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SPECIAL ISSUE ARTICLE

Plant chromatin on the move: an overview of chromatin mobility during transcription and DNA repair

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SUMMARY

It has become increasingly clear in recent years that chromosomes are highly dynamic entities. Chromatin mobility and re-arrangement are involved in many biological processes, including gene regulation and the maintenance of genome stability. Despite extensive studies on chromatin mobility in yeast and animal systems, up until recently, not much had been investigated at this level in plants. For plants to achieve proper growth and development, they need to respond rapidly and appropriately to environmental stimuli. Therefore, understanding how chromatin mobility can support plant responses may offer profound insights into the functioning of plant genomes. In this review, we discuss the state of the art related to chromatin mobility in plants, including the available technologies for their role in various cellular processes.

Keywords: ***Arabidopsis*, chromatin mobility, DNA repair, gene tagging, MSD, transcription.**

INTRODUCTION

Chromatin is a complex structure comprised of numerous proteins and nuclear DNA found in eukaryotic cells. The nucleosome is the fundamental unit of chromatin and consists of approximately 200 bp of DNA wrapped around a histone octamer containing two copies of each of the four core histones (H2A, H2B, H3 and H4). An important feature of histone proteins is that their N-terminal tails are not directly bound to DNA and protrude out of the nucleosome. These N-terminal tails can undergo numerous post-translational modifications, including acetylation, methylation, phosphorylation, ubiquitination and sumoylation (Millán-Zambrano et al., 2022). These modifications modulate the interaction of histones with DNA, regulate the accessibility to regulatory proteins, and affect the compaction and stiffness of the chromatin fiber. Similarly, there are also a wide number of histone variants that can replace canonical histones within nucleosomes and equally affect the structure and regulation of the underlying DNA sequences (Weber & Henikoff, 2014). The interaction between nucleosomes eventually causes chromatin to fold into large structures ranging from the 30-nm fibers to higher-order structures such as the condensed mitotic chromosomes. The primary function of chromatin is to pack long DNA molecules into compact, dense structures enabling them to fit within the limited volume of the cell nucleus. For many years, chromatin was therefore considered as a static entity. Chromatin motion in interphase cells was first demonstrated 25 years ago using live imaging of GFP-tagged loci (Cremer et al., 1982) and later confirmed by (Marshall et al., 1997), who showed that chromatin moves in the nucleus randomly within a constrained space. These experiments have demonstrated high levels of local dynamics and challenged the static view of chromatin. Importantly, mobility does not appear to be simply a passive structural feature of chromatin. Recent studies have now started to show that chromatin movement is an essential part of chromatin dynamics and is involved in several nuclear processes, including gene expression and DNA damage repair. Although most studies have focused on yeast and animal systems (Soutoglou & Misteli, 2007), recent studies have begun to demonstrate that chromatin mobility also plays an important role in plant genome function. In this review, we describe the state-of-the-art concerning chromatin mobility, from the technologies available to the role that chromatin mobility plays in different cellular processes.

MEASURING CHROMATIN MOBILITY

An understanding of how chromatin is organized in the nucleus requires the visualization of nuclear structures and

specific genomic sequences. Fluorescence *in situ* hybridization of DNA (DNA-FISH) was a revolutionary approach for visualizing the arrangement of chromosomes and genes inside the nucleus (Gall & Lou, 1969; Speicher et al., 1996). For example, it convincingly confirmed the existence of chromosome territories first proposed by Carl Rabl (Rabl, 1885) and Theodor Boveri (Boveri, 1909). Later chromatin conformation capture approaches and derivatives, which depend on proximity ligation of DNA ends involved in chromatin contacts, have helped to map chromatin interactions at the genome-wide level (Lieberman-Aiden et al., 2009). However, those methods are snapshots of the chromatin organization and are limited with respect to capturing the spatiotemporal architecture changes.

The observation of chromatin motion requires methods that are compatible with live cell imaging. Different gene tagging systems have been developed that allow tracking the movement of specific chromosome positions in living cells in real-time to measure chromatin mobility at the locus level. Two main gene tagging systems are currently available: bacterial-based- or clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9)-based methods (Figure 1a). The bacterial-based gene tagging approaches include the *lacO*/LacI and *tetO*/TetR systems, which are dependent on the insertion of repeat binding sequences (*lacO* or *tetO*) into the genome, to which fluorescently tagged proteins (LacI or TetR) interact specifically (Marshall et al., 1997; Michaelis et al., 1997; Robinett et al., 1996). In these methods, the repeat sequence can be 10 kb long and the GFP fused repressors bind at their cognate repeat sites, producing a fluorescent spot within the nucleus, making it possible to track the movement of tagged chromosomal loci accurately. In *Arabidopsis thaliana* (Arabidopsis), *lacO*/LacI gene tagging was the only gene tagging system available until recently (Kato & Lam, 2001; Matzke et al., 2003). Using this system, it was possible to determine the position of loci in plant nuclei in a three-dimensional context, providing valuable information about chromosome arrangements in interphase and gene repositioning during gene repression and DNA damage (Hirakawa et al., 2015; Kato & Lam, 2001; Matzke et al., 2008; Rosa et al., 2013). More recently, another system based on the ParB/*parS* bacterial operon called ANCHOR has been engineered. In the ANCHOR system, the repeat sequence (parS) is much shorter (up to 1 kb) and the ParB protein tagged with a fluorescent protein extends over the surrounding chromatin generating a spot visible by fluorescence (Meschichi et al., 2021; Saad et al., 2014) (Figure 1a). The *parS* sequence is composed of four repeats allowing oligomerized ParB proteins to bind and propagate over the *parS* sequence and adjacent DNA (Meschichi et al., 2021). The loose interaction between ParB proteins and *parS* sequences avoids disturbances with other types of machinery, such as transcription or DNA repair (Saad et al., 2014).

The introduction of the CRISPR/Cas9 system brought new opportunities also for gene locus labeling. Tagging DNA sequences with CRISPR/Cas9 is possible by fusing a deadCas9 with eGFP (dCas9-eGFP) and by co-expressing guide RNAs (Chen et al., 2013, 2016, 2018) (Figure 1a). CRISPR locus tagging system has already been used in plants, namely in *Nicotiana benthamiana*, *Scadoxus multiflorus* and *Zea mays*, where it was used to visualize and measure the mobility of repeat sequences such as telomers (Dreissig et al., 2017; Němečková et al., 2019).

Other methods that track chromatin in living cells rely on the accumulation of other fluorescence-labeled proteins that interact with specific genomic regions or on the expression of photoactivatable fluorescent proteins linked to histones (Kruhlak et al., 2006; Wiesmeijer et al., 2008). For example, fluorescently tagged HR components have been used upon damage via radiation or genotoxic chemicals to track the mobility of DNA double-strand breaks (DSBs) (Caridi et al., 2018; Lottersberger et al., 2015; Meschichi et al., 2022; Miné-Hattab & Rothstein, 2012).

The movement of a locus marked with these gene tagging systems can be captured using time-lapse microscopy, and then quantified by mean square displacement (MSD) analysis (Caridi et al., 2018; Meschichi & Rosa, 2021; Spichal & Fabre, 2017). MSD analysis is a standard method for examining and characterizing the physical space explored by a specific locus within the nucleus. It calculates the average distance traveled by a particle over all points of the individual trajectory by increasing the time interval (Figure 1b–d) (Michalet & Berglund, 2012; Oswald et al., 2014). As a result of the large number of data points collected over many cells, this analysis allows the generation of accurate parameters that describe the nature of the chromatin movement, such as the diffusion coefficient and radius of constraint (*Rc*). On an MSD graph, the diffusion coefficient is directly proportional to the initial slope and the *Rc* is determined from the plateau reached by the curve over time (Figure 1d). In yeast, excised extrachromosomal rings have an *Rc* that is identical to the radius of the nucleus (Gartenberg et al., 2004), indicating that flanking chromosomal DNA and the context of tagged loci limit chromatin mobility.

CHROMATIN MOBILITY AND GENE TRANSCRIPTION

The dynamic movement of chromatin has been linked in several instances to transcriptional regulation. Sites with higher levels of transcription were shown to explore larger regions within the nucleus (Bystricky et al., 2009; Rosa et al., 2006), although the functional significance of these observations is still not well understood. Also, genes can move to a favorable location in the nucleus for regulatory purposes, such as activation or repression. Studies on the immunoglobulin loci (Kosak et al., 2002) provided one of the first examples of genes moving within the nucleus

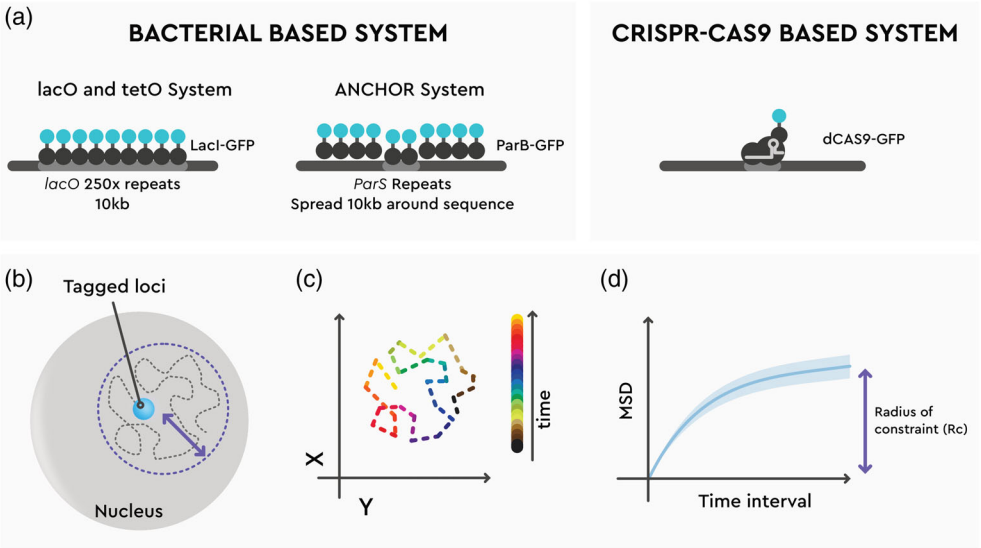

Figure 1. Gene-tagging systems for labeling genomic loci in living cells. (a) Schematic representation of gene tagging systems. Gene tags based on bacterial systems rely on the insertion of a repeat binding sequence into the genome, where proteins fused to a florescent protein will specifically interact. Schematic representation of the *lacO*/LacI system: LacI protein fused to GFP interacts with *lacO* repeats. In the case of ANCHOR system, the repeat sequence is smaller and the ParB-GFP oligomerizes along the flanking genomic region. The CRISPR/Cas9 based system uses a catalytically dead Cas9 (dCas9) fused to a fluorescent protein. By designing specific guideRNAs, particular sites can be visualized. (b) Illustration of single locus mobility inside a subnuclear compartment (purple circle). (c) Schematic representation of a 2D projection trajectory during time acquisition. (d) Trajectories extracted from 2D projection data are examined using mean square displacement (MSD) analysis, which generates an MSD curve allowing quantification of locus motion and calculation of the radius of constraint (*Rc*).

linked to their activity. Immunoglobulin loci were shown to move from the nuclear periphery in hematopoietic precursors towards the nuclear interior as they become activated in B-cells. In Arabidopsis, the inverse effect was observed with the light-inducible chlorophyll a/b-binding proteins (CAB) locus, which moves from the nuclear interior to the nuclear periphery during transcriptional activation by light (Feng et al., 2014) (Figure 2). Co-regulated genes can also associate in the nucleus for transcriptional purposes. In *Drosophila*, particular genes were shown to cluster by moving towards each other during silencing (Bantignies et al., 2011; Cheutin & Cavalli, 2012; Francastel et al., 2001; Wani et al., 2016). In Arabidopsis, it was shown that alleles of the polycomb target *FLOWERING LOCUS C* (*FLC*) physically cluster within the nuclear space during an environmentally triggered epigenetic silencing (Rosa et al., 2013) (Figure 2). The looping out of genes from their main chromosome territories is another example where chromatin motion is associated with changes in gene activity. Examples include the MHC class II locus, epidermal differentiation or the clustering of Hox genes, in which CT looping has been associated with high expression states (Chambeyron & Bickmore, 2004; Morey et al., 2007; Volpi et al., 2000). It is clear from these observations that genes move within the nucleus in relation to their activity. It is less clear, however, whether the observed movement of gene loci is crucial for gene regulation. In human cells, tethering certain genes to a protein of the inner nuclear membrane was sufficient to dampen their expression (Finlan et al., 2008). Studies in *Saccharomyces cerevisiae* indicate that gene repositioning is not required for gene activity *per se*, but rather optimizes gene expression (Taddei et al., 2006). As a result, gene movement may be viewed as an additional layer for fine-tuning gene regulation.

CHROMATIN MOBILITY DURING CELL DIFFERENTIATION AND CELL CYCLE

Changes in chromatin mobility have also been reported during cell differentiation. In Arabidopsis, the *lacO*/LacI and ANCHOR systems were used to measure chromatin mobility in different organs and cells with different degrees of differentiation (Matzke et al., 2019; Meschichi et al., 2021, 2022). The results obtained from studies undertaken in roots are consistent with chromatin mobility being higher in undifferentiated cells compared to fully differentiated ones (Meschichi et al., 2021). Moreover, studies using fluorescence photobleaching of tagged histones have also shown that chromatin in undifferentiated cells is more dynamic with loosely bound histones (Arai et al., 2017; Meshorer & Misteli, 2006; Rosa et al., 2014). The histone acetyltransferase HAG1 has been shown to be involved by increasing histone exchange in undifferentiated meristematic cells (Rosa et al., 2014). Histone acetylation decreases the affinity of histones to DNA, causing the nucleosomes to unfold and increase accessibility to the transcription machinery (Grunstein, 1997), thereby increasing chromatin mobility. Additionally, histone post-

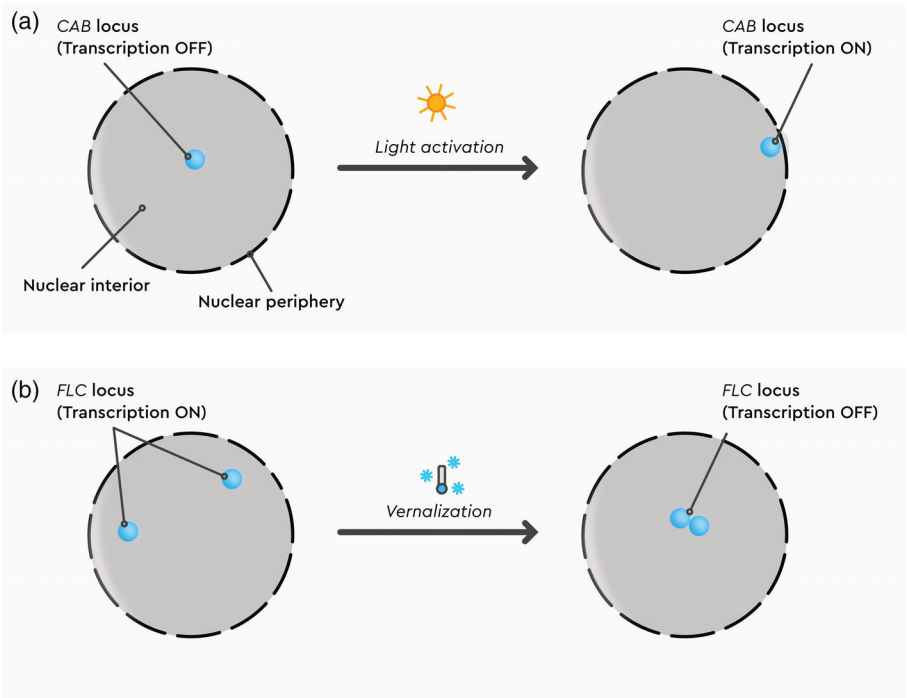

Figure 2. Examples of locus repositioning in the nucleus during transcriptional changes. (a) Schematic representation of the *CAB* gene during transcriptional induction by light. Upon transcriptional activation by light, the transcriptionally silenced *CAB* locus relocates to the nuclear periphery. (b) Vernalization in Arabidopsis involves the physical clustering of *FLC* alleles during polycomb-mediated epigenetic silencing.

translational modifications, such as histone acetylation, also generate docking sites for histone readers (Musselman et al., 2012), which can directly or indirectly modulate chromatin structure and chromatin mobility. Other factors such as polyploidy also appear to affect chromatin mobility. In Arabidopsis, measurements of chromatin mobility using the *lacO*/lacI system in the leaf showed that chromatins of endoreduplicated pavement cells have a greater range of movement compared to that of diploid guard cells (Kato, 2003). These results contrast with the observations carried out in the root, where cells from the differentiation zone showed lower mobility compared to diploid cells from the meristem (Meschichi et al., 2022). Importantly, however, chromatin can be anchored and organized very differently in different cell types (Pecinka et al., 2004; Schubert et al., 2014), which may affect the dynamics of chromatin diffusion. It is therefore important to take into account the effects associated with the cell type when studying chromatin mobility.

Chromatin mobility changes also during the cell cycle. In yeast, tagged loci show less movement in S-phase than in G1-phase (Dion et al., 2012; Heun et al., 2001). Also, in Arabidopsis, our recent study revealed that cells in S/G2 show a lower chromatin mobility (Meschichi et al., 2022). The cohesin complex, which holds sister chromatids together in G2, is likely to account for these observations (Bolaños-Villegas et al., 2017). In mammals, however, the results obtained by visualizing chromosomal regions using a photoactivatable histone fusion suggest that there is no significant difference in cell mobility between mid- and late G1, S or G2. Instead, in mammals, mobility appears to be greater in the early G1-phase (Walter et al., 2003; Wiesmeijer et al., 2008).

In many organisms, chromosome end-led movements have been studied in prophase of meiosis I by combining genetics with *in vivo* imaging of various chromatin or chromosomal markers, such as tagged telomere proteins, Hoechst dyes, *lacO*/LacI-inserted repressor-binding sites, tagged proteins from the synaptonemal complex or chromosome associated proteins (Conrad et al., 2008; Ding et al., 2004; Koszul et al., 2008; Morimoto et al., 2012; Parvinen & Soderstrom, 1976; Scherthan et al., 2007; Sheehan & Pawlowski, 2009). Characterization of both wild-type movement and impaired movement in mutants led to proposed roles for chromosome movement in the homology search process, minimizing undesirable chromosomal interactions and actively promoting recombination (Koszul & Kleckner, 2009; Wanat et al., 2008). However, studies of chromatin mobility during meiosis have yet to be reported in Arabidopsis.

CHROMATIN MOBILITY DURING DNA REPAIR

The most comprehensive studies of chromatin mobility to date have been those associated with DNA damage. DNA

damage can be caused by a variety of factors, including endogenous processes linked to intracellular activity and exogenous factors such as biotic and abiotic stress. DNA damage can be classified into four main categories: base mismatch, single-strand DNA breaks (SDB), DNA adducts and DSBs. DSBs are a particularly deleterious type of DNA damage, potentially leading to chromosome rearrangements or loss of entire chromosome arms. Studies in several systems have analyzed chromatin mobility in the presence of DSBs and found changes in mobility at damaged sites as well as in potentially undamaged chromosomal locations.

Measurements of chromatin mobility in the presence of DSBs have been performed in different ways and measured at different levels: damaged/DSB site, undamaged loci and at the global chromatin level (Miné-Hattab & Chiolo, 2020; Shaban & Seeber, 2020). Chromatin mobility can be studied upon DSB induction using the endonucleases HO or I-*Sce*I (Dion et al., 2012; Miné-Hattab et al., 2017; Miné-Hattab & Rothstein, 2012), the CRISPR/Cas9 system (Emmanouilidis et al., 2021; Wang et al., 2019) or genotoxic agents such as zeocin or bleomycin, or γ radiation. In different model species, it has been shown that, subsequent to DSBs, chromatin becomes generally more mobile (Dion et al., 2012; Dion et al., 2013; Hauer et al., 2017; Herbert et al., 2017; Lawrimore et al., 2017; Miné-Hattab et al., 2017; Miné-Hattab & Rothstein, 2012; Strecker et al., 2016). In our recent study in Arabidopsis, chromatin mobility of *lacO* sites inserted in different positions in the genome also showed an increase in mobility after zeocin treatment (Meschichi et al., 2022).

Recent work in yeast has generated some information on the genetic factors controlling global chromatin mobility in the presence of DSBs (Dion et al., 2012; Hauer et al., 2017; Herbert et al., 2017; Lawrimore et al., 2017; Miné-Hattab et al., 2017; Miné-Hattab & Rothstein, 2012; Seeber et al., 2013; Strecker et al., 2016). In plants, however, we are only now beginning to understand how DSBs affect chromatin mobility and to search for possible factors playing a role in this process. Our recent study has shown that the increase in mobility upon zeocin treatment is lost in the *sog1* mutant, a central transcription factor of the DNA damage response (DDR) in plants, suggesting that the DDR pathway controls the increased chromatin mobility upon DNA damage in plants (Meschichi et al., 2022) (Figure 3a). Additionally, the increase in mobility appears to occur specifically during S/G2 phases of the cell cycle, indicating that cell cycle-related factors may be involved (Meschichi et al., 2022). Future studies are now needed to investigate the mechanisms downstream of SOG1 directly responsible for the increased chromatin mobility after DNA damage.

Assessment of chromatin movement at DSB sites, as performed in yeast by *Sce*I-induced breaks, showed particularly high levels of mobility, with sites adjacent to DSBs exploring a nuclear volume 10 times greater than that before damage (Dion et al., 2012; Miné-Hattab & Rothstein, 2012). Several factors were shown to be involved in this response, including resection factors, chromatin remodellers, checkpoint activators and the homology search factor Rad51 (Dion et al., 2012; Hauer et al., 2017; Horigome et al., 2014; Miné-Hattab et al., 2017; Miné-Hattab & Rothstein, 2012; Neumann et al., 2012; Oza et al., 2009; Saad et al., 2014; Smith et al., 2018). In Arabidopsis, DSB sites were tracked by monitoring two essential factors of homologous recombination (HR), which accumulate at DSBs, RAD51 and RAD54 (Da Ines et al., 2013; Hirakawa et al., 2017). After the resection of the broken dsDNA, RAD51 is first recruited, forming a nucleoprotein filament (Barzel & Kupiec, 2008; Dillingham & Kowalczykowski, 2008; Li & Heyer, 2008). Once the template is found, RAD54 interacts physically with RAD51 and stimulates DNA strand exchange and D-loop formation (Li & Heyer, 2008; Tavares et al., 2019). In Arabidopsis, RAD51 and RAD54 foci formation follow similar action orders after DSB induction (Meschichi et al., 2022). By measuring the mobility of RAD51-GFP and RAD54-YFP foci, it is possible to track chromatin movement specifically at DSB sites (Meschichi et al., 2022). RAD51 foci showed higher mobility than RAD54, suggesting a need for mobility at an early HR stage (Meschichi et al., 2022). Unlike Arabidopsis, yeast RAD54 and RAD51 foci have the same mobility upon zeocin (Dion et al., 2013). However, RAD54 foci mobility varies according to their position within the Arabidopsis nucleus (Meschichi et al., 2022). RAD54 foci in the nucleoplasm have similar mobility as RAD51, in contrast to those in the nuclear periphery, which present a lower mobility (Meschichi et al., 2022). Interestingly, RAD51 foci decrease mobility in the *rad54-2* mutant, reinforcing the role of RAD54 in the early HR and potentially in the homologous search. Overall, our recent work has shown that in Arabidopsis DSB sites have higher mobility during early homologous recombination, which may correspond to the homology search during HR (Figure 3b).

A number of studies have also examined whether in mammalian cells chromatin mobility is altered upon DNA damage (Agarwal et al., 2011; Jakob, Splinter, Durante, & Taucher-Scholz, 2009; Jakob, Splinter, & Taucher-Scholz, 2009; Krawczyk et al., 2012; Nelms et al., 1998; Soutoglou et al., 2007). However, the results obtained have been mixed, with some studies showing no change in mobility after DSB induction. Nevertheless, for most species analyzed so far, the increase in mobility after DNA damage appears to be conserved. This raises the question of the functional role of this increased mobility. Several studies have proposed that the induction of mobility during DNA damage positively correlates with repair efficiency (Gehlen et al., 2011; Herbert et al., 2017; Wiktor

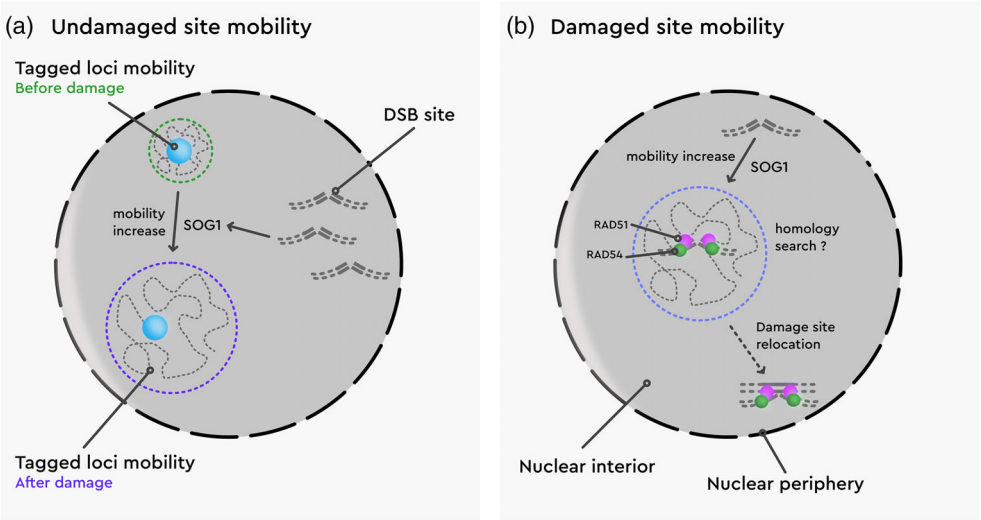

Figure 3. A model of DNA damage-induced chromatin mobility. (a) In response to high levels of DSBs, undamaged genomic sites become more mobile. This increase in mobility is under the control of the DDR master regulator, SOG1. (b) DSB sites loaded with RAD51 show high mobility levels, and their enhanced mobility requires the HR factor RAD54.

et al., 2021). One model suggested that the DSB sites move to find the template, and the increased global chromatin movement helps by increasing the probability of an encounter between broken molecules and the unbroken template (Gehlen et al., 2011; Wiktor et al., 2021). However, these studies are limited because the majority of mutants affecting mobility are also involved in repair functions. On the other hand, the cep3 mutant, which limits chromatin movement and is not a repair protein *per se*, had no impact on recombination frequency. It is therefore still unclear what role chromatin motion plays in homologous recombination and DNA repair. There is also the possibility that increased mobility aids in the movement of damaged sites to nuclear compartments favorable for repair, such as the nuclear periphery (Meschichi et al., 2022; Nagai et al., 2008; Therizols et al., 2006). However, clear evidence of these relationships is still elusive and a full understanding of the exact role of chromatin mobility upon DSBs still awaits additional studies.

FACTORS THAT INFLUENCE CHROMATIN MOBILITY

Chromatin mobility can be influenced by a variety of intrinsic cellular factors. An important factor is the position of a locus in the nucleus. For example, the anchoring of loci to particular nuclear structures appears to significantly affect chromatin movement. In both yeast and animals, chromatin mobility is restricted by proximity to tethering structures such as the nuclear envelope and centromeres (Chubb et al., 2002; Hajjoul et al., 2013; Heun et al., 2001; Verdaasdonk et al., 2013). In Arabidopsis, *lacO*/lacI lines with *lacO* sequences inserted in different chromosomal positions displayed different mobilities (Matzke et al., 2008, 2010; Meschichi et al., 2022). Lines with insertions corresponding to nucleoplasmic regions showed similar levels of chromatin mobility, whereas a locus with subtelomeric localization showed a significant reduction in mobility compared to the other lines. (Meschichi et al., 2021, 2022).

Interestingly, chromatin mobility is highly dependent on ATP levels. In particular, experiments in yeast have clearly shown that ATP synthesis inhibition correlated with a decrease in chromatin motion (Weber et al., 2012). Energy dependence and random motion may appear in contradiction. The likely explanation for this apparent discrepancy is that the chromatin fiber as a whole is constantly transitioning from opening to closing events as a result of ATP-dependent remodeling machineries. Indeed, several nucleosome remodelers contain a SWI2/SNF2-related ATPase subunit. Using their ATPase subunit, these remodelers can alter chromatin structure and accessibility in different ways (removing nucleosomes, altering nucleosome spacing, evicting dimers or modifying octamer composition through the incorporation or removal of histone variants). As an example, the INO80 remodeler, which contributes to the remodeling of nucleosomes at DSBs (Morrison et al., 2004; Tsukuda et al., 2005; van Attikum et al., 2004), increases the mobility of undamaged loci to which it is recruited (Neumann et al., 2012). Moreover, this effect is completely dependent on INO80 ATPase activity because the targeting of a mutant that cannot bind ATP failed to promote chromatin mobility (Neumann et al., 2012). These observations indicate that remodeling chromatin locally by altering nucleosome organization can result in large-scale chromatin movements. In Arabidopsis, a mutation in the chromatin remodeling factor RAD54,

which is also a Snf2-type ATPase, resulted in a decrease in chromatin mobility at DSB sites (Meschichi et al., 2022), thereby potentially linking ATP-dependent chromatin remodeling with chromatin mobility. However, further studies containing ATPase-deficient variants of RAD54 will be required to distinguish the ATP-dependent and independent roles of Rad54 in chromatin mobility in plants.

There is also now some evidence linking the cytoskeleton with chromatin mobility. The first examples came from work carried out on the LINC (Linker of the Nucleoskeleton to the Cytoskeleton) complex. This complex is found in the nuclear envelope and, as the name suggests, connects the cytoskeleton to the nucleoskeleton. In mouse embryonic fibroblast cells, the LINC complex was shown to promote the mobility of damaged telomeres (Lottersberger et al., 2015). Interestingly, this movement was also dependent on microtubules (disrupted in the presence of taxol). In addition, in another study, actin, a constitutive component of the INO80 complex, was also shown to be involved in chromatin motion as judged by MSD analyses of different labeled genomic loci in the presence of latrunculin A, a drug that inhibits actin polymerization (Spichal & Fabre, 2017). However, how cytoskeleton proteins and the nucleoskeleton regulate chromatin motion remains to be explored in the context of plants.

Other physiological parameters such as pH can also affect chromatin mobility. One study carried out in Arabidopsis tested the potential influence of pH on chromatin mobility using the *lacO*/LacI system and the genome-encoded fluorescent pH sensor SEpHluorinA227D (Matzke et al., 2019). This revealed that an ion-based signaling pathway induces alterations in interphase chromatin mobility and the surrounding pH of chromatin-bound proteins (Matzke et al., 2019). Chromatin mobility and function can be altered by pH changes in different ways. For example, as mentioned above, chromatin motion is largely attributed to the activity of ATP-dependent chromatin remodeling complexes (McNally, 2009; Soutoglou et al., 2007). Chromatin remodelers and other transcriptional proteins are probably sensitive to pH. It is also possible that changes in pH function indirectly by inducing alterations in nucleoskeletal elements to affect chromosome mobility. For example, changes in pH are known to affect the integrity and stability of the actin-containing nuclear matrix, which facilitates chromatin remodeling and transcription (Libertini & Small, 1984; Wang et al., 1989).

FUTURE PERSPECTIVES

Chromatin mobility was until recently considered to be a passive structural feature of chromatin. However, with the recent advances in live-cell imaging techniques, it has become increasingly clear that both local and global mobility are functionally relevant. The study of plant chromatin mobility is still in its infancy, althougj it has enormous potential for development in the coming years. In this review, we have summarized the current knowledge regarding chromatin mobility and its relationship with DNA damage and transcription in the plant model system, *A. thaliana*. New technologies that allow visualizing DNA in living cells, such as ANCHOR and CRISPR-based methods, present advantages compared to existing strategies because they do not rely on the use of repeated elements in the target sequence. Because plants have specific silencing systems, this aspect is of particular importance (Grob & Grossniklaus, 2019; Matzke et al., 2015; Watanabe et al., 2005). Combining these methods with PP7 and MS2 RNA-labeling technologies, which allow fluorescence labeling of nascent RNAs in living cells (Alamos et al., 2021; Germier et al., 2017; Hani et al., 2021), will help to expand our understanding of how chromatin mobility and transcription regulation are intertwined. CRISPR-based methods applied as DNA damage tools will provide an inducible and titratable way of targeting DNA damage in desired genomic regions (Emmanouilidis et al., 2021). Also, new live imaging technologies allow the study of whole genome mobility by simultaneously measuring the movement of multiple nano-genome domains using fluorescently tagged chromatin components such as histones (Lou et al., 2019; Nozaki et al., 2017; Shaban et al., 2018; Shaban & Seeber, 2020; Shinkai et al., 2016). It will be therefore interesting to use these methods to confirm and measure genome-wide mobility, such as in relation to DNA damage or other environmental stresses in plants.

As the mechanisms underlying chromatin mobility begin to be uncovered, one major question still remains: is chromatin motion a cause or a consequence of the underlying nuclear processes? Ultimately, this is a very difficult question to answer given the interconnectedness between chromatin remodeling and transcription or DNA repair. To prove causation, it would be interesting to visualize the homology search step live and, at the same time, target remodelers that enhance or limit mobility to the template site (Neumann et al., 2012), as well as examine whether DNA repair occurs more efficiently under these conditions. In the context of transcription, an approach would be to modify locus mobility by targeting chromatin remodeling enzymes (Neumann et al., 2012) and measure transcriptional output via the single molecule RNA FISH method or through systems such as MS2. Even though such studies can be challenging, the tools required to test this and other hypotheses are now readily available and will help us to build a comprehensive understanding of genome organization and function.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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