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Mini Review

Long noncoding RNAs: New regulators in plant development

Yu-Chan Zhang, Yue-Qin Chen [*]

Key Laboratory of Gene Engineering of the Ministry of Education, State Key Laboratory for Biocontrol, School of Life Science, Sun Yat-Sen University, Guangzhou 510275, PR China

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ABSTRACT

Plant long noncoding RNAs (lncRNAs) play important roles in a wide range of biological processes, especially in plant reproductive development and response to stresses. They are transcribed by RNA polymerase II (Pol II), Pol III and Pol V, and exert their functions by a variety of regulation pathways. In this review, we summarized the current knowledge of lncRNAs discoveries in plant, including their identification, functions and regulation pathways as well as production and mediators, with an emphasizing on the novel regulation mechanisms in plant development.

1. Introduction

Emerging evidences showed that there exit a large amount of long noncoding RNAs (lncRNAs), a less well-characterized class of ncRNAs in the plant genome [1]. LncRNAs are greater than 200 nt in length, mRNA-like transcripts, are mainly transcribed by RNA polymerase II (Pol II) and are polyadenylated, spliced, and mostly localized in the nucleus [2]. These novel molecules play important roles in a wide range of biological processes including in developmental regulations and stress responses although the detailed mechanisms remain largely unknown [1]. More intriguingly, several lncRNAs were found involvement in plant reproductive developments [3] and responses to pathogen invasion [4,5]. Further to explore the interaction between lncRNAs and plant development would provide novel and feasible approaches to disclose the complicated regulatory networks of developmental control. Thus, in this review, we summarized the current knowledge of lncRNAs discoveries in plant, with an emphasizing on the novel regulation mechanisms in plant development.

2. LncRNAs discoveries in different plant model species

Although lncRNAs are developmentally regulated and play roles in development and stress responses of plants [1], however, systematic identification of lncRNAs is limited to a few plant species [4–8]. LncRNAs can be classified as natural antisense transcripts (NATs), long intronic noncoding RNAs and long intergenic noncoding RNAs (lincRNAs) according to their characteristics. In Arapdopsis, 6480 intergenic transcripts can be classified as lincRNAs by using a tiling array-based strategy, among which 2708 lincRNAs was detected by RNA sequencing experiments [4]. Interestingly, a subset of lincRNA genes shows organ-specific expression, whereas others are responsive to biotic and/or abiotic stresses. More interestingly, Wu et al. identified a number of lncRNAs as Endogenous Target Mimics (eTM) for microRNAs (miRNAs) in both Arabidopsis and rice, in which the eTMs of several miRNAs, such as miR160, miR166, miR156, miR159 and miR172, can effectively inhibit the functions of their corresponding miRNAs, and the eTMs of miR160 and miR166 play a role in regulation of plant development [6]. Recently, Xin et al. applied computational analysis and experimental approach identifying 125 putative wheat stress responsive lncRNAs, which are not conserved among plant species [5]. Among them, two lncRNAs were identified as signal recognition particle (SRP) 7S RNA variants, and three were characterized as U3 snoRNAs. Furthermore, the wheat lncRNAs also showed tissue dependent expression patterns like the lncRNAs in Arabidopsis [5], suggesting that the highly tissue-specific expression pattern might be a general trait of lncRNAs in plant development.

In addition to Arabdopsis and wheat, Zhang et al. have analyzed global patterns of allelic gene expression in developing maize endosperms from reciprocal crosses between inbreds B73 and Mo17, and found that 38 lncRNAs expressed in the endosperm are imprinted. Among them, 25 are maternally expressed transcripts, whereas 13 are paternally expressed transcripts, and transcribed in either sense or antisense orientation from intronic regions of normal protein-coding genes or from intergenic regions [7]. Subsequently, Boerner et al. identify the potential lncRNAs using the maize full length cDNA sequences. The results showed the noncoding transcription appears to be widespread in the maize genome, and these ncRNAs were predicted to originate from both

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[*] Corresponding author. Fax: +86 20 84036551.
E-mail address: lsscyq@mail.sysu.edu.cn (Y.-Q. Chen).

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genic and intergenic loci. Computational predictions indicated that they may function to regulate expression of other genes through multiple RNA mediated mechanisms [8]. The growing reports of lncRNA identifications in different species indicate that lncRNAs ubiquitously exist in the plant kingdom with conserved roles.

More recently, we developed a computational approach for comprehensive identification of lincRNAs from rice using 40 existing rice RNA-Seq data sets. Genome wide screening identified 2063 lincRNAs in rice, and most of them have a reproductive process preferred expressing pattern. Further functional analyze showed a set of lincRNAs could induce reproductive deficiencies (unpublished data). These studies would provide new insight into the involvement of lncRNAs in the reproductive development of rice. All together suggest that there are a large number of lncRNAs exist in various plant species, which might play a role in regulating the plant development and stress response.

3. LncRNAs are likely related to plant development by a variety of regulation pathways

In contrast to small ncRNAs, much less is known about the functions and regulatory mechanisms of long ncRNAs, and only a few lncRNAs' functions are characterized. The most remarkable function of mammal lncRNAs in regulating gene expressing is to mediate the chromatin remodeling. In plants, lncRNA COLDAIR also functions through this regulation pathway. COLDAIR participates in the repression of *FLC* after vernalization [5]. It could bind PRC2 complex protein CURLY LEAF (CLF), with maximal interaction after 20 days of vernalization/cold exposure, and is required to recruit PRC2 to the *FLC* locus allowing deposition of the repressive H3K27me3 chromatin mark [3]. Although our knowledge of this kind of lncRNAs is still limited, there must be more lncRNAs acting in the regulation of chromatin remodeling way.

The second regulatory mechanism of lncRNAs in plants might work as a decoy of miRNAs [6], these kind of lncRNAs exert their functions by binding miRNAs in a target mimicry mechanism to sequestrate the miRNAs' regulation roles on their target genes, such as lncRNAs IPS1 and at4. IPS1 is complementary to miR399, but contains a mismatch loop which makes it uncleavable when miR399 binding. It is induced by phosphate starvation in plants, and then allows the increased expression of miR399 targets including *PHO2*, consequently alters shoot phosphate content [9–11]. IPS1 has many family members in a number of plant species, including At4, At4–1, At4–2 and At4–3 in *Arabidopsis thaliana*, TPS11 in tomato, Mt4 in Barrel Clover and Alfalfa, Mt4-like in Soybean [9,12]. At4 appears to be functionally redundant with IPS1, overexpression of one of them could result in the same phenotypic change as that when overexpressing both At4 and IPS1 [13]. Moreover, the identification of a number of eTM lncRNAs by Wu et al. also supported universality of lncRNAs working as decoy of miRNAs [6].

Another function of lncRNAs in plant might serve as natural antisense transcripts (NATs) of mRNAs. An example is lncRNA COOLAIR [3,14]. COOLAIR is a cold induced antisense transcripts of FLC gene, and generally shows a positive correlation with FLC transcripts, except upon cold treatment. In early cold induced vernalization, COOLAIR could silence *FLC* transcription [3,14]. However, Helliwell et al. reported that abolishment of COOLAIR does not compromise vernalization-mediated *FLC* silencing, thus the functions of these NATs needs further experimentally addressing [15].

The regulation pathways of most plant lncRNAs are still unclear, but the known models are all similar with that of mammal lncRNAs, suggesting that the regulation pathways of lncRNAs might be conserved in different species.

4. Regulation of reproductive development by lncRNAs

Although more than 9000 plant lncRNAs have been annotated, less than 1% of lncRNAs identified have been characterized [16–18]. Intriguingly, several well studied lncRNAs are reported to be involved in plant reproductive process. An example is the COLDAIR [3]. In Arabidopsis, COLDAIR was a cold induced lncRNA mentioned above, which was identified from the first intron of *FLC*. It is associated with PRC2 both in vitro and in vivo, and recruit PRC2 to the *FLC* locus to repress the *FLC* expression, allowing the unimpeded operation of the photoperiod pathway and starting the rapid flowering (Fig. 1A) [3]. Another cold induced lncRNA COOLAIR might also involved in vernalization-mediated *FLC* repression, although it might not essential in this process (Fig. 1A) [3,15].

Recently, a long-day-specific male-fertility-associated lincRNA (LDMAR) has been found to regulate the photoperiod-sensitive male sterility (PSMS) in rice (Fig. 1B). LDMAR is a lncRNA of 1236 bases in length, and the sufficient expression level of LDMAR transcripts is required for normal pollen development of rice plants grown under long-day conditions. A spontaneous mutation which altered the secondary structure of LDMAR could increase methylation in the putative promoter region of LDMAR, which reduced the transcription of LDMAR specifically under long-day conditions, resulting in premature programmed cell death in developing anthers, thus causing PSMS [19]. Further studies showed that the siRNA named Psi-LDMAR which derived from the promoter region of LDMAR is involved in the DNA methylation in the LDMAR promoter and repressed the expression of LDMAR though a RNA-dependent DNA methylation (RdDM) way (Fig. 1B) [20]. But the detailed biochemical mechanism of this lncRNA remains to be declared. This finding provides a new molecular mechanism of PSMS, and suggests the important regulation roles of lncRNAs in reproductive process in rice.

In addition to these well analyzed lncRNAs, some other lncRNAs were also reported to be associated with the reproductive regulation, although their regulatory mechanisms are still unclear. For examples, CsM10 is expressed preferentially under male expression conditions in cucumber cultivar, which might function in male sex differentiation [21]; Zm401 is expressed primarily in the anther (tapetal cells as well as microspores) in a developmentally regulated manner in Zea mays, and the knockdown of zm401 could significantly affect the expression of several critical genes for pollen development, such as zmMADS2, MZm3–3 and ZmC5, leading to aberrant development of the microspore and tapetum, and finally male-sterility [22]. In our recent study, we also observed most lincRNAs are reproductive process preferred expressing, and the insertion mutant of some of them could induce reproductive deficiencies (unpublished data). These findings indicate that there is a great potential of plant lincRNAs to function in regulating the reproductive process, which might be one of the principal roles of plant lncRNAs.

5. Stress-responsive long non-coding RNAs transcribed in plants

Emerging evidences showed that many lncRNAs also participate in the response to diverse stresses, including biotic stresses and pathogen infections. In Arabidopsis, 1832 lincRNAs have been found to significantly altered after 2 h and/or 10 h of drought, cold, high-salt, and/or abscisic acid (ABA) treatments, whereas treatment by elf18 (EF-Tu), which triggers pathogen-associated molecular pattern responses, could also increase the expression level of one of the representative stress responsive lincRNAs [4]. Similar with those identified in Arapdopsis [23], 125 putative stress (powdery mildew infection and heat stress) responsive lncRNAs were identified in wheat [5], suggesting that diverse sets of plant lncRNA were responsive to pathogen infection and stress induction, and

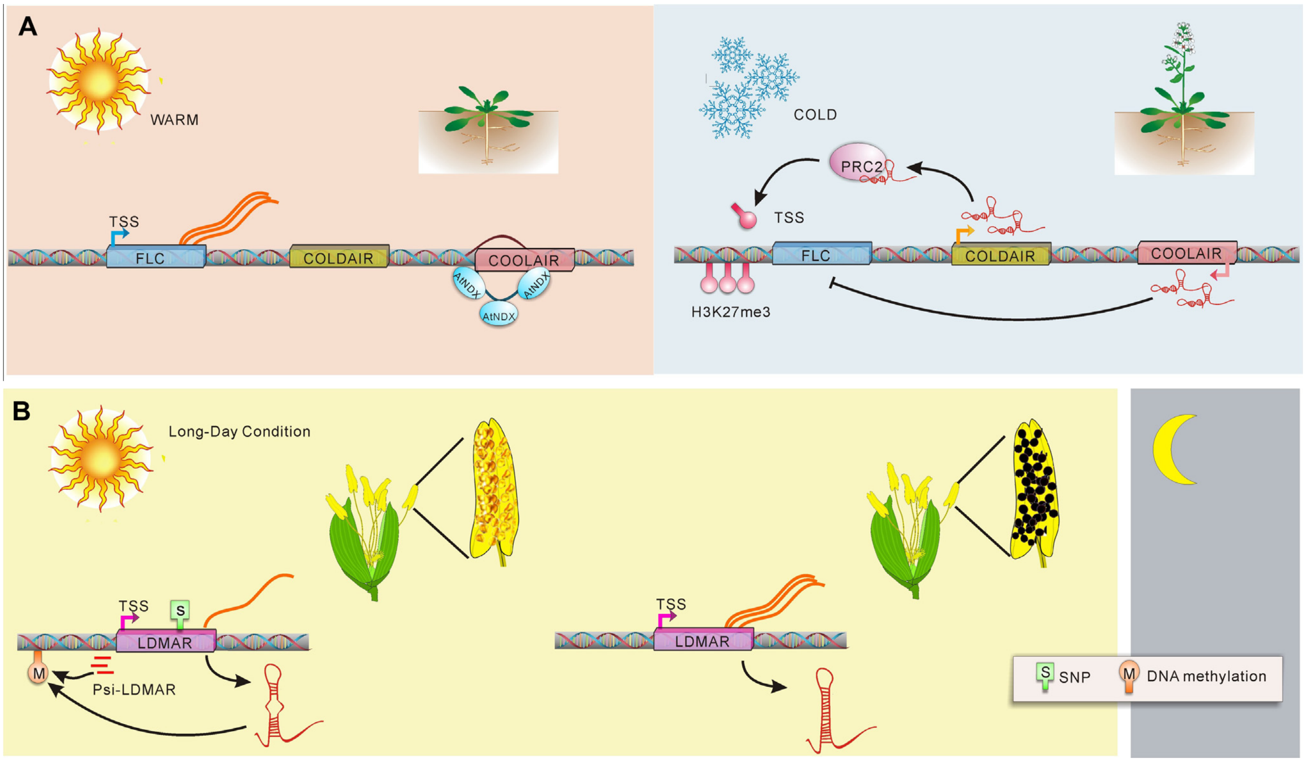

Fig. 1. LncRNAs function in regulation of reproductive development. (A) Schematic representation of the roles of COLDAIR and COOLAIR on the FLC expression and the regulation of COOLAIR by AtNDX during the course of vernalization. (B) Schematic illustrations of the regulation of LDMAR on anther development under long-day conditions regulated by Psi-LDMAR, SNP, RNA secondary structure and DNA methylation.

could function in plant responses to both biotic and abiotic stresses, which would provide a starting point to understand their functions and regulatory mechanisms in the future.

In addition to the large scale identification of stress responsive lncRNAs, several stress responsive lncRNAs have been further studied, such as COLDAIR and COOLAIR. Both are also reproductive process related lncRNAs and positively respond to cold weather, and function in inducing flowering of Arabidopsis under appropriate season [3,14]; Besides, IPS1 and At4 are induced by phosphate starvation, for they could block the repressing role of miR399 on its target gene *PHO2*, which then regulate the dynamic balance of shoot Pi content [9–11],[13]; Npc536 is another NATs lncRNA which up-regulated in roots and leaves of Arabidopsis submitted to stress by phosphate starvation and salt stress, and over-expressing npc536 could promote the root growth under salt stress conditions, with increased primary root growth and secondary root length [24]. Thus lncRNAs might be the important regulators in the biotic and abiotic stress response in plants.

6. The production of lncRNAs and its mediator in plant

As discussed above, the characterization and the functional analysis of lncRNAs are very limited, especially in plants, the mediators in lncRNAs production are also less well studied than that of miRNAs and mRNAs. Until now, the most identified lncRNAs are likely to be transcribed by RNA polymerase II (Pol II), 5' capped and 3' polyadenylated [5] just like that of the mRNAs. In addition, RNA polymerase IV and V (Pol IV/V) are two plant-specific polymerases that evolved from Pol II, which also function in lncRNAs production and exerting their roles [25]. Pol IV is required for siRNA biogenesis [26] and is required for RdDM, which is downstream event triggered by 24-nucleotide siRNAs. Pol V transcripts are proposed to work as scaffold transcripts for siRNAs to recognize their target loci and to mediate repressive chromatin modifications. Pol V-dependent lncRNAs are triphosphorylated or capped, but do not have poly A tails, and they could interact with Argonaute 4 (AGO4), and guide siRNA-AGO4 complexes to their targets in chromatin [25,27]. Interestingly, a recent report also showed that a set of lncRNAs might be the transcripts of Pol III in Arabidopsis, in which At8 is identified by efficient in vitro transcription by Pol III in tobacco nuclear extracts [23]. Pol III usually generates relatively short, high-quantity and stable RNAs, such as 5S rRNAs and tRNAs. Whether the transcription of At8 by Pol III is a special case or a ubiquitous mechanism needs to be further proved.

Mediator, a multi-subunit complex first identified in yeast, promotes the initiation of mRNAs transcription by Pol II in lots of species including plants [28]. Mediator is highly conserved in a wide range of eukaryotes, and the Arabidopsis Mediator contains 21 subunits conserved in yeast and metazoan counterparts and 6 plant-specific subunits [29]. Only a few genes encoding these subunits have been characterized, such as *MED14*, *MED21* and *MED25*[30,31]. Interestingly, Kim et al. found that Mediator also promotes the Pol II-mediated production of long noncoding scaffold RNAs. In the med17, med18 and med20 mutants, the Pol II occupancy decreases and the noncoding scaffold RNAs transcribed by Pol II are reduced, and this supports the role of Mediator in Pol II-mediated lncRNA production [32]. But whether the Mediator also promote the production of Pol V-dependent lncRNAs remains to be determined.

More recently, a novel regulation pathway for lncRNA expression was reported [33]. They identified a homeodomain protein, AtNDX, which is involved in regulating the NATs lncRNA COOLAIR expression. AtNDX associates with single-stranded DNA, which is part of an RNA–DNA hybrid, or R-loop, that covers the COOLAIR promote. R-loops were initially thought to be a rare byproduct of transcription but recently have been found to cause genome instability. This R-loop stabilization mediated by AtNDX inhibits COOLAIR transcription and the following *FLC* regulation (Fig. 1A) [33].

This result suggests the diversity and complex of the regulatory mechanisms influencing lncRNA expression, and more examples need to be explored.

Concluding remarks

Recent studies revealed that lncRNAs function as important regulators in gene regulatory networks and exert crucial roles in various biological processes of plants. Although a number of lncRNAs have been identified from Arabidopsis, wheat, maize and rice, and several lncRNAs' functions have been characterized, however, much still remains to be done. Excavating the regulation role of more and more lncRNAs in plant development and stress response can greatly not only enlarge our understanding of the interaction of lncRNAs in plant cells but also uncover the mechanisms that under plant development. In the near future, it would be necessary to further investigate the functional motifs and secondary or tertiary structure of lncRNAs, to fully elucidate the diverse gene regulatory mechanisms of lncRNAs, and to develop the new and effective methods to investigate target genes of lncRNAs. These endeavors would provide new insights into the complicated gene regulatory network involving lncRNAs during the life cycle of plants.

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