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Organizing DNA repair in the nucleus: DSBs hit the road

Aline Marnef and Gaëlle Legube

In the past decade, large-scale movements of DNA double strand breaks (DSBs) have repeatedly been identified following DNA damage. These mobility events include clustering, anchoring or peripheral movement at subnuclear structures. Recent work suggests roles for motion in homology search and in break sequestration to preclude deleterious outcomes. Yet, the precise functions of these movements still remain relatively obscure, and the same holds true for the determinants. Here we review recent advances in this exciting area of research, and highlight that a recurrent characteristic of mobile DSBs may lie in their inability to undergo rapid repair. A major future challenge remains to understand how DSB mobility impacts on genome integrity.

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Introduction

In eukaryotes, DNA is packaged into an incredibly complex structure, chromatin, which is regulated by mechanisms such as DNA methylation, histone modification, histone variant incorporation and nucleosome remodeling. A plethora of chromatin-binding proteins contribute to chromatin folding thereby allowing the establishment of a complex and multi-scale organization of the DNA, ranging from kilobases (kb) DNA loops, to megabases (Mb) topologically associated domains (TAD) [1]. From the nucleosome scale to the 3D nuclear architecture, chromatin acts as the cornerstone of all DNA organization and regulation machineries, since it directly controls the accessibility of the DNA sequence, the stiffness of the fiber as well as its ability to explore the nuclear volume. While the influence of nuclear organization and chromatin structure on transcription is well established, we just begin to appreciate its importance in the maintenance of genome integrity.

DNA experiences a wide range of damages, among the most detrimental are DNA double-strand breaks (DSBs) which can lead to severe genome rearrangements. DSBs are repaired by two main pathways: homologous recombination (HR) or non-homologous end joining (NHEJ) [2]. While NHEJ directly ligates the two broken ends with no or minimal end processing throughout the cell cycle, homologous recombination depends on the generation of single stranded DNA (ssDNA) by a process called resection in the S and G2 phases of the cell cycle. This is followed by the assembly of a nucleoprotein filament that searches for a homologous sequence to use as a template.

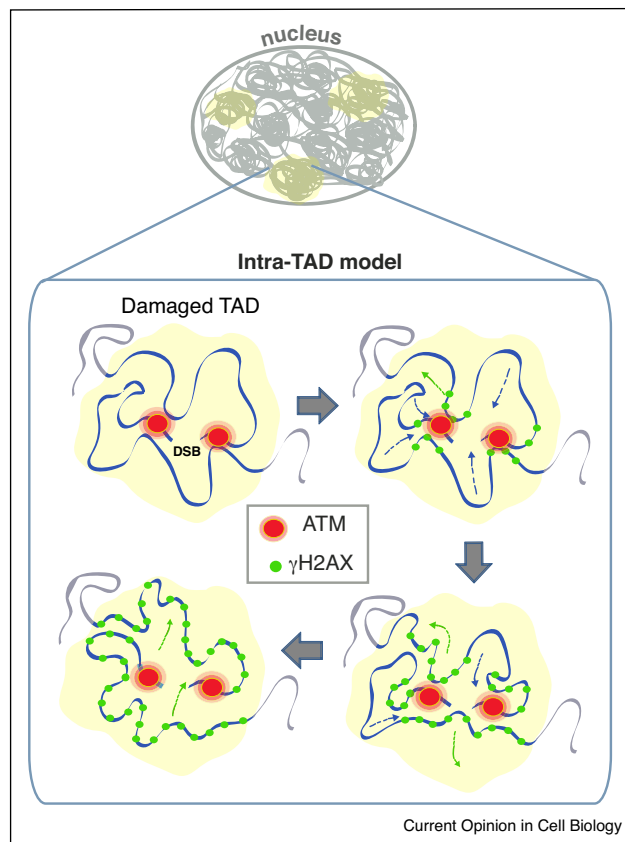
Recent studies established that the initial chromatin properties of the damaged locus and its position within the nucleus impact on the repair pathway and efficiency (nicely illustrated in Refs. [3*,4], and for review see Ref. [5,6]). Equally, the formation of a DSB also influences chromatin compaction and DNA motion in the nucleus [6]. These changes are likely in line with the chosen repair mechanism in order to simultaneously favor the desired outcome (such as homology search) and disfavor potentially deleterious outcomes (such as translocation).

In this review, we will come back on the evidence that pinpoint changes in chromatin motion following damage, from a local, intra-TAD, level to a more global nuclear scale and discuss their potential function during DSB repair.

Alterations of the chromatin fiber within TAD following DSB induction

Following DSB induction, one of the initial events consists in the phosphorylation of the H2AX histone variant in mammals and of H2A in yeast, referred to as γ H2AX and γ H2A respectively. This modification spreads in a bidirectional, although not necessarily symmetrical, manner over large domains surrounding the breakpoint, spanning 1–2 Mb in mammals and 200–300 kb on the smaller yeast genome [7,8], and giving rise to so-called ‘DNA repair foci’. Even though the elements delineating γ H2AX domains remain poorly defined, they correlate with the boundaries of chromosomal domains that exhibit self-association properties, also known as TADs [9]. γ H2AX and γ H2A are respectively generated by the PI3K-like kinases ATM and Tel1/Mec1. In mammals, genome wide mapping unveiled that ATM is activated on relatively restricted regions around DSBs ranging from approximately 2 to 10 kb [10*]. The sharp discrepancy between the distribution of the kinase (ATM) and its product (γ H2AX), led us to propose the ‘intra-TAD model’ for γ H2AX domain establishment (Figure 1;

Figure 1



The «intra-TAD» model for γ H2AX domain establishment. Following DNA damage, ATM (in red) is recruited on small regions flanking the DSB. We propose that the movement of chromatin within a topologically associated domain (TAD) enables H2AX nucleosomes to come in close contact with ATM, which would in turn phosphorylate them (in green) leading to widespread γ H2AX domains.

[11]). In this model, the flexibility and local movements of the chromatin fiber inside the TAD bring distant nucleosomes within spatial proximity of ATM. Such a mechanism would ensure the phosphorylation of any H2AX containing nucleosomes within a TAD, if the signal is sustained (i.e., if the break persists long enough). This model is in agreement with the observation that neither lower levels of H2AX nor a persistent DSB changes the spreading capability of γ H2AX [12]. In addition, the ability of the yeast Mec1/Tel1 kinases to phosphorylate undamaged nucleosomes *in trans* further supports this model [8].

The reasons why such large chromosomal domains need to be modified still remain mysterious, especially given that H2AX is not essential in mice and dispensable for repair [13], suggesting a more subtle function in the maintenance of genome integrity. A potential role could be to restrict DNA ends diffusion and prevent them to drift away from each other. Computational modeling

suggests that folding of the chromatin fiber contributes to maintain the two DNA ends in close proximity [14]. In agreement, the cohesin complex, with an established role in chromosome folding [15], inhibits the joining of distant DNA ends (~ 3 kb), but not close ends (~ 100 bp), suggesting that cohesin, and by extension DNA loops, tightly regulate DNA end trafficking within a chromosomal domain [16]. Another putative role for the wide spreading of γ H2AX could be in promoting liquid demixing, a phase separation event leading to a membrane-free compartmentalisation of repair foci [17]. Since electrostatic interactions are at the heart of these phases separation events [18], extensive accumulation of negative charges caused by the phosphorylation of H2AX on an entire TAD may contribute to the formation of a liquid-like droplet. Liquid demixing could thereby provide a special micro-environment for repair and exclude non-repair proteins. An alternative, non-exclusive, hypothesis is that widespread γ H2AX modification at the TAD level is associated to changes in chromatin compaction/condensation regulating long-range trafficking of the damaged locus, thereby fine tuning repair to maintain genome integrity (see the following sections).

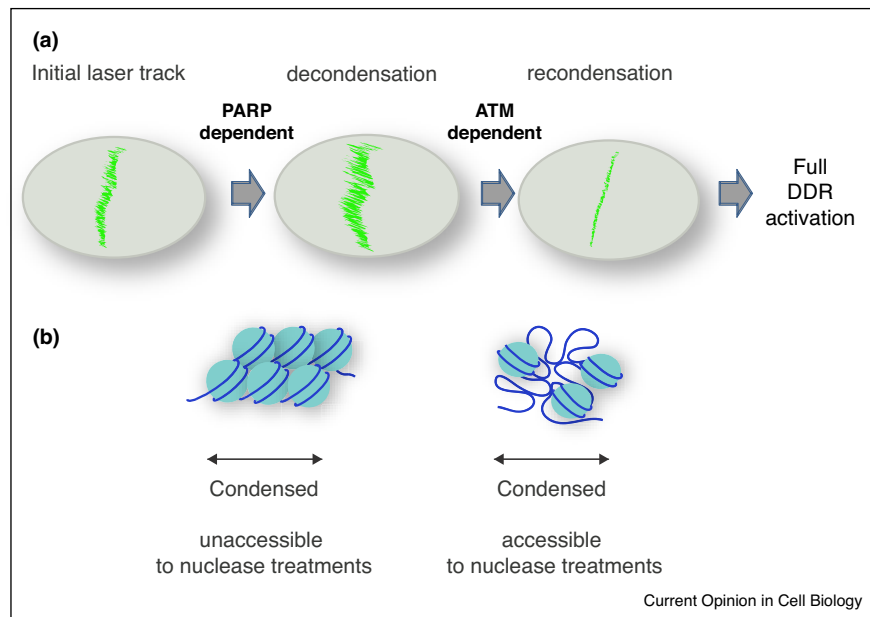
Modification of chromatin condensation around DSBs

The exact links between chromatin modification and DNA condensation are subjected to intense investigations, and have recently benefited from the use of high resolution Hi-C and super-resolution microscopy (see for example [19,20]). Upon DSB induction, multiple lines of evidence pinpoint some changes in chromatin condensation. Chromatin undergoes a quick PARP1-dependent and H2AX/ATM-independent relaxation immediately after DSB induction [21–25,26*,27] followed by a H2AX and ATM-dependent recondensation [21–25]. Importantly, this secondary condensation is required for full DNA damage response (DDR) activation [25]. Together with the reported functions of heterochromatin proteins in the DDR (reviewed in Ref. [28]), this argues for a role of chromatin condensation in DSB repair. Independently, several studies reported that nuclease accessibility is enhanced at damaged chromatin, in yeast and mammals, in an ATM and γ H2A-dependent manner [29,30], and that γ H2AX foci exhibit a decondensed-like structure when observed by electron microscopy [22,31]. A model that reconciles all these observations is still awaited, but it is clear that structural changes of the chromatin fiber are tightly regulated both in time and space to fine tune repair events. Building such a model will necessitate to carefully consider that the nuclear volume occupied by a locus does not necessarily reflect its accessibility to nuclease (Figure 2).

From chromatin structure to DSB mobility

Whether the condensation state of the chromatin fiber directly translates into small or large-scale movement of

Figure 2



Changes in DNA condensation state following DNA damage.

(A) Laser track induced DNA damage (in green) leads to a rapid (within 3 min) PARP-dependent local chromatin expansion at the damaged region. This is followed by an ATM-dependent recondensation phase 20–30 min post-damage induction that is required for full DDR activation. **(B)** DNA condensation, as defined by the nuclear volume occupied in the nucleus, does not necessarily correlate with chromatin accessibility to nuclease treatment. Left panel shows a condensed DNA structure that is inaccessible to nuclease while the right panel shows the same level of condensation that retains accessibility to nuclease treatments.

DNA loci in the nucleus is currently not clear. Nevertheless, previous studies argue for a direct relationship between the compaction state and the ability of an undamaged locus to roam within a large area [32]. In the past decades, the mobility of DSBs has been recurrently investigated. In yeast, it is now well accepted that DSB induction increases both the movement of undamaged chromosomes and of the DSB itself [33–36], even though it may not stand true at very early time points [37]. In higher eukaryotes, the issue of DSB mobility has however been controversial for many years caused by conflicting results ([22,38–41], see also Ref. [42] for a recent review). A plausible explanation for these discrepancies stems in the nature of the broken locus. This was first hypothesized by Aten's team who noticed that DSBs induced by etoposide, a topoisomerase II inhibitor, were substantially more mobile than irradiation-induced DSBs, two methods that likely induce breaks at different positions throughout the genome [41]. Since then a number of reports have supported this hypothesis. Differential motion has been observed between subtelomeric DSBs compared to a more internal DSB [43•] or between DSBs induced in active genes compared to intergenic loci [44]. Hence we believe that the position of the DSB on the genome will dictate immobility, small scale motion or large-scale movement depending on (1) the initial mobility of the considered locus and (2) the repair kinetic and pathway used at this specific locus.

What is the function of such large-scale movements of DSBs? One obvious possibility is to ensure homology search, supported by the fact that DSB mobility is abolished/delayed in mutants impeding ssDNA generation and nucleofilament assembly [33,35] and that long-range mobility triggers contact between homologous sequences [43•,44]. However, DSB mobility may also be required to fulfill additional functions, such as promoting DSB clustering and tethering to subnuclear structures, described below.

DSB clustering: a wanted or unwanted side effect of DSB mobility?

A potential outcome of DSB motion that has been strongly debated, yet recurrently observed, is the ability of DSBs to 'cluster' together. In yeast, the introduction of two DSBs leads to the formation of a single Rad52 focus, indicating that multiple DSBs can indeed coalesce within a 'repair focus' [45]. For a while, the controversy about DSB mobility in mammals fueled the debate on whether or not this phenomenon is conserved in higher eukaryotes, but a significant amount of reports have now clearly established that DSB clustering also exists in higher organisms [10•,43•,46–49].

DSBs clustering is undoubtedly a tightly controlled process, given the increased risk of translocations it elicits [48], but its function remains enigmatic. DSB clustering

may be an unscheduled consequence of the increased DSB motion within the nucleus and the potential stickiness or liquid droplet properties of repair foci (i.e., fusion occurs when two foci encounter by chance). However, DSB clustering could also contribute to the assembly of repair foci by promoting the formation of liquid droplet, thereby reinforcing the recruitment of repair factors. Equally, clustered DSB foci may also reflect 'sequestration bodies' where DSBs exhibiting a delayed repair response accumulate to remain isolated from the rest of the genome while waiting for an adapted molecular and cellular response. Notably, this latter hypothesis echoes the intriguing behavior of persistent breaks in nuclei described in the following section.

DSBs compartmentalization to subnuclear structures

Interestingly, DSB mobility also closely relates to the persistence of a break (reviewed in Refs. [6,42]). Studies in both budding and fission yeast unveiled that unrepairable breaks move towards the nuclear periphery, at the inner nuclear membrane (INM) via the SUN domain containing protein Mps3 and at the nuclear pores [50–53]. Similarly, DSBs induced in sequences inherently difficult to repair, such as heterochromatin and ribosomal DNA, display repositioning by moving towards the border of their initial chromatin environment (i.e., heterochromatin focus and nucleolus respectively) both in yeast and mammals [54,55,56^{••},57,58,59^{••},60,61]. Breaks in heterochromatin can even travel longer distances in *Drosophila* as they can relocate to the nuclear periphery in a Mps3 homologue and nuclear pore proteins-dependent manner [62^{••}]. At present, DSB positioning at the nuclear periphery has yet not been observed in mammals, but this clearly deserves further investigations. Indeed the nuclear envelope invaginates upon damage induction [31]. Moreover, the LINC complex which is embedded within the nuclear envelope and which contains Mps3 homologues (SUN1/2) contributes to DSB mobility in mouse cells [63^{••}].

Why would persistent DSBs or DSBs inherently difficult to repair need to be mobile? Several non-exclusive hypotheses can be envisaged. First, placing a DSB in a 'safe' environment, that is, away from repeated copies of the broken locus, could help to minimize ectopic recombination, as frequently reported ([54,55,56^{••},57,59^{••},62^{••}]; reviewed in Ref. [42]). Second, studies in yeast and mammals have brought to light that subnuclear compartments could be specialized in dedicated types of repair. Indeed, the nuclear envelope and the nuclear pores act as hubs influencing the repair outcome (i.e., either in limiting illicit recombination or favoring error-prone pathways, such as Break Induced Replication (BIR) and Alt-NHEJ) [52,64,65[•],66,67]. Confining error-prone pathways to a dedicated nuclear space could limit their use to non-repairable breaks only, thereby maintaining

genome stability. Third, a hypothesis that has not been given close attention is that targeting a DSB to the nuclear envelope could transmit signals regarding nuclear events to the cytoplasm in order to coordinate cellular responses such as apoptosis. Importantly, the LINC complex mediates the connection between the nucleoskeleton and the cytoskeleton. In this scenario, a direct interaction of DSBs with the LINC complex could trigger modifications of both the nuclear lamina and the cytoskeleton to coordinate the cellular morphological changes associated with apoptosis such as blebbing and cell shrinkage. A precedent in this nuclear/cytoplasm connection already exists since several studies have shown a role for chromatin structure in cell migration, proposed to be mediated in part via the transmission of mechanical forces through the LINC complex (for review [68,69]).

Is DNA motion sustained by random diffusion or by a directed mechanism?

One burning issue in the field is to determine how motion of damaged loci is achieved. Two opposite views face off, which are not necessarily exclusive in our opinion.

Following DSB induction, the chromatin assembled at the damaged locus undergoes massive modifications leading to changes in its condensation state. In the first view, these events contribute to the increased ability of a damaged locus to roam through random diffusion within the nucleus. Increased mobility could be provoked by changes in the entropy of DNA created by the break itself. Modeling the chromatin fiber as a looped-polymer, revealed that faster movement, larger roaming area and even relocation at the periphery of a subcompartment, could be explained by the increased degree of freedom of DNA ends [14]. Another possibility for an increase in random diffusion could arise from alteration of physical constraints through the release of anchoring points. This view is supported by a compelling study from Durocher's group, who showed that disruption of the chromosome anchorage to the spindle pole body is a key determinant of DSB mobility in yeast [70^{••}]. Finally, modifications of chromatin structure could also increase local DNA diffusion, as clearly established by the Gasser's laboratory who demonstrated direct links between ATP-dependent remodeling of the local chromatin structure and large-scale mobility [36,64].

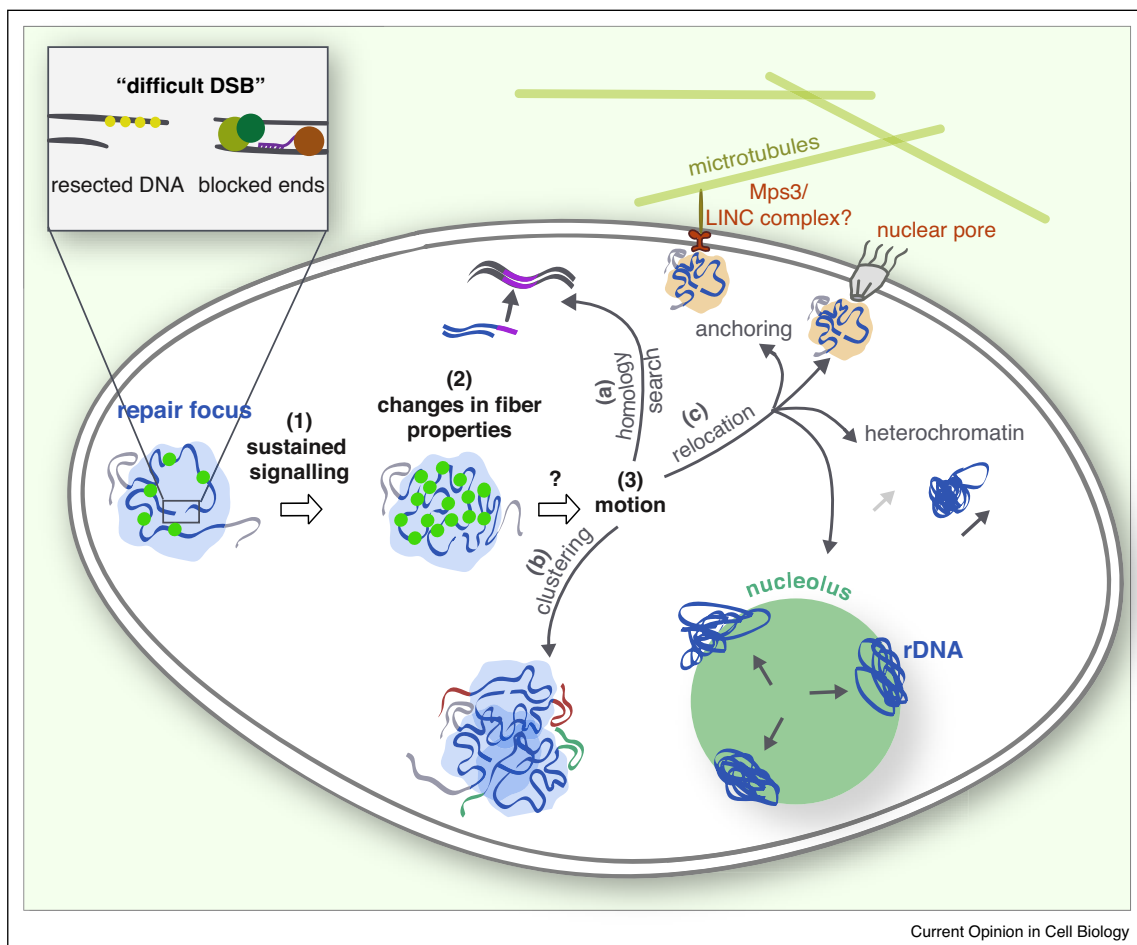
In the second view, DSB mobility is rather driven by a 'directed', filament-driven, mechanism. The most convincing evidence is the directed movement over micron-range distances observed at damaged subtelomeric regions [43^{••}], one of the first directional DNA motions ever identified in nuclei (see also Refs. [71–73]). Here, the directional movement appears to be sustained by a Rad51 filament, whose role is to seek for a homologous sequence and synapse with a stationary 'recipient' telomere. This evokes the RecA (Rad51 bacterial homolog)

bundle recently observed following DSB induction in *Escherichia coli* [74]. Another type of directional movement could involve nuclear actin, previously proposed to sustain directed and micron-range motion of undamaged chromosomes [71,75]. Although its existence as a filamentous form is still vigorously debated (reviewed in Ref. [76]), a recent study reported that actin polymerizes in the nucleus following damage [77^{••}]. Interestingly, a small fraction of DSBs was shown to touch nuclear filaments but their actual movement by live imaging could not be addressed [77^{••}]. Thus, the possible involvement of a nuclear actin network in DSB mobility definitely deserves future investigations. Finally, there are evidences implicating the microtubule network (MT) in DSB motion in *Schizosaccharomyces pombe* and mammalian cells, highlighted through the

use of MT-destabilizing drugs [53,63^{••}]. In addition, several kinesins (MT-motor molecules) are also involved in DSB repair [66,78,79]. It is believed that the MT network functions on DSB mobility through LINC-dependent forces transmission between cytoplasmic MT and chromatin. However, nuclear MT formation has also been reported in yeast outside of mitosis [80], raising the need to investigate more thoroughly whether short nuclear MT (although not yet detected) could also sustain damaged DNA motion.

Overall, while ‘filament’ driven mechanisms in long-range DSB motion represent exciting possibilities, extensive additional work will be required to fully appreciate their contribution, or to definitely exclude their involvement in damaged DNA trafficking.

Figure 3



DSB motion in the nucleus: restricted to break uneasy to repair? Some DSBs can encounter difficulties to be quickly repaired by NHEJ, caused by high protein/RNA occupancy, DNA-ends adducts, ongoing resection . . . (magnification, top left). This leads to sustained ATM activation (1) that triggers consequent chromatin remodeling (including γ H2AX) on large domains such as TADs that likely modifies the condensation of the DNA fiber (2). The mechanisms by which these changes are further translated into the ability of the damaged locus to roam within the nuclear space are still unclear (3). Large-scale DSB motion eventually (A) promotes homology search (if the homologous donor is not in close proximity, for instance when sister chromatid is not available), (B) triggers DSB clustering whose function still needs to be determined and (C) relocates DSB to a more suitable place for repair.

Toward a unified model for DSB motion

Although subjected to intense investigations, our view of the organization of DSB repair within the nuclear space is still fragmented. A fundamental principle of DSB movement may however reside in the ease of particular breaks to be repaired (Figure 3). We propose that loci inherently difficult to repair (such as repeated sequences, over-compacted chromatin, over-transcribed regions . . .) lead to a sustained DDR activation. This triggers profound changes in chromatin properties surrounding the DSB, which further sets off long-range motion. Accordingly, the ATM/Tel1-Mec1 kinases, which are at the heart of chromatin remodeling following DSBs, have been repeatedly involved in the DSB mobility events described in this review [10*,33,36,44,50,54,56**,57,58,70**,81]. Large-scale movements could promote the use of an adequate repair pathway and substrate, the physical sequestration/isolation of unrepaired broken loci, and the signaling to the cytoplasm in order to elicit the adequate cellular response. The use of super-resolution and advanced time-lapse microscopy, as well as high resolution genome-wide approaches such ChIP-seq and Hi-C, combined with the expanded toolbox to induce targeted DSBs at different genomic locations should rapidly give exciting insights into how DSB mobility impacts on genome stability.

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