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Plant chromosomes from end to end: telomeres, heterochromatin and centromeres

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Recent evidence indicates that heterochromatin in plants is composed of heterogeneous sequences, which are usually composed of transposable elements or tandem repeat arrays. These arrays are associated with chromatin modifications that produce a closed configuration that limits transcription. Centromere sequences in plants are usually composed of tandem repeat arrays that are homogenized across the genome. Analysis of such arrays in closely related taxa suggests a rapid turnover of the repeat unit that is typical of a particular species. In addition, two lines of evidence for an epigenetic component of centromere specification have been reported, namely an example of a neocentromere formed over sequences without the typical repeat array and examples of centromere inactivation. Although the telomere repeat unit is quite prevalent in the plant kingdom, unusual repeats have been found in some families. Recently, it was demonstrated that the introduction of telomere sequences into plants cells causes truncation of the chromosomes, and that this technique can be used to produce artificial chromosome platforms.

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Introduction

The genes of plants comprise very little of their chromosomal content, with most sequences being transposable elements and simple repetitive arrays. These components contribute to the chromosomal integrity and function in the form of heterochromatin, centromeres and telomeres. In this review, we summarize the latest findings, made over the past two years, about these three features of plant chromosomes.

Heterochromatin

Traditionally, heterochromatin was thought to contain few if any genes and to have an unknown function. Despite being the major portion of many plant chromosomes, heterochromatin remained the least well characterized and hence least understood portion of the chromosome. Heterochromatin, as revealed by deep staining, is seen at the telomeres and in pericentromeric regions [1]. Some heterochromatin has been associated with centromere or neocentromere function, whereas other regions appear not to have such activities [2]. In plants, heterochromatin is also located at the nucleolar organizers, at knobs and in B chromosomes, for example, those of maize [3–6]. Recent sequence and cytogenetic analyses indicate that plant heterochromatin can have very different origins, composition and dynamics [7]. Heterochromatin plays a key role in repressing transposable elements and as a structural component of chromosomes.

Eleven sequenced bacterial artificial chromosomes (BACs) were annotated and localized using fluorescence *in situ* hybridization (FISH) to tomato pachytene chromosomes, thereby providing global insights into the compositional differences between euchromatin and pericentromeric heterochromatin in this model dicot species [8]. In the euchromatic region, the BACs were gene-rich and had few retrotransposons or other repetitive elements. By contrast, the heterochromatic regions largely consisted of retrotransposons. On the basis of these findings, Wang *et al.* [8] estimated that 90% of the genes in tomato are located in the 25% of the genome that is euchromatic. A sequence comparison of pericentromeric heterochromatin among *Arabidopsis* relatives showed that gene order was conserved in this region but that the distance between genes varied because of the insertion of repetitive elements and pseudo-genes [9].

The maize genome might be more typical of the many flowering plants that have larger genomes. Like tomato genes, maize genes are rare in the pericentromeric heterochromatin [10]. In maize, however, retrotransposons are abundant not only near the centromere but all along the chromosome arms [11,12]. Even in the euchromatin, homologous regions are distinguished among varieties by different repetitive elements and pseudo-genes [13]. This variation is similar to that seen in the pericentomeric regions of different *Arabidopsis* species [9]. In maize and tomato, recent expansion of specific retroelement families is responsible for much of the new DNA in heterochromatic regions. The retrotransposons that compose the heterochromatin of tomato are not abundant in the closely related potato species [8]. Most abundant maize retroelement families are present in low copy numbers in or absent from the related genus *Tripsacum* [12,14]. In maize, unlike tomato, certain retroelement

families have accumulated in gene-rich regions, whereas others have accumulated in the pericentromere [15]. The appearance of retroelements with altered targeting preference might help to explain why some lineages experience expansion of repetitive elements in all intergenic regions whereas others, such as tomato and rice, only have large numbers of repetitive elements in the pericentromeric heterochromatin.

In many locations in the genome, heterochromatin is formed over regions where a single DNA element is present in long arrays. Such arrays are present at centromeres and telomeres, chromosome locations with known functions. In telomeres, the mechanism that generates repeats is known. By contrast, for arrays found at locations such as the subtelomeric regions and various interstitial sites, the roles of the repeat arrays are unclear. In many cases, the individual repeat units are approximately the size appropriate for association with one or two nucleosomes, suggesting that their size has been optimized to create a regular arrangement and to facilitate higher-order packaging.

Retroelements and satellites might both use the RNA interference (RNAi) machinery to form heterochromatin [16–18]. RNAi is the basis of post-transcriptional gene silencing, but it can also lead to heritable transcriptional silencing that is mediated by DNA methylation and histone modification. Plant heterochromatin is less well defined, but studies of histone modification have been performed. The heterochromatic chromocenters of *Arabidopsis* are particularly enriched in H3K9me1,2, H3K27me1,2 and H4K20me1 [19–21]. Clustering of H3K9me2 is typical of, but not essential for, heterochromatin formation in *Arabidopsis* [22]. By contrast, H3K9me2 in maize is abundant in the same chromosomal regions as genes [23]. In other flowering plants that have large genomes, H3K9me2 is also abundant throughout the genome [24]. This arrangement might reflect the abundance of retrotransposons in intergenic regions in these species. H3K27me2 consistently marked maize heterochromatin and H3K4me2 was abundant in complementary locations to H3K27me2 [23]. Thus, histone modifications, and probably chromatin state, can be influenced by the underlying DNA. The formation of heterochromatin also requires the heterochromatin protein HP1 or a homologue. This protein binds specifically to methylated H3K9 in *Schizosaccharomyces pombe* and *Drosophila* [25]. Although HP1 is usually associated with heterochromatin regions, it has also been shown to associate with euchromatin. Different domains control the localization and mobility of Like Heterochromatin Protein 1 (LHP) reported in *Arabidopsis* nuclei [26•].

Centromeres

The presence of a variant of the H3 histone protein, CenH3, is the defining feature of chromatin that composes the kinetochore-forming domain. The centromere region maintains the sister chromatid attachment until metaphase. During metaphase, however, the centromere is separated into at least two distinct parts: one enriched for CenH3 and the other associated with cohesins. Characterizing the chromatin structure of these centromere regions could provide clues as to how they are formed and function.

Recently, direct evidence demonstrating the insufficiency of repeats for plant centromere determination has been uncovered, and the evidence closely parallels observations made in other model systems. First, an example of a new centromere that is devoid of any centromeric elements was reported [27••]. A complementary observation was the efficient inactivation of a maize centromere [28••]. Thus, the primary DNA sequence alone does not determine centromere identity. This suggests that epigenetic factors, such as chromatin structure, play an important role in centromere specification.

Yan and coworkers [29••] took advantage of the small size of the centromeric repeat tract and of the complete sequence of rice centromere 8 to determine the chromatin state at a centromere without a large repeat array. They used chromatin immuno-precipitation with antibodies against various histone modifications, followed by PCR (ChIP-PCR) with primers specific to the centromere regions, to determine the presence of several histone modifications at many positions within the region of recombination repression that surrounds the centromere. This region contained relatively few centromeric repeats and, near the edges of the region, a ratio of retrotransposons similar to that of the remainder of the genome. It also contained many expressed genes. Here, as in other genomic regions, active genes were marked with H3K4me2 and H4-acetylation, whereas chromatin was mostly associated with H3K9me2. Yan *et al.* [29••] concluded that gross histone modifications do not determine centromere identity, and implied that previous observations of distinct patterns of histone modifications at centromeres [30,31] merely reflect the unusual density of DNA repeats found there.

In plants, the region of sister chromatid cohesion is marked by phosphorylation of the H3 histone [32]. In maize, H3 phosphorylation first appears on the CenH3 protein and then on H3 proteins in the flanking region, suggesting that phosphorylation is directed by CenH3 position to the regions that surround this protein [33••]. In *Luzula luzuloides*, centromere activity and the CenH3 protein [34], and phosphorylation of H3 [35], are distributed along the whole chromosome instead of at a single location. Even in this somewhat unusual case, the pattern is consistent with CenH3-directed phosphorylation of H3.

Examining the intensity of immuno-labeled *Arabidopsis* CenH3 at different points in the cell cycle suggests that CenH3 is loaded onto the chromatin primarily during G_2 [36••]. CenH3 quantity was also examined in endoreduplicated cells, where DNA replication has occurred without subsequent cell division. The amount of CenH3 did not increase in proportion to the nuclear DNA. Assuming that centromeric DNA was not under-replicated compared to the rest of the genome, this result shows that replication-coupled CenH3 deposition either does not occur or is insufficient to maintain CenH3 levels in these cells [36••].

Centromere repeat variation and homogenization

Although centromeric repeats are neither sufficient nor necessary for centromere function, they are present exclusively at and around the kinetochore. This suggests a role, or at least a preference, for repeats in centromere function [37]. One likely possibility is that the repeat arrays are optimal for formation of the correct chromatin conformation at the centromere.

Many recent studies have identified centromere repeats in different plant species, allowing repeats among related taxa to be compared and providing new insights into the mechanisms of centromere repeat evolution. Many, but not all, of the relatives of rice share the rice centromeric satellite CentO [38•]. Using chromatin immunoprecipitation, three additional repeats were identified at the kinetochores of *Oryza rhizomatis* and *Oryza brachyantha*, two relatives without CentO. Two of these repeats had an 80-bp region that has homology to CentO and to the centromere repeats of maize and millet [38•]. The third repeat made up most of the *O. brachyantha* centromere and was totally novel. In *O. brachyantha*, several centromeres contained a 366-bp repeat, TrsC, that is also present in the subtelomeric region of *O. brachyantha* and other relatives [39•]. These patterns of centromeric elements demonstrate that repeats can change rapidly, by alterations to existing elements, by development of new elements or by recruitment from other genomic regions.

Among *Arabidopsis*' relatives, there is also variation in centromeric elements. Different blocks of repeats are present at centromeres within a single species and can even vary among homologous centromeres [40,41•]. Yet another type of DNA element was found at tomato centromeres: a tri-nucleotide repeat [42].

Analysis of the centromere 8 region of rice showed a high frequency of unequal recombination among the long terminal repeats (LTRs) of centromere retroelements in the CenH3-binding domain [43•]. Comparisons of this region between *indica* and *japonica* rice identified large duplications and rearranged blocks of centromere repeats [43•]. Centromeric retroelement copy element number was increased primarily by segmental duplications instead of transposition [44•]. Thus, both within and among centromeres, duplications or exchanges of large blocks of sequence are mechanisms of sequence generation and variation.

These studies illustrate the rapid evolution of centromere elements. Such elements can be altered significantly across the genomes of closely related species, which are separated by relatively short evolutionary time frames. These observations suggest a genomic homogenization mechanism of unknown basis.

Telomeres

Telomeres are the end structures of linear chromosomes. Because the very end of the chromosome cannot be fully replicated by the DNA polymerase complex, alternative methods of DNA extension are employed to prevent the chromosome from shortening. The most common mechanism is extension by an enzyme complex that contains a short RNA template and a reverse transcriptase. Successive rounds of extension using the RNA template produces tracts of simple G-rich tandem repeats. For example, the $C_{1\text{-}3}(A/T)G_{1\text{–}3}$ motif in yeast, TTAGG in insects, TTTTAGGG in *Chlamydomonas*, TTGGGG in *Tetrahymena*, TTTTGGGG in hypotrichs, TTAGGG in vertebrates, and TTTAGGG in plants are the dominant forms of telomeres [45]. Exceptions are the telomeres found in *Drosophila*, which consist of two types of specific retroelements [46], and those of some plant species such as *Allium cepa* [47] whose structure is unknown.

The diversity of telomeres in the monocots plant order Asparagales provides an example of evolution in the telomere structure. The Asparagales have three types of telomeres as determined by their primary structure, the *Arabidopsis*-type, the 'human-type' and an unknown type in which the minisatellite telomeric repeat was lost [45,48,49•]. The differentiation of the 'human-type' telomere repeat and its loss in the *Allium* species marked two switch-points in telomere evolution [45]. Analysis of telomerase sequence variation across the first switch point, corresponding to the emergence of the human telomeric motif, and the loss of telomerase activity in *Allium* species at the second switch point suggests co-evolution of telomere primary structure with the telomerase that synthesizes it [49•].

Despite exceptions, the sequences that compose telomeres are conserved. The proteins found at chromosome ends are also remarkably conserved. Telomere proteins have been identified in plants that have functions similar to those of their mammalian, yeast and fly counterparts [50–53]. Recently identified plant proteins include Ataxia Telangiectasia Mutated (ATM) and ATM and Rad3-related (ATR), proteins that repair double strand breaks (DSB). ATM also stabilizes short telomeres, and ATR

also regulates telomere length [50]. The single-strand telomere-repeat-binding proteins, *Protection of telomeres1* (*Pot1*) and *Pot2* [51], which each produce several different splice variants [52], are further examples of plant telomere proteins whose function is conserved in other organisms. Additionally, *Arabidopsis* cells that are deficient for the telomerase catalytic subunit, Tert, can survive in culture, demonstrating that the alternative telomere maintenance mechanisms used by telomerase-deficient cancer cells are also present in plants [54].

Telomere proteins associate with the telomere repeat array to form a specialized chromatin structure at the ends of chromosomes that regulates the length of the telomere, primarily by controlling telomerase activity and accessibility at individual telomeres. The length of the telomere tract is under genetic control and varies widely among species. *Arabidopsis* telomeres are typically 2–5 kb long [55], whereas in tobacco, telomeres are 60–160 kb [56]. In a recent study, telomere length was found to be intermediate in hybrids between *Arabidopsis* ecotypes that have different telomere lengths [57], demonstrating that telomere length is under genetic control. Long-lived species of pine had longer telomeres than shorter-lived relatives, a fact interpreted by the authors as suggesting that telomere length might be related to lifespan [58].

Telomere length might also be regulated by the stability of the telomere complex. Recently, a genome-wide screen for suppressors of the yeast *cdc13-1* mutant, which has a defective telomere-capping protein, identified a KEOPS (Kinase putative Endonuclease and Other Proteins of Small size) complex as a promoter of telomere uncapping [59]. The telomere cap proteins (Cdc13 and Stn1) are dispensable if nuclease activities at the uncapped telomeres are attenuated, demonstrating that alternative strategies (e.g. uncapped telomeres) can be adopted at the telomeres to promote genome stability [60].

Another role for the telomere complex is to prevent the chromosome end from being treated as a DSB by the cellular DNA repair machinery. For example, the non-homologous end-joining (NHEJ) components of the DSB repair pathway were also found in telomeres [61••]. On the other hand, some proteins that were traditionally considered to be telomeric proteins, such as TELOMERIC REPEAT BINDING FACTOR2 (TRF2), have been found in DSBs as soon as 2 s after DNA damage by irradiation [62]. In fact, in telomere-dysfunction mutants, the telomeric ends of linear chromosomes are treated as DSBs by the NHEJ machinery once they are no longer protected. For example, *atm atr* double mutants of *Arabidopsis* have a DNA-damage response that results in telomere fusion and the formation of chromosome bridges during the anaphase of mitosis [50]. The *pot1* mutation in mouse elicited a DNA-damage response and genomic instability, and triggered both NHEJ and homologous recombination (HR) at telomeres [63].

Chromosome healing at DSB sites is another interesting event that results from the interaction between DNA repair and telomere synthesis. Chromosome healing was first described by McClintock [64] who found that the chromosome breakage-fusion-bridge cycle of dicentric chromosomes stopped in the embryos but continued in the endosperms of maize. Unlike repair by HR and NHEJ, DSB repair by chromosome healing through *de novo* telomere addition usually causes large deletions of chromosome fragments. Thus, chromosome healing is a rare event in the genome maintenance of most organisms, the exceptions being *Paramecium*, *Tetrahymena* and *Ascaris*, in which chromosome healing is a normal part of development [65].

A practical application of telomere biology is telomere-mediated chromosome truncation technology for chromosome engineering and the production of artificial chromosomes. When arrays of telomere repeats are transformed into mammalian cells, they can cause chromosomal truncation at the site of insertion [66]. Telomere truncation has also recently been demonstrated in plants [67••]. Transformation constructs that have telomere arrays at only one end were shown to cleave maize chromosomes, most probably during integration [67••]. The presence of telomeric sequences at the transgene insertion DSB [67••] was postulated to increase greatly the efficiency of *de novo* telomere seeding. The telomeric sequences might recruit telomere-binding proteins and the telomerase to the site, thus switching the NHEJ pathway to one of *de novo* chromosome healing [67••]. This technology has been used in our laboratory to generate minichromosomes carrying site-specific recombination cassettes that will possess the desired characteristics for an artificial chromosome platform (W Yu, F Han, Z Gao, J Vega, J Birchler, unpublished).

Conclusions

The organization of repetitive elements in the genomes of plants causes difficulties in analysis because multiple copies of any one sequence are present and because of problems with establishing the DNA sequence of such arrays. However, these features determine the overall structure of the chromosome and ensure its transmission through cell divisions and the life cycle. As such, a thorough understanding of heterochromatin, centromeres and telomeres is crucial to our knowledge of transmission of the genome and its manipulation for biotechnology.

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