Epigenetics in Plant Reproductive Development: An Overview from Flowers to Seeds

Antoine L.F. Gady, Cristiane S. Alves, and Fabio T.S. Nogueira

Abstract Plant development is governed by a wide variety of genetic and epigenetic events that regulate cell fate. Flower to seed developmental transition varies greatly between plants and is of importance in research programs because of its relevance for crop production and human diet. In this chapter, we review the latest research on epigenetics regulation of flower, fruit, and seed development in crop plants. We use tomato (*Solanum lycopersicum* L.) as our reference crop model while referring to *Arabidopsis thaliana* for in-depth studies and look into additional crop model plants such as maize (*Zea mays*), wheat (*Triticum* spp.), and rice (*Oryza sativa*) in order to cover a wide range of flower and fruit/seed types. Tomato is an interesting biological model thanks to its fleshy fruit. Tomato has the second natural epimutation reported, the *Colorless non-ripening* (*Cnr*), as well as newly reported studies on the paramutation SLTAB2, the role of the demethylase DML2 in fruit ripening, and the identification of two long noncoding RNAs (lncRNAs) involved in the ripening process. Altogether, these works make tomato an interesting and important epigenetic model for plants. A variety of epigenetic-based regulations are involved in each stage of the tomato fruit set, development, and ripening. Four epigenetic mechanisms are proved to be involved in flower, fruit, and developmental processes: histone modifications, DNA (de)methylation, small RNA posttranscriptional *locus* regulation, and lncRNA-associated regulatory pathways. Epigenetic mechanisms are involved at all stages of reproductive organs development, from the flower to the mature seed.

Keywords Tomato • Small RNAs • DNA methylation • Epimutation

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Contents

1 Introduction

Plasticity plays a fundamental role in adaptation and resilience of crops allowing cultivars to adjust and grow in different environments. Plasticity is responsible for homeostasis maintenance when environmental conditions change, allowing gene expression to adapt to biotic or abiotic stress or variations like altitude, soil type, seasonal, day length, rain, ambient temperature, disease, plant–plant competition, herbivory, among others (Latzel et al. 2012). To our current knowledge, epigenetic mechanisms are responsible for conferring genetic plasticity to crops by DNA methylation, histone modification, noncoding RNAs, and chromatin modulation. These variations can regulate genome expression leading to new phenotypes in response to environmental changes.

Eukaryotic chromatin is a nucleoprotein complex, where the DNA is packed and condensed enfolding histone proteins. Even being tightly condensed, the chromatin needs to be dynamic in order to allow basic functions like transcription, replication, and DNA repair to occur. For the compaction to happen, 147 base pairs of DNA are wrapped into an octamer of core histones, containing two of each H2A, H2B, H3, and H4, linked by H1 at the entry/exit point. Histones play important roles in metabolic chromatin functions such as chromatin integrity maintenance, DNA recombination, and process of DNA replication in several organisms. In fact, these proteins date back to the dawn of eukaryotic evolution, spanning protozoans, fungi, animals, and plants. Prokaryal and archaeal species are the earliest genomes known to have evolved histone-like proteins (Grove 2011; Sandman and Reeve 2006). In the last 15 years, histones became the focus of research interests thanks to the light shed on the histone code and its implications. This code is a resultant from covalent posttranslational modifications (PTM)—methylation, acetylation, phosphorylation, ubiquitination, and poly-ADP ribosylation—that takes place at the C-

or N-terminal tail of histones and histone variants. There are five histones families—H1, H2A, H2B, H3, and H4—which are subject to PTM. Euchromatin is usually constituted by transcribed *loci* and heterochromatin is enriched with TEs (transposable elements) and is typically transcriptionally silenced through vegetative phases by DNA methylation and histone modifications (Bernatavichute et al. 2008; Law and Jacobsen 2010).

Contrarily to animals, in which cytosine DNA methylation in the CG context is predominant, in plant kingdom cytosine DNA methylation can occur in three contexts: CG, CHG, and CHH (where H=A, T or C) (Law and Jacobsen 2010). Plant DNA methyltransferases are distributed in four families: (1) MET1: similar to DNMT1, which is a CG maintenance DNA methyltransferase; (2) CMT: a plant-specific chromomethylase that lacks the N-terminal extension, and it is a CNG DNA methyltransferase; (3) DRM: a plant de novo DNA methylases of non-CG DNA sequences, which have an unusual arrangement of some conserved catalytic motifs; and (4) DNMT2: highly conserved but enigmatic methyltransferase (Vanyushin and Ashapkin 2009). Recent studies in *Solanum lycopersicum* (tomato) genome reveal the presence of nine genes encoding DNA methyltransferases, comprising the four DNA methyltransferase families: *MET1*, *SlDNMT2*, *SlCMT2*, *SlCMT3*, *SlCMT4*, *SlDRM5*, *SlDRM6*, *SlDRM7*, *SlDRM8* (Kumar et al. 2016).

The DNA methylation marks are reversible, which confer plasticity by facilitating the modulation of RNA transcription from genomic regions or specific *loci*. Alteration in DNA methylation can have meaningful outcomes on chromatin structure, with increases in 5mC marks connected with the arrangement of nucleosomes in plant genomes (Chodavarapu et al. 2010). Based on bisulfite sequencing—where unmodified cytosines are converted to uracil (sequenced as T) and 5mC remains sequenced as C, researchers generated methylome maps for tomato (Zhong et al. 2013), *Arabidopsis* (Zhang et al. 2006), and rice (*Oryza*; Li et al. 2012). Tissue-specific methylomes can provide evidence of epigenetic plasticity by analyzing the changing patterns in gene regulation. Euchromatin is associated with hypomethylated DNA in actively transcribed regions, while heterochromatin contains silenced genes, which were frequently hypermethylated (May 2010).

Noncoding RNAs, RNA molecules that do not code for proteins, have important roles in the regulation of plant reproductive organ development. Small interfering RNAs (siRNA) function as part of the RNA-directed DNA methylation (RdDM) machinery where they direct the RdDM complex to specific DNA locations to be methylated. MicroRNAs (miRNA) provoke transcriptional gene silencing by recruiting the RISC complex to specific target mRNAs by sequence complementarity. Finally, long noncoding RNAs (lncRNA) are transcripts over 200 bp that can act in a multitude of ways to regulate gene expression; depending on their genome location, they can act as *cis*- or *trans*-regulatory elements (Chekanova 2015; Liu et al. 2015b). The implication of ncRNAs in the development of both flower and fruit has been repeatedly proven and have unraveled a vast network of RNA molecules acting as fine regulators of gene expression during plant organ development.

In this chapter, we will highlight the main findings on epigenetic regulation affecting flower, fruit, and seed development. We will discuss the latest findings regarding epigenetic regulations of tomato flower and fruit development that might help to improve the future of tomato breeding.

2 Flowering and Pollen Development

Perennial and overwintering annual crops can respond to the length and severity of the winter thanks to their plasticity, securing that individual plants are adjusted and reaching reproductive maturity. In some cases, this environmental cue causes changes that are mitotically stable throughout the rest of development. To date, the best-characterized example of such epigenetic memory in plants is the vernalization process, namely the acceleration of flowering as a result of exposure to cold temperatures in winter. Flowering program is promoted by the perception of the vernalization signals, including chromatin-based mechanisms, as the winter passes (reviewed in Bloomfield et al. 2014).

FLOWERING LOCUS C (*FLC*) is a major floral repressor that suppresses flowering during exposure to prolonged periods of low temperature. An additive effect of histone modifications at the *FLC* locus takes place in vernalization-sensitive species when the length of winter daytime is noted as temperature degrees below a threshold (Sheldon et al. 2009). Depending on the species, the vernalization signaling can happen at different developmental stages, registered by epigenetic switches maintained during in vitro vegetative propagation and deleted through sexual reproduction (Song et al. 2012). When the winter ends, histone remodification gradually occurs allowing the derepression of *FLC locus* (Song et al. 2012). In response to cold treatment, 5mC (5-methyl cytosine) is significantly reduced both in winter and spring *B. napus* following a gradual DNA re-methylation to pretreatment levels in spring *B. napus*, but only up to 70% in winter *B. napus* (Guzy-Wrobelska et al. 2013). It seems that this primary mechanism of epigenetic regulation is conserved across plant species, as similar results from cold treatment also change 5mC patterning in different crops like cotton, maize, rice, and wheat (Steward et al. 2002; Sherman and Talbert 2002; Pan et al. 2011; Fan et al. 2013). Active repression of *FLC* or *FLC*-like *loci* is required as a standard mechanism until a target threshold is sensed (Sheldon et al. 2009; Xiao et al. 2013). *FLC* is repressed in *Arabidopsis* by decreasing H3 acetylation and demethylation of H3K9 through *REDUCED VERNALIZATION1/VERNALIZATION INSENSITIVE 3* (*VRN1/VIN3*) (Bastow et al. 2004; Sung and Amasino 2004).

Pollen development undergoes a transcriptional and translational reprogramming to promote the production of male gametes from somatic lineages (Calarco et al. 2012). Small RNAs (sRNAs), important components of the plant epigenetic machinery, play an essential role in the pollen reprogramming process, altering the transcriptional and translational dynamics characterizing the individual developmental stages (Borges et al. 2011). Pollen is notably sensitive to elevated

temperatures, and little is known about the mechanisms underlying this stress response. Recently, it has been shown in heat-stressed tomatoes that the accumulation of pollen miRNAs, tRNAs, and snoRNAs is affected by heat stress in distinct pollen developmental stages, especially in post-meiotic and mature stages of male gametophyte development (Bokszczanin et al. 2015). Though still speculative, some of these sRNAs might have important roles in the epigenetic regulation of pollen development in response to environmental cues.

3 Flower and Fruit Development

The final size and shape of the fruit is the result of a multitude of developmental events that go back as far as the floral initiation. The differentiation of the inflorescence meristem into a floral meristem marks the first stage of floral initiation. The size of the floral meristem depends on the number of cells it is composed of, and therefore, it is a parameter that influences the final fruit size (van der Knaap et al. 2014; Xu et al. 2015). Five days after floral initiation, the gynoecium starts to grow. The way the gynoecium will develop pre- and post-anthesis will determine the shape and size of the final fruit. See van der Knaap et al. (2014) for a complete review of the genes influencing tomato fruit weight and shape.

3.1 Histone Acetylation Mediated Regulation

Flower architecture is molecularly determined by the reference ABC model (Bowman et al. 1991). In this model, specific combinations of gene expression and protein interactions determine the geographical limits of each flower whorl (sepals, petals, stamens, and carpel). As demonstrated in *Arabidopsis*, the A class gene *APETALA2* (*AP2*) regulates target gene expression as part of complex it forms together with *TOPLESS* (*TPL*) and *HISTONE DEACETYLASE19* (*HDA19*). That transcription repressing complex negatively regulates *AGAMOUS* (*AG*), a C class gene, and *SEPALATA3* (*SEP3*), an E class gene from the ABC flower architecture model. The gene repression is mediated by deacetylation of H4K16 in regulatory regions of both *AG* and *SEP3* (Krogan et al. 2012). Expression studies in *Arabidopsis* tissues highlighted additional HDACs expressed in reproductive tissues: *HDA5*, HDA6, HDA7, *HDA9, HDA15*, and *HDA18*, but their function in either fruit or flower development remains unidentified. Only for *HDA6* do we know that it is involved in the regulation of flowering time. *HDA6* directly interacts with *FLOWERING LOCUS D* (*FLD*), a histone H3K4 demethylase. The complex removes acetyl and methyl groups from Histone 3 at loci of three repressors of flowering: *FLD*, *MADS AFFECTING FLOWERING 4* (*MAF4)*, and *MAF5*, thus repressing their expression (Yu et al. 2011). From that set of HDACs, *HDA6* is known to be involved in RNA-directed DNA methylation (RdDM), a plant-specific

mechanism to regulate chromatin silencing of developmental genes as well as transposable and repetitive elements.

In plants, the major small-interfering RNA (siRNA)-mediated epigenetic pathway is RNA-directed DNA methylation (RdDM). RdDM is a complex epigenetic machinery that involves a large number of players whose activity can be broken down into a few steps (Matzke and Mosher 2014). Mainly two types of transcripts are involved in the RdDM machinery: Pol IV and Pol V transcripts. Pol IV transcribes long noncoding RNAs (lncRNAs) that are subsequently converted into double-stranded RNAs (dsRNAs) by *RDR2* (Haag et al. 2012). These dsRNAs are processed by *DICER-like3* (*DCL3*) into siRNAs. The siRNAs are exported to the cytoplasm where they are loaded into *AGO4* and reimported into the nucleus. The role of the siRNAs is to guide, by specific base pairing, *AGO4* toward nascent scaffold transcripts of Pol V. The formation of this siRNA, *AGO4*, Pol V-derived lncRNA scaffold ultimately recruits histone deacetylases (HDACs) and DNA methyltransferases that through histone deacetylation and DNA methylation silence the genomic loci transcribed by Pol V. Promoters silenced by RdDM are characterized by histone deacetylation, which in *Arabidopsis* is acted by the RPD3-type histone deacetylase *AtHDA6*, for which the tomato homologue is *SlHDA3*. *AtHDA6* activity results in the deacetylation of histone H3 lysines 9 and 14 which leads to gene expression downregulation (Aufsatz et al. 2007). Functional *AtHDA6* is required to control siRNA-dependent heterochromatin and that deacetylation is a prerequisite for subsequent methylation by HMTs (Aufsatz et al. 2007). Complete loss-of-function mutants for *AtHDA6* exhibit reactivation of RdDM-silenced promoters, despite the continuous presence of the RNA-silencing signal. Moreover, cytosine methylation is reduced, highlighting a function for *AtHDA6* in methylation maintenance. This function of *AtHDA6* might be mediated by the physical association with DMTs, *MET1*, and *CMT3* (Aufsatz et al. 2002).

The roles of histone acetylases and deacetylases in tomato flower or fruit development are poorly understood. Using the sequencing data generated by the international tomato genome sequencing consortium (Tomato Genome C 2012), Cigliano et al. (2013) identified in silico potentially all the histone modifiers of the tomato genome. Next they used the RNAseq data from that same source to look at the expression profile of each histone modifiers in 10 sample tissues. The histone acetylases *SlHAG18* and *SlHAG6* both presented peaks of expression in the flower samples, possibly indicating a role in reproductive development. A recent study (Zhao et al. 2014) identified 15 tomato histone deacetylases. *AtHDA6* tomato homologue, *SlHDA3*, was expressed in all tissues with a maximum expression at the flower stage. *SlHDA1*, the homologue to *AtHDA19*, was as well highly expressed in flowers and then repressed for all fruit samples except for a peak at the ripe fruit stage. In addition, yeast two-hybrid assays showed that *SlHDA1*, *SlHDA3*, and *SlHDA4* interact with MADS-box transcription factors TOMATO AGAMOUS1 (*TAG1)* and TOMATO MADS-BOX29 (*TM29)* (Zhao et al. 2014). *TAG1* is a transcription factor necessary to express ethylene dependent and independent ripening genes (Klee and Giovannoni 2011). *TM29* is a homologue to

SEPALATA which when silenced provokes the formation of parthenocarpic fruits and aberrant flowers (Ampomah-Dwamena et al. 2002).

3.2 DNA Methylation-Mediated Regulation

The *clark kent* hypermethylated epialleles of the *SUPERMAN (SUP)* gene are a clear illustration that DNA methylation can affect flower development through the regulation of gene expression level. The *sup-5 Arabidopsis* mutant which contains a nearly complete deletion of the *SUP* gene produces flowers with an increased number of stamens and carpels: 12 stamens compared to six and three carpels against two in wild-type *Arabidopsis* flowers. The stronger *clk3* epiallele contains an average of eight stamens and three carpels. The *clk* alleles have the exact *SUP* sequence of the wild-type accession but are extensively methylated from the start of transcription and covering most of the gene region (Jacobsen and Meyerowitz 1997). Antisense *MET1* and *ddm1* and *ddm2* mutant plants are hypomethylated but contain methylation-rich regions such as the *SUP* locus that is consistently hypermethylated in the three DNA methylases' mutant or antisense backgrounds (Jacobsen et al. 2000). In addition, *agamous* mutant-like flower phenotypes were identified in the MET1-antisense plants. Reduced levels of *AGAMOUS* mRNA were observed in the transgenic plants. An increase in methylation of the *AG* locus was measured by bisulfite genomic sequencing. Interestingly, hypermetylation of the *AG* locus only occurred in lines with a hypermethylated *SUP* locus, thus suggesting that hypermethylation of *SUP* is necessary to the hypermethylation of *AG*.

To understand the extent of the epigenetic regulation of flower development by DNA methylation, Yang et al. (2015) used high-throughput sequencing of DNA fragments obtained by MspJI digestion to obtain a whole genome profile of DNA methylation patterns. They divided the *Arabidopsis* flower development in three key stages: (1) floral meristem from *ap1 cal* double mutants, (2) wild-type early flowers at stages 1–9, and (3) wild-type late flowers at stages 10–12. In plants, DNA methylation occurs on the Cytosine nucleotide in three distinct sequence contexts: *MET1* transfers a methyl residue to CG sites, *CMT3* to CHG sites, and *DRMs* add methyl groups to CHH sites where H stands for A, T, or C nucleotides. The number of methylated cytosine increased by 8% between floral meristem and early flower stage and then decreased by 0.55% from the early to late flower stages. The initial stages of flower development are marked by numerous de novo methylation: 80,056 new methylation events reported. The study identified 3067 genes out of 24,035 that are co-differential: genes with significant variations in both methylation and gene expression. In the transition from floral meristem to early flower stage, 1048 genes were co-differential at ^{m}CG, 601 at ^{m}CHG, and 509 ^{m}CHH-containing genes. A total of 909 genes were co-differential in the early to late flower transition. Among them are important flower development regulators such as *SEP1*, *LEUNIG* (*LUG*), and *SEEDSTICK*. Moreover, 33 genes associated with flower development,

21 genes involved with pollen development, 201 transcriptional regulators, 29 genes linked to chromatin organization, and 56 to signal transduction have differential gene expression profile along flower development that is linked to DNA methylation variation. While the number of methylated cytosine increased during the meristem to early flower transition in all sequence contexts, only ^{m}CGs' number increased in the early to late flower shift, thus hinting at a role for MET1 methylations in a developmental phase characterized by organ growth. Considering the three studied developmental stages, over 1000 genes for each CHG and CHH methylation were both transcriptionally and epigenetically differentially regulated, suggesting an important role for these methylation types into reproductive organ development. Finally, Yang et al. (2015) also show that DNA methylation does not only regulate protein coding gene expression but as well that other epigenetic players are regulated in this manner: transposable elements, miRNAs, and noncoding RNA also had variation in the methylation status along flower development. This data thus hints toward the idea that the activity of additional epigenetic players is regulated by their methylation state.

3.3 MiRNA Mediated Regulation

MicroRNAs, small 21 nucleotides RNA molecules, regulate gene expression by specifically binding to mRNA with near-perfect sequence complementarity and thereof provoking their degradation. In *Arabidopsis thaliana*, at least eight miRNA families are responsible for the regulation of transcription factors involved in flower development. The miR164, miR169, and miR172 families are participating in setting boundaries between floral organs. The miR164 family regulates transcription factor of the NAC-domain family such as *CUP-SHAPED COTYLEDONS* (*CUC1* and *CUC2*) in *Arabidopsis*. Alteration to *CUC1* and *CUC2* expression results in modified sepal boundaries leading to fused sepals and fewer petals (Baker et al. 2005; Laufs et al. 2004). In addition, miR164 could play a role in carpel development (Baker et al. 2005; Sieber et al. 2007). Through the regulation of NF-YA transcription factors, the miR169 family limits the expression of the C-class family of genes to the inner two whorls of petunia and antirrhinum flowers. MiR172 on the other hand restricts the expression of *AP2* to the two outer whorls of the floral meristem (Chen 2004; Wollmann et al. 2010; Zhao et al. 2007). In addition to its role in flower development in *Arabidopsis*, miR172 was shown to be actively regulating flower formation in both rice and barley (Lee and An 2012; Nair et al. 2010; Zhu et al. 2009). MiR159 targets GAMYB-like genes such as *LEAFY*, *MYB33*, or *MYB65* and thus regulates flowering time but as well anther formation during flower development. Overexpression of miR159 downregulates *MYB33* and results in male sterility. Similarly, to what is observed in *Arabidopsis*, in rice the GAMYB gene expression is restricted to anthers. Resembling the effect of miR159, the overexpression of miR319 as well leads to defects in stamen development and male sterility, but the phenotype is the consequence of the

mis-regulation of a set of TCP transcription factor genes (Palatnik et al. 2007; Schommer et al. 2012). Plants with reduced levels of miR159 and miR319 show similar floral phenotypes as *arf6*/*arf8* double mutants. Auxin Response Factors 6 and 8 through the regulation of Auxin level regulate the extent of cytokinin activity in the developing floral meristem. *ARF6* and *ARF8* are regulated by miR167 (Rubio-Somoza and Weigel 2011), and miR167 is upregulated by either *TCP4* or *MYB33*, the targets of miR159 and miR319, thus forming a complex floral development regulatory network. Another regulator of ARF genes is miR160 which targets *ARF10*, *ARF16*, and *ARF17* genes. Downregulation of miR160 in transgenic *Arabidopsis* plants increases its target expression. These plants have defects in fertility and in floral organ formation and floral organs appeared inside siliques (Liu et al. 2010). MiR165 and miR166 regulate HD-ZIP III genes *ATHB15*, *ATHB8*, *REVOLUTA*, *PHABULOSA*, and *PHAVOLUTA*. Downregulation of these genes through overexpression of miR165 results in plants with carpel developmental defects, enlarged apical meristem, and short sterile carpels (Kim et al. 2005). The involvement of miRNAs in *Arabidopsis* reproductive development from juvenile to the flower producing plant phases is reviewed in Hong and Jackson (2015).

The expression of *SlARF6* and *SlARF8* is also regulated by miR167 in tomato plants. Plants overexpressing AtmiR167a produce female sterile flowers with shortened sepals, stamens, and style which is a consequence of the *SlARF6* and *SlARF8* low expression levels in developing flowers (Liu et al. 2014). MiR160 is abundant in tomato ovaries. In tomato, miR160 preferentially targets ARF10A and to a lesser extent Sl*ARF10B* and Sl*ARF17* (Damodharan et al. 2016). Therefore, the use of a target mimic to sequestrate miR160 and inhibit its natural function provokes an increase in *SlARF10A* accumulation in tomato ovaries which result in perturbed ovary patterning: an excessive elongation of its proximal end and thinning of the placenta. Consequently, postfertilization, the fruit is pear shaped. This fruit shape phenotype is the result of a mis-distribution of auxin in the early stages of ovary development regulated by SlmiR160 (Damodharan et al. 2016). In rice, Huang et al. (2016) showed that OsmiR160 regulates *OsARF18* and thereby auxin signaling. mOsARF18 transgenic plants express a modified allele of *OsARF18* that is not recognized and thus not regulated by OsmiR160. These transgenic rice plants had overall growth and development defects such as dwarfism, rolled leaves, small seeds, and abnormal flowers. mOsARF18 plants were impaired in reproductive organ development: the lemma and palea did not contain flowers and stamens remained attached to developing seeds when fertilization did occur, suggesting abnormal senescence of stamens, reminiscent of the senescence phenotypes observed in tomato (Damodharan et al. 2016).

SlmiR396 targets 8 out of the 13 tomato Growth Regulating Factors (GRF): *SlGRF1*, *SlGRF2*, *SlGRF3, SlGRF4, SlGRF5, SlGRF7, SlGRF8*, and *SlGRF12*. GRFs are a class of transcription factors expressed in most developing organs. GRFs regulate cell number, and their overexpression in *Arabidopsis* results in enlarged organs (Cao et al. 2016). On the other hand, overexpression of miR396 in *Arabidopsis* plants developed flowers with a single carpel; in rice a similar approach led to similar results: altered floral organ morphology (Cao et al. 2016).

Strong downregulation of miR396a and miR396b with STTM396a/396a-88 in tomato produced plants with increased cell number and cell size in both flowers and fruits leading to larger sepals and larger fruits (Cao et al. 2016). Fruits from STTM396a/396a-88 transgenic lines were 39 and 45% larger than control fruits. This might prove to be a new way to improve yield. Overexpression of miR172 was shown as well to increase fruit size in tomato (Yao et al. 2016). MiR172 overexpressing tomato plants contain numerous flower defects such as sepal to petal transformation, poorly developed stamen that produce sterile pollen, and the development of seedless parthenocarpic fruits with ectopic ovaries inside the fruits and occasionally fruit in fruit phenotypes. Similar phenotypes were also observed by our group in Micro-Tom tomato plants overexpressing miR156 (Silva et al. 2014). The altered fruit morphology, fruit-like structures emerging from the main fruit, was correlated with accumulation of miR156 in meristematic tissues such as placenta and ovules of developing ovaries and immature fruits. miR156 overexpression plants (miR156-OE) had flower buds with extra whorls and meristem-like structures that developed into ectopic structures instead of ovaries and ovules. The overexpression of miR156 prolonged the phase of floral meristem proliferation, and when organs finally formed, they produced flowers with additional partly fused carpels that likely account for the appearance of the miR156-OE fruits (Silva et al. 2014). We have identified five miR156-targeted SQUAMOSA promoter binding protein like (*SPL*/*SBP-box*) genes that are differentially expressed in pre- and post-anthesis ovaries. Our data show that the *LeT6*/*TKn2* and *GOBLET* (*GOB*) are repressed by Sl-SBPs to control meristem maintenance and cell proliferation at the onset of flower organs' initiation and differentiation, thereby controlling proper carpel and ovule development. In addition, we showed that *MACROCALLIX* (*MC*), *FRUITFUL1* (*FUL1*), and *FALSIFLORA* (*FA*) may act under the control of the miR156/*Sl-SBP* node to regulate floral meristem identity and specification of organ whorls, but while *GOB* is controlled by the miR156/*Sl-SBP* node through miR164, it remains unknown how miR156/*Sl-SBP* controls *MC*, *FUL1*, and *FA*.

The epigenetic factors controlling the expression profile of genes involved in both flower and fruit development as reviewed in this chapter are all schematically overviewed in the Fig. 1.

4 Fruit Ripening

The ripening development process is a unique feature to plants bearring fleshy fruits. Its function is to help the dissemination of the plants seeds through animal consumption. Therefore, the fruit undergoes important changes in, for example, color: making them more visible, in metabolite composition, such as production of sugars and volatiles. In tomato, this transition from a mature green to a ripe red fruit is induced at the breaker stage by a concomitant burst in ethylene production and by a sharp increase in differential expression of transcription factors. Our

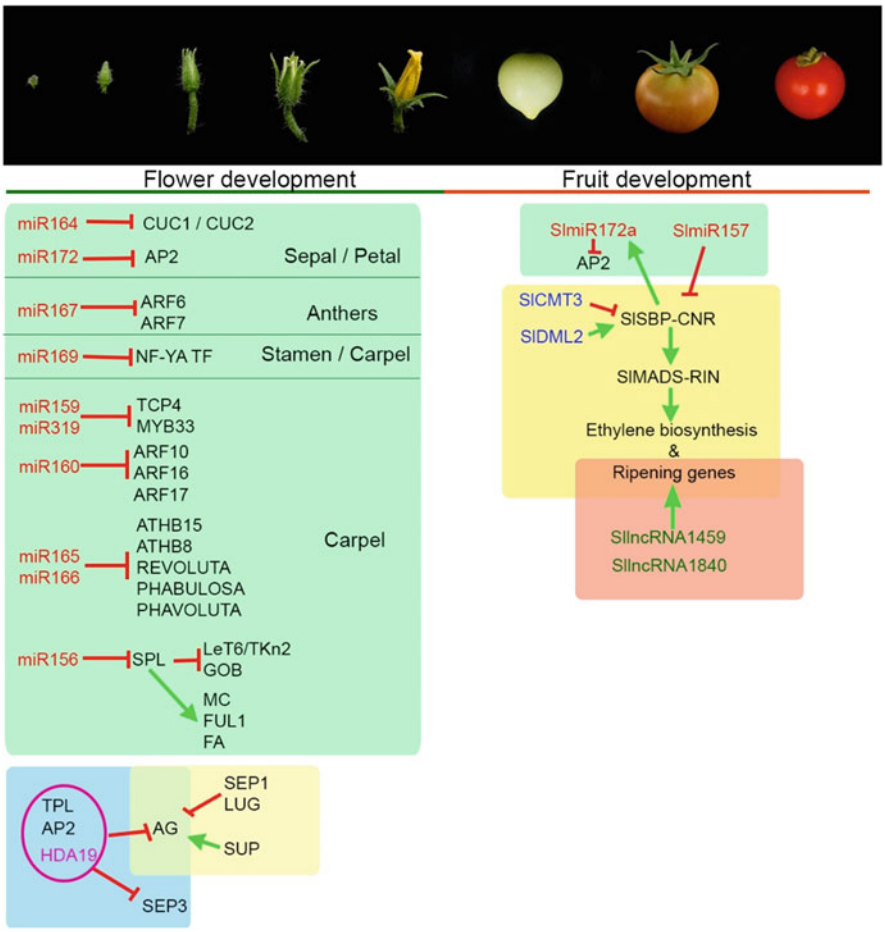

Fig. 1 Epigenetic regulation of genes governing flower and fruit development. Flower development, on the *left*, is monitored by a combination of histone acetylation, DNA methylation, and miRNA posttranscriptional gene silencing. The *green* backgrounds encapsulate regulations monitored by miRNAs. The list of miRNA directed regulations is divided according to organ location specificity. On the *yellow* backgrounds are genes whose expression is dependent on DNA methylation status: *SEPALATA1* (*SEP1*), *LEUNIG* (*LUG*), and *SUPERMAN* (*SUP*), all of which regulate *AGAMOUS* (*AG*). On the *blue* background are represented flower development genes regulated by *HISTONE DEACETYLASE 19 (HDA19)*. Regulation of the transition from *green* to maturing fruit is regulated by the pivotal gene *SLSBP-CNR* which is controlled (1) by promoter methylation status thanks to *SlCMT3* and *SlDML2* and (2) by SlmiR157 (*green* background). lncRNA1459 and lncRNA1840 that are involved in the regulation of fruit ripening genes are shown on the *red* background

understanding of the role of epigenetic players in fruit ripening regulation is a recent addition to this complex development process.

4.1 DNA Methylation-Mediated Regulation

The regulation of the fruit transition from green to ripe is epigenetically regulated by DNA methylation of cytosine nucleotides. Progress in understanding the molecular regulations of the ripening process in tomato have been achieved by the study of ripening mutants such as ripening inhibitor (*Rin*) and Colourless non-ripening (*Cnr*). The *Cnr* mutation was mapped to a SlSBP3-like gene (Manning et al. 2006), and since the nucleotide sequence of the *SlSBP-CNR* mutant was identical to the one of the wild-type plant, the authors hypothesized that it could be an epimutation. Bisulfite sequencing of 2.4 kb upstream of the *SlSBP-CNR* start site revealed a 286 bp region rich in cytosine methylation. Further VIGS experiment confirmed the role of *SlSBP-CNR* as regulator of fruit ripening. The *Rin* mutation mapping and sequencing characterized the Rin locus as a MADS box transcription factor gene whose expression in the *Rin* background restored ripening (Vrebalov et al. 2002). *SlMADS-RIN* acts upstream of ethylene in the ripening regulatory chain. *SlMADS-RIN* regulates, through interaction with their promoters, the expression of numerous genes involved in various ripening-related pathways such as ethylene biosynthesis, perception, and response; cell wall metabolism; and carotenoid biosynthesis (Martel et al. 2011). In addition, the binding of *SlMADS-RIN* to its target promoters cannot occur in the hypermethylated *Cnr* mutant (Martel et al. 2011); thus, transcription activation by *SlMADS-RIN* is impaired by methylation of these promoters and can only occur in plants with proper *SlSBP-CNR* activity. Inhibition of 5-cytosine DNA methyltransferases in green immature fruits leads to early fruit ripening, before seeds are mature. Whole genome methylome showed that, in genic regions, differentially methylated regions were located on the 5′-end of genes, therefore likely to be associated with promoter regulatory regions (Zhong et al. 2013). While whole genome methylome showed that a substancial epigenome reprogramming is occurring during fruit development, it also identified 292 genes that are regulated by the *SlRIN* MADS box transcription factor. These genes, in the hypermethylated *Cnr* background, have methylation in their promoter region that prevent binding and activation by *SlRIN*. This work confirmed 16 previously identified RIN targets. RIN-regulated genes are demethylated in the control plants, thus allowing normal production of ethylene, volatiles, sugar metabolites, carotenoids, and fruit softening. Genome methylation is thus the third key determining factor to the transition to ripening in fleshy fruit plants in addition to ethylene hormonal control and fruit-specific transcription factors (Zhong et al. 2013).

To identify which DNA methyltransferase is responsible for the methylation of the promoter of the *Cnr* locus Chen et al. (2015b) used the VIGS system to silence a range of DNA methyltransferases in *Cnr* fruits. *Cnr* fruits with silenced *SlDRM7*, *SlMET1*, *SlCMT2*, and *SlCMT3* ripened to various degrees. *SlCMT3*-silenced fruits

ripened almost completely. The expression of *SlCNR* and of the ripening genes *SlRIN*, *SlAP2a*, *SlTAGL1*, of the ethylene biosynthesis, and signaling pathway were all upregulated in the red sections of the VIGS-SlCMT3 fruits. Bisulfite sequencing determined that 8 out of 18 methylated cytosine in the *Cnr* promoter were demethylated in the VIGS-SlCMT3-silenced fruits. This reduction in methylation was sufficient to alter the expression level of the CNR gene. In addition, the bisulfite sequencing data showed that hypomethylation in *SlCMT3*-silenced tissues also occurred at the promoter sites of *SlRIN* and RIN-targeted promoters directing the expression of ripening genes. Thus, *SlCMT3* is essential to the maintenance of the stable *Cnr* epiallele. In wild-type plants, at the time when the fruit is ready to switch into its ripening phase, the promoters of genes discussed above have to be demethylated. The tomato genome contains four DEMETER-like DNA demethylases (DMLs), but only *SlDML2* is expressed from the onset of fruit ripening and further on until the fruit is fully ripe (Liu et al. 2015a). *SlDML2* RNAi silencing lines showed delayed initiation of ripening, from 10 to 20 days, and the ripening was never fully completed in fruits of these lines. Thus, active demethylation is a prerequisite to tomato fruit ripening. *SlDML2* is the only demethylase expressed at the developmental stage corresponding to demethylation of ripening genes characterized in *Rin* and *Cnr* mutants such as *SlPSY1* (Liu et al. 2015a).

4.2 MiRNA Mediated Regulation

Deep sequencing of tomato short RNAs and comparative genomics have contributed to the identification of hundreds of miRNA expressed in tomato tissues (Din and Barozai 2014; Karlova et al. 2013; Moxon et al. 2008; Zuo et al. 2012). These large-scale projects showed that miRNAs are involved in most aspects of plant and fruit development and along all stages of fruit growth and ripening. But few of the predicted miRNA/target gene interaction have been experimentally validated. Moxon et al. (2008) predicted that miR157 and miR156 play a role during fruit ripening through an interaction with *SlSBP-CNR*. This prediction was experimentally validated, and it was demonstrated that SlmiR157 regulates the expression of *SlSBP-CNR* and thereby participates in the fine-tuning of the ripening process (Chen et al. 2015a). SlmiR156 on the other hand does not take part in ripening regulation, but on top of its role in fruit development (Silva et al. 2014), it has a function in fruit softening (Chen et al. 2015a). While *SlSBP-CNR* is to some extent regulated by SlmiR157, *SlRIN* on the other hand controls the expression of multiple miRNAs (Gao et al. 2015). Out of 33 identified miRNA families in the *rin* mutant, 14 of them were differentially regulated in ripening fruits of the mutant plants. *SlRIN* CArG-box binding sites were identified in four out of ten looked into promoters of SlmiRNA precursors. A ChiP-qPCR assay experimentally proved that indeed *SlRIN* binds to the promoter region of miR172a.

4.3 LncRNA Mediated Regulation

Besides small RNAs, long noncoding RNAs (lncRNAs) are also important for epigenetic regulation in plants. LncRNAs are defined as RNA molecules over 200 bp that do not contain an open reading frame coding for a polypeptide longer than 100 amino acids. Around 40,000 lncRNAs were identified in *Arabidopsis* (Liu et al. 2012; Wang et al. 2014). lncRNAs are transcribed by Pol II, Pol IV, and PolV polymerases. Pol II lncRNA transcripts have a 5′-cap and a 3′polyadenylated tail, similarly to mRNA. Long noncoding RNAs function as epigenetic regulators through various mechanisms. Functions as target mimics of miRNA were identified in *Arabidopsis* and Rice (Franco-Zorrilla et al. 2007; Wu et al. 2013); in this role, the lncRNA is a decoy that capture specific miRNA in place of the miRNA target gene, thus regulating the miRNA activity. Additional functions include posttranscriptional modification of transcription factors, regulation of mRNA alternative splicing, regulation of the Pol II transcription machinery, and working as enhancers or super-enhancers of mRNA transcription, and lncRNAs have a central role in the plant-specific RdDM epigenetic machinery [lncRNA roles in plants are reviewed in Chekanova (2015) and Liu et al. (2015b)]. Pol IV and Pol V transcripts are long noncoding RNAs (lncRNAs) essential to the RdDM machinery: Pol IV transcripts are transformed into double-stranded RNA (dsRNA) by RDR2 and broken down into siRNA by DCL3. They direct, through sequence complementarity to Pol V lncRNA, the DNA and histone epigenetic modifiers to their target genome location. Therefore, both types of lncRNA are involved in the direction of the RdDM complex to the genome's target sites. A G to C SNP mutation in the rice lncRNA, long-day-specific male fertility-associated RNA (LDMAR), was sufficient to affect the RNA secondary structure which in turn led to increased methylation in the promoter of LDMAR. Promoter methylation decreased the accumulation of LDMAR levels in anthers under long day condition, thus leading to male sterility (Ding et al. 2012). lncRNAs, like mRNAs, are differentially expressed depending on location and developmental stages, thus when aiming at systematic identification of expressed transcripts, it is important to carefully select the tissues under examination. From RNAseq data generated from rice anthers, pistils, seeds, and shoots, 2224 lncRNAs were identified (Zhang et al. 2014). Among them, the authors identified several lncRNAs acting as target mimics for miR160 and miR164. In addition, the T-DNA insertion mutant of lncRNA XLOC_057324 had earlier flowering and low seed set phenotypes, pointing to a role for that lncRNA in the formation of rice panicule and flower fertility. In the rice and maize cereals, lncRNAs are very probably contributing to agronomic traits because the combination of results from Genome Wide Association Studies (GWAS) with the position of about 29,000 lncRNAs in rice and maize showed that 234 SNPs associated with 34 morphological, developmental, and agronomical traits were mapping to lncRNA genomic positions (Wang et al. 2015a). In tomato, Wang et al. (2015b) generated RNAseq datasets from control and TYLC virus-infected leaves to identify lncRNAs involved with the defense of tomato against TYLCV. They predicted 1565

lncRNAs as potentially involved in TYLCV infection. Results from differential expression analysis were confirmed by qRT-PCR, and the implication of one lncRNA into the response to TYLCV virus infection was confirmed using a VIGS approach (Zhu et al. 2015), focused on the fruit ripening process. They compared lncRNAs identified in a RNAseq dataset from breaker stage (the transition from green to ripe tomato fruit) in Aisla Craig control plant to a RNAseq dataset from the same ripening stage but from the *ripening inhibitor* (*rin*) mutant. A total of 3679 lncRNAs were identified in these samples from which 677 were differentially expressed between the two conditions. The involvement of lncRNAs in the ripening transition process was validated for two lncRNAs (SllncRNA 1459 and SllncRNA1840) using the VIGS method to silence the target lncRNAs. VIGS plants for both lncRNAs showed delayed ripening compared to the control, thus validating the function of lncRNA in the ripening process. The mechanisms by which these lncRNAs operate to produce the observed phenotype remain to be deciphered. Using publicly available RNAseq datasets, Wang et al. (2016) looked into the evolution of lncRNAs by analyzing noncoding transcripts in both cultivated *Solanum lycopersicum* and the wild accessions *Solanum pimpinellifolium* and *Solanum pennellii*. The authors identified 413 lncRNAs from *S. lycopersicum* Heinz1706 and confirmed by qRT-PCR that the transcripts they identified match the expression profile of the RNAseq data. The datasets from Heinz1706 used in this study were generated by the tomato genome sequencing consortium. They cover a vast range of tomato tissues: from roots to fruits. Wang et al. (2016) analyzed the expression profile of 413 lncRNAs in developing flowers and fruits and confirmed with qRT-PCR that some lncRNAs are differentially regulated throughout fruit development, thus leading to the hypothesis that lncRNAs are involved in this process.

5 Seed Development

Seeds are the keystone of human development. Plants evolved several strategies and a wide range of adaptations to preserve successful germination of its genetic content and to conquer several different environments. Angiosperm seed development initiates with the double fertilization of the megagametophyte, where the pollen tube delivers two haploid sperm cells to the embryo sac. One sperm cell fuses with the haploid egg to generate a diploid embryo, and the other sperm cell fuses with the diploid central cell to form the triploid endosperm. The resulting embryo and endosperm are genetically identical except for their ploidy level with the endosperm having two maternal doses of the genome and one male dosage (reviewed in Bai and Settle 2014). The fertilized egg and central cell go on to form the embryo and the endosperm, respectively, by multiplying and expanding through several cell cycles. Core cell cycle factors play important roles in the regulation of the cell division cycle during seed development and its coordination with cell differentiation and maturation. Diverse aspects of the seed development such as seed dormancy and embryo and endosperm development involve epigenetic

mechanisms (Kohler and Makarevich 2006; Wollmann and Berger 2012). Although seed development is regulated through physiological and transcriptional regulation, in this chapter we are going to focus only on the epigenetic aspects of seed development.

5.1 Seed Dormancy

Seed dormancy is a process that allows germination delay until a favorable environment arrives. Pieces of evidence for an epigenetic regulation of gene expression in controlling dormancy and germination in cereal seeds have emerged only recently. Genes associated with histone and chromatin structure are overrepresented among *loci* transcriptionally induced at the whole-seed level during germination of non-dormant barley seeds, specifically during the phase of late germination (An and Lin 2011). Moreover, the SET family—transcription factors that play role in histone methylation—are consistently expressed in the embryos during germination of non-dormant rice seeds (Malagnac et al. 2002; Xiao et al. 2003; Howell et al. 2008). Recent study analyzing the whole wheat seed transcription showed several genes activated during imbibition of after-ripened samples that were enriched in the chromatin assembly gene ontology (Gao et al. 2012). Such genes include those encoding for histone proteins such as H4, HTA11, HTA12, HTB11, HTB9, and FASCIATA 1 (FAS1), a histone-binding protein, which are important for nucleosome and chromatin formation, and thus gene expression regulation. Furthermore, orthologues of DNA methylation-related genes, including *CHROMOMETHYLASE 3* (*CMT3*) and *METHYLTRANSFERASE 1* (*MET1*), exhibit transcriptional induction in embedded, after-ripened dormant seeds. Such findings suggest an epigenetic role in regulating gene expression and modulating after-ripening-induced developmental switch of wheat seeds from dormant to non-dormant state (Fig. 2) (Gao and Ayele 2014). Additional studies are required to identify more dormancy-related epigenetic regulators and define how epigenetic mechanisms are involved in the control of wheat seed dormancy and germination.

In *Arabidopsis thaliana*, seed dormancy-specific genes include the *DELAY OF GERMINATION* (*DOG*) family. *DOG1* is expressed in seeds during the maturation stage; the transcript accumulates during seed maturation stage with peaks around 14–16 days after pollination (DAP) (Bentsink et al. 2006) and then is downregulated around 20% in freshly harvested seeds, vanishing during imbibition. Loss of function of *DOG1* results in the absence of dormancy (Bentsink et al. 2006). Wheat transcription factor Histone Binding Protein-1b (HBP-1b) displays the highest similarity with *Arabidopsis* DOG1 (Bentsink et al. 2006). The leucine zipper class transcription factor HBP-1b binds to the H3 hexamer motif ACGTCA in the promoter regions of wheat histone H3 loci (Mikami et al. 1989). This motif is required for transcription of wheat *H3 histone locus* (Nakayama et al. 1989).

A suitable candidate for a seed dormancy-imposing gene is *HUB1* (*histone mono-ubiquitination1*) since *hub1* seeds exhibit reduced dormancy (Liu et al.

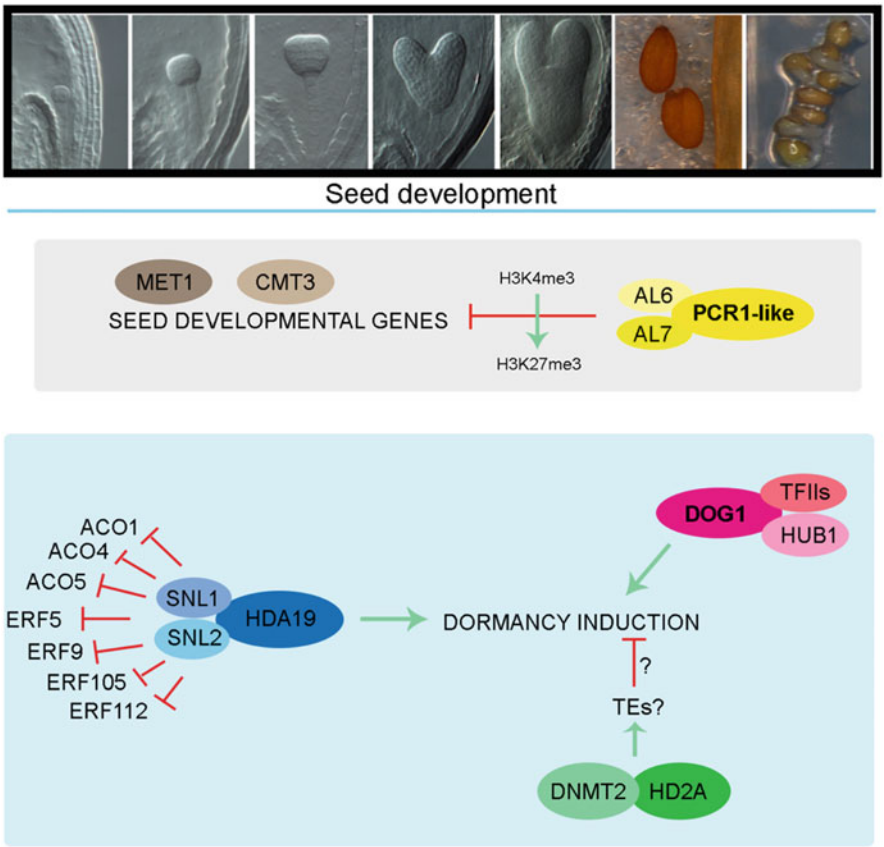

Fig. 2 Epigenetics of *Arabidopsis thaliana* seed development. Embryo developmental stages shown as octant stage, globular stage, early-heart stage, heart stage, torpedo stage, mature dry seed, and imbibed seeds, respectively. *CHROMOMETHYLASE 3* (*CMT3*) and *METHYLTRANSFERASE 1* (*MET1*) regulate developmental seeds genes by methylating DNA during embryogenesis. AL (*Alfin1-like*) proteins *AL6* and *AL7* interact with the Polycomb repressive complex 1 (PRC1)-like complex repressing seed developmental genes by switching from active H3K4me3 to inactive H3K27me3 marks of seed genes during seed germination. *DELAY OF GERMINATION1* (*DOG1*) targets *HUB1* (histone mono-ubiquitination 1) and *TFIIS* (transcription elongator factor IIS) controlling seed dormancy. *SIN3-LIKE1* (*SNL1*) and *SNL2* interact with *HDA19* (histone deacetylase19) positively regulating seed dormancy, targeting *1-AMINOCYCLOPROPANE-1-CARBOXYLATE OXIDASE 1* (*ACO1*), ACO4, and ACO5 and ethylene responsive genes, such as ETHYLENE RESPONSE FACTOR 9 (ERF9), ERF105, and ERF112, triggering seed dormancy by suppressing the ethylene pathway, affecting positively the seed germination. HISTONE DEACETYLASE2A (HD2A) and DNA METHYLTRANSFERASE2 (DNMT2) are possibly working together in the germination process affecting early embryogenesis stages

2007). *ABA INSENSITIVE 4* (*ABI4*), *DOG1*, *NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 9* (*NCED9*), and other genes have been identified as possible targets of *HUB1* (Liu et al. 2007). *TFIIS* (transcription elongator factor IIS) and *HUB1*, two positive regulators of transcription, are induced during the same stages of seed maturation (∼18–19 DAP). There is a significant overlap of differentially expressed genes in *tfIIs* and *hub1* mutants. This finding suggests that *TFIIS* and *HUB1* might share common targets. One of the genes commonly downregulated in both two mutants is *DOG1* (Liu et al. 2011). Therefore, chromatin remodeling and transcriptional elongation might activate *DOG1* through a primary mechanism for seed dormancy. A recent analysis of the *tfIIs* mutant, in which seed dormancy is decreased but returned to the wild-type level by an extra copy of *DOG1,* supports the hypothesis that seed dormancy is controlled by the efficiency of transcription elongation of *DOG1* (Fig. 2) (Mortensen and Grasser 2014). More analyses of the specific targets of epigenetic modification and transcriptional elongation will be necessary to shed more light on seed dormancy regulation through these processes.

While activation of dormancy *loci* through transcription elongation seems to be critical for dormancy induction, continuous repression of seed germination-associated genes is perhaps an essential part of dormancy maintenance. There is evidence that histone deacetylation is imperative for repression of loci positively affecting seed germination. In mammals and yeast, histone deacetylase (HDAC) interacts with SWI-INDEPENDENT3 (SIN3), an amphipathic helix-repeat protein, to remove acetyl groups from lysine in the histone tails, creating a transcriptionally inactive state of the chromatin (Lai et al. 2001; Grzenda et al. 2009). In *Arabidopsis*, SIN3-LIKE1 (SNL1) physically interacts with HDA19, an *Arabidopsis* HDAC orthologue, both in vitro and in planta (Wang et al. 2013). The *Arabidopsis* genome contains also *SNL2*, which is partially redundant to *SNL1*. Seeds from *snl1*/*snl2* double mutant exhibit decreased dormancy. A decreased dormancy phenotype is also observed in *hda19* mutant seeds (Wang et al. 2013). These data imply that *SNLs* and *HDA19* are seed dormancy positive regulators. It seems that suitable repression of SNL-HDA19 complex targets, which are most likely germination-inducing *loci*, through histone deacetylation, is essential for typical seed dormancy. Acetylation of H3K9/18 and H3K14 is increased in the *snl1*/*snl2* double mutant (Wang et al. 2013), corroborating that in wild-type seeds, the SIN3-HDAC complex deacetylates histones and therefore adds repressive marks on the chromatin (Richon and O'Brien 2002). Global gene expression analysis of *snl1*/*snl2* double mutant and wild-type seeds identified possible targets for *SNL-HDA19* such as *1-AMINOCYCLOPROPANE-1-CARBOXYLATE OXIDASE 1* (*ACO1*), *ACO4*, and *ACO5* and ethylene responsive genes, such as *ETHYLENE RESPONSE FACTOR 9* (*ERF9*), *ERF105*, and *ERF112* (Wang et al. 2013). Quantitative PCR combined with chromatin immunoprecipitation employing H3K9/18 acetylation-specific antibodies confirmed that *ACOs* and *ERFs* genes were indeed hyper-acetylated in the mutant, mostly in the promoter region, although hyperacetylation was also found in coding regions (Wang et al. 2013). These results indicate that *SNL-HDA19* triggers seed dormancy by suppressing the

ethylene pathway, affecting positively the seed germination in *Arabidopsis* (Chiwocha et al. 2005; Arc et al. 2013) (Fig. 2).

Another family of plant histone deacetylase, the HD2s (HD2A, HD2B, HD2C, HD2D), are probably part of the seed dormancy-regulated pathways. HD2A can mediate transcriptional repression (Ueno et al. 2007) and is correlated with early stages of somatic embryo development (Zhou et al. 2004). HD2s and DNMT2 (an intriguing enzyme that holds a DNA methylation motif, but methylates specific tRNAs instead) proteins interact in *Arabidopsis* nucleus; then one of the hypothesis is that they are working together in epigenetic pathways, playing role in plant development (Song et al. 2010). Unpublished data from our group on *Arabidopsis hd2a/dnmt2* double mutants show a consistent difference at the germination and seedling growing rates in the first 48 h compared to wild-type seeds, where double mutant seeds germinate earlier and seedlings grow faster than the wild type. It seems that HD2A and DNMT2 work together, most likely as a complex (Song et al. 2010), in the germination process affecting male gametogenesis and/or early embryogenesis stages (Alves 2015). This conclusion is supported by data on mice, suggesting that DNMT2 is required for male gametogenesis (Kiani et al. 2013). Furthermore, HD2A is already known to be a seed germination and fine-tune growing regulator in *Arabidopsis* (Zhou et al. 2004; Colville et al. 2011). Further studies are necessary to unravel the epigenetic mechanisms by which DNMT2-HD2A complex regulates seed germination and early seedling development.

Histone modification may be partially inherited through cell division and epigenetic reprogramming should take place at fertilization, but these processes remain far from being completely understood. DNMT2 targets transcripts other than tRNAs (Alves 2015), and it could work as a cofactor together with HD2A to remove histone acetylation and, as a result, some *loci* that should be repressed during seed germination may be activated or partially activated in the *hd2a/dnmt2 Arabidopsis* double mutant. Moreover, methylome profiling of pollen indicates dynamic DNA methylation changes during male gametogenesis, but information regarding methylation enzymes acting at this stage is unknown. Non-CG methylation increases inn pollen vegetative cells on transposable element (TE)-rich regions, probably to regulate these elements (Borges et al. 2012). Normally, LTR (long terminal repeats) retrotransposons are regulated by RdDM pathway. Low levels of siRNAs associated with LTR retrotransposons were found in the pollen vegetative cell (Slotkin et al. 2009). However, 21-nucleotide (nt) siRNAs are found at this stage and not 24-nt siRNAs. The current model is that noncanonical RdDM pathways take place at this stage to control these elements.

5.2 Embryo–Endosperm Interaction

In *Arabidopsis* endosperm, genes and TEs are regulated by both DNA and histone (H3K37me3) methylation (Schmidt et al. 2013), with substantial variation between endosperm and embryo tissues (Gehring et al. 2009). In rice, endosperm

hypomethylation occurs in all sequence contexts (CG and non-CG), although CG methylation is not similar to CHG and CHH, which are hypomethylated similarly across the genome (Zemach et al. 2010). For seed viability, MET1 and CMT3 activity is required during *Arabidopsis* embryogenesis (Xiao et al. 2006), leading to preferential maternal hypomethylation in the endosperm, while paternal methylated alleles are maintained. However, the function of the remaining methylated *loci* is largely unknown (Zhang and Xue 2013).

Epigenetic regulation is crucial for leading tissue differentiation into distinct *primordium* cell lineages and driving inheritance of each transcriptional programmed through mitosis at early stages of embryo development (Bantignies and Cavalli 2006). In the egg cell, MET1 is expressed following gametogenesis, yet the developing embryo, endosperm, and seed coat also contain its transcripts (Schmidt et al. 2013). The jmjC domain-containing histone demethylase from BcJMJ30 in *Brassica rapa* is associated with pollen development and fertilization (Li et al. 2012). Although *Helianthus LEAFY COTYLEDON1-LIKE* (*HaL1L*) is involved in early stages of zygotic and somatic embryogenesis, with multiplexed transcriptional regulation by DNA methylation, TFs (Transcription Factors), auxin, and ABA (Salvini et al. 2012).

The *MATERNALLY EXPRESSED LOCUS 1* (*MEG1*) in maize is expressed only in the basal nutrient transfer region of the endosperm (Gutiérrez-Marcos et al. 2004), where the genomic imprinting (an epigenetic event that silence one allele from one of the parents; see next section) of *MEG1* supports nutrient transfer from endosperm to the newly developing embryo (Costa et al. 2012). AL (Alfin1-like) proteins are PHD-containing proteins, and there are seven AL proteins in *Arabidopsis*, identified by AL1–AL7 (Lee et al. 2009; Molitor et al. 2014). The AL proteins are named following their homologue Alfin1 in alfalfa, which participates in salt tolerance (Winicov 2000). Functional studies have uncovered that AL6 and AL7 interact with the Polycomb repressive complex 1 (PRC1)-like complex (a complex that contains Polycomb group-like/PcG-like proteins) responsible for the methylation of Lys 27 of histone H3 (Deleris et al. 2012), in *Arabidopsis* to repress seed developmental genes by switching from active H3K4me3 to inactive H3K27me3 marks of seed genes during seed germination and early seedling growth. Also, this function depends on the interaction between AL6/AL7 and H3K4me3 (Molitor et al. 2014). A delay in seed germination under osmotic treatments but not under normal conditions is manifested in Al6 and Al7 double mutants, in agreement with the function of their homologue Alfin1 (Molitor et al. 2014; Winicov 2000). However, the single mutants of *al6* or *al7* show a normal phenotype under any conditions, indicating that AL6 and AL7 act redundantly in seed germination (Molitor et al. 2014).

5.3 Genomic Imprinting

Imprinting is an epigenetic phenomenon by which one of the alleles is silenced through methylation and histone modification mechanisms (Raissig et al. 2011). Imprinted alleles can be inherited maternally or paternally, and the imprinting takes place at the germline and is maintained through mitosis in somatic cells. It is an important mechanism to ensure the correct information transmission to the offspring. Imprinting also guarantees that TEs stay epigenetically silenced during reprogramming of plant gametogenesis, facilitating seed germination events (Wollmann and Berger 2012). Exposure to pathogens can initiate differential 5mC (5-methylcytosine) patterning, activating *NON-EXPRESSOR OF PR GENE* (*NPR1*), a defense regulatory gene (Dowen et al. 2012; Luna and Ton 2012). The transgenerational genomic imprinting of *NPR1* is probably due to posttranslational histone modifications and expression of RNA Polymerase V acting along with siRNAs to recruit methylation machinery (Luna and Ton 2012; You et al. 2013). In developing embryo genomic imprinting, sRNAs produced maternally in plant reproductive tissue can be mobile and may target specific genes, providing the first evidence for a link between genomic imprinting and RNA silencing in plants (Gutierrez-Marcos et al. 2012; Mosher et al. 2009). A variety of microRNAs, including at least four associated with nutrient homeostasis (miR169, miR395, miR398, and miR399), are indeed mobile and graft transmissible and detected in the phloem (Marín-González and Suárez-López 2012). These findings reinforced that maternally produced small RNAs (miRNAs or siRNAs) may be present in the next generation. sRNA-based regulation found in fertilization can take place during seed maturation and possibly during seed dormancy as well (Mosher et al. 2009). It is possible that the female sRNAs may influence gene regulation during germination to assist seedling establishment. This female-specific genomic imprinting mechanism may have evolved from the advantage fitness from the maternal niche germinated offspring (Gorecki et al. 2012). Throughout plant embryogenesis, hypomethylation is less prominent when compared to mammalian systems, with a higher proportion of parental DNA methylation events carried to the following generation (Reinders et al. 2009). An example is the different methylated profile in the *Arabidopsis PHE1* (*PHERES1*), in which the male *PHE1* allele is methylated, and the female allele is hypomethylated (Kohler and Makarevich 2006; Makarevich et al. 2008).

A few years back, plant imprinting was believed to occur only in the triploid endosperm, so, gymnosperms were presumed to lack imprinting mechanisms (Garnier et al. 2008). Nonetheless, more recently, it has been shown that genomic imprinting can occur in angiosperm and gymnosperm embryos (Scholten 2010). The exposure to different temperatures during embryo development can store epigenetic memory during embryogenesis, fixing epigenetic marks before seed maturation, leading to modified germination time and seedling development in the gymnosperm Norway spruce (Yakovlev et al. 2010). The epigenetic memory in long-lived plant species may confer adaptive plasticity to environmental drift in a

single generation, with significant consequences for perennial and clonally propagated crops (Bloomfield et al. 2014).

6 Conclusions and Future Prospects

The epigenetic mechanism most amenable to plant breeding programs is gene expression regulation through DNA methylation. Epigenetic variability in crop plants can be either induced through chemical treatment using methyltransferase inhibitors or induced by exposure to specific stressful growing environments. Individual plants showing desired phenotypes as well as stable methylation profiles can be selected to be part of selection programs (Rodriguez Lopez and Wilkinson 2015). Thus, fixed epimutations as described for the *Cnr* mutant are not the only source of epigenetic diversity. Using a population of isogenic *Arabidopsis* lines that segregate for differentially methylated regions, Cortijo et al. (2014) showed that two agronomical traits, flowering time and primary root length, are controlled by epigenetic quantitative trait loci. Thus, forward epigenetic approaches could be actually implemented as part of crop breeding for the improvement of significant traits.

The *MutS* HOMOLOGUE1 (*MSH1*) gene is known in *Arabidopsis* to influence plant growth behavior. *msh1* mutant plants have affected vigor and development reprogramming linked to altered genome methylation. The increased plant vigor is characterized by rapid growth and earlier flowering, and greater aboveground biomass was also identified in tomato plants silenced for the *MSH1* gene (Yang et al. 2015). In tomato, the most relevant phenotype in a plant breeding perspective was the increased flower and fruit set that resulted in increased yield. *MSH1* RNAi plants were crossed with the wild-type Rutgers parent and −/− plants not carrying the RNAi transgene were selected as epi-lines. The enhanced hybrid vigor and fruit yield was increased until the epiF4 generation. Such heritable increased methylation in *msh1* plants was observed in multiple plant species (*Arabidopsis*, tomato, sorghum). This work, together with the studies demonstrating the role of methylation status of ripening genes, proves the relevance of considering the methylome as part of breeding programs.

Methylations and miRNAs are both involved in the regulation of the tomato fruit ripening process. Another group of epigenetic regulators involved in relevant traits is the lncRNAs. Zhu et al. (2015) showed that lncRNAs are another layer of regulation to the ripening process. In addition, lncRNAs are involved in tomato defense against pathogens such as TYLCV (Wang et al. 2015b) and *phytophthora infestans* (Cui et al. 2017). Together, the works on the identification of lncRNAs implicated in plant quality traits and disease resistance traits have permitted the identification of thousands of lncRNAs in a large array of tissues, developmental stages, and disease treatments. lncRNAs, as genes, are being mapped to the genome sequence and, therefore like genes, can and should be looked at in association studies, forward and reverse genetic approaches. Discovery of tomato lncRNAs is

at its early stage and much of their functions remains to be identified. One particular type of lncRNA is of interest for future fundamental and applied studies: lncRNAs coding for micropeptides (miPEPs). In *Medicago truncatula* and *Arabidopsis thaliana*, miPEPs coded by pri-miRNA of miR171b and miR165a are involved in root development through positive feedback regulation of their own pri-miRNAs (Lauressergues et al. 2015). Treatment with 0.1 μM of miPEP172c increased soybean root nodulation through the stimulation of miR172c and thus *AP2* downregulation (Couzigou et al. 2016). This illustrates the potential of this novel type of epigenetic regulator to regulate agronomical traits and to be able to regulate gene networks through a simple treatment with a synthetic micropeptide.

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