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Review

Long non-coding RNAs: Fine-tuning the developmental responses in plants

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Plant developmental biology is associated with various gene regulatory pathways involved in different phases of their life cycle. In course of development, growth and differentiation of different organs in plants are regulated by specific sets of gene expression. With the advances in genomic and bioinformatic techniques, particularly high-throughput sequencing technology, many transcriptional units with no protein-coding potential have been discovered. Previously thought to be the dark matters of genome, long non-coding RNAs (lncRNAs) are gradually gaining importance as crucial players in gene regulation during different developmental phases. Some lncRNAs, showing complementarity to microRNAs (miRNAs), are used as endogenous target mimics of specific miRNA family. A number of lncRNAs can also act as natural antisense transcripts to attenuate the expression of coding genes. Although lncRNA-mediated regulations have extensively been studied in animals, plant lncRNA research is still in its initial phase. The present review highlights the regulatory mechanism and different physiological aspects of lncRNAs in plant development. In plants, lncRNAs are found to be associated with a number of plant developmental functions such as lateral root development, vernalization, photomorphogenesis, pollen development, fiber development and nodulation. Understanding these potent roles of lncRNAs in plant development can further provide novel tools for crop improvement programs in future.

Keywords. Fiber development; long non-coding RNAs; nodule development; photomorphogenesis; pollen development; root development

Abbreviations: ABA, abscisic acid; APOLO, auxin-regulated promoter loop; ARF, auxin response factor; ASCO-RNA, alternative splicing competitor long non-coding RNA; CBP, cap-binding protein; eTM, endogenous target mimic; FLC, FLOWERING LOCUS C; FT, FLOWERING LOCUS T; HID1, hidden treasure 1; LDMAR, long day (LD)-specific male-fertility associated RNA; lincRNA, long intergenic non-coding RNA; lncRNA, long non-coding RNA; miRNA, microRNAs; NAT, natural antisense transcript; ncRNA, non-coding RNA; NMD, nonsense-mediated-mRNA decay; NSR, nuclear speckle RNA-binding protein; PID, PINOID; PIF, phytochrome-interacting factor; PRC1, polycomb recessive complex 1; RBP1, RNA binding protein 1; RdDM, RNA-dependent DNA methylation; siRNA, small interfering RNA; sncRNA, small non-coding RNA; snRNA, small nuclear RNA; UBP1, ubiquitin specific protease 1; UPF, UP-frameshift protein

1. Introduction

The growth and development of plants under particular environmental conditions are delicately regulated by various signaling molecules. These signaling molecules are of two types: external and internal. The external signaling molecules induce a change in the cellular gene expression leading to the development of internal signaling molecules. The internal signaling molecules can then act as an inducer for the second level of gene expression which promotes downstream signaling cascade and initiates a particular developmental phenomenon. The role of sugars, proteins and lipid molecules as signal transducers in numerous signaling pathways has been well deciphered. Nowadays, emerging evidence suggests the role of non-coding RNAs (ncRNAs) as important internal signaling modulators (Morange 2008; Axtell 2013; Dong *et al.* 2016). The ncRNAs can be classified into two groups. One group comprises small non-coding RNAs (sncRNAs) and the other includes long non-coding RNAs (lncRNAs). The sncRNAs are

generally less than 40 nucleotides in length and consist of small nuclear RNAs (snRNAs), small nucleolar RNAs, signal recognition particle RNAs, microRNAs (miRNAs) and small interfering RNAs (siRNAs). Throughout the plant kingdom, the role of miRNAs and siRNAs in controlling different physiological responses has already been demonstrated widely (Axtell 2013; Chitwood and Sinha 2014; Xie *et al.* 2015).

In contrast to sncRNAs, the second group comprises lncRNAs, which are more than 200 nucleotides long. Based on their origin, the lncRNAs can be further categorized into long intronic non-coding RNAs, natural antisense transcript (NAT) region, overlapping lncRNAs (that partially overlaps the coding gene) and long intergenic non-coding RNAs (lincRNAs; that lies between two protein coding genes) (Rinn and Chang 2012). Recent findings suggest that the lincRNA transcripts are 5′-capped, 3′-polyadenylated and many of them contain spliced introns without encoding proteins (Liu *et al.* 2012; Guttman *et al.* 2013). These observations suggest that, like mRNA, the lincRNAs also possess RNA polymerase II (RNA pol II)-mediated origin. However, unlike mRNA, they are without a long open reading frame (Rymarquis *et al.* 2008). The translational mechanism for lncRNAs also distinctly differs from that of mRNA (Chew *et al.* 2013; Zhou *et al.* 2013). Recently, a growing body of evidence has revealed the contribution of lncRNAs in gene regulation mechanisms in different plant species (Kim and Sung 2012). The role of lncRNAs as NATs and scaffolds for various protein complexes has been reported (Kim and Sung 2012; Lu *et al.* 2012). Intriguingly, in some plant species, they have also been reported to act as precursors for miRNAs (Xin *et al.* 2011). Recent advancements in molecular biology research have deciphered the role of lncRNAs as endogenous target mimics (eTMs) for miRNAs to regulate gene expression (Franco-Zorrilla *et al.* 2007; Meng *et al.* 2012; Yan *et al.* 2012; Wu *et al.* 2013). In addition, their multiple functions in the development of roots and reproductive organs, photomorphogenesis and fiber development in cotton have recently been elucidated (Xin *et al.* 2011; Di *et al.* 2014; Shuai *et al.* 2014; Zhu *et al.* 2014; Wang *et al.* 2015). However, the genome-wide identification of lncRNAs has only been explored in *Arabidopsis*, *Medicago*, maize and cotton to date (Wen *et al.* 2007; Song *et al.* 2009; Yan *et al.* 2012; Li *et al.* 2014; Wang *et al.* 2015).

Recently, the role of lncRNAs in sexual reproduction has been delineated in different plant species. Because precise gene regulation and cell division during sexual reproduction is common in plants, lncRNAs are thought to be involved in regulating sexuality by altering chromatin organization and making epigenetic changes (Golicz *et al.* 2018). In this review, the biogenesis of lncRNAs, mechanism of gene expression regulation and their role in different developmental phenomena have been delineated. The present review will help us to understand the diverse molecular mechanisms of lncRNA-mediated regulations of the specific developmental event in plants, like lateral root development, vernalization, photomorphogenesis, pollen development, fertility, fiber development and nodule formation, which may be used to develop novel biotechnological strategies for crop improvement programs in future.

2. Biogenesis of lncRNAs

Evidence of 5′-capped and 3′-polyadenylated lncRNAs in *Arabidopsis* favors an RNA pol II-mediated transcription from lncRNA genes, such as mRNA (Liu *et al.* 2012; Ulitsky and Bartel 2013; Blevins *et al.* 2014). The lncRNAs are also transcribed from various regions of the genome which includes transcription start and termination sites, enhancer or intron splicing regions. However, most of them are unstable (Jensen *et al.* 2013; Ntini *et al.* 2013). In some species, lncRNAs are also found to be transcribed by RNA pol III, IV and V (Wierzbicki *et al.* 2008). RNA pol IV and V generate noncoding RNAs through RNA-dependent DNA methylation (RdDM) which act as precursors for siRNAs and scaffold RNAs, respectively (He *et al.* 2014). The association of three protein complexes such as the transcription factor mediator complex, histone modification complex and transcription elongation factor complex facilitates the transcription of polyadenylated lncRNAs (Di *et al.* 2014). To maintain the quality, different RNA degradation pathways are involved for different groups of lncRNAs after transcription (van Dijk *et al.* 2011; Flynn *et al.* 2011; Ntini *et al.* 2013). Among them, the nonsense-mediated mRNA decay (NMD) pathway is common where the UP-frameshift (UPF) protein plays an important role. Interestingly, the *upf1-1* and *upf3-1*, the two NMD pathway deficient *Arabidopsis* mutants, accumulate high levels of non-coding transcripts which are distinctly different from lincRNAs (Kurihara *et al.* 2009). This finding confirms the involvement of other controlling pathways for sustaining the lincRNA transcript levels. In *Arabidopsis*, the mutant deficient of exosome activities revealed the higher accumulation of lncRNAs thus suggesting the role of the exosome in lncRNA degradation (Chekanova *et al.* 2007). Recently, a number of lincRNAs have been found to be associated with SERRATE and Cap-binding proteins (CBPs) such as CBP 20 and CBP 80 during transcription (Liu *et al.* 2012). The deficiency of these proteins leads to the accumulation of primary transcript of lincRNAs in an unspliced form (Liu *et al.* 2012). The presence of introns in 5′-untranslated region (UTR) of some primary lincRNA transcripts accelerates the nuclear export of RNAs for translation. On the contrary, repression of RNA silencing is induced by intron splicing (Christie *et al.* 2011; Akua and Shaul 2013).

In comparison with polyadenylated lncRNAs, non-polyadenylated lncRNAs are 50–300 nucleotides in length and has also been identified from *Arabidopsis* and rice (Liu *et al.* 2013; Wang *et al.* 2014b). They are known as intermediate-sized ncRNAs and exhibit low levels of expression. They are evolutionarily diverse in nature and are generally

confined to the 5′-UTR genomic regions (Wang *et al.* 2014b).

3. Role of lncRNAs in different developmental phenomena

lncRNAs play various physiological functions and have emerged as signaling molecules during different physiological responses in plants (Singh *et al.* 2018). The role of lncRNAs in plant development can be broadly categorized into several groups based on their functional properties (table 1). In different plant species, the lncRNAs are found to be associated with a number of plant developmental functions such as lateral root development, vernalization, photomorphogenesis, pollen development, plant fertility, fiber development and nodule formation. Moreover, lncRNAs regulate the plant development by four possible mechanisms: histone and chromatin modification, transcriptional regulation, target mimicking of miRNA and alteration of post-translational changes.

3.1 *lncRNAs in root development*

Auxin, a major growth hormone in plants critically regulates the primary growth of roots and lateral root formation in plants. The growth and development of roots depend on the concentration of auxin which is modulated by transport and auxin signaling responses. Recently, the role of lncRNAs in the regulation of auxin transport has been investigated in roots of *Arabidopsis* (Ariel *et al.* 2014). The changes of chromatin topology significantly affect the transcription. The lincRNA viz. *auxin-regulated promoter loop* (*APOLO*) has been found to be transcribed from a gene located at 5 kb upstream of *PINOID* (*PID*) gene which ensures the polar auxin transport. A reduced level of APOLO and PID has been found to be associated with altered primary root growth. The *APOLO* gene is transcribed by two types of RNA pols (RNA pol II and RNA pol IV/V) which finely control the *PID* gene expression via the formation of the chromatin loop surrounding the *PID* promoter. The increased auxin content in roots helps in the unwinding of the *APOLO–PID* chromatin loop by triggering demethylation and facilitates RNA pol II-mediated transcription from both the genes (figure 1). The increased RNA pol II activity recruits heterochromatic protein 1 (LHP1) for loop formation. Activated RNA pol IV/V facilitates siRNA-mediated DNA methylation on the *APOLO* locus which is necessary to stabilize the loop. This down-regulates the *PID* transcription. The accumulated *APOLO* gradually employs the polycomb recessive complex 1 (PRC1) to close the loop. In the closed state, RNA pol IV/V plays a vital role in the transcription of *APOLO* gene (Ariel *et al.* 2014). In addition, *cis*-acting elements of two auxin response factors (ARFs), ARF7 and ARF19, have been found to be present within the *APOLO* and *PID* genes suggesting the dynamic regulation of *PID* gene expression upon auxin treatment.

lncRNAs are also considered as a splicing regulator in plants. In *Arabidopsis*, a lncRNA family member, alternative splicing competitor long non-coding RNA (ASCO-RNA), along with nuclear speckle RNA-binding protein (NSR)

Table 1. Developmental functions of various lncRNAs identified in different plant species

Roles	Category-wise functions	Plants	lncRNAs	Molecular mechanisms	References
Growth and development	Photomorphogenesis	*Arabidopsis*	NATs *HID1*	Antisense-mediated gene silencing Histone modification, *PIF3* regulation	Wang *et al.* (2014c)
	Male sterility	*Oryza sativa*	*LDMAR*	RDRM-mediated DNA methylation	Ding *et al.* (2012); Zhou *et al.* (2012)
		B. Campestris	*MF*11	Not characterized	Song *et al.* (2007, 2013)
		Zea mays	Zm401	Not characterized	Ma *et al.* (2008)
	Polar auxin transport	*Arabidopsis*	*APOLO*	Regulation of methylation and demethylation	Ariel *et al.* (2014)
	Regulation of auxin concentration during developmental phase	*Arabidopsis*	*ASCO*	Splicing regulator	Bardou *et al.* (2014)
	Fruit ripening	*Lycopersicon esculentum*	lncRNAs 1549, 1840	Not confirmed	Zhu *et al.* (2015)
	Vernalization	*Arabidopsis*	*COLDAIR* *COOLAIR*	Histone modification Transcriptional regulation	Heo and Sung (2011); Sun *et al.* (2013)
	Nodule development	*M. truncatula*	*Enod40*	Nuclear traffic (post-translational changes)	Campalans *et al.* (2004)
	Fiber development	*Gossypium* spp.	lincRNAs	miRNA precursors, miR397-mediated regulation, point mutation and transposon element insertion	Wang *et al.* (2015)

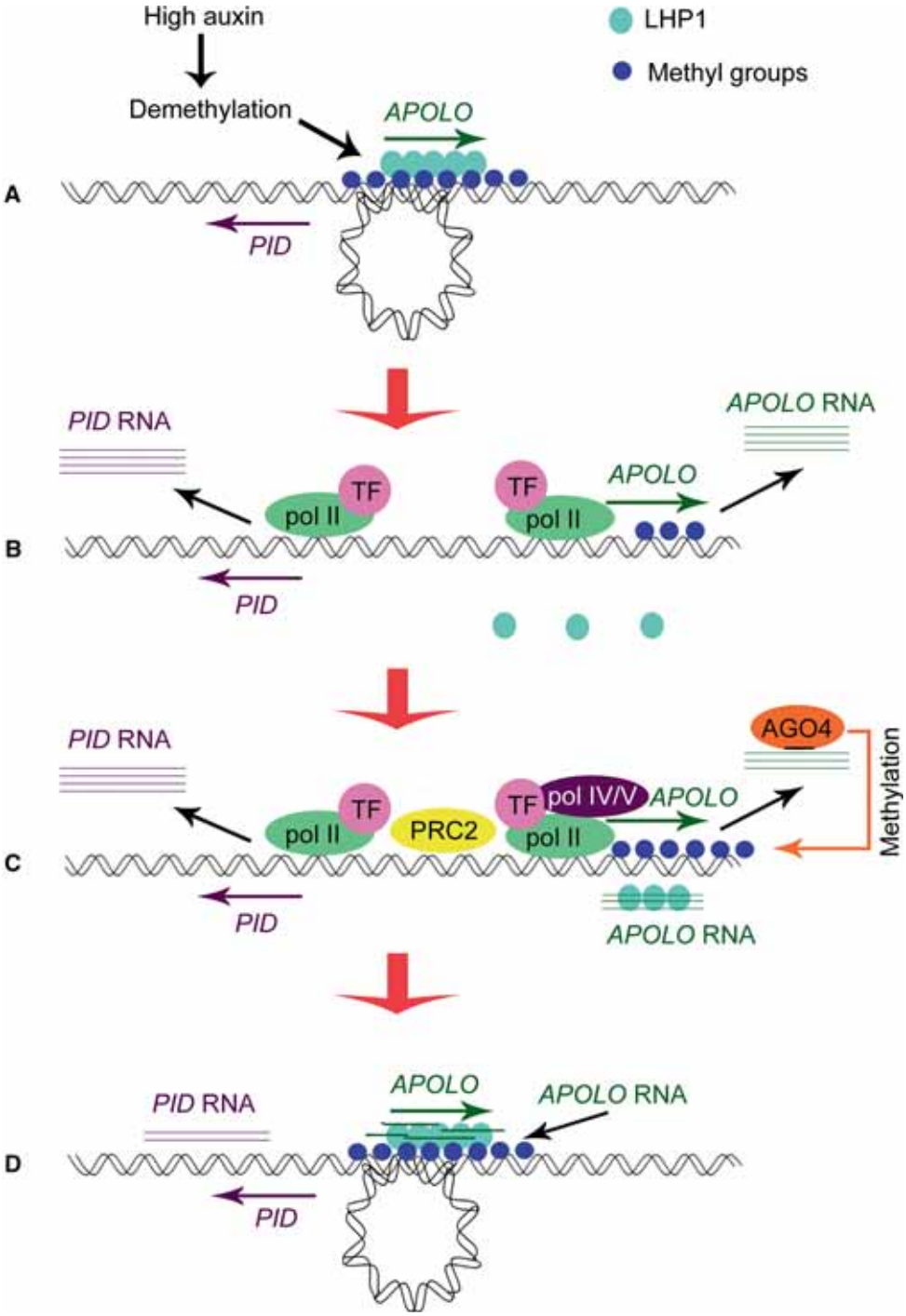

Figure 1. Regulation of PID biogenesis by lncRNAs. lncRNAs can alter *PID* gene expression by modulating histone methylation and transcriptional regulation. (**A**) Auxin induces demethylation of *APOLO* (an lncRNA member) gene and opens the loop encompassing *PID* promoter and RNA pol II activity recruits LHP1 protein, (**B**) RNA pol II starts the transcription from the *PID* gene and also increases *APOLO* transcript, (**C**) RNA pol IV/V enhances AGO4-mediated methylation of the *PID* promoter (**D**) and recruits PRC1 on the *APOLO* locus to close the loop which inhibits the PID biosynthesis.

regulates the alternative splicing pathway of the intron splicing mechanism (Bardou *et al.* 2014). The NSR family proteins such as AtNSRa and AtNSRb have been found to be confined in the root meristems and lateral roots. The mutation of both the genes resulted in shorter and fewer lateral root production and showed less sensitivity to auxin-mediated lateral root growth. The NSR binds to the target mRNA to form an ASCO-NSR complex during RNA processing. The ASCO-RNA displaces the target mRNA from the NSR–mRNA target association complex and modulates

the pattern of splicing of a specific subset of genes which are associated with the lateral root formation (figure 1). The changes in auxin concentration in roots also influence the modulation of alternative splicing in roots.

Another novel lncRNA member, *MIKKI* associated with the transposable element in rice helps in root development by regulating the expression of *Scarecrow like* (*SCL*) gene (Cho and Paszkowski 2017). Generally, MIKKI, derived from retrotransposon, consists of four introns. The splicing of the fourth intron of *MIKKI* generates the exon–exon junction which specifically binds with miR171 during root development and may be considered as a fascinating example of eTMs in plants. In plants, miR171 cleaves its target *SCL* gene, which encodes the SCL transcription factor, a critical regulator of root growth (Wang *et al.* 2010). The overexpression of *MIKKI* resulted in attenuation of miR171 and subsequent enhancement of SCL proteins while an elevated amount of miR171 was found in the knockout mutants of *MIKKI*, thus deciphering its role in root growth (Cho and Paszkowski 2017).

3.2 *lncRNAs in floral development during vernalization*

Flowering is an essential developmental phenomenon of plants which is regulated by various complex mechanisms. Photoperiod regulates flowering temporally. In addition, vernalization includes the response of flowering upon prolonged low temperature or cold treatment. This is the best studied physiological pathway for the development of floral organs in plants where the distinct role of lncRNAs has been observed. During the transition from the vegetative phase to the reproductive phase, *FLOWERING LOCUS C* (*FLC*) gene plays the major regulatory role in flowering (Irish 2010). *FLC* encodes an MADS-box transcription factor which represses the expression of *SUPPRESSION OF OVEREXPRESSION OF CO*1 and *FLOWERING LOCUS T* (*FT*). The FT protein crucially controls the flowering by initiating floral development in the apical meristem. None-the-less, the two lncRNAs viz. *COOLAIR* and *COLDAIR* have been found to be present in the locus of *FLC* genes that critically regulates the *FLC* gene expression by transcriptional regulation and histone modification, respectively (Heo and Sung 2011; Sun *et al.* 2013). *COOLAIR* is an antisense of *FLC* and are overlapped. It originates from the 3′ end of the *FLC* gene in an antisense orientation (figure 2). The accumulation of *COOLAIR* transcript minimizes the *FLC* expression during vernalization (Sun *et al.* 2013; Wahba and Koshland 2013). Interestingly, the polyadenylation properties of *COOLAIR* transcript regulate the *FLC* expression. The proximal 3′-end polyadenylation lowers the expression of *FLC* whereas distal polyadenylation activates the accumulation of *FLC* transcripts. During the cold period, the proximal 3′-polyadenylation of *COOLAIR* induces H3K4 demethylation of *FLC* (Liu *et al.* 2010). The repression of *FLC* thus initiates flowering. The transcription of *COOLAIR* is critically regulated by the formation of single-stranded R-loop to its promoter. The transcription factor, *At*NDX, localizes to a heterochromatic region of *COOLAIR* promoter *in vivo* and binds to the single-stranded DNA of the R-loop maintaining the DNA in an open conformation. The open form of DNA, in turn, facilitates the formation of RNA–DNA hybrid once *COOLAIR* transcription initiates (figure 2). Therefore, the attenuation of *COOLAIR* increases the *FLC* gene expression and regulates flowering. During the cold period, both lncRNAs activate the PRC2 protein for epigenetic regulation of the *FLC* promoter while *At*NDX-mediated suppression of *COOLAIR* has been reported during warmer days thus promoting the *FLC* expression.

Moreover, *COLDAIR* is an intronic lncRNA originating from the first intron of *FLC* in a sense orientation (figure 2). The *COLDAIR* associated with the PRC2 protein facilitates histone trimethylation. The histone deacetylation of H3K9 and trimethylation of H3K27 are generally associated with FLC inhibition (Crevillén *et al.* 2014). In addition, two proteins, FCA and FPA, also regulate the chromatin modification and negatively modulate the FLC gene expression. In previous reports, it has been demonstrated that the FCA protein along with FY directly interacts with the *FLC* locus, but not with the transcript processing, thus suggesting the role in chromatin modification. However, the influence of FCA and FPA on *FLC* is still obscure and requires further investigation in future. In addition, the initial suppression of *FLC* transcripts during vernalization is regulated solely by *COLDAIR* whereas maintenance of repression depends on *COOLAIR* accumulation (Helliwell *et al.* 2011).

In a later study, genome-scale RNA-Seq analysis of flower and fruit tissues from *Fragaria vesca* has identified lncRNAs exhibiting tissue-specific expression thus indicating their probable role in flower and fruit development (Kang and Liu 2015). RNA-Seq analysis has been performed from eight successive stages of floral development as well as from vegetative stages in chickpea which demonstrated a possible regulatory role of lincRNAs in flowering (Khemka *et al.* 2016). Several lncRNAs regulating floral development has also been reported from trifoliate orange, *Poncirus trifoliata* (Wang *et al.* 2017).

3.3 *lncRNAs in photomorphogenesis*

Photomorphogenesis is a major light-sensitive developmental phenomenon, which differentiates the vegetative phase from the reproductive phase. In addition, seedling photomorphogenesis is characterized by the development of short hypocotyls and the opening of cotyledons after germination of seeds. Light induces the chlorophyll biosynthesis when seedlings grow during the day. The photoreceptors such as phytochromes (for red and far-red light), phototropins and cryptochromes (for blue light) capture different wavelengths of light and initiate light-induced signaling pathways (Chen and Chory 2011). The molecular

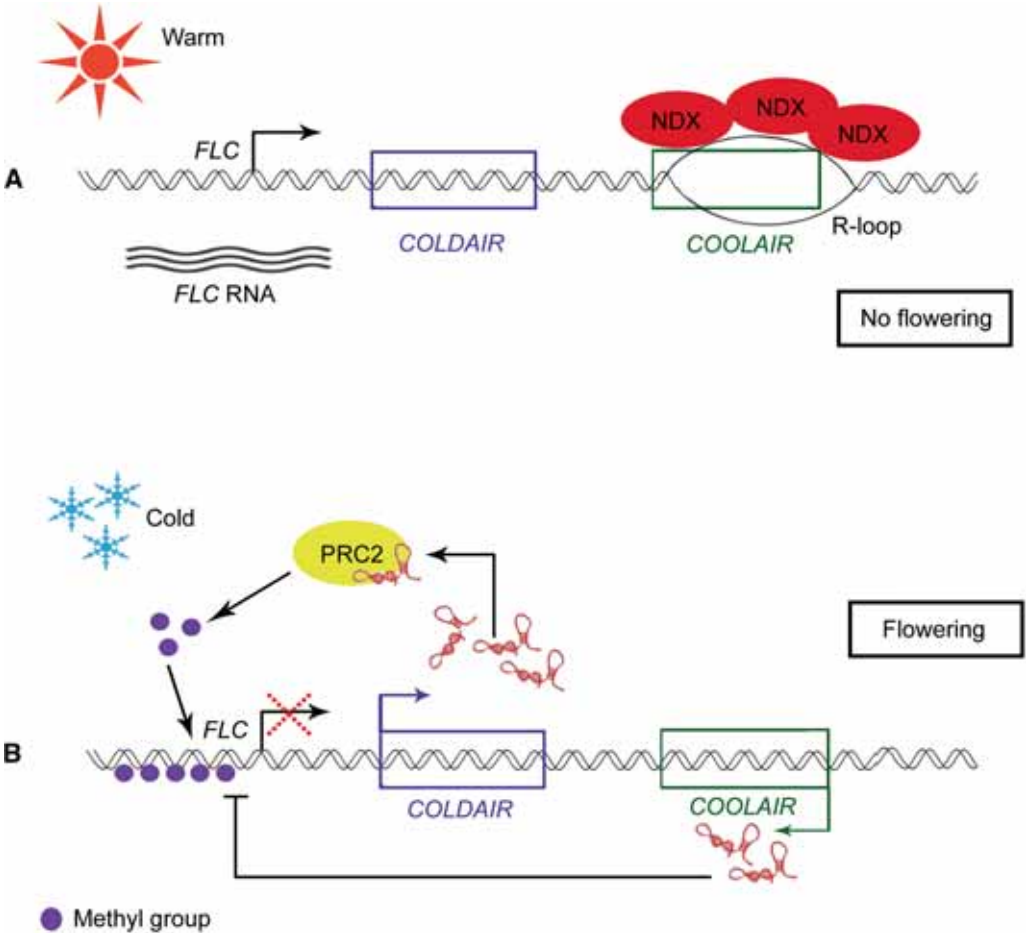

Figure 2. Regulation of *FLC* gene expression by lncRNAs. (**A**) Under warm conditions, the R-loop forms within the promoter region of *COOLAIR* and binds to the NDX protein initiating open conformation. The *COOLAIR* transcript binds to the single-stranded DNA and remains *FLC* gene free for transcription. (**B**) During vernalization in the cold period, *COOLAIR* binds to PRC2 and acts as a natural antisense transcript of *FLC* gene whereas *COLDAIR*, synthesized from the first intron of *FLC* gene induces methylation in the *FLC* promoter to suppress *FLC* transcription. Red color loops denote lncRNAs.

signal-sensing mechanism of photomorphogenesis has been extensively studied in various plant species. A number of proteins are associated as primary and secondary signaling molecules during this phenomenon. Among these proteins, a family of basic helix loop helix group of transcription factors, known as phytochrome-interacting factor (PIF) has been identified as a repressor of seedling photomorphogenesis during the dark (Kim *et al.* 2003). Recently, a novel lncRNA viz. *hidden treasure* 1 (*HID1*) has been functionally characterized and has been found to be involved in the regulation of *PIF*3 gene expression (Wang *et al.* 2014b). *PIF*3 is a key signaling molecule of photomorphogenesis that negatively regulates red-light responses. *HID*1 associated with chromatin directly interacts with the first intron of *PIF*3 and inhibits its expression. The mutation of *HID*1 in *Arabidopsis* increases the expression of *PIF*3 which results in elongated hypocotyls under dark. The homolog member of *At*HID1 has been found in rice which confirms the conserved nature of lncRNAs through different plant species. In another investigation, 626 concordant and 766 discordant NATs have been found to be up-regulated in *Arabidopsis* in response to light (Wang *et al.* 2014a). Most of them are related to histone modifications.

3.4 *lncRNAs in pollen development and male sterility*

The development of normal pollen grain is crucial for fertilization and seed development. The anther acts as a parental tissue of pollen grain and regulates its development. In early stages of pollen development, tapetum renders protection to the young pollen grain whereas supplies nutrition to the matured pollen grain during a later stage. Abnormal pollen grain may result in the development of male sterile plants. The signaling network that functions in male sterile plants has not been properly recognized so far. Recently, the lncRNA, known as long day (LD)-specific male-fertility associated RNA (*LDMAR*), has been identified which determines fertility in rice (Ding *et al.* 2012). In male sterile plants, the diminution of *LDMAR* transcripts has been

reported which induces the programed cell death of anther cells under LD conditions (Ding *et al.* 2012; figure 3). In addition, a spontaneous point mutation due to methylation has been identified in the 5′-*cis*-acting region of *LDMAR* gene. This methylation follows the RdDM pathway. Furthermore, Zhou *et al.* (2012) also demonstrated the biosynthesis of the unique 21 nucleotide long small RNA, *osa-smR5864w*, from *LDMAR* genes attributing the origin of siRNAs or miRNAs to lncRNAs. It has been hypothesized that the small RNA restores fertility and a C to G point mutation in *osa-smR5864w* seizes the function of this small RNA and eventually develops the male sterile plants. In *Brassica campestris*, *BcMF*11, an 828 nt lncRNA, is transcribed throughout the pollen developmental stages. It maintains the degradation of tapetum tissues and regulates male fertility by producing normal pollen grains. The attenuation of *BcMF*11 hampers tapetum degradation and results in aborted pollen grain formation (Song *et al.* 2007, 2013). *Zm*401 is another lncRNA found in maize which critically regulates anther and microspore development. The *Zm*401 activates anther specific *ZmC*5 and *ZmMADS*2 whereas minimizes the *MZm*3-3 gene expression. Silencing of *Zm*401 follows the reversal of regulatory gene expression leading to the development of sterile pollen grains (Ma *et al.* 2008). Recently, RNA-Seq analysis has been performed from five developmental stages during pollen development as well as three different time points after pollination in *Brassica rapa*. The study has identified 14 lncRNAs that are highly co-expressed with 10 known protein-coding genes which are crucial players in pollen development. Among them, two lncRNAs have been shown to act as eTMs for miR160 and regulate pollen development

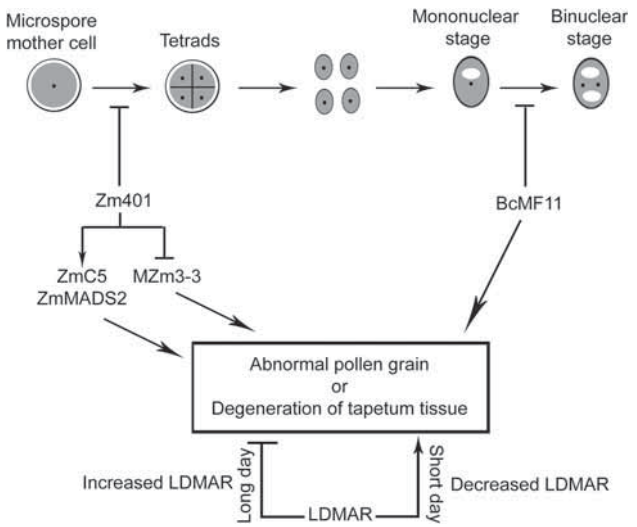

Figure 3. Role of different lncRNAs in plants during pollen grain development. *Zm*401 inhibits tetrad formation whereas *BcMF*11 thwarts binucleate stages of pollen grain development. *LDMAR* under long day initiates tapetum wall formation and thus helps in pollen grain development. Under short day, the decreased amount of *LDMAR* shows degenerated tapetum tissue.

(Huang *et al.* 2018). As the present scenario depicts, studying the comprehensive signaling mechanism of lncRNA-mediated fertility regulation will be a major breakthrough in future.

3.5 *lncRNAs in fiber development in cotton*

Cotton is an economically important crop plant which provides two types of fibers. The developmental time period during the initiation of cotton fiber determines the type of fiber. Lint fiber appears at the beginning of flowering (0-day post-anthesis) while fuzz fiber develops on the fourth day after anthesis (Wang *et al.* 2015). Several lncRNAs have been found to be involved in the development of lint and fuzz fibers. Furthermore, the detailed functional characterization of lncRNAs in fiber elongation and secondary cell wall synthesis has been performed. The differential regulation of lncRNAs between At and Dt subgenomes of cotton species confirms the pattern of fiber development. In addition, lignin deposition is very crucial for secondary wall biosynthesis. Laccase is the key enzyme for lignin biosynthesis which is finely controlled by miR397 (Wang *et al.* 2015). One lncRNA pair has been reported in cotton which generates miR397. The expressional differences between two copies of *LAC* genes viz. *LAC*4a and *LAC*4b determine the lignin biosynthesis. The variation in the expression levels of both the genes depends on the subgenome types and anthesis period. The relative lower expression of *LAC*4a is associated with the miR397-mediated cleavage of the transcript. The single-nucleotide polymorphism mutation in the 10th nucleotide of *LAC*4b gene reduces the cleavage (Wang *et al.* 2015). The expressional transition of Dt subgenome is also associated with transposon element insertion within the *LAC*4b gene from At subgenome. In cotton, the methylation levels of lncRNAs have been found to be higher over protein-coding genes which maintain the lincRNAs in the silenced state in developing ovule (Wang *et al.* 2015). In another study, transcriptome analysis of cotton fibers and leaves revealed dynamic changes in lncRNAs expression during fiber and rapid elongation stages (Zou *et al.* 2016). Recently, the identification of different lncRNAs from three representative lines with different fiber quality and silencing of three different lncRNAs increased the number of fibers initially in the ovule, while reducing the fiber length, thereby confirming their regulatory activity in lint and fuzz fiber development in cotton (Hu *et al.* 2018). However, deeper investigations are necessary to dissect the molecular regulation mechanism of lignin biosynthesis by lncRNAs during cotton fiber development.

3.6 *lncRNAs in modulation of nodule development*

lncRNAs play a diverse role in the modification of protein structures and functions. Intriguingly, they can facilitate the

sub-cellular localization of the target protein molecules (Batista and Chang 2013). In *Medicago truncatula*, the *ENOD40* RNA, a similar to candidate lncRNA, binds with the RNA binding protein 1 (RBP1) to facilitate the transport of RBP1 to the cytoplasm from the nucleus during nodule development (Campalans *et al.* 2004). The deficiency of *ENOD40* retains RBP1 in the nucleus (figure 4). Detailed investigations may elucidate the clear scenario of *ENOD40*-mediated molecular traffic in future. It has been suggested that *ENOD40* also encodes two short peptides, ENOD40-I (13 amino acids) and ENOD40-II (27 amino acids) which presumably play crucial roles in nodule development (Sousa *et al.* 2001). In addition, some RBPs play an essential role in the abscisic acid (ABA) signaling pathway during development and stress responses in *Arabidopsis*. Upon ABA treatment, the transport or localization of some RBP members such as ubiquitin-specific protease 1 (*At*UBP1) and *At*UBP2 into nucleus speckles is thought to be associated with lncRNAs which needs to be investigated further.

3.7 *lncRNAs in fruit ripening*

Fruit ripening is a complex developmental phenomenon regulated by different exogenous and endogenous modulators and their coordinate signaling networks. The role of various protein coding genes, transcription factors and miRNAs has been determined during different developmental stages of the developing fruit and embryo. Recently, genome-wide identification of several lncRNAs from ripening tomato has suggested the regulatory role during fruit development (Zhu *et al.* 2015; Wang *et al.* 2018). Suppression of lncRNA1459 and lncRNA1840 showed delayed ripening in tomato confirming their regulatory role in fruit development (Zhu *et al.* 2015). In addition, DNA methylation of differentially regulated lincRNAs in tomato was thought to be involved in fruit ripening (Wang *et al.* 2018). In another study, 118 differentially regulated lncRNAs have been identified from sea buckthorn fruits collected on different post anthesis days (Zhang *et al.* 2018). Among them, silencing of two lncRNAs (LNC1 and LNC2) exhibited differential regulation of *SPL*9 and *MYB*114 transcripts thus augmenting anthocyanin biosynthesis. This finding suggests novel insights into lncRNA-mediated quality improvement in fruit.

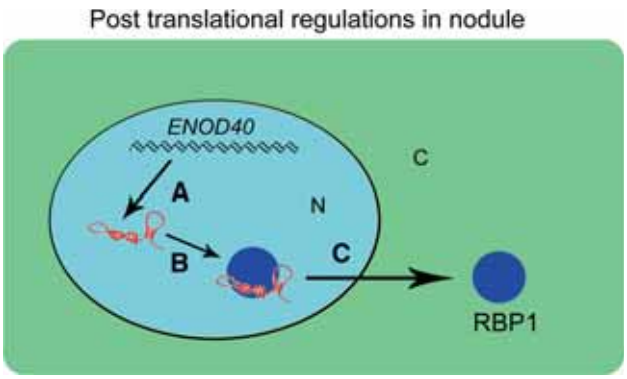

Figure 4. Post-translational changes by lncRNAs during nodule formation. (**A**) lncRNA, *enod40* synthesized from its coding region, (**B**) binds to RBP1 protein and (**C**) facilitates its transport to cytoplasm from the nucleus favoring nodule formation in *M. truncatula*. Red color loop denotes lncRNA. N and C specify nucleus and cytoplasm, respectively.

4. Conclusion and future perspective

The discovery of plant lncRNAs in controlling various developmental pathways is a new milestone in developmental biology research. The fine regulation of gene expression by lncRNAs during different developmental phases is a common phenomenon and more investigation in different plant species will provide further valuable information in future. In flowering, only vernalization-associated lncRNAs have been characterized so far. More lncRNAs and their regulation during photomorphogenesis and reproduction may be explored to open up a novel arena of plant reproductive development research. Recently, two lincRNAs, lncRNA 1459 and lncRNA 1840, showed their regulatory role in fruit ripening in tomato which adds an extra level of complexity to the signaling mechanism of fruit ripening (Zhu *et al.* 2015).

Several lincRNAs and NATs can act as precursors of small RNAs and can regulate diverse metabolic pathways. They can alter the miRNA expression by interacting with the corresponding miRNAs (Wu *et al.* 2013). The sequences of the binding sites of lincRNAs are found to be conserved and thus, single lincRNA can effectively bind to multiple miRNAs. This eTM is an additive strategy for gene expression studies *in vitro*. The transcript sequences with three nucleotide bulges, used as snare, exhibit partial nucleotide homology of the target gene. The miRNAs bind with the snare transcript in place of its target gene due to its complementarities resulting in the suppression of the interaction of the miRNA and its target gene. This follows the enhancement of the expression of the corresponding target gene. Genome-wide analysis and prediction of eTMs in different plant species have revealed the role of lincRNAs as eTMs (Meng *et al.* 2012). Although, the eTM-mediated regulation of gene expression is mainly confined to phosphate homeostasis of plants, investigating their roles in other developmental phenomena may establish novel strategies for crop improvement in future. For example, this eTM technology can be applied to modulate the expression of specific miRNAs to reduce the flowering time of crop plants such as rice which will effectively shorten the harvest period. Cutting down the harvest period in this way can be economically beneficial for the farmers particularly in the developing countries. Apart from these developmental aspects, the lncRNA-mediated eTM technology has wide application in the generation of stress tolerant plants. In addition,

identification of different lncRNAs related to various developmental phenomena and analysis of their *cis*-acting regulatory regions will provide novel findings in molecular signal-sensing mechanisms involved in different developmental pathways.

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