SEMINAR REPORT

Epigenetic regulation of transposable elements in plants

By

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(2018-11-032)

Presented on 22-11-2019

Submitted in partial fulfillment of requirement of the course

GP 591: Masters Seminar (0+1)

DEPARTMENT OF PLANT BREEDING AND GENETICS

COLLEGE OF HORTICULTURE

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CERTIFICATE

This is to certify that the seminar report entitled **"Epigenetic regulation of transposable elements in plants"** has been solely prepared By AKHIL K P (2018-11-032) under my guidance and has not been copied from seminar reports of any seniors, juniors or fellow students.

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This is to certify that the seminar report entitled **"Epigenetic regulation of transposable elements in plants"** is a record of seminar presented by Akhil K P (2018-11-032) on 22nd November, 2019 and is submitted for the partial requirement of the course GP 591.

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1. Introduction

In plants, transposable elements (TEs) represent considerable fraction of the genome, with potential to alter gene expression and produce genomic rearrangements. They were discovered in the 1940s by Barbara McClintock because of their propensity to damage chromosomes, and numerous other active transposons in plants were subsequently identified because they inserted into and interrupted the function of host genes. Transposable elements in crop plants are the powerful drivers of phenotypic variation that has been selected during domestication and breeding programs.

TEs are activated by stress through motifs embedded in their promoters, which can lead to bursts of transposition that increase their copy number. Since plants commonly experience stress throughout their life cycle, activated TEs can potentially jump to new genomic locations leading to gene-altering effects that can have positive or negative consequences. Insertions that fall inside genes typically inactivate gene function, although many of these insertions are never observed since they can result in lethality if the gene is essential. Insertions inside introns can trigger alternative splicing patterns, while insertion in adjacent gene regions can generate new regulatory functions that modify gene expression and function. Given the risk for lethality or significant modification of gene expression, plant genomes possess mechanisms to stop indiscriminate genome expansion and alterations by TEs. One of these mechanisms is based on epigenetic control, which allows the recognition and silencing of TE sequences.

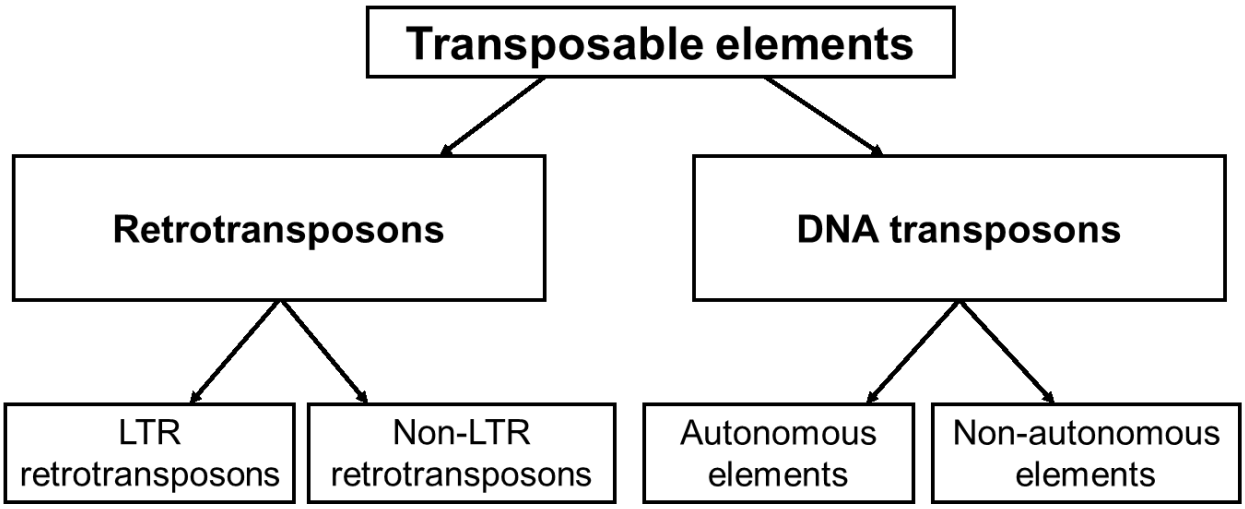
2. Transposable elements

Transposable elements (TEs) are DNA sequences that move through the genome via a cut and paste mechanism using a DNA intermediate (Class II TEs - DNA transposons), or a copy and paste mechanism with an RNA intermediate (Class I TEs - retrotransposons). In plants, TEs account for an important proportion of genomes, although the proportional representation range varies: Fourteen percent in the genome of *Arabidopsis thaliana* (L.) to more than 80% in the genome of maize. TEs are usually represented by numerous families corresponding to different super families, orders and classes, each of which have a specific set of characteristics including

their mode of transposition, presence of promoter sequences, order of genes coding for proteins and mechanisms of replication.

3. Classification of transposable elements

Transposable elements represent one of several types of mobile genetic elements. TEs are assigned to one of two classes according to their mechanism of transposition, which can be described as either *copy and paste* (Class I TEs) or *cut and paste* (Class II TEs) (Feschotte *et al*., 2002).



3.1 Retrotransposon

Class I TEs are copied in two stages: first, they are transcribed from DNA to RNA, and the RNA produced is then reverse transcribed to DNA. This copied DNA is then inserted back into the genome at a new position. The reverse transcription step is catalysed by a reverse transcriptase, which is often encoded by the TE itself. The characteristics of retrotransposons are similar to retroviruses, such as HIV.

Retrotransposons are commonly grouped into three main orders:

- Retrotransposons, with long terminal repeats (LTRs), which encode reverse transcriptase, similar to retroviruses
- Retroposons, long interspersed nuclear elements (LINEs, LINE-1s, or L1s), which encode reverse transcriptase but lack LTRs, and are transcribed by RNA polymerase II

- Short interspersed nuclear elements (SINEs) do not encode reverse transcriptase and are transcribed by RNA polymerase III

3.2 DNA transposons

The cut-and-paste transposition mechanism of class II TEs does not involve an RNA intermediate. The transpositions are catalysed by several transposase enzymes. Some transposases non-specifically bind to any target site in DNA, whereas others bind to specific target sequences. The transposase makes a staggered cut at the target site producing sticky ends, cuts out the DNA transposon and ligates it into the target site. A DNA polymerase fills in the resulting gaps from the sticky ends and DNA ligase closes the sugar-phosphate backbone. This results in target site duplication and the insertion sites of DNA transposons may be identified by short direct repeats (a staggered cut in the target DNA filled by DNA polymerase) followed by inverted repeats (which are important for the TE excision by transposase).

Cut-and-paste TEs may be duplicated if their transposition takes place during S phase of the cell cycle, when a donor site has already been replicated but a target site has not yet been replicated. Such duplications at the target site can result in gene duplication, which plays an important role in genomic evolution.

Not all DNA transposons transpose through the cut-and-paste mechanism. In some cases, a replicative transposition is observed in which a transposon replicates itself to a new target site (e.g. helitron) (Lish, 2009).

Class II TEs comprise less than 2% of the human genome, making the rest Class I.

3.3 Autonomous and non-autonomous

Transposition can be classified as either "autonomous" or "non-autonomous" in both Class I and Class II TEs. Autonomous TEs can move by themselves, whereas non-autonomous TEs require the presence of another TE to move (Kapitonov *et al*., 2008). This is often because dependent TEs lack transposase (for Class II) or reverse transcriptase (for Class I).

Activator element (*Ac*) is an example of an autonomous TE, and dissociation elements (*Ds*) is an example of a non-autonomous TE. Without *Ac, Ds* is not able to transpose.

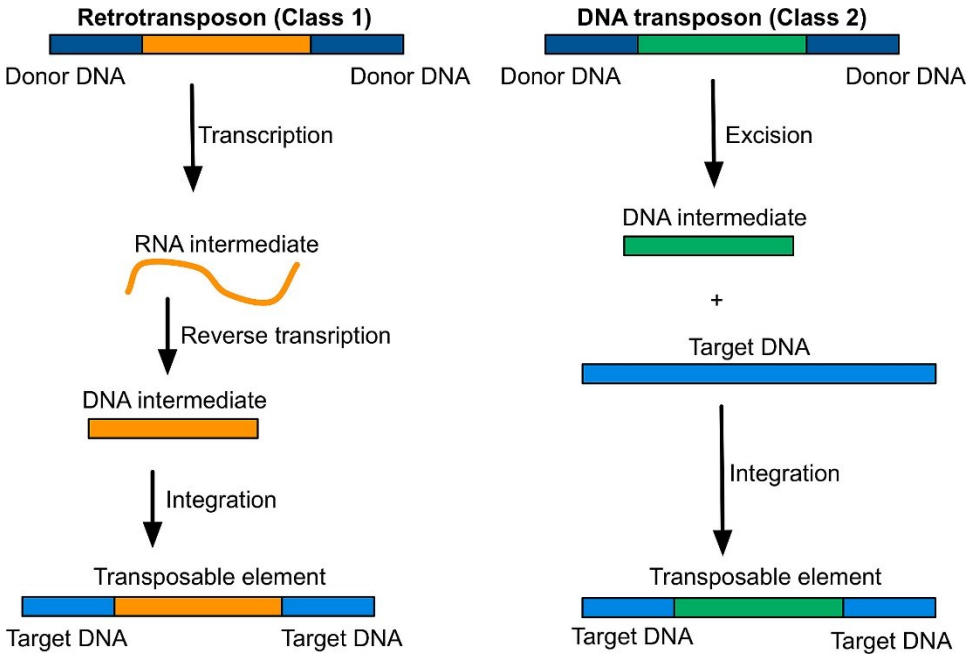


Plate 1: Class I and class II transposons

4. Epigenetics

Epigenetics is the study of heritable phenotype changes that do not involve alterations in the DNA sequence. The Greek prefix *epi-* in *epigenetics* implies features that are "on top of" or "in addition to" the traditional genetic basis for inheritance. Epigenetics most often involves changes that affect gene activity and expression, but the term can also be used to describe any heritable phenotypic change. Such effects on cellular and physiological phenotypic traits may result from external or environmental factors, or be part of normal development.

Epigenetic changes modify the activation of certain genes, but not the genetic code sequence of DNA. The microstructure (not code) of DNA itself or the associated chromatin proteins may be modified, causing activation or silencing. This mechanism enables differentiated cells in a multicellular organism to express only the genes that are necessary for their own activity. Epigenetic changes are preserved when cells divide. Most epigenetic changes only occur within the course of one individual organism's lifetime; however, these epigenetic changes can be transmitted to the organism's offspring through a process called trans-generational epigenetic inheritance. Moreover, if gene inactivation occurs in a sperm or egg cell that results in fertilization, this epigenetic modification may also be transferred to the next generation.

5. Molecular mechanism of epigenetic regulation

In eukaryotes, the structure of chromatin regulates the accessibility of genes to the transcriptional machinery, thereby controlling gene expression. Structurally, DNA is packaged by means of nucleosomes, where histones (H2A, H2B, H3, and H4) are present as octamers, around which 147 bp of DNA are wrapped in almost two turns. The positioning and spacing of nucleosomes as well as post-translational histone modification, together with DNA methylation, affect the overall packaging of DNA and the accessibility of the transcription unit to specific regulatory elements, which results in altering gene expression. In plants three main epigenetic mechanisms have been described: DNA methylation, histone modifications and RNA-interference (RNAi).

6. Epigenetic regulation of transposable elements

6.1 DNA methylation

DNA methylation occurs specifically over cytosine nucleotides that are followed by a guanine and sometimes by other nucleotides; in many cases, DNA methylation stops interaction with transcription factors and impairs gene activation (Saze *et al*., 2012).

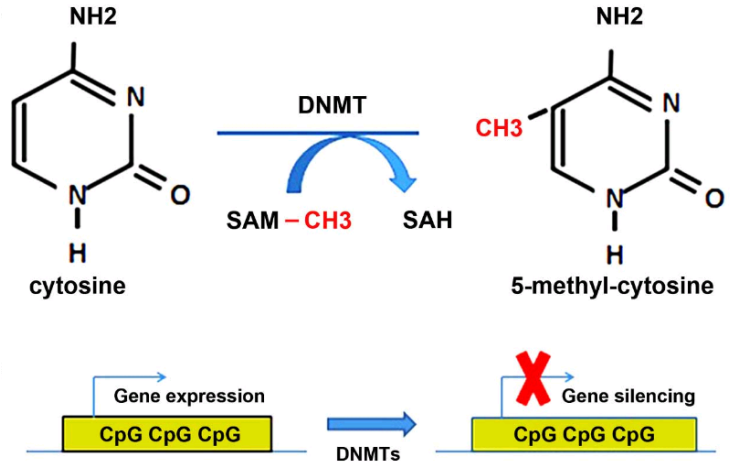


Plate 2: DNA methylation

Case study: Activation and Epigenetic Regulation of DNA Transposon nDart1 in Rice

Eun *et al*., (2012) studied activation and epigenetic regulation of DNA transposon nDart1 in rice. From the study they arrived at following conclusions. A large part of the rice genome is composed of transposons. Since active excision/reintegration of these mobile elements may result in harmful genetic changes, many transposons are maintained in a genetically or epigenetically inactivated state. However, some non-autonomous DNA transposons of the nDart1-3 subgroup, including nDart1-0, actively transpose in specific rice lines, such as pyl-v which carries an active autonomous element, aDart1-27, on chromosome 6. Although nDart1-3 subgroup elements show considerable sequence identity, they display different excision frequencies. The most active element, nDart1-0, had a low cytosine methylation status. The aDart1-27 sequence showed conservation between pyl-stb (pyl-v derivative line) and Nipponbare, which both lack autonomous activity for transposition of nDart1-3 subgroup elements. In pyl-v plants, the promoter region of the aDart1-27 transposase gene was more hypomethylated than in other rice lines. Treatment with the methylation inhibitor 5-azacytidine (5-azaC) induced transposition of nDart1-3 subgroup elements in both pyl-stb and Nipponbare plants; the new insertion. Sites were frequently located in genic regions. 5-AzaC treatment principally induced expression of Dart1-34 transposase rather than the other 38 aDart1-related elements in both pyl-stb and Nipponbare treatment groups. Our observations show that transposition of nDart1-3 subgroup elements in the nDart1/aDart1 tagging system is correlated with the level of DNA methylation.

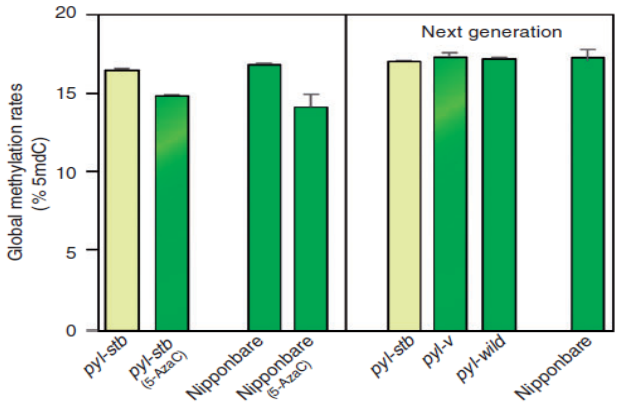


Figure 1: HPLC analysis of rice plant genomes treated with 5-azaC

6.2 Histone modification

Histone modifications are more diverse and include methylation, phosphorylation, acetylation, and ubiquitination of mostly histone H3, but post-translational modifications upon histones H4, H1, and H2A have also been described. These protein modifications constitute the "histone code" of chromatin epigenetic marks (Gonzalez *et al*., 2018).

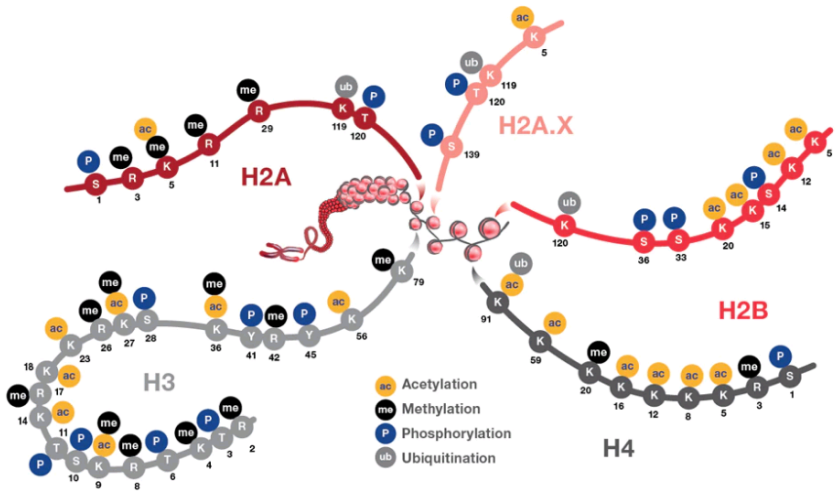


Plate 3: Histone modifications

6.2.1 Histone methylation

Histone methylation is a process by which methyl groups are transferred to amino acids of histone proteins that make up nucleosomes, which the DNA double helix wraps around to form chromosomes.

6.2.2 Histone acetylation

Acetyl group is added to histone tail. Acetylation of histones alters accessibility of chromatin and allows DNA binding proteins to interact with exposed sites to activate gene transcription and downstream cellular functions.

6.2.3 Histone phosphorylation

Histone phosphorylation is associated with local chromatin opening and transcriptional activation. Histone phosphorylation is also linked with chromatin condensation during mitosis. Histone phosphorylation marks are important for regulation of the DNA damage response.

6.2.4 Ubiquitination

Histone ubiquitination is a reversible modification whose steady state is determined by two enzymatic activities involved in addition and removal of the ubiquitin moiety from histones.

Case study: Control of transposon activity by a histone H3K4 demethylase in rice

Cui *et al*., (2012) studied Control of transposon activity by a histone H3K4 demethylase in rice. Using both in vivo and in vitro assays, they identified JMJ703 as a unique functional histone H3K4 demethylase in rice. Rice protein JMJ703 is an active H3K4-specific demethylase required for TEs silencing. Impaired JMJ703 activity led to elevated levels of H3K4me3, the mis-regulation of numerous endogenous genes, and the transpositional reactivation of two families of non-LTR retrotransposons. Genome-wide RNA-seq analysis has revealed that the loss-of function mutant *jmj703* depresses thousands of genes, especially those involved in chromatin assembly. *jmj703* displays pleiotropic defects that could be due to ectopic expression or up-regulation of direct targets of JMJ703. Using a combination of ChIP-seq and RNA-seq analysis, we have identified the non-LTR retrotransposable elements Karma and *LINE1* as direct targets of JMJ703. In *jmj703*, *Karma* displays increased levels of histone H3K4me3; reduced DNA methylation at CpG and, to some extent, CHG; and up-regulated gene expression. These changes enhanced the movement of transposons that are normally silenced and immobile. Their findings show that plants use H3K4me3 demethylase to constitutively remove active chromatin marks and maintain the silent status of a subset of retrotransposons to preserve genome integrity.

6.3 RNA interference

Regarding RNA-interference mechanisms, small RNAs, together with factors commonly associated with (RNAi) processes, target complementary DNA sequences and recruit factors that

can induce chromatin modifications, specifically, the formation of heterochromatin, to silence targeted genes.

To initiate silencing, the host genome must first recognize transposons as such. A central feature of this recognition process is the production and processing of double-stranded RNAs (dsRNAs). Although the mechanism has diversified in different species, its essential components are remarkably well conserved. Aberrant RNA begins as, or is converted into, dsRNA. In many eukaryotes, including plants, this dsRNA is processed into short, 21–26-nucleotide duplexes by the activity of a dicer endonuclease. The size of these small RNAs varies depending on the specific processing pathway being used. One strand of these duplexes (small interfering RNA, siRNA) is then loaded onto a RNA-induced silencing complex (RISC), which invariably includes a member of the Argonaute (AGO) family. The AGO-associated siRNA is then guided to a target mRNA, which is cleaved by the RISC complex. This degradation of target mRNAs is often referred to as posttranscriptional gene silencing (PTGS).

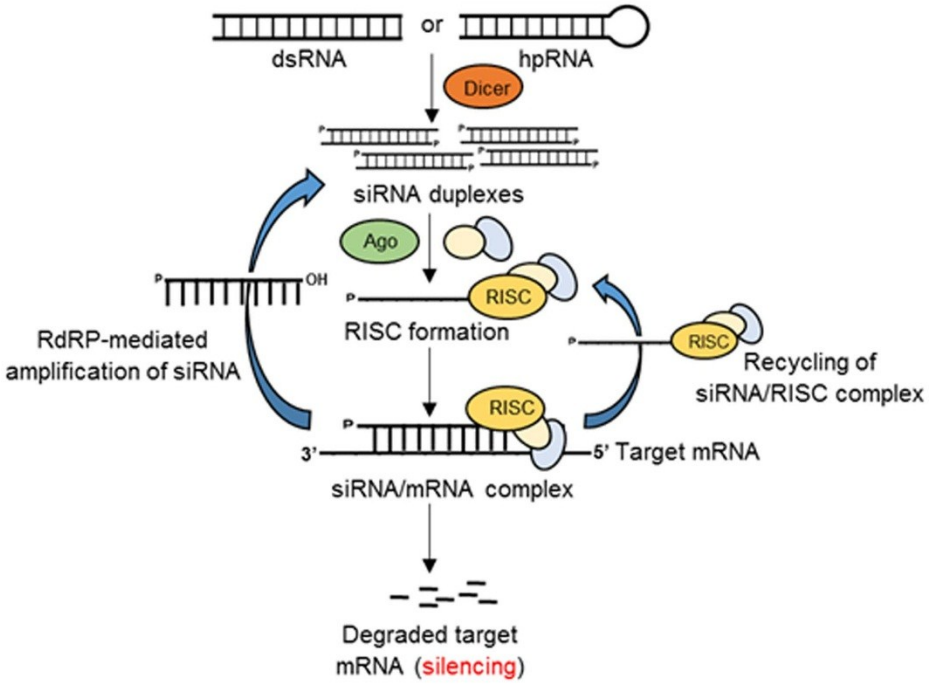


Plate 4: RNA interference

In plants, in addition to triggering degradation of target mRNAs, dsRNAs can also lead to transcriptional gene silencing (TGS) via RNA-directed DNA methylation (RdDM), a process that results in both DNA and histone modifications. These modifications can lead to both mitotically and meiotically heritable silencing, even in the absence of the initial trigger.

Case study: Environmental and epigenetic regulation of Rider retrotransposons in tomato

Benoit *et al*., (2019) studied Environmental and epigenetic regulation of Rider retrotransposons in tomato. They observed that *Rider* transcription is also restricted by RNA directed DNA methylation. Tomato seedlings were grown on media supplemented with 5-azacytidine, an inhibitor of DNA methyltransferases. Rider transcript steady-state levels increased in plants treated with 5-azacytidine compared to controls (Fig 2A). Comparison of Rider transcript accumulation in 5-azacytidine-treated and ABA-treated plants revealed similar levels of transcripts and the levels were similar when the treatments were applied together (Fig 2A).

To further examine the role of DNA methylation in controlling *Rider* transcription, Benoit *et al*., (2019) took advantage of tomato mutants defective in crucial components of the RdDM pathway, namely SlNRPD1 and SlNRPE1, the major subunits of RNA Pol IV and Pol V, respectively. These mutants exhibit reduced cytosine methylation at CHG and CHH sites (in which H is any base other than G) residing mostly at the chromosome arms, with slnrpd1 showing a dramatic, genome-wide loss of 24-nt siRNAs. To evaluate the role of RdDM in Rider transcript accumulation, they first assessed the consequences of impaired RdDM on siRNA populations at full-length Rider elements. Deficiency in SlNRPD1 resulted in a complete loss of 24-nt siRNAs that target *Rider* elements (Fig 2B). This loss was accompanied by a dramatic increase in 21-22-nt siRNAs at Rider loci (Fig 2B). In contrast, the mutation in SlNRPE1 triggered increases in both 21-22-nt and 24-nt siRNAs targeting *Rider* elements (Fig 2B). Compilation of the genomic positions and siRNA data in RdDM mutants didn't reveal preferential accumulation of 21-22-nt siRNAs or 24-nt siRNAs over specific *Rider* classes. Subsequently, examined whether loss of SlNRPD1 or SlNRPE1 was sufficient to increase levels of *Rider* transcripts and observed increased accumulation of Rider transcripts in both *slnrpd1* and *slnrpe1* compared to WT (Fig 2C).

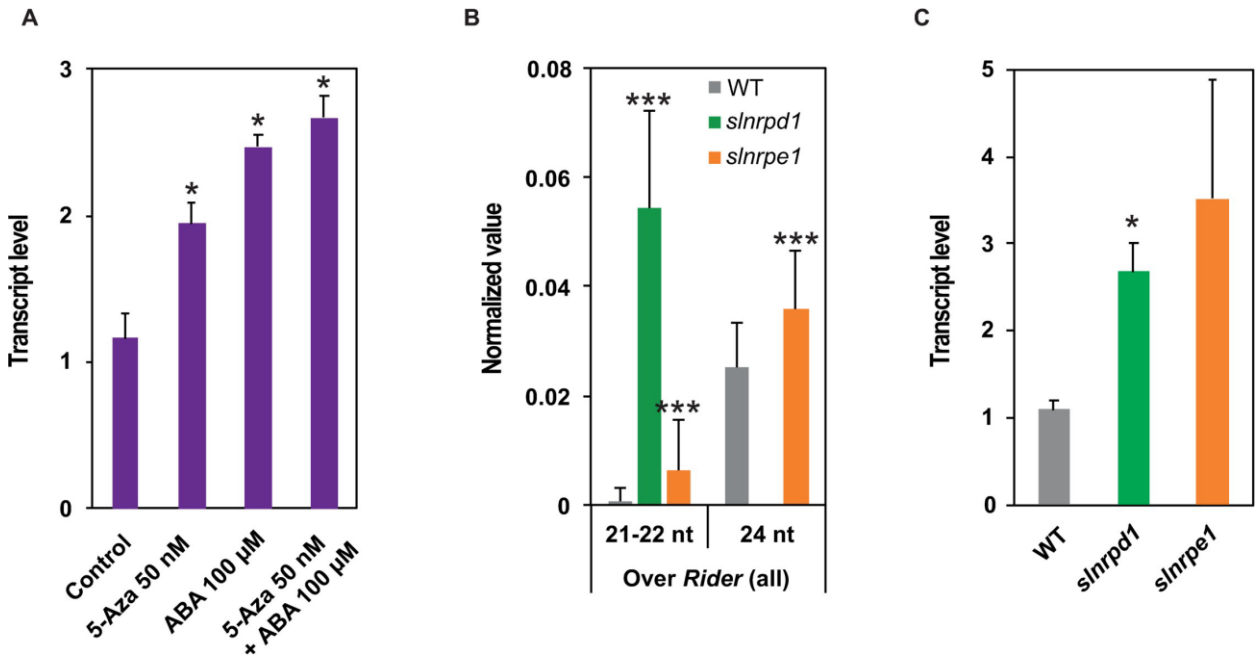


Figure 2: Accumulation of Rider transcripts in tomato plants deficient in epigenetic regulation.

7. Analysis of epigenetic modification

DNA methylation can be studied by Bi-sulfite sequencing and Methylation Sensitive Amplification Polymorphism (MSAP). Chromatin immune-precipitation (ChIP) is the technique used to analyzing histone modification.

7.1 Bi-sulfite sequencing

Bisulfite sequencing is the use of bisulfite treatment of DNA before routine sequencing to determine the pattern of methylation. DNA methylation was the first discovered epigenetic mark, and remains the most studied. In animals it predominantly involves the addition of a methyl group to the carbon-5 position of cytosine residues of the dinucleotide CpG, and is implicated in repression of transcriptional activity.

Treatment of DNA with bisulfite converts cytosine residues to uracil, but leaves 5-methylcytosine residues unaffected. Therefore, DNA that has been treated with bisulfite retains only methylated cytosines. Thus, bisulfite treatment introduces specific changes in the DNA sequence that depend on the methylation status of individual cytosine residues, yielding single-nucleotide resolution information about the methylation status of a segment of DNA. Various analyses can be performed on the altered sequence to retrieve this information. The objective of

this analysis is therefore reduced to differentiating between single nucleotide polymorphisms (cytosines and thymidine) resulting from bisulfite conversion.

7.2 Methylation Sensitive Amplification Polymorphism (MSAP)

Methylation Sensitive Amplification Polymorphism (MSAP) is one of the most commonly used methods for assessing DNA methylation changes in plants. This method involves gel-based visualization of PCR fragments from selectively amplified DNA that are cleaved using methylation-sensitive restriction enzymes. In this study, we developed and validated a new method based on the conventional MSAP approach called Methylation Sensitive Amplification Polymorphism Sequencing (MSAP-Seq). We improved the MSAP-based approach by replacing the conventional separation of amplicons on polyacrylamide gels with direct, high-throughput sequencing using Next Generation Sequencing (NGS) and automated data analysis. MSAP-Seq allows for global sequence-based identification of changes in DNA methylation. This technique was validated in *Hordeum vulgare*. However, MSAP-Seq can be straightforwardly implemented in different plant species, including crops with large, complex and highly repetitive genomes. The incorporation of high-throughput sequencing into MSAP-Seq enables parallel and direct analysis of DNA methylation in hundreds of thousands of sites across the genome. MSAP-Seq provides direct genomic localization of changes and enables quantitative evaluation. We have shown that the MSAP-Seq method specifically targets gene-containing regions and that a single analysis can cover three-quarters of all genes in large genomes. Moreover, MSAP-Seq's simplicity, cost effectiveness, and high-multiplexing capability make this method highly affordable. Therefore, MSAP-Seq can be used for DNA methylation analysis in crop plants with large and complex genomes.

7.3 Chromatin immune-precipitation (ChIP)

Chromatin immune-precipitation (ChIP) is a type of immune-precipitation experimental technique used to investigate the interaction between proteins and DNA in the cell. It aims to determine whether specific proteins are associated with specific genomic regions, such as transcription factors on promoters or other DNA binding sites, and possibly defining cistromes.

ChIP also aims to determine the specific location in the genome that various histone modifications are associated with, indicating the target of the histone modifiers.

Briefly, the conventional method is as follows:

- DNA and associated proteins on chromatin in living cells or tissues are cross-linked (this step is omitted in Native ChIP).
- The DNA-protein complexes (chromatin-protein) are then sheared into ~500 bp DNA fragments by sonication or nuclease digestion.
- Cross-linked DNA fragments associated with the protein(s) of interest are selectively immune-precipitated from the cell debris using an appropriate protein-specific antibody.
- The associated DNA fragments are purified and their sequence is determined. Enrichment of specific DNA sequences represents regions on the genome that the protein of interest is associated with in vivo.

8. Conclusion

Epigenetic control of TEs through methylation adds another layer of TE-dependent regulation in the genome. The tight control of TEs through methylation can be both beneficial and detrimental to genomic stability. Lifting silencing transiently during events of stress, development or genomic shock, would in fact give some flexibility to TEs to generate certain amount of change without totally scrambling the genome. This allows the genome to have some room for restructuring and adaptation in response to different elicitors. Additionally, the incorporation of TEs in promoter regions provides a mechanism to modulate stress response in certain genes. Such insertions can be positively selected if these genes become effective in responding to stress and if their modulation provides better energy balance for the cell. Epigenetic regulation of TEs alters the genomic landscape which could improve long term adaptation to environmental challenges.

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10. Discussion

1. Is this epigenetic regulation occurs in plants only?

   No. Epigenetic regulation occurs in all eukaryotic organism.

2. What is CpG islands?

   The CpG sites or CG sites are regions of DNA where a cytosine nucleotide is followed by a guanine nucleotide in the linear sequence of bases along its 5' → 3' direction. CpG sites occur with high frequency in genomic regions called CpG islands (or CG islands). Cytosines in CpG dinucleotides can be methylated to form 5-methylcytosines.

3. Is there any site other than CpG islands where methylation occurs?

   In plants and other organisms, 'DNA' methylation is found in three different sequence contexts: CG (or CpG), CHG or CHH (where H correspond to A, T or C). In mammals however, DNA methylation is almost exclusively found in CpG dinucleotides, with the cytosines on both strands being usually methylated. Non-CpG methylation can however be observed in embryonic stem cells and has also been indicated in neural development.

4. What is RNA directed DNA methylation?

   RNA-directed DNA methylation (RdDM) is an epigenetic process first discovered in plants. During RdDM, double-stranded RNAs (dsRNAs) are processed to 21-24 nucleotide small interfering RNAs (siRNAs) and guide methylation of homologous DNA loci.

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GP 591: Masters Seminar

Name	: Akhil K.P.	Venue	: Seminar hall
Admission No.	: 2018-11-032	Date	: 22-11-2019
Major advisor	: Dr. Biju S.	Time	: 10.00 a.m.

Epigenetic regulation of transposable elements in plants

Abstract

When the genetic code was first deciphered, genomes were expected to be an amalgamation of genes. The reality has proved to be considerably more complex. In plants, transposable elements (TEs) represent considerable fraction of the genome, with potential to alter gene expression and produce genomic rearrangements. Proportion of TEs varies from 14 per cent in the genome of *Arabidopsis thaliana* (L.) to more than 80 per cent in the genome of maize. Transposons of various classes, including retrotransposons (class I) and DNA transposons (class II), are abundant in plant genomes (Feschotte *et al*., 2002).

In nature, most TEs are silent and rarely transpose due to the point mutation, deletion, or recombination that abolish their activities. Host plants have evolved various types of epigenetic regulation to transposition activity. Epigenetic control of TEs is often used to stop unrestricted movement of TEs that would result in detrimental effects due to insertion in essential genes.

Epigenetics is the study of heritable changes in gene expression that do not involve changes to the underlying DNA sequence. DNA methylation, histone modifications, and RNA interference (RNAi) are the three epigenetic mechanisms for transposon regulation. DNA methylation occurs specifically over cytosine nucleotides that are followed by a guanine. Methylation stops interaction with transcription factors and impairs gene activation. Histone modifications are post-translational modifications upon histone tails. These are more diverse and include methylation, phosphorylation, acetylation, and ubiquitination of mostly histone protein, H3 (Gonzalez *et al*., 2018). RNA-interference is a post transcriptional silencing of target mRNA. In addition to triggering degradation of target mRNAs, dsRNAs can also lead to transcriptional gene silencing (TGS) *via* RNA-directed DNA methylation (RdDM), a process that results in both DNA and histone modifications.

The interplay between silencing, transient TE activation and purifying selection allows the genome to use TEs as a reservoir of potential beneficial modifications but also keeps TEs under control to stop uncontrolled transposition. In tomato, transpositions of the LTR (long terminal repeat) retrotransposon family *Rider* have contributed to various phenotypes of agronomical interest, such as fruit shape and colour (Benoit *et al*., 2019). Decreased levels of cytosine methylation as well as H3K9 methylation at the *Tos17* locus made activation of a retrotransposon which is widely used for the generation of rice mutants (Cheng *et al*., 2006).

Epigenetic control of TEs through methylation adds another layer of TE-dependent regulation in the genome. The tight control of TEs through methylation can be both beneficial and detrimental to genomic stability. Lifting silencing transiently during events of stress, development or genomic shock, would in fact give some flexibility to TEs to generate certain amount of change without totally scrambling the genome. This allows the genome to have some room for restructuring and adaptation in response to different elicitors. Additionally, the incorporation of TEs in promoter regions provides a mechanism to modulate stress response in certain genes. Such insertions can be positively selected if these genes become effective in responding to stress and if their modulation provides better energy balance for the cell. Epigenetic regulation of TEs alters the genomic landscape which could improve long term adaptation to environmental challenges.

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