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DNA in motion during double-strand break repair

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DNA organization and dynamics profoundly affect many biological processes such as gene regulation and DNA repair. In this review, we present the latest studies on DNA mobility in the context of DNA damage. Recent studies demonstrate that DNA mobility is dramatically increased in the presence of double-strand breaks (DSBs) in the yeast *Saccharomyces cerevisiae*. As a consequence, chromosomes explore a larger nuclear volume, facilitating homologous pairing but also increasing the rate of ectopic recombination. Increased DNA dynamics is dependent on several homologous recombination (HR) proteins and we are just beginning to understand how chromosome dynamics is regulated after DNA damage.

Exploring DNA mobility in living cells

The genetic information contained in DNA molecules is packaged into chromatin and is highly condensed in the cell nucleus. In the early 1900s, the cell biologist Theodor Bovery proposed the idea that chromosomes are not randomly organized in the nucleus but occupy distinct areas named 'chromosome territories' [1]. The first experimental evidence for such an organization came in the 1980s when Cremer *et al.* observed that a laser beam directed on a particular area of mammalian nuclei always reaches the same chromosomes [2]. Over the past 30 years, the organization of chromosomes inside the nucleus has been extensively studied using model systems such as budding yeast [3–5], *Drosophila* [3,6], mice [7,8], and humans [9,10]. Those studies revealed that genome organization plays an important role in many biological processes such as gene regulation [11], genome integrity [10,12], and cell differentiation [13–15]. For example, there is a correlation between the distance two genes are apart from one another and their probability of translocation [16]. As a consequence, the spatial organization of genes influences which chromosomes recombine to form cancer-promoting translocations [17,18]. In addition, gene positions differ in tumor cells compared with cells from normal tissue, suggesting that the analysis of gene positioning may become a powerful tool for early-stage detection of cancers [19].

Advances in microscopy and image analysis have made it possible to visualize not only the static organization of chromosomes but also their mobility in real time [20]. Despite a higher-order spatial organization, chromosomes can be highly mobile under certain conditions and the movement of genomic loci is associated with a wide range of DNA processes, such as transcription [21], replication [22], and repair [23–25]. Recently, two studies revealed that the mobility of damaged loci is dramatically increased in the presence of DNA damage in budding yeast [24,25]. One consequence of increased chromosome mobility (ICM) after damage is to facilitate homology search during inter-homolog recombination [24]. Interestingly, undamaged loci are also more mobile, albeit at lower levels, indicating that there is a global increase in DNA mobility following DSB formation [24]. Increased DNA mobility must be controlled because it might promote unwanted translocations between chromosomes and endanger genome stability [26]. In mammalian cells, it is unclear whether chromosomes are more mobile following DSB induction (Table 1). Several studies report increased chromatin mobility after damage [6,8,23,27,28], whereas others do not [7,29,30]. Additionally, when mobility is observed along with local chromatin perturbations, chromatin is rapidly decondensed at the site of the DSB, whereas the DSB site remains relatively immobile over time [31,32].

In this review, we describe recent studies of chromosome mobility focusing on the context of DNA repair. First, we review results that describe the nature of chromosome motion and discuss its dependence on genome localization as well as effects of the cell cycle. Next, we present recent evidence that DNA damage dramatically modifies genome organization by causing transient ICM. Finally, we discuss the role that different genes play in DNA mobility after DNA damage.

Evidence for constrained diffusion of chromosomes

Chromosome movement can be followed inside the nucleus of living cells using genomic integration of *tetO* and *lacO* arrays at specific loci, which are bound by fluorescently tagged repressors [20,33]. From measurements of its position over time, the nuclear space explored by a locus can be calculated using mean-square displacement (MSD) analysis, a standard tool to analyze the motion of particles [34] (Box 1). When a single locus is tracked relative to a fixed point in the nucleus, it is referred to as MSD. When two

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Table 1. Different methods used to induce DSB *in vivo* and study DNA mobility

Type of DSB	Properties	Organism
Endonuclease-induced DSBs		
I-*Sce*I	The I-*Sce*I cut-site can be inserted anywhere into the genome and allows the induction of a single DSB in the genome. In budding yeast, the efficiency of the cutting varies between 10% and 75% depending on the locus where the cut-site is inserted.	Budding yeast [24,25]; NIH 3T3 cells and MEFs [7]
HO	Used to create a DSB at the endogenous HO cut-site on budding yeast chromosome III, or its recognition site can be inserted into the genome at any locus. The efficiency of cutting in budding yeast is 60–80%.	Budding yeast [24]
Chemically induced DSBs		
Zeocin	Creates random single-strand breaks and DSBs in DNA	Budding yeast [25]
Radiation-induced DSBs		
UV laser microirradiation	Cells need to be treated with 10 μM bromodeoxyuridine for 24 h before irradiation to sensitize them to DSB generation by UV-A laser (λ = 337 nm).	MEFs [31]
X-rays	Create localized DSBs.	*Drosophila* [6]
γ-rays	Create random single-strand breaks and DSBs. The density of lesions can be scaled according to the time of irradiation.	Budding yeast [24]; U2OS [28]; human MCF7 mammary carcinoma cells; human lung fibroblasts [32]; MEFs [31]
α-particles	Create localized DSBs. The density of DSBs can be scaled according to the energy and charge of the particle used.	HeLa cells, human primary fibroblasts and Chinese hamster ovary [23]; U2OS [30]

moving loci are tracked simultaneously, it is referred to as mean-square change in distance (MSCD) [3]. In the case of confined motion, the locus cannot escape a certain nuclear subvolume, implying that, for long time intervals, the MSD/MSCD covered by the locus is independent of the

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Box 1. Different methods to analyze chromosome motion *in vivo*

MSD: Chromosome mobility can be measured by calculating MSD = $<\Delta x^2(t)>$ from the position of a tagged locus over time.

In simple liquids, the diffusion of a particle is purely thermal and its MSD depends linearly on time, $<\Delta x^2(t)> \sim Dt$, where D is the diffusion coefficient of the particle (Brownian motion). In viscoelastic materials, such as polymers, the particle is moving in a more complex environment, which slows it down. In this case, its motion is subdiffusive (or constrained) and $<\Delta x^2(t)> \sim t^\alpha$ with $\alpha < 1$. It is thought that a chromosomal locus exploring the nucleus is confined because of DNA entanglement [70]. A highly confined locus moves in a low elastic environment with very entangled chromatin, whereas a less-confined locus moves in a more elastic chromatin environment. By contrast, a particle transported along a substrate shows directive motion and $<\Delta x^2(t)> \sim t^\alpha$ with $\alpha > 1$. In some cases, the motion of a particle can be a combination of directive motion and thermal diffusion; for example, when a particle moves freely before attaching to a substrate and sliding on it.

To compensate for possible drift during the acquisition of images, the position of a locus has to be corrected for each frame using the 3D position of a fixed point in the nucleus. Many studies use the center of the nucleus, measured by fitting the tagged nuclear membrane to a circle [20]. Other studies use the spindle pole body to serve as a marker of relative nuclear position [24]. Because the center of mass of individual dots can be determined with greater accuracy than the center of mass of an entire nucleus, the second method offers high spatial resolution while minimizing the effects of nuclear translation or rotation.

MSCD: Chromosome mobility can also be quantitated by measuring the distance between two moving loci as a function of time [3,24,38]. This method is named MSCD or distance MSD. An important assumption of this method is that the two moving spots have the same behavior, with similar confinement radii and diffusion coefficients. The confinement radius can be reconstructed from the MSCD plateau if the confining regions are sufficiently far from each other [20].

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elapsed time interval. Consequently, the plateau reached by the MSD/MSCD curve is proportional to the confinement volume explored by the locus. In addition, the initial slope of the MSD/MSCD curves is proportional to the diffusion coefficient.

Consistent with the higher-order structure of the genome, chromosomes undergo confined Brownian motion within a small nuclear subvolume and this confinement is evolutionarily conserved from budding yeast and *Drosophila* to mammalian cells [4,35–38]. However, the properties of chromatin mobility differ quantitatively between organisms. Human loci have smaller diffusion coefficients than budding yeast, indicating that they are more resistant to motion (Table 2) (4.8×10^{-5} to 1.8×10^{-4} μm^2/s for human cells [39–41], 5×10^{-4} to 10^{-3} μm^2/s for budding yeast [3,24,25]). Because human nuclei are around 80 times larger than yeast nuclei, the percentage of the nuclear volume that chromosomes can explore in human cells is much smaller than in yeast. As a consequence, DNA organization is more constrained in mammalian nuclei than in yeast.

Chromosome mobility is highly dependent on the locus observed and the stage of the cell cycle. In budding yeast, centromeres and telomeres explore 3–5% of the nuclear volume in the S and G1 phases of haploid or diploid cells [3,4]. Several lines of evidence show that telomeres are confined due to their tethering to the nuclear envelope [5,42]. For example, yeast telomeres interact with the nuclear envelope through the Ku heterodimer and chromosome mobility is greater in Ku-mutant strains [43]. Similarly, centromeres are highly confined, probably due to their tethering to the spindle pole body (SPB) through microtubules [5]. Unlike centromeres and telomeres, loci in the middle of a chromosome arm explore 4% of the nuclear volume in S-phase cells but up to 43% in G1 haploid cells [4] (Table 1). It is hypothesized that middle-arm regions are more confined during replication due to the presence of multiple replication forks [4,42].