Plant 3D Chromatin Organization: Important Insights from Chromosome Conformation Capture Analyses of the Last 10 Years

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Over the past few decades, eukaryotic linear genomes and epigenomes have been widely and extensively studied for understanding gene expression regulation. More recently, the three-dimensional (3D) chromatin organization was found to be important for determining genome functionality, finely tuning physiological processes for appropriate cellular responses. With the development of visualization techniques and chromatin conformation capture (3C)-based techniques, increasing evidence indicates that chromosomal architecture characteristics and chromatin domains with different epigenetic modifications in the nucleus are correlated with transcriptional activities. Subsequent studies have further explored the intricate interplay between 3D genome organization and the function of interacting regions. In this review, we summarize spatial distribution patterns of chromatin, including chromatin positioning, configurations and domains, with a particular focus on the effect of a unique form of interaction between varieties of factors that shape the 3D genome conformation in plants. We further discuss the methods, advantages and limitations of various 3C-based techniques, highlighting the applications of these technologies in plants to identify chromatin domains, and address their dynamic changes and functional implications in evolution, and adaptation to development and changing environmental conditions. Moreover, the future implications and emerging research directions of 3D genome organization are discussed.

Keywords: Chromatin conformation capture (3C)-based techniques • Chromatin positioning • Configurations • Domains • Genome structures • Three-dimensional (3D) chromatin organization

Introduction

As the main carrier of genetic information, genomic DNA is packed with histone octamers to form the chromatin (Kouzarides 2007). Recent research has revealed that the information and function of a genome are not only modulated via epigenetic marks in the linear DNA sequence but also by the occupancy and three-dimensional (3D) chromatin organization within the nucleus (Doğan and Liu 2018, Grob 2020). This occupancy and 3D organization have important functional implications for DNA replication, DNA repair and transcription regulation (Ouyang et al. 2020). The chromatin structure is highly dynamic in response to environmental conditions and developmental cues (Teresa Avelar et al. 2013, Probst and Mittelsten Scheid 2015). Over the past decades, 3D nuclear architectures in plants and animals have been unveiled and described at a rapid speed due to the development and improvement of bioimaging and biochemical methods (Sexton and Cavalli 2015, Ouyang et al. 2020). By combining microscopy methods, the first technology to study chromosome conformation with fluorescent in situ hybridization (FISH) boosted progress in uncovering how the spatial position and organization of chromosome territories affect gene expression within the nuclear space (Paweletz 2001). Chromosomes occupy distinct nuclear spaces, exhibiting radial and relative positioning (Parada and Misteli 2002). Additionally, chromosome arrangements can be arranged into different configurations including Rab1, Bouquet and Rosette configuration (Grob and Grossniklaus 2017). The emergence of high-throughput chromatin conformation capture (3C) techniques and their derivatives further allows the quantification of chromosomal interactions and addresses the complicated interplay between local chromosome organization and genome functionality (Sexton and Cavalli 2015). Recent advances have identified and characterized 3D genome organization in both animal and plant fields, in which chromosomes are structurally subdivided into different functional domains at multiple scales (Sexton and Cavalli 2015), ranging from A/B compartments with hundreds to thousands of kilobases to small chromatin loops with tens of kilobases (Fullwood et al. 2009, Lieberman-Aiden et al. 2009). Topologically associated
domains (TADs) are megabase-sized, in which genomic interaction shows high frequencies within a domain but are relatively insulated between two TADs (Sexton et al. 2012). It should be noted here that chromatin TAD domains of some plants are less prominent compared with animal clear 3D organization TADs (Sexton and Cavalli 2015). Although technical advances have facilitated rapid progress of research on the mammal 3D genome architecture, research in plants is more limited. Moreover, previous reports focus on the transcriptional regulation involving the 3D genome positioning and architecture within the nucleus. It still remains elusive whether chromosome conformation is the consequence or cause of many factors’ activities, particularly in plants. In this review, we further summarize 3D genomic features and how various factors including transcription factors, histone modifications and RNA, shape the 3D genome in eukaryotic cells. We highlight recent developments in the application of 3C-related approaches to 3D genome research in plants. We discuss current knowledge in the field of plant chromatin structures as well as their relation to gene expression to effect physiological processes during evolution and development and in response to environmental cues.

Chromosomal Architecture Features

Distinct spatial features and epigenetic modification of chromatin are major factors in the regulation of gene expression (Strahl and Allis 2000). Each chromosome is not randomly arranged within the nuclear space, but occupies a discrete territory in the nucleus to represent a structured unit, referred to as a chromosome territory (CT) (Fig. 1) (Parada and Misteli 2002, Meaburn and Misteli 2007, de Wit and de Laat 2012, Fraser et al. 2015, Grob and Grossniklaus 2017). This was originally proposed by Rab1 and Boveri more than a century ago and confirmed in the 1980s by Cremer and colleagues using elegant ultraviolet-laser micro-irradiation experiments (Cremer et al. 1982). Furthermore, the application of FISH using fluorescently labeled probes enabled the examination of arrangements of chromosome territories in the nuclei (Habermann et al. 2001). These observations, together with reports by Cremer and colleagues, substantiated the concept of chromosome territories to reflect the distinctly physical nature of chromosomes in the interphase nucleus. Chromosome territories are formed at the top of hierarchical structures in most eukaryotic genomes (Pecinka et al. 2004). Circular chromosome conformation capture (4C) and chromosome high-throughput sequencing (Hi-C) technologies further revealed that chromosome territories can be partitioned into chromosome-arm territories (Grob et al. 2013), which appear to represent the major organization units of Arabidopsis thaliana (A. thaliana) (Feng et al. 2014, Grob et al. 2014, Wang et al. 2015). Most interestingly, structural features of chromosomes occupying CTS turned out to be distinct in different species. A. thaliana and rice, for instance, exhibit obvious differences in their structural features without considering the details of the local chromatin organization patterns (The Arabidopsis Genome Initiative 2000; Wu et al. 2003).

Chromosome configuration and positioning

Chromosome organization exhibits different configurations in the nucleus of different eukaryotes, associated with the fold and contact of chromosome arms, telomeres and centromere (Fig. 1A) (Sotelo-Silveira et al. 2018). Firstly reported a polarized arrangement of interphase chromosomes in the nuclei of salamander larvae, which thereafter became known as the ‘Rabl’ configuration (Grob and Grossniklaus 2017). It refers to separate clustering of centromeres and telomeres in opposite poles of the nucleus, which has been adopted by drosophila, mouse, human, wheat, rye, barley and oats (Dong and Jiang 1998, Cowan et al. 2001, Tiang et al. 2012, Rodriguez-Granados et al. 2016, Stevens et al. 2017). The Rabl configuration ensures the orientation of chromosomes in a nucleus to maintain chromosomal integrity and facilitates the alignment of homologues during meiosis (Zickler and Kleckner 1999, Parada and Misteli 2002). Some higher-order structures of chromatin, and domains with various epigenetic marks in plants, are organized in a manner that is different from mammals with variations even existing between different plant species. In rice, chromosomes present a Rabl conformation in the xylem vessel cell nuclei (Prieto et al. 2004). However, strong interactions among the centromeres, which are key features of Rabl, disappear in leaf tissues of rice indicating the existence of non-Rabl chromosome organizations (Liu et al. 2017). The Rabl configuration is commonly present in plants with larger genomes than A. thaliana, such as barley and rice, whereas small genome plants such as A. thaliana adopt a Rosette-like configuration (Dogan and Liu 2018). A 'Bouquet' chromosome configuration has been identified during meiosis in maize, wheat and rice cells and characterized
by telomeres clustering to a limited area beneath the nuclear envelope while the rest of the chromosomes spread throughout the nucleoplasm (Schwarzacher 1997, Zhang et al. 2017, Doğan and Liu 2018). As mentioned, the ‘Rosette’ configuration is adopted by A. thaliana chromosomes, in which megabase-size euchromatin loops emanate from condensed chromocenters (CCs) (Fransz et al. 2002). CCs are formed through highly condensed centromeres and their flanking pericentromeric heterochromatin (Fransz et al. 2002). Chromosome configurations can be different even in the same species and in the same cell type due to its highly dynamic and flexible nature, which allows euchromosomes to adapt to changing environmental conditions and development cues (Doğan and Liu 2018).

In mammalian cells, Rabl configurations are relatively rare in the interphase nucleus. Instead, there exists a non-random arrangement of chromosomes in a radial distribution (Fig. 1B) (Finch et al. 1981, Schwarzacher et al. 1987, Tanabe et al. 2005, Li et al. 2015). In humans, chromosome 18 (with lowest gene density) was noticed to be preferentially positioned toward the nuclear periphery, whereas the high gene-dense chromosome 19 was preferentially located near the nuclear center, which is a characteristic feature of radial positioning, suggesting that radial positioning is correlated with gene density (Tanabe et al. 2005).

In addition to gene density, radial positioning shows a strong correlation with chromosome size. The smaller chromosomes tend to be centrally located during metaphase and the larger chromosomes are preferentially located toward the periphery (Finch et al. 1981, Schwarzacher et al. 1987). In tetraploid cotton, chromosomes of the A subgenome are overall larger than chromosomes of the D subgenome (Li et al. 2015, Zhang et al. 2015). Chromosomes of the A subgenome appear scattered toward the periphery on the metaphase plate, but smaller chromosomes of D subgenome concentrate at the center (Han et al. 2015).

In addition to radial positioning, relative positioning has been observed with respect to each other (side-by-side arrangements) (Fig. 1B). Evidence for relative positioning comes from observations in human cells, in which chromosomes occupy preferential positions relative to each other within the nucleus (Nagele et al. 1995, Allison and Nestor 1999). However, other studies show that some species adopt chromosome spatial arrangements distinct from the preferential patterns. The relative positions in the nuclei were less clear in alien rye and barley chromosomes (Kolářková et al. 2019). One explanation may be that chromosome arrangements are highly correlated with different organisms, tissues and different cell types and states (Parada and Misteli 2002).

**Association between chromosome territories and gene expression**

The correlation between chromosome territory structure and gene expression has received increasing attention. Early findings reveal that transcriptionally active genes tend to be mostly located at the surface of the chromosome territory due to its impermeability, which fosters transcription factors accessible to promoters (Dietzel et al. 1999). However, subsequent studies suggest that these active sites are not only confined to the surface of chromosome territories, since most nuclear proteins have been found to be of high mobility in vivo, which is critical for gene expression and nuclear architecture (Misteli 2001).

Moreover, through high-resolution light and electron microscopy it can be clearly observed that chromosome territories are more akin to a sponge, which is capable of protein permeability (Verschure et al. 1999, Parada and Misteli 2002). Therefore, active genes are more likely to be scattered within CTs (Abranches et al. 1998, Verschure et al. 1999, Williams 2003).

In rice, a higher interaction frequency most likely occurs at the outer layer of Cts (Liu et al. 2017, Sotelo-Silveira et al. 2018). However, the Hi-C study of barley reveals that the frequency of different loci interactions is primarily correlated with their position along the genome sequence (Houben et al. 2003, Mascher et al. 2017). Recent studies have revealed that gene expression is associated with chromatin positioning in the nuclear space. In animals, nuclear periphery tends to be enriched with repressed chromatin, which is associated with lamin fibers, named as lamina-associated domains (LADs). In addition, some chromatin domains are associated with nucleolar periphery, named as nucleolus-associated chromatin domains (NADs) (Pontvianne and Grob 2020). Similarly, in plants LADs and NADs have been detected, which are both transcriptionally inactive. In A. thaliana, the specific nuclear lamin candidate protein, crowded nuclei 1 (CRWN1), has been reported to interact with PWWP interactor of polycombs 1 (PWO1), mediating chromatin tethering at the nuclear periphery (Poulet et al. 2017, Hu et al. 2019, Pontvianne and Liu 2020). This is consistent with the previous reports that plant nuclear periphery provides a repressive environment (Bi et al. 2017). Moreover, recent studies further reveal that the interaction between CRWN1 with the copper-associated (CA) gene locus enable the locus to localize at the nuclear periphery under excess copper conditions (Sakamoto et al. 2020). Until now, research into LADs and NADs in plants is still limited due to a lack of knowledge of the proteins required for the formation of these chromatin domains (Pontvianne and Liu 2020, Sakamoto et al. 2020).

**Chromosome compartments, topologically associating domains and gene body loops**

Mammalian genomes have been shown to be spatially organized in three levels: compartments, domains and loops (Fig. 2) (Lieberman-Aiden et al. 2009, Doğan and Liu 2018, Kim and Shendure 2019, Pontvianne and Liu 2020). Inside the CTS, the chromosomes can be divided into active A compartments with open and euchromatic regions, and inactive B compartments with closed, silent and heterochromatic regions on the megabase scale, roughly 1–10 Mb in size (Lieberman-Aiden et al. 2009). Each of them has distinct genetic and epigenetic features, preferentially associating with other compartments of the same identity (Lieberman-Aiden et al. 2009, Ryba et al. 2015).
Fig. 2 Hierarchical chromatin organization. (A) Each chromosome is precisely positioned within the nucleus in ‘chromosome territories’. Inside CTs, chromosomes can be divided into active A and repressed B compartments. A high frequency of interactions occurs within TADs, while the interactions are decreased with neighboring regions outside of the TADs. (B) A variety of factors and modifications are involved in the formation of DNA looping that connects regulatory elements to their target loci in plants.
et al. 2015, Zhu et al. 2017), and also in certain circumstances, such as before Drosophila zygotic genome activation (Hug et al. 2017). The lack of TADs in A. thaliana may be due to its small genome size, as prominent TADs cannot be detected in species with genomes smaller than 400 Mb (Dong et al. 2017, Stam et al. 2019). However, the effect of genome size on TAD formation is still under debate. TADs can be clearly observed in Drosophila with a genome size of 180 Mb (dos Santos et al. 2014, Pont-vianne and Grob 2020). This raises another explanation that the rather uniform distribution of epigenetic landscape in chromosome arms of A. thaliana might lead to the un-prominent features of TAD as significant changes in epigenetic marks of chromatin appears in Drosophila (Sexton and Cavalli 2015, Rowley et al. 2017, Stam et al. 2019). Moreover, the linear genome exhibits a smooth transcription density (Rowley et al. 2017). In addition, other speculation is that TADs are likely to be displayed in plant with lower gene density of genomes (larger genome size) (Doğan and Liu 2018).

In animals, TAD boundaries are often bound by the insulator protein CTCF-binding factor (CTCF), housekeeping genes, transfer RNAs, short interspersed element retrotransposons and specific epigenetic marks (Dixon et al. 2012, Sexton et al. 2012, Rao et al. 2014, Tang et al. 2015). So far in plants, CTCF homologues have not been identified, indicating that it might be not required for the formation of 3D boundary (Pont-vianne and Grob 2020). Growing evidence reveals that loop extrusion by cohesin is coupled with CTCF blocking cohesin in TAD establishment in mammal (Sanborn et al. 2015, Fudenberg et al. 2016, Nora et al. 2017, Rao et al. 2017, Nuebler et al. 2018). The deletion of chromatin-associated cohesin and of the TAD boundary protein CTCF can weaken TADs in humans (Nuebler et al. 2018). Some experiments show that CTCF may block cohesin in a directional fashion (Rao et al. 2014, Guo et al. 2015, 2018, Sanborn et al. 2015). However, the molecular mechanism underlying CTCF blocking cohesin remains to be elucidated (Kim and Shendure 2019). Cohesin proteins are conserved between plants and animals, and several cohesin subunits have also been identified in rice (Zhang et al. 2004). However, whether these cohesins have similar functions is still unclear (Ouyang et al. 2020). It would also be interesting to identify CTCF-like insulators in plants using cohesion antibodies and to investigate whether CTCF-like insulators of plants also function in TAD boundaries.

TAD establishment also involves other factors. Recent super-resolution chromatin tracing techniques suggest that TAD-like structures still exist in single cells even upon cohesin depletion in mammals (Bintu et al. 2018). Another factor, internal ERCE, is sufficient to determine TAD structure's strength like CTCF does in mammals due to similar effects of ERCE deletion on the TAD structure to CTCT deletion (Sima et al. 2019). Mammalian TAD borders are enriched with chromatin loops on the hundreds of kilobase scale which can link promoters and cis-regulatory elements together to mediate gene transcription by recruiting transcription factors (TFs) to the target genes (Li et al. 2012, Rao et al. 2014, 2017). In animals, the compartmentalization within single TAD may protect the promoters from ectopic contact with distant enhancers (Szabo et al. 2019). By contrast, plant TADs may play different roles due to regulatory contact between putative enhancers and promoters occurring across TAD boundaries (Dong et al. 2017, Stam et al. 2019). Furthermore, plant TADs are composed of four categories with different epigenetic features, including active, repressive, polycomb silenced and intermediate type in which specific features are absent (Dong et al. 2017). In Marchantia polymorpha, TADs are enriched for transcription factor TEOSINTE BRANCHED 1, CYCOIDEA and PCF1 (TCP1), which is dispensable for TAD formation. In tcp1 mutants, genes located in TCP1-rich TADs show larger changes in expression in comparison with genes outside of these TADs (Karaaslan et al. 2020). In plants, chromatin loops are often formed between distal regulatory elements and promoters to exert function, providing opportunities for enhancers directly contacting with their genes at the tens of kilobase-pair scale (Doğan and Liu 2018, Li et al. 2019). In A. thaliana, the disruption of the loop between gene promoter and transcription termination site results in a decrease in flowering locus C (FLC) expression (Crevillén et al. 2013). Adenosine phosphate-isopentenyltransferases (IPTs) are responsible for the biosynthesis of cytokinins. The gene loops in IPT3 and IPT7 loci enhance transcription to promote cytokinin production (Jégu et al. 2015, Gagliardi and Manavella 2020). Extensive chromatin loops are observed in the large genomes of plants, such as maize and tomato (Dong et al. 2017). Recently, in sunflower a gene looping formation encompassing the whole of the HaWRKY6 gene was found to enhance the expression of HaWRKY6 (Gagliardi et al. 2019).

Cutting-edge Techniques to Explore 3D Genomes and Their Applications in Plants

FISH and its derivatives

Intensive research over the past years have traced and visualized different configurations of chromosome organization using microscopic techniques, such as FISH. This method as a macroscopic recognition technology has been greatly improved in terms of sensitivity, specificity and resolution (Cui et al. 2016). 3D-FISH (Solovie et al. 2002, Cremer et al. 2008) and FISH using Oligopaint (Beliveau et al. 2012) or molecular beacon probes (Ni et al. 2017) have been extensively used for examining chromatin organization. Combining FISH with super-resolution microscopy boosts the characterization of structural chromatin domains in detail (Boettiger et al. 2016). Chromatin domains have been labeled using dCRISPR-Cas9 reporter proteins guided by RNA sequence or green fluorescent protein-tagged m6A-tracer proteins, allowing tracking of the location of chromatin domains in the nucleus (Qin et al. 2017, Ye et al. 2017, Hong et al. 2018).
Table 1 The chromosomal architecture of plants

| Arabidopsis thaliana | Solanum lycopersicum | Brassica | Zea mays | Oryza sativa | Gossypium hirsutum | Sorghum bicolor | Triticum aestivum | Secale cereale | Hordeum vulgare | Avena sativa | Setaria italica |
|----------------------|-----------------------|----------|----------|-------------|------------------|----------------|-----------------|-----------------|----------------|-------------|----------------|
| Rabl configuration   | + (xylem)             |          |          |             |                  |                |                 |                 |                |             |                |
| Rosette configuration| +                     |          |          |             |                  |                |                 |                 |                |             |                |
| Bouquet configurations| + (meiotic)          | + (meiotic) |          |             |                  |                |                 |                 |                |             |                |
| Chromatin loop       | +                     | +        | +        | +          |                  |                |                 |                 |                |             |                |
| TADs                 | −                     | +        | +        | +          |                  |                |                 |                 |                |             |                |
| More repressive      | +                     |          | +        | +          |                  |                |                 |                 |                |             |                |
| domains              |                        |          |          |            |                  |                |                 |                 |                |             |                |
| Polycomb domains     |                        |          |          |            |                  |                |                 |                 |                |             |                |

3C, its derivatives and their applications

3D genome organization displays an ordered and hierarchical pattern (Sexton and Cavalli 2015). To analyze 3D chromatin interactions in the nuclei, Dekker and colleagues developed novel molecular techniques—3C technology, to analyze the interaction frequencies between two genomic loci (Dekker et al. 2002). This has facilitated the development of a wide range of 3C-based technologies, and this field has rapidly progressed in recent years (Fig. 2) (Grob and Cavalli 2018). Moreover, the development of 3C-based techniques provides increasing resolution and uncovers a large catalogue of interaction domains between chromatin (Hug et al. 2017, Sewitz et al. 2017, Stevens et al. 2017), which affects gene transcription (Schubert and Shaw 2011, Wang et al. 2016). Moreover, 3C technology and its derivatives have been employed in plants to address the DNA loops and global features of genome architecture (Table 1) (Louwers et al. 2009, Moisissi et al. 2012, Crevillén et al. 2013, Grob et al. 2013, 2014, Liu et al. 2013, 2016, Ariel et al. 2014, Feng et al. 2014, Jégu et al. 2014, Wang et al. 2015).

The first step of 3C and 3C-derived methods is to fix the chromatin using a fixative agent, usually formaldehyde (Fig. 3) (Dekker et al. 2002). The fixed chromatin is cut using restriction enzymes targeting 6 bp, cutting the genome every 4,096 bp (de Wit and de Laat 2012). This has been used to demonstrate the existence of chromatin loops in vivo between regulatory sequences and their target genes (Tolhuis et al. 2002, Vormmenn et al. 2007). The first application in plants identified the characterization of chromatin looping involved in long-distance cis and trans interactions in maize (Louwers et al. 2009). Later, 3C was employed to discover a gene loop linking 5′- and 3′-flanking regions of the flowering regulator FLC for the correct expression (Crevillén et al. 2013). Additionally, 3C has demonstrated that auxin-regulated promoter loop (APOL, a long non-coding RNA (lncRNA)) expression modulates local chromatin loop dynamics to determine the expression patterns of pinoid (PID) that is a kinase and controls the polar localization of the auxin transporter pin-formed (PIN) 2 in the root cells (Ariel et al. 2014). However, reliable and correct measurements of contact frequencies by 3C are difficult (Hagège et al. 2007, Simonis et al. 2007). Another limitation of 3C is to only allow the study of chromosome interactions between two chosen loci (one-to-one) in the genome.

Later, the 3C method was developed into 4C known as a ‘one to all’ strategy, which enabled genome-wide screening for interactions between one specific locus (viewpoint) with all other loci in the genome (Fig. 3) (Zhao et al. 2006). 4C-seq refers to the same strategy, but utilizes next-generation sequencing (NGS) instead of microarrays to determine long-range chromatin interactions (Splinter et al. 2012). 4C studies have also been applied to address chromosomal architecture in A. thaliana nuclei from a genome-wide perspective. In addition, it reveals the basic principles of chromosomal interactions and their correlations with epigenetic marks in A. thaliana (Grob et al. 2013). However, 4C may only be suitable for studying long-range interactions with larger regions of approximately more than 50 kb in size (Grob and Cavalli 2018).

Another powerful 3C derivative is 5C (3C carbon copy) as a ‘many to many’ detection, in which interactions among thousands of selected genomic loci are detected in a single run (Fig. 3) (Dostie et al. 2006, Simonis et al. 2007). Not only does 5C provide interaction information between specific pairs of sites, but it also builds a matrix of interaction frequencies for entire genomic regions. 5C technology is only used for detecting multiple selected sequences and is not suitable for use on relatively small genomes, such as those of yeast, Drosophilal. or A. thaliana, due to the high cost of primers in the design and generation.

Hi-C and its application in plants

The Hi-C approach (all-to-all) with the most far-reaching impact offers the advantage of detecting interactions between any chromosomal locus with all others (Fig. 3) (Lieberman-Aiden et al. 2009). This detection is achieved by employing a restriction enzyme leaving a 5′-overhang, which is then filled with biotin-labeled nucleotides. The recovered ligation products are analyzed by high-throughput sequencing, investigating both short- and long-range genomic interactions in a whole genome (Lieberman-Aiden et al. 2009). Today, Hi-C...
is extensively applied to plant research for characterizing general chromosomal architecture in species such as Arabidopsis, rice, foxtail millet, sorghum, tomato, Brassica, cotton and maize (Table 1) (Feng et al. 2014, Grob et al. 2014, Wang et al. 2015, 2017, 2018, Dong et al. 2017, Liu et al. 2017, Grob and Cavalli 2018, Sotelo-Silveira et al. 2018, Ting et al. 2019). Recent studies by Hi-C have demonstrated that TADs are not obvious in A. thaliana (Feng et al. 2014, Wang et al. 2015). However, hundreds of ‘insulator-like’ regions in A. thaliana have instead been identified as being analogous to ‘TAD boundaries’ in animals (Wang et al. 2015). By contrast, TADs are common structures in other plants as shown by Hi-C studies in rice, cotton and Brassica (Dong et al. 2017, 2018, Liu et al. 2017, Wang et al. 2018, Ting et al. 2019). Moreover, TADs are highly conserved between Brassica rapa and Brassica oleracea (Ting et al. 2019). TAD boundaries are enriched in open chromatin with euchromatic histone marks (H3K4me3, H3K27me3 and H3K9ac), while DNA methylation (CG, CHG and CHH contexts) is depleted around the TAD boundaries in B. rapa and B. oleracea (Ting et al. 2019). In cotton, genome allopolyploidization leads to...
the switching of A/B compartments and the reorganization of TADs in both subgenomes whose boundaries preferentially form in open chromatin (Wang et al. 2018). The KNOT, in which intra- and inter-chromosomes interact with all chromosomes in A. thaliana, has been proposed to play a role in protecting the genome from the disruptive potential of some transposable elements (TEs) (Feng et al. 2014, Grob et al. 2014). Recently, the KNOT structure was also found in rice and the Brassicaceae family, but it is not conserved between B. rapa and B. oleracea (Dong et al. 2018, Ting et al. 2019). KNOT engaged elements (KEEs) or interactive heterochromatic islands are highly enriched with heterochromatic islands within euchromatin and exhibit strong long-range interactions in A. thaliana (Feng et al. 2014, Grob et al. 2014, Grob and Grossniklaus 2019). Furthermore, a local chromatin-packing feature, termed positive strip, has been identified in Hi-C map (Wang et al. 2015, Sotelo-Silveira et al. 2018). The positive strip refers to kilobase-sized intrachromosomal region with higher frequencies of interaction than the nearby regions (Sotelo-Silveira et al. 2018). At the gene-level resolution, small self-loops of chromatin have been identified in A. thaliana and are characterized by transcription start sites (TSSs) associated with downstream regions and transcription termination sites (TTSs) loop with upstream regions (Liu et al. 2016). Many self-loops tend to be associated with high gene expression (Liu et al. 2016). In addition, the 3D genome organization and the relationship between proteins and chromosome architectures have been assessed by Hi-C. ATPases of the Microchidia (AtMORC) family are localized to nuclear bodies near A. thaliana chromocenters. In the first Hi-C experiment of its kind, a mutation in AtMORC was shown to disrupt the packing of CCs and affect the pattern of long-range interactions that maintain TE silencing (Moissiard et al. 2012).

Another example in A. thaliana highlights the role of genome duplication in the regulation of chromatin organization and epigenetic marks through Hi-C. Genome doubling contributes to the switching of chromatin looping and H3K27me3 histone modification in FLC (Zhang et al. 2019). In maize, chromatin loops and epigenetic states of open chromatin regions can affect the different architectures and identities of the ear and tassel through influencing target gene expression (Sun et al. 2020b).

In addition, the Hi-C method has been improved for its scaled-down use for single cells to reveal the specific 3D genome structures of rice gametes and unicellular zygotes. A compact silent center is found in eggs and unicellular zygotes but is absent in sperm cells, which appears to function in the regulation of zygotic genome activation (Zhou et al. 2019). Further, Hi-C reveals that global and local chromatin rearrangement occurs upon perceived environmental cues. The chromosomes of rice seedlings decondense when the temperature changes from 30 to 16°C (Liu et al. 2017). In A. thaliana, heat stress causes the activation of TEs and the global reorganization of the 3D genome with reduced interactions between KEEs (Sun et al. 2020a). To date Hi-C has already provided a variety of information regarding chromatin domain interactions. However, Hi-C is not sufficient to detect all the loops on the upper kilobase scale due to limited sensitivity. The full identification of chromatin architectural features requires an increased depth of sequencing (Grob and Cavalli 2018).

Hi-C derivatives

Chromatin Interaction Analysis by Paired-End Tag Sequencing (ChIA-PET) as site-specific chromosome conformation capture strategy has been developed and is proving suitable for achieving enhanced specificity (Fig. 3). ChIA-PET is a technology combining chromatin immunoprecipitation (ChIP) with 3C-type analysis, identifying the comprehensive long-range chromatin contacts bound by a protein (e.g. promoters) at the lower-kilobase resolution (Li et al. 2010, 2014). The first ChIA-PET study was utilized to show long-range interaction networks between human estrogen receptor α binding sites and target gene promoters (Fullwood et al. 2009). Recent ChIA-PET analyses of plant genome organization further point to long-range chromatin interaction patterns associated with transcriptional regulation in maize and rice (Table 1) (Li et al. 2019, Peng et al. 2019, Zhao et al. 2019). Two ChIA-PET studies in maize highlight epigenetic features of these regulatory elements. From these, RNA Polymerase II, H3K4me3 and H3K27ac were shown to be enriched at promoter proximal regions, promoter and enhancer, respectively. These works identified novel networks of promoter proximal–proximal interactions and proximal–distal interactions in maize, which affect some metabolic phenotypes and important agronomic traits (Li et al. 2019, Peng et al. 2019). Similarly, one rice ChIA-PET study unveiled the spatial connections between expression quantitative trait loci (QTLs) and their target genes (Zhao et al. 2019). However, the current limiting factors for ChIA-PET are that it only allows the interaction analysis of genomic regions between DNA sites bound by the same factor and that it requires up to 100 million cells per experiment to generate a small fraction of informative data output (Tang et al. 2015).

Another newly developed technology is Proximity Ligation-Assisted ChIP-seq (PLAC-seq), which improves the efficiency and accuracy over ChIA-PET by putting the proximity ligation step prior to chromatin shearing and immunoprecipitation (Fig. 3). With this method, biotin-tagged nucleotide fill-in and in situ proximity ligation are performed in formaldehyde-fixed cells. This is followed by nuclei lysis and chromatin shearing by sonication and immunoprecipitation. This method has been conducted in mammalian cells (Fang et al. 2016). Another protein-directed genome architecture approach, named HiChiP, has been established (Fig. 3), which improves conformation-informative output with lower input material compared with ChIA-PET (Mumbach et al. 2016). HiChiP has not been widely used so far in plants due to its novelty. One recent study using a combination of HiChiP with other sequencing methods revealed that gene–distal loci exist and act as long-range transcriptional cis-regulatory elements related to agronomic QTLs in the maize genome (Ricci et al. 2019). Nowadays, functional characterization of the chromatin regions is...
usually investigated by combining 3D-related methods identifying the physical interaction networks of chromatin and 1D methods exploring features of interest of chromatin regions. Various sequencing approaches, such as ChiP-seq, MethylC-seq, DAP-seq and Starr-seq, ATAC-seq, and RNA sequencing (RNA-seq), have been widely applied together with these 3C-based methods in plants (Li et al. 2019, Ricci et al. 2019).

The capture Hi-C approach obtains genome-wide chromosomal contacts belonging to a certain annotation category (e.g. promoters), such as long-range chromatin contacts of single-nucleotide polymorphisms (Fig. 3) (Dryden et al. 2014, Jäger et al. 2015, Mifsud et al. 2015). In this method, Hi-C is used together with hybridization-based capture of targeted genomic regions (Dryden et al. 2014, Jäger et al. 2015, Mifsud et al. 2015). Mutation analysis in pools by chromosome conformation capture explores the effect of simultaneous mutations of numerous cis or trans on chromosome conformation and demonstrates TFs required for a chromosomal contact of interest (Kim et al. 2019). ChromEMT combining electron microscopy tomography (EMT) with a labeling method (ChromEM) can enable the visualization of the 3D chromatin ultrastructure and compaction in interphase and mitotic cells (Ou et al. 2017). Moreover, development of the 4D nucleome project has enabled scientists to investigate the structure and dynamics of genomes in space and time, which will greatly help scientists gain deeper mechanistic insights into the organization and functions of the nucleus (Dekker et al. 2017). Recently, methods capturing genome-wide RNA–DNA interactions have been developed, such as mapping RNA–genome interactions, global RNA interactions with DNA by deep sequencing and chromatin-associated RNA sequencing (Ouyang et al. 2020). Due to the development of these technologies, various roles of RNAs in shaping local chromatin structures have been identified, such as R-loops, RNA–DNA triplexes, co-transcriptional processing complexes and RNA-binding protein scaffolds (Ouyang et al. 2020).

Factors Associated with Plant Chromatin Loops

Numerous studies demonstrate that dynamic 3D genome structure can determine TF activity and regulate gene expression (Lupiáñez et al. 2015, Giorgetti et al. 2016). In return, the TFs and other factors can also shape DNA looping. The relationship is complicated between the DNA looping and various factors. TFs are capable of direct interaction with DNA, proteins and even RNAs, which have the potential to affect DNA looping in plants (Fig. 2B) (Rodriguez-Granados et al. 2016, Lambert et al. 2018, Kim and Shendure 2019). The formation of some DNA loops come from mediation of the direct oligomerization or cofactor oligomerization, which is formed through TFs directly binding DNA (Weintraub et al. 2017) or by TFs recruiting cofactor proteins (Deng et al. 2014, Monahan et al. 2019), respectively. For example, Yin Yang 1 as a ubiquitously expressed TF mediates DNA looping via directly binding both promoters and enhancers in mammals (Weintraub et al. 2017). In plants, short-range loops formed via protein–protein interaction were detected in high-resolution 3D genome maps. The interaction between transcription factors, agamous and terminal flower 2, with the TSS and TTS flanking regions of wuschel facilitating the formation of a loop (Guo et al. 2018, Gagliardi and Manavella 2020, Huang et al. 2020). Additionally, TFs together with RNA polymerase II (Pol II) are responsible for the promoter–enhancer and promoter–promoter interactions in mammals. In contrast, in rice only extensive promoter–promoter interactions organized by Pol II can be detected, suggesting that Pol II might play different roles in higher-order chromatin structure between rice and mammals. And, recent studies have reported that RNAs and polycomb repressive complexes (PRCs) confer the formation of plant 3D genomes. PRCs enable the establishment of H3K27me3 patterns across the genome (Doğan and Liu 2018). In A. thaliana, H3K27me3 are associated with long-range chromatin interactions (Doğan and Liu 2018). The interaction between H3K4me3/RNAPII and RNAs forming the loop promotes efficient gene expression, and heterochromatin histone modifiers (H3K9me2 and H3K27me3) drive the formation of silenced chromatin loops in rice (Zhao et al. 2019). MED25, a subunit of the mediator transcriptional co-activator complex, is involved in the formation of enhancer–promoter loop responsible for jasmonate signaling in A. thaliana. MED25 recruits Pol II to MYC2 targets and enhances the H3K9 acetylation (Wang et al. 2019). It has been found that the formation of R-loops-triple strands involves DNA–RNA duplex, and R-loops are enriched in IncRNA regions in some cases. In A. thaliana, the loop encompassing the promoter region of PID and APOLO has been reported to regulate the PID expression through Pol IV/V-directed DNA methylation and the PRC2-associated repressive mark (Huang et al. 2020). To date, many RNAs have been found to influence local chromatin conformation in both animals and plants, but RNAs associated with global chromatin 3D structure have not been reported. In this regard, it will be most interesting in the future to explore genome-wide RNA–DNA interactions, identify ncRNAs related to chromatin 3D architecture and reveal the functional implications of RNAs in shaping 3D organization in plants. In addition, multiple TFs and co-activators have been proposed to be enriched among intrinsically disordered regions (IDRs) to form condensates in eukaryotic cells, exhibiting properties of liquid–liquid phase separation (LLPS) to regulate gene expression (Banani et al. 2017). These LLPS mechanisms might drive protein- and RNA-mediated chromatin loops and chromatin compartmentalization (Ouyang et al. 2020). It would be interesting to further study how other factors modulate the chromatin looping in plants, especially in crop species.

There is also evidence to illustrate that the disruption of physical proximity affects their function. This has been demonstrated by using CRISPR interference to perturb enhancer function at scale to alter gene expression, coupled with single-cell RNA-seq in human (Gasperini et al. 2019) and Flow-FISH techniques (Fulco et al. 2019). It should be noted that in some
cases, promoters are not activated even when in proximity to strong enhancers. Moreover, the activation of target promoters by enhancers may be affected by the timing and stability of enhancer–promoter loops (Kim and Shendure 2019). In addition, the spatial organization of the genome, nuclear microenvironments, nuclear architecture and chromosome conformation can shape the temporal dynamics of TF activity and subsequent transcription. However, whether chromosome structure is a reason or consequence of genomic functions still remains to be further explored (Sexton and Cavalli 2015, Sotelo-Silveira et al. 2018). Much work concerning the implications of plant chromatin loops is needed for further exploitation, and a comprehensive understanding of the role played by plant chromatin folding in transcriptional regulation could pave the way for molecular breeding programs (Ouyang et al. 2020).

Conclusions and Future Perspectives

The 3C-based methods allow scientists to analyze genome architecture at an unprecedented resolution to address fundamental biological questions of plant growth and development, as well as plant response to the environment. We envisage that in the near future, there will be much work to describe plant epigenomes and transcriptomes in the 3D context, and many key regulators of plant chromatin shaping and positioning will be identified.

Although knowledge about nuclear structure, chromatin architecture and gene regulation are being deepened and refined, research into 3D genome organization in plants to date has only focused on several plants: Arabidopsis, rice, barley, maize, tomato, sorghum, foxtail millet, Brassica and cotton. Therefore, there is a large field for chromatin architecture research in plants to be explored, such as Medicago and mosses.

The shaping of chromatin folding in plants involves a unique class of factors, such as transcription factors, PRCs and RNA molecules. The function of RNAs in forming chromatin architecture has received increasing attention (Hall and Lawrence 2016). However, we still need to further explore which RNAs act as trans-regulatory elements to modulate genome organization. In addition, multiple of factors in animals have been identified in shaping chromatin structure, such as cofactor proteins, cohesin, structural maintenance of chromosome complexes, nuclear landmarks, RNA and coactivators in an IDR-dependent manner (Kim and Shendure 2019). Considering the growing evidence in animals, it is tempting to speculate that some of them may play a similar role in regulating plant chromatin looping and compartmentalization. In this regard, it will be important for future research to decipher the detailed molecular mechanisms that determine how chromatin is organized in plants. Moreover, to date little is known about RNA–DNA interaction mapping strategies, the mechanism and function of LLPS, and the connection between 3D genome architecture and the control of plant traits. It is therefore tempting to further improve high-resolution mapping technologies in order to deepen our understanding of the structure and function of plant genome organization. In addition, new techniques for studying how the simultaneous interactions among RNA, DNA and proteins affect chromatin structure remain to be further developed (Quinodoz et al. 2018).

Data Availability

No new datasets were generated or analyzed in this study.

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Disclosures

The authors have no conflicts of interest to declare.

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