76]. Although *in vivo* tissue-specific levels of arabidopsis circRNAs have been described [77], it would be important to both validate their accumulation and demonstrate whether plant circRNA can also affect protein translation of a miRNA target.

Tissue specificity and environmental conditions can modulate lncRNA–miRNA interactions. In mammals, the lncRNA maternally expressed gene 3 (*MEG3)* binds to seven miRNAs depending on cancer cell type and hypoxia conditions [63,78,79]. In plants, specific conditions, such as cold, drought, or heat, potentially affect the interactions between lncRNAs and miRNAs (reviewed in [80]). Nevertheless, the potential occurrence of multiple plant lncRNA–miRNA interactions has been based on colocalization, co-expression, and predicted binding capabilities, with only a few interactions being confirmed *in planta*. Therefore, further functional assays are required to assess the potential biological role of these lncRNA–miRNA interactions.

Interestingly, besides the described lncRNA–miRNA association, mammalian lncRNAs can also interact with mRNAs and other lncRNAs, and this appears to be a frequent and common molecular mechanism [81]. Some of these lncRNAs, such as metastasis-associated lung adenocarcinoma transcript *1* (*MALAT1*) and nuclear enriched abundant transcript 1 (*NEAT1*) can interact with hundreds of RNAs [81]. These lncRNAs are considered hub RNAs because they associate with numerous other transcriptional units, most of which are transcribed from coding genes across the genome. *HOTAIR* is another example of a mammalian lncRNA that has multiple confirmed interactions, including with both miRNAs [82] and mRNAs [83,84]. RNA immunoprecipitation (RIP) pulldown of *HOTAIR* revealed that it interacts directly with target mRNAs and affects their expression levels [83]. This is achieved through interaction with heterogeneous nucleus ribonucleoprotein (hnRNP) B1, a so-called ‘matchmaker protein’ that facilitates RNA–RNA interactions [83,84] (Figure 3). This dsRNA formation appears to associate with higher PRC2 activity, because it was inhibited by single-stranded RNA compared with a no-RNA control [84]. It

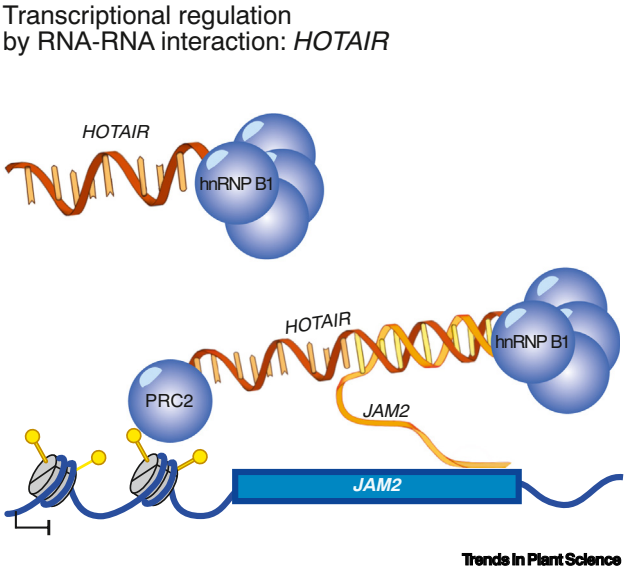

Figure 3. *HOTAIR* can engage in RNA–RNA interactions and promotes PRC2 function. The human lncRNA *HOTAIR* can interact with other RNAs (the mRNA of *JAM2* shown here). This interaction is facilitated by the matchmaker protein hnRNP B1. *HOTAIR* also binds to the H3K27me3 writer complex PRC2. *HOTAIR* interaction with *JAM2* promotes PRC2 function, with the consequent deposit of H3K27me3 at the *JAM2* locus. Abbreviations: *HOTAIR*, Hox antisense intergenic RNA; hnRNPB1, heterogeneous nucleus ribonucleoprotein B1; lncRNA, long noncoding RNA; PRC2, polycomb repressive complex 2.

might be that dsRNA could target chromatin remodelers to specific locations, and that lncRNAs could have an important role in the formation of these double-stranded structures. Nevertheless, their role in guiding PRC2 complexes requires further investigation.

Thus, these results from the mammalian field show that many different types of lncRNA–RNA interaction are possible. Although none have yet been confirmed *in vivo*, several plant lncRNAs are predicted to interact with mRNAs [85]. Therefore, it would be of interest to assess how widespread these lncRNA–RNA interactions are in plants compared with what has been found in mammals [81].

LncRNAs involved in splicing regulation

Alternative splicing (AS) is a process by which a single pre-mRNA can be converted into a multitude of individual mRNAs with different stability and functions or that encode different protein isoforms [86]. The process is mediated by the spliceosome, a multiprotein complex including various splicing regulators [87], a conserved core of **splicing factors** [88], hundreds of peripheral splicing factors, and splicing-associated proteins [89]. Several studies revealed that plant lncRNAs can interact with some of these splicing factors and affect splicing (reviewed in [90]). This is the case of the *ALTERNATIVE SPLICING COMPETITOR* (*ASCO*) lncRNA in arabidopsis, which can bind to splicing factors, such as PRP8a, SMD1b, NSRa, and NSRb [91–93]. Both knockdown by RNAi and overexpression of *ASCO* resulted in altered AS events of protein-coding mRNAs [92,93]. Furthermore, *ASCO* overexpression resulted in increased competition with specific pre-mRNAs normally bound by PRP8a, leading to differential splicing outcomes for these targets [93] (Figure 4). These observations suggest that *ASCO* binds to multiple splicing factors to fine-tune AS of a defined set of mRNAs modulating transcriptome reprogramming in response to biotic stress.

LncRNA interaction with specific splicing factors also occurs in animals. This is the case of the lncRNA *GOMAFU*, which interacts with multiple splicing factors in the nuclear compartment, namely SF1, Celf3, SRSF, and QKI [94,95]. The authors hypothesized that *GOMAFU* hijacks these splicing factors to inhibit their ability to splice pre-mRNA and that they are released when

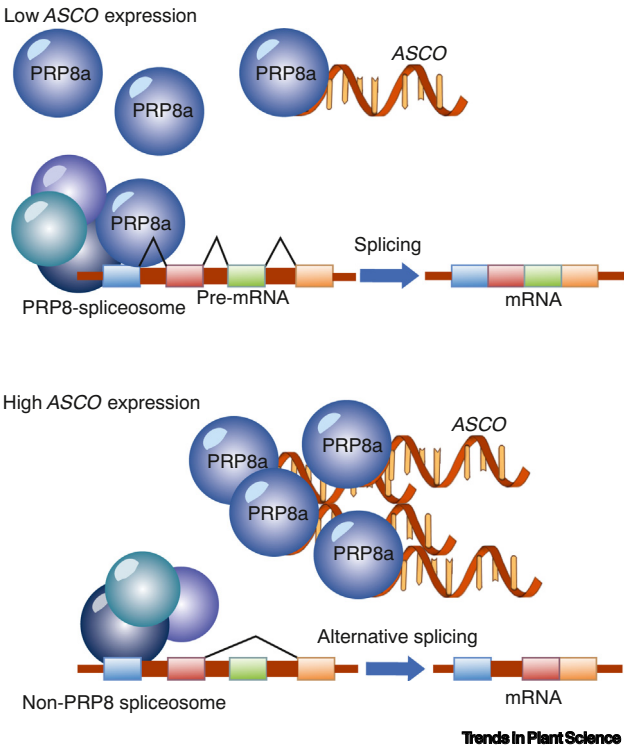

Figure 4. *ASCO* competes with pre-mRNAs for interaction with splicing factor PRP8a and affects pre-mRNA splicing. The lncRNA *ASCO* can interact with multiple splicing factors, such as PRP8a, SMD1b, NSRa, and NSRb (PRP8a is depicted here as a specific example). Through this interaction, *ASCO* competes with pre-mRNAs, the splicing of which depends on PRP8a. At regular *ASCO* transcription levels, enough PRP8a is present to affect the splicing of its pre-mRNA targets. When *ASCO* is overexpressed, it outcompetes the pre-mRNAs, resulting in alternative splicing of PRP8a target genes. Abbreviations: *ASCO*, ALTERNATIVE SPLICING COMPETITOR; lncRNA, long noncoding RNA; pre-mRNA, precursor mRNA.

splicing is necessary through the downregulation of the *GOMAFU* transcript [94]. Interestingly, splicing regulation in animals involving lncRNAs is also affected by post-transcriptional modifications. The lncRNA nuclear enriched abundant transcript 1 (*NEAT1*) modulates the AS of *PPARg* pre-mRNA by regulating the post-translational modification of the SRp40 splicing factor through protein phosphorylation [96,97]. Finally, the lncRNA *MALAT* not only interacts with SR proteins and regulates their phosphorylation, but also stabilizes the crucial splicing factor complex components polypyrimidine tract-binding protein-associated splicing factor (PTB1) and PTB-associated splicing factor (PSF) [98,99]. Such mechanisms have not yet been described in plants, but it would be interesting to confirm whether these functionalities are conserved among kingdoms. Nevertheless, these results reveal that splicing can be affected by lncRNAs in multiple ways, either via direct competition with pre-mRNAs for binding to proteins of the splicing machinery or by altering splicing factors, through post-transcriptional modifications. It will be of interest to explore whether plant lncRNAs have also evolved this range of ways to influence AS.

Natural antisense lncRNAs act as multitasking regulators

NAT-lncRNAs are a group of lncRNAs that originate from the antisense strand in relation to their targets (e.g., other lncRNAs or protein-coding genes). Plant NAT-lncRNAs are some of the most functionally characterized and include *COOLAIR* (recently reviewed in [100]), *MAS* [32] (Figure 1; see also section 'LncRNAS in epigenetics and chromatin interactions'), *SVALKA* [101], *asCBF1* [102], FLORE [103], and *asDOG1* [104,105]. These NAT-lncRNAs may associate with chromatin remodelers, as exemplified by the aforementioned *COOLAIR*-PRC2 complex mediating

H3K27me3 accumulation at the *FLC* locus upon prolonged cold exposure, and the associated silencing of *FLC* expression. Alternatively, NAT-lncRNAs have been shown to promote RNA Pol II collision and inhibition of transcription, as is the case for *SVALKA*-mediated regulation of the *CBF1* locus. Despite these diverse mechanisms of action, some shared patterns emerge, such as the specific accumulation of NAT-lncRNAs in response to certain abiotic stresses, including short or prolonged cold exposure and drought. Furthermore, many NAT-lncRNAs have the opposite biological function in relation to their target transcripts; for example, the NAT-lncRNA *asDOG1* regulates the antisense encoded gene *DELAY OF GERMINATION 1* (*DOG1*) and, thus, seed dormancy levels and germination [105].

An anti-phasically expressed transcript pair in arabidopsis, *CDF5 Long non-Coding RNA* (*FLORE*) and *CYCLING DOF FACTOR 5* (*CDF5*), display a mutual inhibitory relationship, which is reflected in their opposite roles in regulating photoperiod-dependent flowering [103]. Moreover, *FLORE* was shown to act both *in cis* and *in trans* and each component of this NAT pair was found to be required to ensure the proper oscillation of its counterpart, suggesting a connection between circadian perception and lncRNA *FLORE* function [103]. A similar lncRNA/StCDF1 transcript pair was also identified in potato. This locus appears to affect water homeostasis, resulting in drought tolerance in *StCDF1-RNAi* plants with higher *StFLORE* expression and drought susceptibility in the natural *StFLORE* mutants represented by the *StCDF1.3* allele. These results indicate a similar mutual regulation of the lncRNA *StFLORE* and the sense *StCDF1* transcript [106].

Interestingly, the NAT-lncRNA *SVALKA* was shown to modulate cold stress responses [101]. Unlike *COOLAIR*, which regulates the *FLC* locus upon prolonged cold exposure, *SVALKA* is involved in the downregulation of C-REPEAT/DEHYDRATION-RESPONSIVE ELEMENT BINDING FACTOR 1 (*CBF1*) after short-term cold stress to prevent its overaccumulation and growth inhibition. Detailed molecular analyses showed that *SVALKA* run-through transcription by RNA Pol II generates an additional cryptic antisense lncRNA to *CBF1* (*asCBF1*). Accumulation of *asCBF1* promotes Pol II collision at the 3′ end of the *CBF1*, resulting in early transcription termination and an overall reduction in *CBF1* expression. Similarly to *COOLAIR*, this mechanism operates *in cis* since *SVALKA* and *asCBF1* fail to modulate the expression of other components of the *CBF* regulon located in their vicinity [101]. Recently, a longer isoform of *SVALKA* was shown to accumulate under optimal temperature (22°C) conditions, which extends over the antisense strand of *CBF1*a. This leads to the formation of dsRNA, which is recognized by DICER2 and/or DICER4. Ultimately, these result in *CBF1* mRNA downregulation via AGO1 [102]. Although this mechanism is fully independent of regulation occurring after short cold exposure, it appears to modulate plant biomass under normal temperatures. Interestingly, these studies show that one NAT-lncRNA can regulate its target antisense gene through multiple processes, with opposite biological effects based on different environmental conditions.

Recently, a novel lncRNA locus encoding *FLOWERING-ASSOCIATED INTERGENIC lncRNA* (*FLAIL*) was shown to modulate flowering time by modulating gene expression *in trans* similarly to the *FLORE* NAT-lncRNA [107]. *FLAIL* is transcribed from both sense and antisense strands, but only its sense isoform appears to regulate flowering by modulating both AS events, as well as the accumulation of H3K4me1 and H3K4me3 marks in specific target loci. Thus, *FLAIL* constitutes one of the first plant lncRNAs shown to regulate these two cotranscriptional processes.

NAT-lncRNAs have been extensively studied in animals, with *HOTAIR* being one of the best characterized. Their widespread functions have been closely associated with cancer, where they could act as either oncogene factors (e.g., *HOTAIR*) or tumor suppressors (e.g., growth arrest-specific 5; *GAS5*) (reviewed in [108]). Detailed analyses of their mechanisms of action reveal

common biological functions, including sponging of specific miRNAs, association with chromatin remodelers at specific loci (e.g., *KCNQ1OT1/LIT1* [109] and HOX11-AS [110]), and regulation of protein stability by modulating ubiquitination and proteasome-dependent degradation of targets (e.g., *KCNQ1OT1* and hexokinase 2 [111]). This ability to fulfil diverse biological functions is best shown in antisense ncRNA in the INK4 locus (*ANRIL*), which performs different biological functions according to its intracellular localization [108]. Cytoplasmic *ANRIL* engages mostly in miRNA sponging, whereas, in the nucleus, it acts both *in cis* and *in trans*. *ANRIL* recruits PRC1 and PRC2 components to silence *CDKN2B*, its antisense protein-coding transcript. This regulation appears to involve chaperones with RHA helicase activity, and the formation of a triple helix at this locus (see Box 1 for an explanation of triple helixes). *ANRIL* could also associate with PRC2 components to inhibit specific targets *in trans*. Interestingly, *ANRIL* was shown to induce NADPH oxidase 1 (*NOX1*) transcription via its interaction with WD-40 repeat-containing protein 5 (WDR5) and histone deacetylase 3 (HDAC3) in the nucleus [112]. In addition to these functions, *ANRIL* could regulate AS events via an, as yet unknown, mechanism [108]. Together, these results suggest that NAT-lncRNAs perform a range of biological functions according to their intracellular localization and physical interactions. This finding and the previously mentioned multifaceted and dynamic activity of the plant NAT-lncRNA *SVALKA* show that multitasking activity by NAT, or other lncRNAs, is likely to be a frequent occurrence. This makes it interesting and relevant to study the functionality of plant lncRNAs with an open mind and to consider multiple roles for these dynamic molecules.

LncRNAs as mobile signals

In all multicellular organisms, communication between cells, tissues, and organs is vital and requires unique mobile signals traveling from cell to cell or over long distances. Interestingly, mammalian lncRNAs were shown to travel intercellularly and act as messenger molecules in neighboring cells. This transport is achieved through different forms of extracellular vesicle trafficking, including exosomes, microvesicles, and apoptotic bodies (reviewed in [113,114]). These membrane-derived extracellular vesicles are of different sizes, with exosomes being the smallest (<100 nm) and apoptotic bodies the largest (>1000 nm). Extracellular vesicle-based lncRNA movement was shown to occur between the same and different cell types [115,116]. For example, *MALAT1* is transcribed in bone marrow-derived mesenchymal stem cells and transported through exosomes to osteoblasts. Furthermore, it is involved in sponging specific miRNAs and, thus, regulating downstream genes, strengthening the notion that lncRNAs can act as signaling molecules between cell types [117].

In addition to this short-distance travel, mammalian lncRNAs are frequently excreted into the circulatory system and transported through blood serum [118–120]. For example, *LINC00470* is present in exosomes in blood serum samples and can be absorbed by different cell lines *in vitro* [118]. However, whether serum-transported lncRNAs remain functional in their destination tissues *in vivo* remains to be established.

In plants, lncRNAs were also shown to travel long distances. In cucumber, 22 lncRNAs were identified in the phloem and appeared to be transmissible between cucumber and watermelon heterografts. These lncRNAs seemed to be specifically targeted for transport since their movement in the phloem was not concentration dependent [121]. The transport mechanisms of lncRNAs are similar to those of mRNAs. Both types of RNA contain the same CU-rich motifs that are required for binding to POLYPYRIMIDINE TRACT BINDING PROTEIN (PTB) and to facilitate phloem transport [121,122]. Although these findings provide strong support for lncRNA transport in plants, their functionality at the destination was not shown until very recently, when it was reported that the lncRNA *ELF18-INDUCED LONG NONCODING RNA 1 (ELENA1)* is

transcribed in roots under low nitrogen conditions and systemically transported to the shoot, where it acts to attenuate leaf senescence in the event of nitrogen deficiencies [123]. At its destiny, *ELENA1* limits the activity of the master transcription factor of plant senescence ORESARA1 (ORE1) by dissociating the MEDIATOR SUBUNIT 19a (MED19a)–ORE1 transcriptional complex. This spectacular finding reveals for the first time a plant lncRNA that is specifically transported and shows specific interactions in the target tissue; thus, it could even suggest a hormone-like role for *ELENA1* in plants. All-inclusive definitions of hormones are lacking, since, for each proposed definition, there will be canonical hormones that fail to adhere to all requirements. Nevertheless, it is commonly accepted that hormones are molecules produced by multicellular organisms to carry specific signals to target tissues. Interestingly, these common definitions would not separate hormones from other signaling molecules [124]. For example, the florigen (FLOWERING LOCUS T) protein is considered a plant hormone, although it binds an intracellular transcription factor complex to effect transcription rather than binding to extracellular receptors [125]. By contrast, reactive oxygen species (ROS), which are sensed by specific receptors and affect specific signaling cascades [126], are not considered bona fide plant hormones. However, wider definitions that consider hormones as organic molecules that regulate physiological processes at low concentrations [127] could also classify other molecules as hormone-like, such as lncRNAs.

Considering the specificity of lncRNA transport and tissue accumulation, and the recent identification of the long-distance functioning of *ELENA1*, some plant lncRNAs might be considered hormone-like molecules. This novel function of plant lncRNAs significantly affects our understanding of within-plant communication systems and opens new research opportunities, such as the study of source to sink transport and signaling during growth, in development, or in systemic abiotic and biotic stress responses.

Concluding remarks and future perspectives

Rapid advances in lncRNA research and sequencing technology have resulted in the discovery and annotation of thousands of novel lncRNAs from a multitude of plant species under a variety of environmental conditions. However, due to several technical challenges in functionally characterizing lncRNAs, only a relatively small number have been assigned a molecular and biological function. This has resulted in a 'numbers game' dilemma, where it is difficult to see the wood, namely the vast numbers of lncRNAs found, for the trees, which are the molecular functions of specific lncRNAs. Here, we explored several insights from the mammalian field and built on the similarities in lncRNA molecular functionality across kingdoms, to provide guidance for future studies and to predict further lncRNA functions in plants. While it is likely that lncRNA-mediated regulation of biological processes evolved early in the tree of life, it is possible that the different kingdoms have evolved in parallel and that there are species-specific lncRNA mechanisms. Nevertheless, as with other regulatory processes, it is useful to compare the broadest possible diversity of mechanistic links and, thus, learn from what is known in mammals. The growth in fundamental knowledge of lncRNA functionality in mammals provided researchers with more tools to tackle the 'numbers game' problem and reinforced the notion that lncRNAs are not 'just transcriptional noise' [128]. We also believe this to be the case in plants, where lncRNAs have been associated with distinct functions, including regulation of genome dynamics and the fine-tuning of regulatory processes related to particular developmental transitions.

Despite all the challenges, the lncRNA field has seen much progress over the past few years. Many types of interaction have been uncovered and a range of molecular processes have been characterized, leading to a new view of eukaryotic genomes as dynamic entities where

Outstanding questions

Why is such a large part of the genome transcribed into lncRNAs?

Do lncRNAs fulfil a specific biological need by saving the energy costs of protein translation?

How do we functionally characterize the large number of lncRNAs found in plants in an efficient way?

Are there any lncRNA molecular mechanisms specific to plants?

How universal is the relationship between lncRNAs and the deposition of chromatin marks?

Do lncRNAs commonly affect activating chromatin marks in plants?

How do lncRNAs in R-loops affect local chromatin and transcription?

How can we validate plant lncRNA participation in triple helices?

How much can research on secondary structures and conservation of lncRNA help us understand their function across kingdoms?

Do lncRNAs contribute substantially to plant stress adaptation and, if so, how?

transcriptional regulation occurs at different levels. As discussed earlier, the plant lncRNA field can benefit from the mammalian research field. However, an interesting facet of animal and plant biology is worth highlighting. Unlike animal cells, which can be isolated and generally retain their cellular identity, which makes them amenable for detailed study, plant cells withdrawn from their tissue context and their cellular compartment tend to revert to a stem cell-like status. This makes developmental analysis at a single cell level challenging, especially for the exquisite fine-tuning processes that involve lncRNAs. Thus, specific *in planta* technologies are required to study these processes in all their detail. However, there are also advantages and opportunities for plant sciences in this respect. The generation of mutant, overexpression, and reporter lines is routine and allows easy comparison between different tissues in the same genotype, facilitating, for example, the investigation of lncRNAs with putative hormone-like function at the organism level, of which the recently published research on *ELENA1* is an excellent example.

Considering the vast progress made in bioinformatics and available molecular tools, we anticipate that lncRNA studies will improve in their complexity and accuracy over the next few years. For example, recent predictions of lncRNA secondary structures *in vivo* show how different isoform structures can be associated with specific environmental conditions that likely correspond to different functionalities [129]. Research into the secondary structure of lncRNAs could prove especially useful. Recently, it was shown that two sequence-unrelated lncRNAs from arabidopsis and humans can bind to the same arabidopsis chromatin remodelers, probably due to a similar domain in their secondary structures [13]. This observation once again underlines the usefulness of comparing plant and animal lncRNAs and how this could advance our fundamental understanding of lncRNA biology.

Many of the characterized lncRNAs in model plants and crops modulate important agricultural traits, such as flowering time, root development, seed dormancy, and biotic and abiotic stress responses [103,106,130–135]. Therefore, further research into plant lncRNAs has the potential to uncover novel fine-tuning mechanisms, expanding their identification and functional characterization from model species to crops [136]. This regulation could become even more relevant as a tool to uncover the molecular mechanisms providing tolerance to climate change and extreme weather events [137]. Recently, the link between lncRNAs, the regulation of complex traits, and the associated potential for targeted crop improvement has been recognized [138,139]. An urgent and immediately emerging question that follows is how this knowledge can be applied in breeding programs, such as biomarkers for particular traits or as regulators of abiotic stress responses.

In conclusion, the plant research field is on the brink of transitioning from large-scale lncRNA identification and a 'numbers game' to their functional characterization and full harnessing of the enormous potential that these regulators offer for breeding approaches (see Outstanding questions).

Acknowledgments

S.B. and R.G.H.I. wrote this review as part of the TTW-VICI research program entitled 'Climate-proof plant reproduction' with project number 16129, financed by the Dutch Research Council. C.W.B.B. received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 862858, as part of the ADAPT project. R.H.'s lncRNA research was supported by grants from the Spanish Ministerio de Economía y Competitividad (MINECO) (BIO2015-72161-EXP, BIO2015-70812-ERC, and RYC-2011-09220) and by start-up funding from the School of Biological, Earth, and Environmental Sciences, University College Cork. L.R.G. is a recipient of a fellowship from FONDECYT – CONCYTEC (grant contract number 090-2016-FONDECYT) (Lima, Perú).

Declaration of interests

None declared by authors.

References

1. Mercer, T.R. *et al.* (2009) Long non-coding RNAs: insights into functions. *Nat. Rev. Genet.* 10, 155–159
2. Volders, P.-J. *et al.* (2013) LNCipedia: a database for annotated human lncRNA transcript sequences and structures. *Nucleic Acids Res.* 41, D246–D251
3. Rai, M.I. *et al.* (2019) Classification and experimental identification of plant long non-coding RNAs. *Genomics* 111, 997–1005
4. Jin, J. *et al.* (2021) PLncDB V2. 0: a comprehensive encyclopedia of plant long noncoding RNAs. *Nucleic Acids Res.* 49, D1489–D1495
5. Budak, H. *et al.* (2020) Long non-coding RNA in plants in the era of reference sequences. *Front. Plant Sci.* 77, 1055–1065
6. Lucero, L. *et al.* (2021) Functional classification of plant long noncoding RNAs: a transcript is known by the company it keeps. *New Phytol.* 229, 1251–1260
7. Yang, J. *et al.* (2023) Plant long noncoding RNAs: biologically relevant and mechanistically intriguing. *J. Exp. Bot.* 74, 2364–2373
8. Chen, Q. *et al.* (2021) From 'dark matter' to 'star': insight into the regulation mechanisms of plant functional long non-coding RNAs. *Front. Plant Sci.* 1009, 650926
9. Mattick, J.S. *et al.* (2023) Long non-coding RNAs: definitions, functions, challenges and recommendations. *Nat. Rev. Mol. Cell Biol.* 24, 430–447
10. Xiao, J. *et al.* (2016) Tug of war: adding and removing histone lysine methylation in *Arabidopsis*. *Curr. Opin. Plant Biol.* 34, 41–53
11. Feng, S. *et al.* (2010) Conservation and divergence of methylation patterning in plants and animals. *Proc. Natl. Acad. Sci. U. S. A.* 107, 8689–8694
12. Greenberg, M.V. and Bourc'his, D. (2019) The diverse roles of DNA methylation in mammalian development and disease. *Nat. Rev. Mol. Cell Biol.* 20, 590–607
13. Fonouni-Farde, C. *et al.* (2022) The *Arabidopsis* APOLO and human UPAT sequence-unrelated long noncoding RNAs can modulate DNA and histone methylation machineries in plants. *Genome Biol.* 23, 181
14. Taniue, K. *et al.* (2016) Long noncoding RNA UPAT promotes colon tumorigenesis by inhibiting degradation of UHRF1. *Proc. Natl. Acad. Sci. U. S. A.* 113, 1273-1268
15. Grimanelli, D. and Ingouff, M. (2020) DNA methylation readers in plants. *J. Mol. Biol.* 432, 1706–1717
16. Ariel, F. *et al.* (2014) Noncoding transcription by alternative RNA polymerases dynamically regulates an auxin-driven chromatin loop. *Mol. Cell* 55, 383–396
17. Du, Z. *et al.* (2010) DNMT1 stability is regulated by proteins coordinating deubiquitination and acetylation-driven ubiquitination. *Sci. Signal.* 3, ra80
18. Zhang, S. *et al.* (2020) LncRNA HOTAIR-mediated MTHFR methylation inhibits 5-fluorouracil sensitivity in esophageal cancer cells. *J. Exp. Clin. Cancer Res.* 39, 131
19. Song, H. *et al.* (2021) Depleting long noncoding RNA HOTAIR attenuates chronic myelocytic leukemia progression by binding to DNA methyltransferase 1 and inhibiting PTEN gene promoter methylation. *Cell Death Dis.* 12, 440
20. Wang, S.-L. *et al.* (2019) Silencing long non-coding RNA HOTAIR exerts anti-oncogenic effect on human acute myeloid leukemia via demethylation of HOXA5 by inhibiting Dnmt3b. *Cancer Cell Int.* 19, 114
21. Cheng, D. *et al.* (2018) LncRNA HOTAIR epigenetically suppresses miR-122 expression in hepatocellular carcinoma via DNA methylation. *EBioMedicine* 36, 159–170
22. Price, R.L. *et al.* (2021) HOTAIR beyond repression: in protein degradation, inflammation, DNA damage response, and cell signaling. *DNA Repair* 105, 103141
23. Finnegan, E. and Kovac, K. (2000) Plant DNA methyltransferases. *Plant Mol. Biol.* 43, 189–201
24. Heo, J.B. *et al.* (2013) Epigenetic regulation by long noncoding RNAs in plants. *Chromosom. Res.* 21, 685–693
25. Davidovich, C. and Cech, T.R. (2015) The recruitment of chromatin modifiers by long noncoding RNAs: lessons from PRC2. *RNA* 21, 2007–2022
26. Heo, J.B. and Sung, S. (2011) Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. *Science* 331, 76–79
27. Wu, H.W. *et al.* (2018) A noncoding RNA transcribed from the AGAMOUS (AG) second intron binds to CURLY LEAF and represses AG expression in leaves. *New Phytol.* 219, 1480–1491
28. Bure, I.V. *et al.* (2022) Histone modifications and non-coding RNAs: mutual epigenetic regulation and role in pathogenesis. *Int. J. Mol. Sci.* 23, 5801
29. Fang, J. *et al.* (2019) PIRCh-seq: functional classification of non-coding RNAs associated with distinct histone modifications. *Genome Biol.* 20, 292
30. Wang, K.C. *et al.* (2011) A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. *Nature* 472, 120–124
31. Yang, Y.W. *et al.* (2014) Essential role of lncRNA binding for WDR5 maintenance of active chromatin and embryonic stem cell pluripotency. *Elife* 3, e02046
32. Zhao, X. *et al.* (2018) Global identification of *Arabidopsis* lncRNAs reveals the regulation of MAF4 by a natural antisense RNA. *Nat. Commun.* 9, 5056
33. Wang, Y. *et al.* (2018) Overexpressing lncRNA LAIR increases grain yield and regulates neighbouring gene cluster expression in rice. *Nat. Commun.* 9, 3516
34. Ganguly, P. *et al.* (2021) The natural antisense transcript DONE40 derived from the lncRNA ENOD40 locus interacts with SET domain protein ASHR3 during inception of symbiosis in *Arachis hypogaea*. *Mol. Plant-Microbe Interact.* 34, 1057–1070
35. Liu, X. *et al.* (2018) A novel antisense long noncoding RNA, TWISTED LEAF, maintains leaf blade flattening by regulating its associated sense R2R3-MYB gene in rice. *New Phytol.* 218, 774–788
36. Bernard, L.D. *et al.* (2022) OCT4 activates a Suv39h1-repressive antisense lncRNA to couple histone H3 Lysine 9 methylation to pluripotency. *Nucleic Acids Res.* 50, 7367–7379
37. Scarola, M. *et al.* (2015) Epigenetic silencing of Oct4 by a complex containing SUV39H1 and Oct4 pseudogene lncRNA. *Nat. Commun.* 6, 7631
38. Monnier, P. *et al.* (2013) H19 lncRNA controls gene expression of the Imprinted Gene Network by recruiting MBD1. *Proc. Natl. Acad. Sci. U. S. A.* 110, 20693–20698
39. Xi, J. *et al.* (2022) LncRNA SOX1-OT V1 acts as a decoy of HDAC10 to promote SOX1-dependent hESC neuronal differentiation. *EMBO Rep.* 23, e53015
40. Arunkumar, G. (2023) LncRNAs: the good, the bad, and the unknown. *Biochem. Cell Biol.*, Published online August 14, 2023. https://doi.org/10.1139/bcb-2023-0155
41. Xu, W. *et al.* (2017) The R-loop is a common chromatin feature of the *Arabidopsis* genome. *Nat. Plants* 3, 704–714
42. Sanz, L.A. *et al.* (2016) Prevalent, dynamic, and conserved R-loop structures associate with specific epigenomic signatures in mammals. *Mol. Cell* 63, 167–178
43. Fang, Y. *et al.* (2019) Characterization of functional relationships of R-loops with gene transcription and epigenetic modifications in rice. *Genome Res.* 29, 1287–1297
44. Kim, A. and Wang, G.G. (2021) R-loop and its functions at the regulatory interfaces between transcription and (epi) genome. *Biochim. Biophys. Acta Gene Regul. Mech.* 1864, 194750
45. Tan-Wong, S.M. *et al.* (2019) R-loops promote antisense transcription across the mammalian genome. *Mol. Cell* 76, 600–616
46. Moison, M. *et al.* (2021) The lncRNA APOLO interacts with the transcription factor WRKY42 to trigger root hair cell expansion in response to cold. *Mol. Plant* 14, 937–948
47. Ariel, F. *et al.* (2020) R-Loop mediated trans action of the APOLO long noncoding RNA. *Mol. Cell* 77, 1055–1065
48. Conn, V. *et al.* (2017) A circRNA from SEPALLATA3 regulates splicing of its cognate mRNA through R-loop formation. *Nat. Plants* 3, 17053
49. Liu, X. *et al.* (2021) Genome-wide profiling of circular RNAs, alternative splicing, and R-loops in stem-differentiating xylem of *Populus trichocarpa*. *J. Integr. Plant Biol.* 63, 1294–1308
50. Memczak, S. *et al.* (2013) Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 495, 333–338
51. Arab, K. *et al.* (2019) GADD45A binds R-loops and recruits TET1 to CpG island promoters. *Nat. Genet.* 51, 217–223

52. Kienhöfer, S. *et al.* (2015) GADD45a physically and functionally interacts with TET1. *Differentiation* 90, 59–68
53. Bartel, D.P. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116, 281–297
54. Franco-Zorrilla, J.M. *et al.* (2007) Target mimicry provides a new mechanism for regulation of microRNA activity. *Nat. Genet.* 39, 1033–1037
55. Huntzinger, E. and Izaurralde, E. (2011) Gene silencing by microRNAs: contributions of translational repression and mRNA decay. *Nat. Rev. Genet.* 12, 99–110
56. Ameres, S.L. and Zamore, P.D. (2013) Diversifying microRNA sequence and function. *Nat. Rev. Mol. Cell Biol.* 14, 475–488
57. Iwakawa, H.-O. and Tomari, Y. (2013) Molecular insights into microRNA-mediated translational repression in plants. *Mol. Cell* 52, 591–601
58. Liu, Q. *et al.* (2014) Analysis of complementarity requirements for plant microRNA targeting using a Nicotiana benthamiana quantitative transient assay. *Plant Cell* 26, 741–753
59. Rhoades, M.W. *et al.* (2002) Prediction of plant microRNA targets. *Cell* 110, 513–520
60. Cho, J. and Paszkowski, J. (2017) Regulation of rice root development by a retrotransposon acting as a microRNA sponge. *Elife* 6, e30038
61. Wu, H.-J. *et al.* (2013) Widespread long noncoding RNAs as endogenous target mimics for microRNAs in plants. *Plant Physiol.* 161, 1875–1884
62. Todesco, M. *et al.* (2010) A collection of target mimics for comprehensive analysis of microRNA function in Arabidopsis thaliana. *PLoS Genet.* 6, e1001031
63. He, J.H. *et al.* (2017) Overexpression of long non-coding RNA MEG3 inhibits proliferation of hepatocellular carcinoma Huh7 cells via negative modulation of miRNA-664. *J. Cell. Biochem.* 118, 3713–3721
64. D'Angelo, D. *et al.* (2019) RPSAP52 lncRNA is overexpressed in pituitary tumors and promotes cell proliferation by acting as miRNA sponge for HMGA proteins. *J. Mol. Med.* 97, 1019–1032
65. Gu, J. *et al.* (2018) Effect of the LncRNA GAS5-MiR-23a-ATG3 axis in regulating autophagy in patients with breast cancer. *Cell. Physiol. Biochem.* 48, 194–207
66. Luo, Y. *et al.* (2020) LncRNA DANCR promotes proliferation and metastasis in pancreatic cancer by regulating miRNA-33b. *FEBS Open Biol.* 10, 18–27
67. Liu, J. *et al.* (2004) Argonaute2 is the catalytic engine of mammalian RNAi. *Science* 305, 1437–1441
68. Meister, G. *et al.* (2004) Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. *Mol. Cell* 15, 185–197
69. Chekulaeva, M. *et al.* (2011) miRNA repression involves GW182-mediated recruitment of CCR4–NOT through conserved W-containing motifs. *Nat. Struct. Mol. Biol.* 18, 1218–1226
70. Zekri, L. *et al.* (2009) The silencing domain of GW182 interacts with PABPC1 to promote translational repression and degradation of microRNA targets and is required for target release. *Mol. Cell. Biol.* 29, 6220–6231
71. Kong, P. *et al.* (2019) circ-Sirt1 controls NF-κB activation via sequence-specific interaction and enhancement of SIRT1 expression by binding to miR-132/212 in vascular smooth muscle cells. *Nucleic Acids Res.* 47, 3580–3593
72. Cao, P. *et al.* (2022) hsa_circ_0003410 promotes hepatocellular carcinoma progression by increasing the ratio of M2/M1 macrophages through the miR-139-3p/CCL5 axis. *Cancer Sci.* 113, 634–647
73. Westholm, J.O. *et al.* (2014) Genome-wide analysis of drosophila circular RNAs reveals their structural and sequence properties and age-dependent neural accumulation. *Cell Rep.* 9, 1966–1980
74. Bachmayr-Heyda, A. *et al.* (2015) Correlation of circular RNA abundance with proliferation–exemplified with colorectal and ovarian cancer, idiopathic lung fibrosis and normal human tissues. *Sci. Rep.* 5, 8057
75. Rybak-Wolf, A. *et al.* (2015) Circular RNAs in the mammalian brain are highly abundant, conserved, and dynamically expressed. *Mol. Cell* 58, 870–885
76. Zhou, J. *et al.* (2021) Efficient deletion of multiple circle RNA loci by CRISPR-Cas9 reveals Os06circ02797 as a putative sponge for OsMIR408 in rice. *Plant Biotechnol. J.* 19, 1240–1252
77. Ye, J. *et al.* (2019) AtCircDB: a tissue-specific database for *Arabidopsis* circular RNAs. *Brief. Bioinform.* 20, 58–65
78. Xing, Y. *et al.* (2019) Long noncoding RNA-maternally expressed gene 3 contributes to hypoxic pulmonary hypertension. *Mol. Ther.* 27, 2166–2181
79. Zhang, J. *et al.* (2016) Long noncoding RNA MEG3 is downregulated in cervical cancer and affects cell proliferation and apoptosis by regulating miR-21. *Cancer Biol. Ther.* 17, 104–113
80. Biswas, A. *et al.* (2021) Co-regulatory functions of miRNA and lncRNA in adapting biotic and abiotic stress in economically important dicot plants. *Plant Gene.* 26, 100275
81. Cai, Z. *et al.* (2020) RIC-seq for global in situ profiling of RNA–RNA spatial interactions. *Nature* 582, 432–437
82. Cantile, M. *et al.* (2021) Functional interaction among lncRNA HOTAIR and microRNAs in cancer and other human diseases. *Cancers* 13, 570
83. Meredith, E.K. *et al.* (2016) An RNA matchmaker protein regulates the activity of the long noncoding RNA HOTAIR. *RNA* 22, 995–1010
84. Balas, M.M. *et al.* (2021) Establishing RNA-RNA interactions remodels lncRNA structure and promotes PRC2 activity. *Sci. Adv.* 7, eabc9191
85. Szcześniak, M.W. *et al.* (2016) CANTATAdb: a collection of plant long non-coding RNAs. *Plant Cell Physiol.* 57, e8
86. Graveley, B.R. (2001) Alternative splicing: increasing diversity in the proteomic world. *Trends Genet.* 17, 100–107
87. Will, C.L. and Lührmann, R. (2011) Spliceosome structure and function. *Cold Spring Harb. Perspect. Biol.* 3, a003707
88. Shi, Y. (2017) The spliceosome: a protein-directed metalloribozyme. *J. Mol. Biol.* 429, 2640–2653
89. Barbosa-Morais, N.L. *et al.* (2006) Systematic genome-wide annotation of spliceosomal proteins reveals differential gene family expansion. *Genome Res.* 16, 66–77
90. Romero-Barrios, N. *et al.* (2018) Splicing regulation by long noncoding RNAs. *Nucleic Acids Res.* 46, 2169–2184
91. Bazin, J. *et al.* (2018) Nuclear speckle RNA binding proteins remodel alternative splicing and the non-coding *Arabidopsis* transcriptome to regulate a cross-talk between Auxin and immune responses. *Front. Plant Sci.* 9, 1209
92. Bardou, F. *et al.* (2014) Long noncoding RNA modulates alternative splicing regulators in *Arabidopsis*. *Dev. Cell* 30, 166–176
93. Rigo, R. *et al.* (2020) The *Arabidopsis* lncRNA ASCO modulates the transcriptome through interaction with splicing factors. *EMBO Rep.* 21, e48977
94. Barry, G. *et al.* (2014) The long non-coding RNA Gomafu is acutely regulated in response to neuronal activation and involved in schizophrenia-associated alternative splicing. *Mol. Psychiatry* 19, 486–494
95. Ishizuka, A. *et al.* (2014) Formation of nuclear bodies by the lnc RNA Gomafu-associating proteins Celf3 and SF 1. *Genes Cells* 19, 704–721
96. Cooper, D.R. *et al.* (2014) Long non-coding RNA NEAT1 associates with SRp40 to temporally regulate PPARγ2 splicing during adipogenesis in 3T3-L1 cells. *Genes* 5, 1050–1063
97. Hutchinson, J.N. *et al.* (2007) A screen for nuclear transcripts identifies two linked noncoding RNAs associated with SC35 splicing domains. *BMC Genomics* 8, 39
98. Tripathi, V. *et al.* (2010) The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. *Mol. Cell* 39, 925–938
99. Miao, H. *et al.* (2022) MALAT1 modulates alternative splicing by cooperating with the splicing factors PTBP1 and PSF. *Sci. Adv.* 8, eabq7289
100. Jin, Y. and Marquardt, S. (2021) Winter fields antisense RNAs to kick off flowering. *Genes Dev.* 35, 785–786
101. Kindgren, P. *et al.* (2018) Transcriptional read-through of the long non-coding RNA SVALKA governs plant cold acclimation. *Nat. Commun.* 9, 4561
102. Zacharaki, V. *et al.* (2023) The non-coding RNA SVALKA locus produces a cis-natural antisense transcript that negatively regulates the expression of CBF1 and biomass production at normal temperatures. *Plant Commun.* 4, 100551

103. Henriques, R. *et al.* (2017) The antiphasic regulatory module comprising CDF5 and its antisense RNA FLORE links the circadian clock to photoperiodic flowering. *New Phytol.* 216, 854–867
104. Yatusevich, R. *et al.* (2017) Antisense transcription represses *Arabidopsis* seed dormancy QTL DOG 1 to regulate drought tolerance. *EMBO Rep.* 18, 2186–2196
105. Fedak, H. *et al.* (2016) Control of seed dormancy in *Arabidopsis* by a cis-acting noncoding antisense transcript. *Proc. Natl. Acad. Sci. U. S. A.* 113, E7846–E7855
106. Ramírez Gonzales, L. *et al.* (2021) Potato CYCLING DOF FACTOR 1 and its lncRNA counterpart StFLORE link tuber development and drought response. *Plant J.* 105, 855–869
107. Jin, Y. *et al.* (2023) LncRNA FLAIL affects alternative splicing and represses flowering in *Arabidopsis*. *EMBO J.* 42, e110921
108. Sanchez, A. *et al.* (2023) The long non-coding RNA ANRIL in cancers. *Cancers* 15, 4160
109. Cagle, P. *et al.* (2021) KCNQ1OT1: an oncogenic long noncoding RNA. *Biomolecules* 11, 1602
110. Lu, C.W. *et al.* (2018) HOXA 11 antisense long noncoding RNA (HOXA 11-AS): a promising lnc RNA in human cancers. *Cancer Med.* 7, 3792–3799
111. Chen, C. *et al.* (2020) Long noncoding RNA KCNQ1OT1 promotes colorectal carcinogenesis by enhancing aerobic glycolysis via hexokinase-2. *Aging* 12, 11685
112. Zhang, C. *et al.* (2020) LncRNA ANRIL acts as a modular scaffold of WDR5 and HDAC3 complexes and promotes alteration of the vascular smooth muscle cell phenotype. *Cell Death Dis.* 11, 435
113. Zhang, W.I. *et al.* (2020) Extracellular vesicle long non-coding RNA-mediated crosstalk in the tumor microenvironment: Tiny molecules, huge roles. *Cancer Sci.* 111, 2726–2735
114. Nie, H. *et al.* (2021) Exosomal long non-coding RNAs: Emerging players in cancer metastasis and potential diagnostic biomarkers for personalized oncology. *Genes Dis.* 8, 769–780
115. Qu, L.E. *et al.* (2016) Exosome-transmitted lncARSR promotes sunitinib resistance in renal cancer by acting as a competing endogenous RNA. *Cancer Cell* 29, 653–668
116. Bian, E.B. *et al.* (2019) Exosomal lncRNA-ATB activates astrocytes that promote glioma cell invasion. *Int. J. Oncol.* 54, 713–721
117. Yang, X. *et al.* (2019) LncRNA MALAT1 shuttled by bone marrow-derived mesenchymal stem cells-secreted exosomes alleviates osteoporosis through mediating microRNA-34c/SATB2 axis. *Aging* 11, 8777
118. Ma, W. *et al.* (2021) Long non-coding RNA LINC00470 in serum derived exosome: a critical regulator for proliferation and autophagy in glioma cells. *Cancer Cell Int.* 21, 149
119. Lan, F. *et al.* (2021) Serum exosomal lncRNA XIST is a potential non-invasive biomarker to diagnose recurrence of triple-negative breast cancer. *J. Cell. Mol. Med.* 25, 7602–7607
120. Ding, X.-Z. *et al.* (2021) Serum exosomal lncRNA DLX6-AS1 is a promising biomarker for prognosis prediction of cervical cancer. *Technol. Cancer Res. Treat.* 20, 1533033821990060
121. Zhang, Z. *et al.* (2019) Plant lncRNAs are enriched in and move systemically through the phloem in response to phosphate deficiency. *J. Integr. Plant Biol.* 61, 492–508
122. Ham, B.-K. *et al.* (2009) A polypyrimidine tract binding protein, pumpkin RBP50, forms the basis of a phloem-mobile ribonucleoprotein complex. *Plant Cell* 21, 197–215
123. Cheng, S.L.H. *et al.* (2023) Systemic movement of long non-coding RNA ELENA1 attenuates leaf senescence under nitrogen deficiency. *Nat. Plants* 9, 1598–1606
124. Stárka, L. and Dušková, M. (2020) What is a hormone? *Physiol. Res.* 69, S183
125. Taoka, K.-I. *et al.* (2011) 14-3-3 proteins act as intracellular receptors for rice Hd3a florigen. *Nature* 476, 332–335
126. Mittler, R. *et al.* (2022) Reactive oxygen species signalling in plant stress responses. *Nat. Rev. Mol. Cell Biol.* 23, 663–679
127. Davies, P.J. (2010) The plant hormones: their nature, occurrence, and functions. In *Plant Hormones: Biosynthesis, Signal Transduction, Action!* (Davies, P.J., ed.), pp. 1–15, Springer
128. Unfried, J.P. and Ulitsky, I. (2022) Substoichiometric action of long noncoding RNAs. *Nat. Cell Biol.* 24, 608–615
129. Yang, M. *et al.* (2022) In vivo single-molecule analysis reveals COOLAIR RNA structural diversity. *Nature* 609, 394–399
130. Yu, Y. *et al.* (2020) Transcriptional landscape of pathogen-responsive lnc RNA s in rice unveils the role of ALEX 1 in jasmonate pathway and disease resistance. *Plant Biotechnol. J.* 18, 679–690
131. Jiang, H. *et al.* (2019) Identification and characterization of long non-coding RNAs involved in embryo development of *Ginkgo biloba*. *Plant Signal. Behav.* 14, 1674606
132. Liu, S. *et al.* (2018) Identification and characterization of long non-coding RNAs involved in the formation and development of poplar adventitious roots. *Ind. Crop. Prod.* 118, 334–346
133. Eom, S.H. *et al.* (2019) Identification and functional prediction of drought-responsive long non-coding RNA in tomato. *Agronomy* 9, 629
134. Fu, L. *et al.* (2020) Genome-wide discovery and functional prediction of salt-responsive lncRNAs in duckweed. *BMC Genomics* 21, 212
135. Sun, X. *et al.* (2020) Comparative transcriptome analysis reveals new lncRNAs responding to salt stress in sweet sorghum. *Front. Bioeng. Biotechnol.* 8, 331
136. Bolha, L. *et al.* (2017) Long noncoding RNAs as biomarkers in cancer. *Dis. Markers* 2017, 7243968
137. Yang, H. *et al.* (2023) Long non-coding RNAs of plants in response to abiotic stresses and their regulating roles in promoting environmental adaption. *Cells* 12, 729
138. Zheng, X.M. *et al.* (2019) Genome-wide analyses reveal the role of noncoding variation in complex traits during rice domestication. *Sci. Adv.* 5, eaax3619
139. Parvathaneni, R.K. *et al.* (2020) The regulatory landscape of early maize inflorescence development. *Genome Biol.* 21, 165
140. Kunkler, C.N. *et al.* (2019) Stability of an RNA•DNA–DNA triple helix depends on base triplet composition and length of the RNA third strand. *Nucleic Acids Res.* 47, 7213–7222
141. Li, Y. *et al.* (2016) RNA-DNA triplex formation by long noncoding RNAs. *Cell Chem. Biol.* 23, 1325–1333
142. Greifenstein, A.A. *et al.* (2021) RNA: DNA triple helices: from peculiar structures to pervasive chromatin regulators. *Essays Biochem.* 65, 731–740
143. Trembinski, D.J. *et al.* (2020) Aging-regulated anti-apoptotic long non-coding RNA Sarrah augments recovery from acute myocardial infarction. *Nat. Commun.* 11, 2039
144. Zhou, Z. *et al.* (2019) DNA· RNA triple helix formation can function as a cis-acting regulatory mechanism at the human β-globin locus. *Proc. Natl. Acad. Sci. U. S. A.* 116, 6130–6139
145. Kalwa, M. *et al.* (2016) The lncRNA HOTAIR impacts on mesenchymal stem cells via triple helix formation. *Nucleic Acids Res.* 44, 10631–10643
146. Buske, F.A. *et al.* (2012) Triplexator: detecting nucleic acid triple helices in genomic and transcriptomic data. *Genome Res.* 22, 1372–1381
147. Kuo, C.-C. *et al.* (2019) Detection of RNA–DNA binding sites in long noncoding RNAs. *Nucleic Acids Res.* 47, e32
148. Tao, X. *et al.* (2021) Neofunctionalization of a polyploidization-activated cotton long intergenic non-coding RNA DAN1 during drought stress regulation. *Plant Physiol.* 186, 2152–2168
149. Zhang, Y.-C. *et al.* (2022) Genome-wide analysis and functional annotation of chromatin-enriched noncoding RNAs in rice during somatic cell regeneration. *Genome Biol.* 23, 28
150. Sentürk Cetin, N. *et al.* (2019) Isolation and genome-wide characterization of cellular DNA: RNA triplex structures. *Nucleic Acids Res.* 47, 2306–2321
151. Lubitz, I. *et al.* (2010) Specific high-affinity binding of thiazole orange to triplex and G-quadruplex DNA. *Biochemistry* 49, 3567–3574
152. Derick, S. *et al.* (2017) LUCS (Light-Up Cell System), a universal high throughput assay for homeostasis evaluation in live cells. *Sci. Rep.* 7, 18069
153. Brown, J.A. (2020) Unraveling the structure and biological functions of RNA triple helices. *Wiley Interdiscip. Rev. RNA* 11, e1598