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## **Chromatin mobility upon DNA damage: state of the art and remaining questions.**

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### **Abstract**

Chromosome organization and chromatin mobility are central to DNA metabolism. In particular, it has been recently shown by several labs that Double Strand Breaks (DSBs) in yeast induce a change in chromatin mobility at the site of the damage. Intriguingly, DSB also induces a global mobility of the genome, at others, potentially undamaged positions. How mobility is regulated and what are the functional outcomes of these global changes in chromatin dynamics are however not yet fully understood. We present the current state of knowledge in light of recent literature and discuss some perspectives opened by these discoveries towards genome stability.

### **Keywords**

Chromatin, Double strand breaks, mobility, polymer physics, yeast

### ***Assessing chromatin mobility***

Chromatin of any living organism is under constant motion and is probably essential for most, if not all, DNA transactions including replication, transcription and repair. To address functional outcomes, defining how a moving particle is displaced is important, since it permits to apprehend the way this particle explores its surrounding space and the time it might take to create contacts with functional molecular partners.

Mean Square Displacement (MSD) is the common measure to define the space explored by a randomly diffusing particle ((Albert et al. 2012; Spichal and Fabre 2017) for recent reviews). When a particle diffuses freely, MSD curve increases proportionally with time. When particle free diffusion happens in a constrained space, the MSD curve shows a plateau defining a radius of confinement ( $R_c$ ) for the moving particle. An increased  $R_c$ , visible at long time points, is often interpreted as the result of a faster diffusion or a less constrained diffusion although only the slope at the origin of the curve, at short time points, can define this. In yeast, initial time lapse particle tracking studies described motion of genomic loci as free diffusion in a restricted space (Marshall et al. 1997; Heun 2001). However, MSD of a moving particle scales as  $Dt^\alpha$ .  $D$  values inform on the speed with which the particle moves and  $\alpha$  values inform on the space explored by the particle: the smaller  $\alpha$ , the more often the particle will explore the same area. The motion is named subdiffusive when  $\alpha$  values are below 1. Several studies in yeast have now established that  $\alpha$  ranges from  $\sim 0.4$  to  $\sim 0.76$  and the prefactor  $D$  lies between  $1.2$  and  $2 \cdot 10^{-3} \mu\text{m}^2/\text{sec}^\alpha$  (Cabal et al. 2006; Hajjoul et al. 2013; Backlund et al. 2014; Spichal et al. 2016; Hauer et al. 2017; Miné-Hattab et al. 2017; Herbert et al. 2017). Contrary to confined diffusion, particle subdiffusive motion reflects a constrained motion without boundaries. Subdiffusion can be explained by crowding, viscoelastic properties of the environment and/or behavior of a locus embedded within chromatin and retained by juxtaposing neighbors in a polymeric chromatin chain (Weber et al. 2010). The fact that subdiffusive motion of chromatin was observed in bacteria and mammalian cells, suggests a universal dynamic behavior of chromatin in non challenging conditions (Weber et al. 2010; Bronshtein et al. 2015).

### ***Factors involved in chromatin motion***

To follow chromatin motion, the most popular tool remains the tracking of bacterial repeats LacO or TetO inserted into the genome, although interesting alternatives are developed {Belmont:2001tj}; {Bystricky:2015fj}. When bound by their cognate repressor fused to a fluorescent protein, MSD can be calculated at different time scales. Different physiological parameters are known to affect chromatin motion in yeast. First, locus position in the nucleus is important; proximity to tethering

structures like the nuclear envelope, as in mammals, will restrict movements (Heun 2001; Chubb et al. 2002; Hajjoul et al. 2013; Spichal et al. 2016). In particular, centromere tethering by a nuclear microtubule clearly restricts mobility of an undamaged locus located 8.8 kb from the centromere (Marshall et al. 1997; Verdaasdonk et al. 2013; Lawrimore et al. 2017). Induction of transcription through the centromere – which corresponds to a single nucleosome in budding *S.cerevisiae* yeast – detaches it from kinetochore and leads to a dramatic increase in mobility. Furthermore, the use of nocodazole that inhibits microtubules polymerization leads to larger  $R_c$  of a centromere proximal locus (Marshall et al. 1997; Verdaasdonk et al. 2013; Lawrimore et al. 2017). A second factor is ploidy; chromatin mobility can appear faster in haploids than diploid cells for certain loci – for example *URA3*, which is located in the middle of chromosome VR (Miné-Hattab et al. 2017), but not for others – like *GAL1-GAL10*, which is close to the chromosome II centromere (Backlund et al. 2014). Cell cycle is a third factor; chromatin motion is slower in S phase than in G1 in haploid cells (Heun 2001; Dion et al. 2012). Fourth, growth conditions, like the nature of the carbon sources are important to consider. Mobility is observed to be slower in galactose than glucose in some cases (Backlund et al. 2014). In addition to these factors, chromatin remodeling, ATP driven, complexes are involved in energy consuming motion. For instance, direct targeting of catalytically active Arp8, the ATPase subunit of the chromatin remodelling INO80 complex, drives enhanced mobility of undamaged chromatin, through a mechanism proposed to involve nucleosome eviction (Neumann et al. 2012). Surprisingly, in this study, only INO80 remodelling complex, and not other complexes such as SWI/SNF also able to remove nucleosomes, were shown to be able to drive chromatin motion, as visualized by lacO tracking of the fiber (Neumann et al. 2012). Of note, actin, a constitutive component of the INO80 complex is involved in chromatin motion as judged from the MSD analyses of different labeled genomic loci in the presence of latrunculin A, a drug that inhibits actin polymerization (Spichal et al. 2016). How cytoskeleton proteins, chromatin remodeling complexes or cell physiology regulate chromatin motion in these undamaged conditions remains to be fully understood.

### ***Polymer physics and chromatin motion***

Polymer theory is of great help to understand the mechanism underlying chromatin motion. Polymer chains can be defined by their contour length  $C$  in (nm) and their Kuhn Length  $L_k$ , which is twice the persistence length  $L_p$ .  $L_p$  defines the bending rigidity of the chain. By definition, the orientation between the segments linking two monomers becomes uncoupled if the distance between these

monomers is greater than  $L_p^{-1}$ . The Rouse model describes the dynamics of an ideal chain. This chain is composed of monomers connected by elastic segments subjected to springs oscillation. Each monomer, subjected to Brownian random motion, will define the conformational dynamics of the chain. This simple Rouse polymer dynamics predicts  $\alpha$  scaling of 0.5;  $MSD(t) \approx L_k(Dt)^{0.5}$ . This is the  $\alpha$  value found at several time scales for different chromosomal loci examined in yeast in a pioneering study (Hajjoul et al. 2013). Interestingly, chain properties like its compaction or its rigidity are expected to influence  $\alpha$ . The Rouse model predicts a reduction in chromatin mobility upon decondensation for times above the Rouse relaxation time  $\tau_R$  and an increased mobility for stiffer chromatin for times below the Rouse time (Steinhauser et al. 2009; Arbona et al. 2017; Herbert et al. 2017). Yet, in an exact Rouse model,  $\alpha$  can gradually increase from 0.5 to 1 for increased time intervals, corresponding to the transition between a global subdiffusive behavior of the chain to a normal diffusion. There are different extensions of this model, for instance when the concentration of polymer chains that can topologically restrict chain motion is taken into account (*i. e.* entanglement described in the reptation model proposed by P-G. de Gennes for long chains, in this case an  $\alpha = 0.75$  is predicted) or when solvent properties are considered (*i. e.* hydrodynamic interactions in the Zimm Model).

### **DNA Double strand breaks and chromatin motion**

Double strand breaks (DSBs) are a major threat for genomic integrity. Left unrepaired, cells will die, while repair between repeated sequences or mis-rejoining from different break sites could create chromosome aberrations with a potential risk for the cell. Studies in yeast have analyzed chromatin mobility upon DNA damage and found increased motion at the damaged locus and elsewhere in the potentially undamaged genome.

In these studies, DSBs were created either through the controlled induction of homing endonucleases HO or I-SceI (Miné-Hattab and Rothstein 2012; Dion et al. 2012; Miné-Hattab et al. 2017) or through the action of genotoxic chemotherapeutic agents, like antibiotics from the bleomycin glycopeptide family (including Zeocin or Phleomycin) or  $\gamma$ Rays. *In vitro*, bleomycin was found to create specific concerted DNA strand scission through oxygenation of the C-3'--C-4' bond of

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<sup>1</sup> Stiffening is used with apparently contradictory meanings in the literature. In the case of an ideal polymer, oscillating springs connects monomers. On one hand, stiffness is defined by the tension exerted by the entire polymer when pulling on its ends. In this case, spring constant is expressed as  $1/L_p$ . If  $L_p$  is high, the polymer will moderately resist to elongation and will behave as a soft polymer (Verdaasdonk et al. 2013). On the other hand, stiffening is also used to directly define the bending rigidity of the polymer. If  $L_p$  is high, the polymer will bend moderately and appear as stiff (Herbert et al. 2017). Therefore it is important to clearly address the meaning of stiffening when polymer properties are examined. In the present review we use the second definition.

deoxyribose. Enzymes create “clean” breaks with 3’OH and 5’Phosphate DSB ends directly accessible to repair enzymes, while  $\gamma$  irradiations can give rise to complex and heterogeneous breaks. Bleomycin creates 3’phosphoglycolate termini to be cleaned before repair. Once the DSB generated, at the G1/S transition, the cell cycle dependent checkpoint Cdc28 kinase controls a single stranded resection initiated at the DSB. Indeed, the MRX complex (Mre11, Rad50, Xrs2), in cooperation with Sae2, engages 5’ to 3’ resection and stimulates further resection by Exo1 and Sgs1-Dna2 (Figure 1). Single stranded DNA can extend up to several kb and is required for subsequent repair by homologous recombination. Single stranded DNA is bound by the Replication factor A complex RPA, Rad52 and bacterial recA orthologue, Rad51 (Tsukuda et al. 2005; Gibb et al. 2014) (Figure 1). When DSBs are generated either enzymatically, or by genotoxic means, changes in chromatin mobility are observed. Locally, chromatin next to an HO induced DSB cut shows a faster motion, after a transient mobility reduction (Saad et al. 2014). In diploid cells, the Rc of a locus close to an I-SceI generated DSB increases at longer time points (Miné-Hattab and Rothstein 2012). Furthermore, tracking at short time scales, at 10 ms, evidenced that damaged DNA was less mobile than undamaged DNA (Miné-Hattab et al. 2017). Interestingly this motion is Rad51 dependent, suggesting a role in the single-stranded bound chromatin structure in regulating DNA motion. Rad51 bound chromatin, proposed to act as a “needle to help search in the chromatin mesh”, deserves super resolution microscopy observations (Miné-Hattab et al. 2017). Surprisingly, not only the broken chromosome shows increased mobility: several labs found that other chromosomes move as well, both in haploid and diploid yeast cells. How mobility is regulated and what is the purpose of this mobility is not well understood. However recent work summarized below, has generated some information about the genetic control, and about the different apparent behavior of chromatin at different scales (Miné-Hattab and Rothstein 2012; Dion et al. 2012; Seeber et al. 2013; Strecker et al. 2016; Hauer et al. 2017; Lawrimore et al. 2017; Miné-Hattab et al. 2017; Herbert et al. 2017).

### ***Potential factors driving global chromatin movement upon DNA damage***

Given our knowledge on chromatin motion, factors that may be at play in the global genome mobility can be: i) modification of internal components of the chromatin or of chromatin remodeling activities that can change the intrinsic physical properties of the chromatin upon damage; ii) factors, viewed as external forces, that can free constrained chromatin motion after DSBs; iii) modification of the viscoelastic properties of the nucleoplasm, a possibility not yet explored and not discussed here (figure 2).

#### ***A. Intrinsic components of the chromatin fiber***

1. *Checkpoint proteins* One of the first steps in the DNA damage response (DDR) is the sensing of damaged DNA. Checkpoint kinase proteins and DSB repair proteins themselves are key for this DDR. As seen above, after a DSB, MRX complex can bind DSB ends. It then promotes the recruitment of kinase Tel1 (ATM in mammals) whose activation occurs prior to 5' to 3' DNA end resection. Single stranded DNA induces checkpoint signaling by Mec1 (ATR in mammals). Mec1 associates with Ddc2 (ATRIP in mammals), while the 9-1-1 complex (Ddc1 - Rad17- Mec3) clamp binds independently to single stranded RPA coated DNA (Finn et al. 2011). Direct Ddc1 and Ddc2 recruitment to chromatin was shown to promote checkpoint activation and local chromatin mobility, independently of a DSB (Bonilla et al. 2008; Seeber et al. 2013). Both Tel1 and Mec1 phosphorylate H2A on Ser129; local recruitment of Ddc1 and Ddc2 also induces H2A phosphorylation (Bonilla et al. 2008; Seeber et al. 2013) . Domains of H2A phosphorylation can extend for ~50kb around the DSB, up to a third of the length of a chromosome arm (Shroff et al. 2004; Lee et al. 2014). Rad9 (53BP1 in mammals) is recruited through binding to phosphorylated H2A and constitutive methylated H3K79 (Grenon et al. 2007; Hammet et al. 2007). Phosphorylated Rad9 induces checkpoint Rad53 and Chk1 activation, resulting in several responses, including cell cycle arrest (Weinert and Hartwell 1993).

Evidences for checkpoint protein involvement in chromatin motion come from studies where the effect of *mec1*, *rad9* and *rad53* deletions on chromatin mobility was studied<sup>2</sup>. Chromatin mobility was examined through measurement of  $R_c$  at long time points (5 minutes) by tracking a genomic locus or alternatively, a Rad52-GFP focus taken as a marker of genomic break (Dion et al. 2012; Seeber et al. 2013). In all mutants,  $R_c$  did not increase upon Zeocin treatment (Lisby et al. 2001; Dion et al. 2012; Seeber et al. 2013). Consequently, a role for Rad9 induced motion in homologous recombination was tested in G2/M arrested cells (arrest is used to avoid subsequent cell cycling due to Rad9 deletion), full repair by homologous recombination was found decreased in  $\Delta rad9$ , implicating Rad9 in long range homology search (Dion et al. 2012).

2. *Histone modifications*. It is not surprising that mutations of structural chromatin components can cause motion changes at the site of damage. Effects elsewhere in the genome are less intuitive. In our recent study, we showed that genomic damage by Zeocin resulted in increased intra-chromosomal distances concomitant to increased chromatin subdiffusion, an observation that polymer modeling could explain by chromatin stiffening (Herbert et al. 2017). This stiffening was further supported by super-resolution STORM/PALM microscopy observations (Herbert et al. 2017). One possible explanation for stiffening could be the negative charges due to H2A S129

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<sup>2</sup> Study of  $\Delta mec1$  or  $\Delta rad53$  is hampered by the fact that these genes are essential, unless *Sml1* is also deleted. The increased uptake of dNTPs in this mutated  $\Delta sm1$  background renders cells less sensitive to the lack of Mec1 or Rad53

phosphorylation, since the Zeocin dependent increase in intrachromosomal distances was reduced H2A S129A mutant (Herbert et al. 2017). Interestingly, a concomitant study analyzed chromatin behavior by multi-scale tracking after exposure to  $\gamma$ -rays, leading to  $\sim 4$  random DSBs across the genome, or after an I-SceI induced DSB (Miné-Hattab et al. 2017). MSD was reduced at small time scales but MSD increased at longer time scales (Miné-Hattab et al. 2017). These different patterns of chromatin mobility are consistent with a global increase in chromatin stiffening (Faller and Müller-Plathe 2001). Intriguingly, in the presence of Zeocin, an impressive, nhp6 dependent, degradation of histones H3 and H4 (up to 20-40%) is observed (Hauer et al. 2017). Whereas a different polymer modeling interprets chromatin structural changes as chromatin decompaction, rather than stiffening (Amitai et al. 2017), chromatin MSD is shown to similarly increase with an  $\alpha$  value rising from 0.5 to up to 0.7. Although the changes in the physical parameters of compaction and/or stiffening are not yet fully understood, these concomitant studies all point to the important global chromatin structural alterations challenged by DNA damages (Miné-Hattab et al. 2017; Hauer et al. 2017; Herbert et al. 2017).

2. *Repair proteins* Factors involved in the repair process itself (*sae2*, *rad54*, *rad52*) are shown to be involved in the pronounced changes of local motion of damaged chromatin, but no clear role has been established in global chromatin dynamics, with the exception of Rad51 (Miné-Hattab and Rothstein 2012; Miné-Hattab et al. 2017). Upon zeocin treatment or  $\gamma$  ray irradiations, global mobility is increased in a Rad51 dependent manner through a mechanism that remains to be elucidated (Seeber et al. 2013; Miné-Hattab et al. 2017) in agreement with observations made in *drosophila*
3. *Chromatin remodelers and cytoskeleton proteins.* It was shown that Arp8 is at least in part involved in enhanced mobility of damaged chromatin, since mobility of a genomic locus (2.8kb from I-SceI cutting site) is decreased at long time points in a  $\Delta arp8$  strain (Neumann et al. 2012). Arp8 and Arp5, both members of the INO80 complex are also involved in DSB induced global mobility (Seeber et al. 2013). Intriguingly, a role for nuclear microtubules in chromatin mobility upon I-SceI cleavage has been recently shown for an interstitial locus far from tethering structures (Lawrimore et al. 2017). It is not known how microtubules act on damaged chromatin. Perhaps nuclear dynein is important in this process, as suggested (Chung et al. 2015).

Altogether these studies show that global mobility, observed in *trans* upon DNA damage, is regulated by multiple factors whose interplay has to be deciphered. Understanding how trans-mobility is established remains an exciting and important challenge.

#### *B. Chromosome organization and tethering features of the nucleus.*



Combination of high-throughput imaging of genomic loci in individual cells, chromosome capture techniques and chromosome modeling based on polymer physics has established that yeast chromosomes follow a Rabl-like configuration (Jin et al. 2000; Schober et al. 2008; Therizols et al. 2010; Duan et al. 2010; Cournac et al. 2012; Wong et al. 2012; Arbona et al. 2017). In this configuration, anchoring to nuclear microtubules tethers centromeres close to the Spindle Pole Body — which in yeast is embedded in the nuclear envelope — and telomeres are found close to the nuclear envelope in a position dependent on the chromosome arm size. By searching for factors phosphorylated by Mec1 upon DNA damage, Strecker et al found Cep3, a kinetochore protein (Strecker et al. 2016). If *Cep3* is mutated for its phosphorylation site (*S175A*), increase of  $R_c$  generated by HO endonuclease is inhibited, for loci both in *cis* and *trans* of the break (Strecker et al. 2016). It is proposed that Cep3 phosphorylation by Mec1 mediates chromatin mobility through relaxation of the kinetochore-centromere link (Strecker et al. 2016). The nature of this relaxation remains to be further comprehended. Lawrimore et al., observed an expanded chromatin at centromeres and implicated microtubules in the enhanced movement of damaged chromatin (Lawrimore et al. 2017). Zeocin treatment also led to similar increase in distances between SPB and a centromere proximal loci is found in WT cells strains in WT cells and *cep3* mutated cells (Herbert et al. 2017). These observations are complicated by the concomitant G2/M checkpoint arrest. Of note, another checkpoint, the spindle assembly checkpoint (SAC), prevents aberrant chromosome segregation and aneuploidy. SAC controls chromosome separation through inactivation of the Securin/Pds1 (George and Walworth 2016). Pds1 is known to inhibit the separase /Esp1 itself involved in the cleavage of the cohesin that holds sister chromatids after replication. Cleavage by Esp1 is required for sister chromatid segregation (Uhlmann et al. 1999). It has been recently shown that SAC is active even in the absence of Mec1 and Tel1 (Palou et al. 2016). Analysis of chromatin mobility in this mutated context should certainly clarify how G2/M checkpoint controls chromosome mobility. Tethering of telomeres might also be modified upon DSB, since deletions of *SIR4*, or *KU70*, both proteins involved in telomere anchoring at the nuclear periphery, mirror the mobility increase observed upon HO cleavage (Strecker:2016jj; Lawrimore et al. 2017). One explanation for this observation could come from work showing that upon DSB, Ku70 relocates from telomeres to DSB ends (Martin et al. 1999; Walker et al. 2001), but mutants for which telomere tethering would not be modified upon DNA damage are not characterized to date.

### ***Functional roles for chromatin mobility?***

What could be the functional role for increased chromatin mobility? In yeast, several studies have shown that enhanced DNA mobility positively correlates with repair efficiency, as judged by transformation frequencies or molecular recombination events upon a DSB, as compared to the absence of DSB. For instance, targeting Arp8 or Ino80, increases the rate of homologous recombination. On the contrary, delayed motion due to latrunculin or mutation in the catalytic subunit of INO80 complex, decreases homologous recombination rates (Neumann et al. 2012; Spichal et al. 2016). Defect in mobility due to rad9 deletion also delays the appearance of recombination intermediates (Dion et al. 2012). A limitation in these studies remains in the difficulty to find mutants where mobility function is distinct from the repair function. In this regard, cep3 mutant, that limits chromatin motion and is not a repair protein *per se*, had no impact on recombination frequency (Strecker et al. 2016). The role of chromatin motion in repair by homologous recombination therefore remains an open question. It is however consistent to find a correlation between increased subdiffusion and increased repair efficiency, since it could ensure a greater probability of encounter between broken molecules. But because the temporal windows are difficult to reconcile, since mobility can be observed minutes after breakage, and repair events are followed days after it, it leaves open the possibility that increased mobility does not directly impact homology search and repair. Another possibility could be that an increased mobility helps moving the damaged chromatin towards nuclear compartments prone to repair (Therizols et al. 2006; Nagai et al. 2008). Conversely, it cannot be excluded that, as proposed for telomeres mobility in mammals, increased mobility could favour unwanted translocations, if not properly regulated (Cho et al. 2014; Lottersberger et al. 2015). Could it also be that increased motion is an inherent response of cells to DNA damage, with no obvious function in repair? Understanding the how and why of chromatin mobility upon double strand breaks thus still awaits fascinating studies.

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**Figure 1** Molecular scenario at the site of a DSB

Involvement of checkpoint, histone H2A phosphorylation, remodeling complexes, and repair proteins at the site of DNA damage are depicted.

**Figure 2:** Possible mechanisms for chromatin mobility upon DNA damage.

Four chromosomes in a Rabl configuration of a haploid yeast cell are shown (A). In undamaged conditions, chromosomes are tethered to the spindle pole body (SPB) by their centromere (CEN) via a nuclear microtubule (MT) and to the nuclear envelope (NEV) by their telomere (TEL). (B) Possible scenarios of enhanced chromatin dynamics following DNA damage at the site of DNA damage is shown. Proposed mechanism for global mobility include (C) stiffening (or alternatively, decondensation) of the chromatin fiber and (D) relaxation of the link between centromere and SPB through microtubule, loss of telomeres tethering and impaired function of nuclear actin.

**Table 1** Effects of mutants in *cis* (local) or *trans* mobility (global) are recapitulated. Increase in motion upon DNA damage is indicated as “inhibited”, when it is similar to the WT motion without DNA damage. Effects upon Zeocin treatment are also shown. Note that all these studies follow locus motion for  $\Delta t$  around 180 seconds after 3 hours of DNA damage induction. Studies in haploid and diploid cells are distinguished, since ploidy has an effect in chromatin mobility. Local mobility refers to the motion of a locus close to an enzymatic cutting site. *Trans* mobility can refer to the motion of a locus on a chromosome distinct from the chromosome carrying the enzymatic cutting site or to the motion due to a genotoxic random agent like Zeocin.

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