The Homologous Recombination Machinery Regulates Increased Chromosomal Mobility

After DNA Damage in *Saccharomyces cerevisiae*

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# ABSTRACT

## The Homologous Recombination Machinery Regulates Increased Chromosomal Mobility After DNA Damage in *Saccharomyces cerevisiae*

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It is incumbent upon cellular life to ensure the faithful transmission of genetic material from mother cell to daughter cell and from parent to progeny. However, cells are under constant threat of DNA damage from sources both endogenous and exogenous, such as the products of metabolism and genotoxic chemicals. Thus, cells have evolved multiple systems of repair to ensure genome integrity. The DNA double-strand break (DSB) is among the most lethal forms of DNA damage, and a critical pathway to resolve these lesions is homologous recombination (HR). During HR, information lost at the cut site of one locus is repaired when the damaged site locates a homologous sequence in the nucleus to use as template for repair. The process by which a cut chromosome finds its homolog is known as homology search, and, while the enzymatic steps of HR have been well studied in recent years, the coordination of cell biological events like HS in the context of the crowded nucleus has remained poorly understood. Recently, our laboratory and others have studied a phenomenon known as DNA damage-induced increased chromosomal mobility, in which chromosomal loci, both damaged and undamaged, explore larger areas of the nucleus after the formation of DSBs. The increase in the mobility of cut loci is known as local mobility, and the increase in mobility of undamaged loci in response to a break elsewhere in the

nucleus is known as global mobility. Here, I report that the recombination machinery and the DNA damage checkpoint cooperate in order to regulate global mobility of chromosomes following DSB formation. The RecA-like recombinase Rad51 is required for global mobility, and exerts its effect at single-stranded DNA (ssDNA), but its canonical homology search and strand exchange functions are not required. I find that Rad51 is ultimately required to displace Rad52, which is revealed to be an inhibitor of mobility when bound to ssDNA in the absence of Rad51. Thus, recombination factors can serve as DNA damage sensors, and relay information to the checkpoint apparatus in order to govern the initiation of increased mobility after DSB formation. I have also studied how the baseline confinement of loci is established, and assessed the contributions of several genes involved in repair to increased mobility. These observations offer novel insight into previously unappreciated regulatory functions performed by the recombination machinery, and demonstrate how the progression of DNA repair pathways influences nuclear organization.

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

# LIST OF ABBREVIATIONS

HR, homologous recombination
DSB, double-strand break
HS, homology search
MMS, methylmethane sulfonate
NHEJ, non-homologous end joining
SCE, sister chromatid exchange
PCNA, proliferating cell nuclear antigen
SDSA, synthesis dependent strand annealing
SPB, spindle pole body
MSCD, mean square change in distance
MSD, mean square displacement
ALT, alternative lengthening of telomeres
LINC complex, linker of nucleoskeleton and cytoskeleton
ATP, adenosine triphosphate
YFP, yellow fluorescent protein
RFP, red fluorescent protein
CFP, cyan fluorescent protein
VPA, valproic acid
LatA, latrunculin A
B/M Ratio, bud to mother ratio
RPA, replication protein A

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"The fact that the scientific investigator works 50 percent of his time by nonrational means is, it seems, quite insufficiently recognized."

-Neal Stephenson

*Cryptonomicon*

**CHAPTER 1:**

**INTRODUCTION**

Adapted from *Poetry in motion: Increased chromosomal mobility after DNA damage*

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**OVERVIEW**

Homologous recombination (HR) requires a multitude of carefully orchestrated steps to accomplish the repair of DNA double-strand breaks (DSBs) (Figure 1-1). Besides a complex cascade of signaling molecules, chromatin remodelers and strand exchange factors, the cell must coordinate the contact of damaged sequences with template, a process known as homology search (HS). While the biochemical steps of recombination have been well studied in recent years, the connections between these reactions and cell biological events like HS are just now beginning to be uncovered. Recent studies in yeast, mammalian cells and other model systems, have revealed that chromosomal loci undergo dramatic changes in mobility in response to DSB formation in mitotic cells.

Previous studies of this phenomenon have revealed the importance of two major pathways: the DNA damage checkpoint and the recombination machinery. The DNA damage checkpoint is engaged following the formation of a DSB in order to pause the cell cycle and mobilize the cellular response to the break. The activation of this pathway is synchronized with the processing of the break, which eventually yields resected ssDNA substrates that are bound by both the ATR/Mec1 checkpoint pathway and homologous recombination machinery such as Rad51 and Rad52. Crosstalk between these elements appears critical for the regulation of the mobility of loci, both damaged and undamaged, during HR.

In this manner, the induction of increased mobility is tied to the progression of HR, thus enabling a stringency mechanism to avoid inappropriate increases in the mobility of loci. Cells may tightly regulate mobility to avoid spurious or deleterious contacts between DNA sequences during replication and repair, and in yeast, this regulation is seemingly tied

2

to ploidy status. Several models have been developed to explain the mechanisms of locus confinement and increased mobility, including local changes to the qualities of the DNA polymer at break sites, chromatin remodeling, and modulation of centromeric and telomeric attachments of chromosomes. While the relative contributions of these various models have not yet been determined, the basic phenomenology of mobility and its regulation has been observed in diverse organisms, from fission yeast to human cells, suggesting that these pathways are highly conserved throughout evolution. Therefore, understanding the precise mechanisms that govern mobility, how it pertains to homology search, repair, and nuclear organization, may offer critical insights into disease states such as cancer.

To this end, I have studied the role of the recombination machinery in regulating the mobility of chromosomes following DNA damage, particularly the mobility that occurs at undamaged loci in the context of damage elsewhere in the nucleus (global mobility). I have found that the RecA recombinase Rad51 is essential for this process throughout the cell cycle, and exerts its regulatory role at resected ssDNA. Moreover, the recombination mediator, Rad52, also impinges on this process, and, inhibits mobility in the absence of recombinase recruitment. Thus, the role of Rad51 at ssDNA includes the displacement of Rad52. I propose that this regulatory circuit limits increased mobility to contexts of checkpoint activation in the presence of a fully assembled recombination apparatus. These findings illustrate that the machinery of recombination also participates in the signaling of damage, linking the large-scale reorganization of chromosomal loci during the damage response with the factors directly responsible for the enzymatic steps of repair.

## 1. DSBs: their repair and resolution

In yeast, DSBs can be generated by a variety of endogenous and exogenous sources, including genotoxic agents like ionizing radiation, as well as products of metabolism such as reactive oxygen species and as a consequence replication stress.[1,2] Depending on the context in which DSBs form, different repair mechanisms are mobilized to resolve the lesion. The two main repair mechanisms are HR and non-homologous end joining (NHEJ). In NHEJ, the ends of the DSB are ligated together, which can cause deletions or additions to the DNA sequence at the newly formed junction. HR, however, uses a homologous template elsewhere in the genome to restore the information lost at the break site. The template can be either a replicated sister chromatid or the homologous chromosome in diploid cells (interhomolog repair).

Figure 1-1 outlines many of the steps in HR. Binding of the MRX complex (Mre11, Rad50, Xrs2) to ends, along with the interaction of Mre11 and Sae2, catalyzes initial 5' end resection and commits the cell to HR as opposed to NHEJ[3]. Next, Exo1, Dna2 and the helicase Sgs1 cooperate to promote more extensive resection. Once ssDNA is generated, it is bound by the heterotrimeric single-strand binding complex, RPA (Rfa1, Rfa2, Rfa3)[4]. In addition to these initial DNA processing events, MRX binding and resection mediate DNA damage checkpoint response signaling through checkpoint kinases Tel1 and Mec1, homologs of mammalian ATM and ATR PI3K-like kinases[5]. The activation of Tel1 is promoted by an interaction with Xrs2[6], while the generation of RPA-coated ssDNA by the resection machinery leads to the recruitment of Ddc2-Mec1[7,8] and subsequent phosphorylation of Rad9[9]. Rad9 promotes the activation of Rad53, a major checkpoint

effector kinase, which then phosphorylates many downstream targets[9]. The PCNA-like

sliding clamp complex 9-1-1 (Ddc1, Mec3, Rad17) also binds to newly generated ssDNA

junctions and assists in checkpoint activation[10].

The strand exchange reactions of HR are mediated by recombinase enzymes,

frequently homologs of bacterial RecA[11]. Yeast have two such enzymes—Rad51 and

Dmc1[12]. While Dmc1 is solely required for meiosis, Rad51 is required for both mitotic and

meiotic HR. Rad51 exerts its role in DSB repair through the formation of structures on

resected ssDNA known as presynaptic filaments. These structures require the assembly of

oligomeric complexes of Rad51, coordinated by the ATP binding domains such as Walker

A[13], and, once formed, bind dsDNA complexes, assess homology and then promote strand

invasion and repair. Rad51 interacts with resected ssDNA through one of its two DNA

binding sites, Site I, and associates with donor dsDNAs through Site II[14]. Additionally, two

loop elements, L1 and L2, also influence DNA binding, with L2 being implicated in dsDNA

association[15]. While binding of RPA to ssDNA protects it from additional lesions, RPA is

inhibitory to nucleation of Rad51 filaments[3]. Therefore, mediators, such as RecFOR[16] in

bacteria and Rad52[17] in yeast are needed to help overcome the inhibition of RPA and

promote the binding and extension of recombinase filaments[17]. Other proteins involved in

filament formation and stabilization in yeast include Rad51 paralogs such as Rad55, Rad57,

and the Shu complex[18]. In mammalian cells, BRCA2 is the dominant mediator, but RAD52

and RAD51 paralogs also play a role in filament formation and stability[19]. Once formed,

presynaptic filaments seek out homology and perform strand exchange reactions. Proteins

such as Rad54 interact with Rad51 and facilitates displacement of strands within the target

molecule, forming D-loops[20,21]. D-loop structures can be dissolved after limited polymerase

extension of the invading strand, generating non-crossover products (SDSA)[1]. Alternatively, the second end of the break may be captured and processed into double Holliday junctions, which can later be resolved by the Sgs1-Top3-Rmi1 complex[22], or by structure selective nucleases such as Mms4-Mus81[23] and Yen1[24]. The final resolution of these structures and the mismatch repair reactions that follow determine whether or not recombination events yield crossover or non-crossover products and whether gene conversion occurs.

## 2. Chromosomes move dynamically within defined territories

Over the past 20 years, cell biological tools have been developed to explore various aspects of chromosome biology and HR. The use of fluorescent protein tags has enabled cell biological approaches to be used to investigate the timing of protein loading to specific chromosomal sites during repair. In the case of HR, binding of repair proteins to the sites of DSBs often forms bright foci that can be easily distinguished by microscopy[25,26]. In addition to tags on individual proteins, systems have been developed to permit the visualization of entire chromosomal regions[27]. Operator sequences from bacteria can be concatenated into long multiple tandem arrays and inserted into sites of interest within the yeast genome. Their cognate repressor proteins (TetR for TetO arrays, LacI for LacO arrays) can then be fluorescently tagged. When these repressors bind to operator sequences, the entire array becomes visible. These methods, along with others, have led to the realization that the positions of yeast chromosomes within the nucleus are ordered, with centromeres clustered around the spindle pole body (SPB) and the various chromosome arms radiating outwards, confirming the early studies of Rabl[28]. Telomeric sequences form clusters at the

nuclear periphery, with Yku70, Sir4 and nuclear pore components such as Mps3 participate in this tethering[29]. The organization of specific loci within the nucleus is well-studied, revealing that chromosomes tend to occupy distinct territories in mammalian as well as yeast nuclei[30]. This static conception of loci was at odds with the dynamic behaviors known to occur within the nucleus, as many processes, such as HR, are dependent upon distant sequences coming into contact with one another.

Pioneering studies of the *LEU2* locus in yeast using chromosome tagging technologies and 4D imaging provided the resolution for this apparent contradiction[31]. Marshall and colleagues studied the interphase movements of two homologous loci within diploid cells and modeled the relationship between the change in distance between the loci and the time interval over which they were observed. This mean square change in distance analysis (MSCD) revealed that while chromosomal loci are indeed confined to territories, collisions with charged solvent particles drive dynamic Brownian diffusion within these territories. Interestingly, the *LEU2* loci become less confined after cells are treated with the microtubule depolymerizing agent nocodazole. These results raise the possibility that active cellular processes regulate the nuclear space that chromosomes can explore. Later studies in haploid yeast further characterized the behavior of chromosomal loci throughout the cell cycle. Heun and colleagues[32] found that chromosomes become more confined during S phase, demonstrating that the radius of confinement of a locus varies with the cell cycle.

## 3. Chromosomal mobility: linking nuclear organization and repair

*3.1 Increased mobility of chromosomal loci in response to damage*

Over the last several years, chromosome mobility after DNA damage has been examined in a variety of systems. While under some circumstances mobility was not observed[33-36], many studies have shown that nuclear loci are capable of large-scale reorganization following DSB formation[37-42]. A 2003 study by Lisby and colleagues showed that, although multiple breaks in a cell could be generated by γ-irradiation, these breaks do not generate a commensurate number of Rad52 foci[37]. Instead, multiple breaks coalesce to just a few repair centers in the cell. In 2004, Aten and colleagues[38] found that γ-H2AX labeled DSBs in mammalian cells form clusters after alpha particle irradiation. Other studies showed that chromosome breaks, under some circumstances, can translocate to the nuclear periphery and recruit telomere addition machinery[39]. Furthermore, Torres-Rosell and colleagues also reported that breaks that occur in the nucleolus migrate outside of this specific nuclear subdomain for repair[40]. Later, similar observations were made in *Drosophila* heterochromatic regions (see section 4)[41]. In mammalian cells, the Rad9 homolog 53BP1 has been implicated in the regulation of increased mobility of uncapped telomere ends, structures analogous to DSBs[42]. Thus, damaged DNA appears highly dynamic and undergoes programmed movements throughout the nucleus during repair.

In 2012, two studies, one performed in haploid cells[43], and one in diploids[44], explored the behavior of single loci in various damage contexts.  Both studies made use of

mean square displacement (MSD) analysis of bacterial operon arrays to reveal that I-*Sce*I cut loci increase in mobility by expanding their radius of confinement. Regions close to a cut locus undergo a large expansion in mobility, referred to as *local* mobility, to allow the damaged locus to explore more than 40% of the nuclear volume (Figure 1-2). Dion and colleagues, working in haploids, observed that this increase occurred in various genomic locations, and was specific to the formation of a DSB. Engineered collapsed replication forks did not induce this response. In the diploids, Miné-Hattab and Rothstein found that not only did damaged loci increase in radius of confinement, but that undamaged loci also increased in mobility after the formation of a DSB elsewhere in the nucleus, referred to as *global* mobility. This global effect leads to a modest (~20%) increase in explored volume and was observed in three different situations: on the intact homolog of a cut chromosome, on chromosome V after HO cutting the *MAT* locus on chromosome III and on undamaged loci in cells randomly damaged with ionizing radiation. Miné-Hattab and Rothstein also reported that cut chromosomes pair with their homolog after DSB formation. This paired configuration later dissolves, and the timing of dissolution correlates with the disappearance of the repair focus and production of the repaired product. These observations suggest that increased mobility following DSB induction facilitates HS.

*3.2 Mobility after DNA damage is genetically controlled*

The studies in haploid and diploid yeast just described (section 3.1) implicated a number of genes in the regulation of increased mobility after DNA damage. Both studies found that the recombinase Rad51 is required for mobility. Dion and colleagues also

reported that the strand exchange factor Rad54 as well as the DNA damage checkpoint components Mec1 and Rad9 are required for increased mobility[43]. The deletion of Rad53 also depresses mobility following break induction. Miné-Hattab and Rothstein reported that the deletion of Sae2, a protein involved in initial end resection of DSBs, delays the initiation of mobility[44]. Interestingly, a later study in haploids reported that cut loci display transient confinement during the very early steps of resection at the *MAT* locus[45]. These results suggest that the essential events that initiate mobility are related to the formation of ssDNA at the site of a break. This signaling platform attracts both Mec1-directed checkpoint components as well as recombination machinery[1], two pathways that coordinate the activation of mobility (Figure 1-3). Thus, the DNA damage checkpoint and the HR machinery coordinate a dynamic change in chromosome mobility and alter the territories that loci can explore. This increase in explored territory makes the random contact between the broken chromosome and a homologous sequence far more likely, and may promote timely repair after DNA damage.

While local mobility was observed similarly in haploids and diploids, global mobility was not initially observed in haploids[43]. However a subsequent study[46] reported that global mobility could be induced in haploid cells following treatment with the radiomimetic drug zeocin, or by direct activation of the checkpoint through forced colocalization of the DNA damage sensors Ddc1 and Ddc2[47]. In contrast to the large amount of zeocin required to induce global mobility in haploids, global mobility in diploids occurs even after the formation of a few DSBs[44]. The increase in global mobility observed in haploids requires the actin-related proteins Arp5 and Arp8, members of the INO80 chromatin-remodeling complex, which may affect chromosome mobility via histone eviction[48] (depicted in Figure

1-3). Intriguingly, the requirement for Rad51 in mobility is bypassed in haploids after zeocin treatment[46]. Chromatin remodeling following checkpoint activation is a persuasive mechanism for the control of global mobility[49], but results from haploid and diploid cells suggest that the recombination machinery must also have some influence on this process, either at the level of checkpoint control or on chromatin remodelers themselves.

The differences between haploid and diploid cells offer insight into the genetic regulation of increased mobility in budding yeast. Ploidy affects DNA damage survival[50,51] and the activity of many individual repair proteins, including Rad51[13], Rad55/57[52,53] and Rdh54[54]. In addition, mating-type heterozygosity leads to the production of the **a**1-α2 transcriptional repressor complex, which strongly suppresses NHEJ in diploids[55]. These differences might reflect the repair strategies used in haploid versus diploids. In diploids, a homologous template for repair is always available, even in interphase, while, in contrast, haploid DSBs are frequently "dead-end" events unless a sister chromatid or an ectopic donor sequence is utilized. Global mobility may therefore be primarily required to promote interhomolog repair in diploids, and is thus downregulated in haploids. Furthermore, as DSBs in haploids are irreparable without the use of NHEJ or an ectopic donor, local mobility in these cells may be required to move broken ends towards domains of the nucleus where their potential harm can be mitigated. For example, the nuclear periphery has been proposed to serve as a site for the repair of persistent DNA ends and/or *de novo* telomere repeat addition[39,56]. Increased mobility in haploids may also reflect a process that prevents inappropriate contacts between DNA sequences by "shaking" them apart, as has been proposed in mammalian cells (see section 4)[57]. Understanding these important differences will be a key area of future research.

*3.3 Centromeric tethering and chromosome confinement*

Although many details of the phenomenology of mobility have been reported, the nature of confinement has remained somewhat elusive. One explanation for confinement is the tethering of chromosome arms to nuclear sub-domains, such as the spindle pole body and the nuclear envelope[29]. In 2013, the Bloom group investigated the mobility of various loci along chromosome arms to evaluate what role centromeric tethering plays in mobility[58]. They found that sites close to the centromere are the most tightly tethered and are confined to the smallest volumes. By releasing centromeric tethering through transcription, this confinement could be relaxed. In addition, both the Bloom and the Gasser groups found that cohesin loading on DNA also contributes to confinement[58,59]. Studies by the Fabre group also raised the possibility that both nuclear and cytosolic actin contribute to chromosome mobility[60]. Thus, the changes in confinement observed during recombination may be related to tightening and relaxation of centromeric tethering and/or other modifications of the cytoskeleton.

Another recent study in haploids by the Durocher group reports that untethering of chromosomes from centromeric as well as telomeric attachments reduces confinement[61]. Taking into account the known role of the checkpoint in promoting mobility, the authors then analyzed proteins at the kinetochore known to undergo changes in phosphorylation state following checkpoint activation. One such protein, Cep3, is phosphorylated at S575 and phosphorylation at that site is required for altered mobility following DNA damage. The authors hypothesize that this modification promotes transient uncoupling of the

kinetochore and centromere after DNA damage to promote increased local and global

mobility, which they argue are both the result of altered tethering (Figure 1-3).


*3.4 Mobility and recombination outcomes*


Interestingly, *cep3-S575A* cells, which cannot undergo Cep3 phosphorylation at

S575, do not show defects in ectopic recombination frequencies despite their defect in

mobility, thus raising the question of whether or not mobility is directly required for repair.

Since *cep3-S575A* mutant strains also have an increase in chromosome loss, the authors

argue alternatively that mobility may function to preserve chromosome transmission

fidelity. Again, these experiments are performed in haploid cells where the formation of

DSBs may lead to mobility in order to engage salvage repair pathways (see section 3.2), and

may not reflect the same mobility observed in diploid cells where interhomolog repair is

available. Moreover, this model does not totally account for the influence of the

recombination machinery[44], or chromatin remodelers[46]. Lee and colleagues[62] also raised

the question of whether mobility is directly related to recombination. They report that the

likelihood of repair between two ectopic sites in haploids is not sensitive to the deletion of

either of two genes involved in mobility, *SAE2* and *HTZ1*, but is dependent upon the contact

frequency of the two loci as determined by their relative position in the nucleus. Thus, both

the Durocher and Haber groups argue that increased mobility may not drive homology

search.

The requirement for the recombination machinery during increased mobility in

diploid as well as haploid cells, however, strongly argues that mobility is consequential for

recombination outcomes. In addition, the recombination outcomes measured in the Durocher and Haber reports are determined by plate assays performed days after the mobility studies, which are performed in the seconds and minutes following DSB formation. It is difficult to definitively know whether or not an increase in mobility ever occurred. The *SAE2* deletion analyzed in the Haber study is a case in point, as that particular mutation, likely due to its delay of DNA end resection, delays increased mobility[44]. If DSBs are induced longer in this background, increased mobility is observed. Furthermore, it is possible, that mobility is primarily necessary to promote interhomolog recombination in diploids. Thus, ectopic recombination events studied in haploids may not require increased mobility, as they are likely driven by random, proximity-based contacts in cells suffering irreparable breaks. It will be interesting to examine the contributions of *CEP3* and chromosome contact frequency in interhomolog repair in diploid or pseudodiploid cells.

## 4. Mobility is an evolutionarily conserved process

Increased chromosome mobility after DNA damage is not confined to budding yeast. Studies in fission yeast and metazoans have revealed that mobility is a conserved pathway regulated by functionally similar genes. In flies, breaks that form in heterochromatic nuclear domains migrate out of that compartment in order to undergo repair[41]. Interestingly, this migration requires ATR checkpoint activation. Like in yeast, this compartment restriction is enforced by Smc5/6 complexes[40]. In fission yeast, persistent DSBs migrate to the nuclear periphery and become associated with microtubule linked

KASH domain proteins[63]. Cells may direct these breaks to the periphery in order to provide access to alternative HDR templates.

In mammalian cells, the Aten group has observed that loci explore larger regions of the nucleus after ionizing radiation exposure[64]. This increase is blocked upon treatment with drugs that inhibit various chromatin modifications. Mobility has also been well studied in telomeric sequences where either damage has been induced or protective protein complexes have been removed. The de Lange lab, working with cells in which telomeres have been uncapped by the conditional deletion of components of the shelterin complex, has shown that mobility at these sites is dependent on the Rad9 homolog, 53BP1[42,57]. Interestingly, they report that modulation of microtubule attachments through drugs such as nocodazole strongly inhibits increased mobility, an inverse finding to what has been observed in budding yeast[31]. However, like fission yeast[63], mammalian microtubules in the cytoplasm link to the nucleoplasm through the LINC complex, and deletions of SUN1 and SUN2, members of the LINC complex, suppress increased mobility at unsheltered telomeres. These results suggest that forces are transduced from cytoplasmic microtubules to chromatin after DNA damage to promote increased mobility. As end-to-end fusions of uncapped telomeres are also suppressed in these mutants, the de Lange group argues that mobility functions to promote NHEJ. While it is possible that the effect these genes have on chromosome fusions and on mobility are separable, this model is intriguing given the frequency of NHEJ events in higher eukaryotes. The Greenberg group, studying the alternative lengthening of telomeres, or ALT, a recombination-mediated method of telomere extension, have recently reported that damaged telomeric sites undergo directional movement prior to association with other damaged sites[65]. These

movements are under the control of Rad51, as well as two genes normally only active in the meiotic developmental program, Hop2 and Mnd1. Remarkably, these experiments were able to capture the critical moment of association between damaged sites.

## 5. Conclusion and future directions

The management of chromosome positions throughout the life of the cell through alterations in territory, confinement and mobility are central to many cellular processes. Recent work has elucidated the complex network of factors that manage these states, especially during the repair of DNA damage. The conservation of mobility among many organisms suggests that managing the positions of chromosomal loci after DNA damage is important for DNA repair. While extensive progress has been made in understanding chromosome confinement and DNA damage induced chromosomal mobility, many important questions remain. How do the checkpoint and the recombination machinery cooperate to control mobility? What types of DNA lesions promote increased mobility? At present, in yeast, it appears that only resected DSBs promote mobility[43,44,46,61], however, work in mammalian cells suggests that mobility may also be required for NHEJ[57]. In addition, the behavior of collapsed forks or single-ended breaks formed during replication have not been well studied. During the budding yeast meiotic mid-prophase, telomere led, dynamic movements of chromosomes have been observed and are proposed to be required as a stringency mechanism during pairing[66]. Similarly, in *S. pombe* meiosis, the telomeres of chromosomes reorganize, and the entire nucleus moves dramatically to promote homologous pairing[67]. It will also be interesting to determine whether the phenomena

studied during mitotic repair have parallels in meiosis, and assess the importance of the alternative RecA homolog Dmc1 in their regulation, especially since Rad51 has been shown to serve as a cofactor for Dmc1 activity in meiosis[14]. Lastly, the mechanics of increased mobility are still fairly cryptic. For both local and global mobility, the precise contributions of the recombination machinery, chromatin remodeling, checkpoint activation and centromeric tethering need to be determined. How do repair proteins contribute to damage signaling? Does the Rad51 filament encourage changes in exploration through signaling (like LexA and RecA in bacteria[68]), changes to the physical characteristics of the DNA end or mechanically via the formation of RecA-like bundles as seen in bacteria[69]? What are the nature of the chromatin changes that promote mobility? By answering these critical questions, the field will get closer to the fundamental physical and cellular events that occur during DSB repair.

The work described in this thesis explores how the mobility of loci is defined, and how connections between the checkpoint signalling apparatus and the recombination machinery regulate global mobility following DSB induction. In Chapter 2, a regulatory circuit between the checkpoint, Rad51, and Rad52 that governs global mobility is described. I find that the recruitment of Rad51 to ssDNA is necessary for mobility, as retained Rad52 in the absence of presynaptic filament loading blocks increased mobility after damage and checkpoint activation. In Chapter 3, the mechanisms of chromosomal confinement are investigated, and the contributions of additional genes important for DNA repair to increased mobility are assessed. Confinement is most affected at the URA3 locus by mechanisms that influence chromatin state. In addition, Rad54 and Htz1 influence global and local mobility, and Rad51 is important for the colocalization of repair foci after

induction of breaks. This work uncovers a critical link between the mechanical factors that repair DNA and the systems that govern recognition and signalling of the damaged state. The results detailed in this thesis add materially to the understanding of how the mobility behavior of chromosomal loci is defined and how it is altered globally during HR.
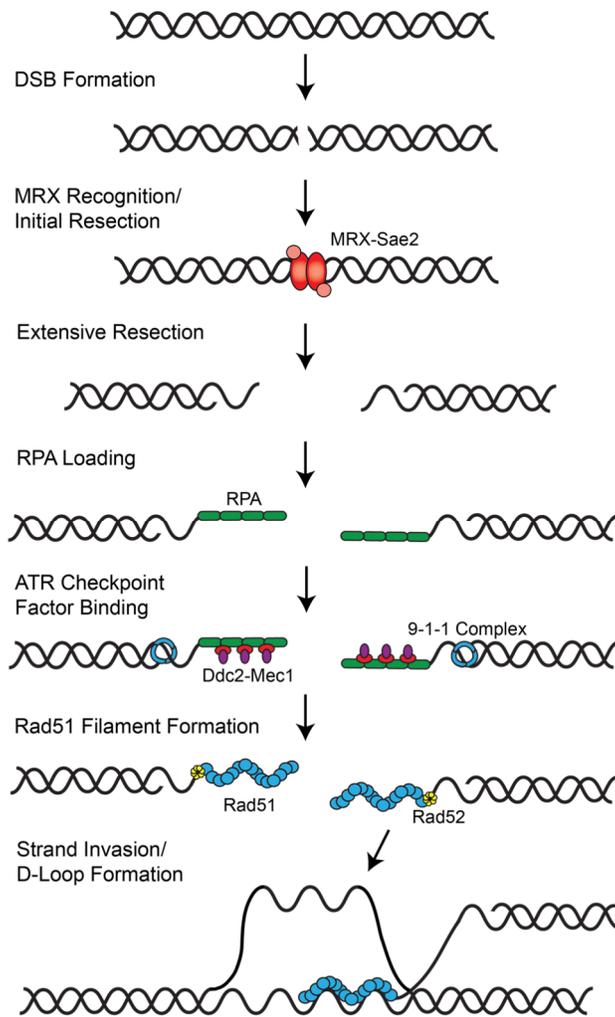
**Figure 1-1**

Major steps in the HR pathway in budding yeast. After DSB formation, the break is recognized by the MRX (Mre11, Rad50, Xrs2) complex along with the cofactor Sae2 to initiate initial 5' resection. Then, Sgs1, Dna2 and Exo1 (not shown) cooperate to catalyze more extensive resection, revealing 3' ssDNA tracts, which are bound by the single-strand binding protein RPA. Proteins involved in checkpoint signaling bind to RPA, such as the 9-1-1 complex and the ATR homolog Mec1 and its DNA binding partner Ddc2. Rad52, a recombination mediator, catalyzes the exchange of RPA for the recombinase Rad51, which forms filaments on ssDNA. These filaments invade dsDNA sequences to find homology, and form a D-Loop structure. Later, the second end can be captured to form double Holliday junctions, which can be resolved following repair to yield both crossover and non-crossover products.
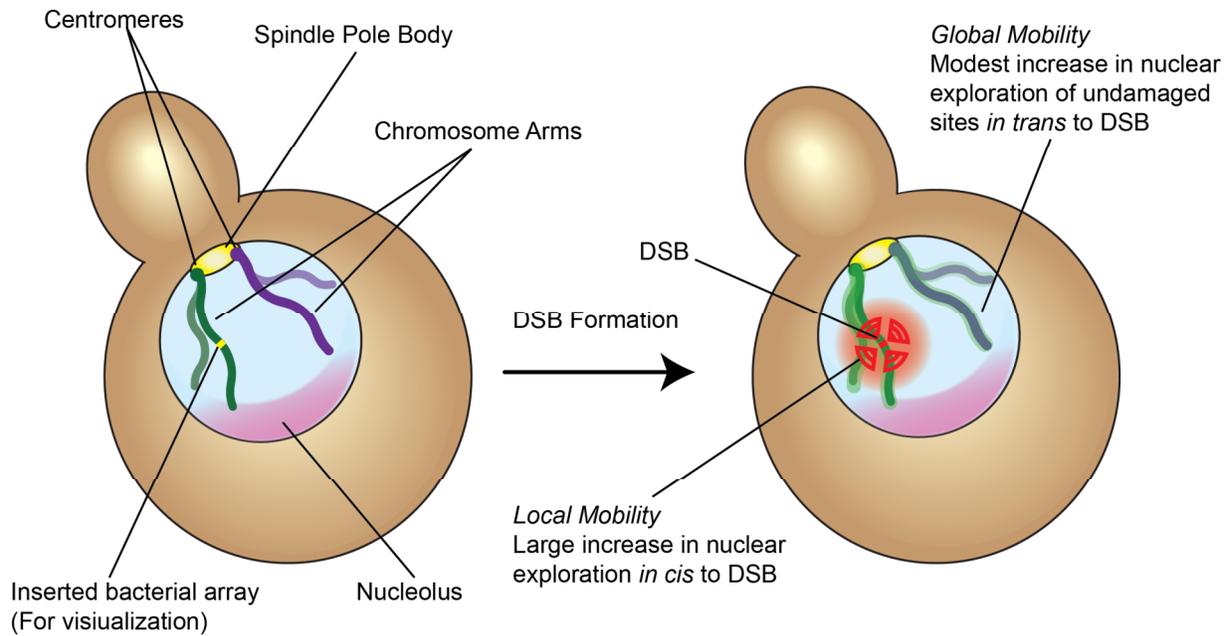
**Figure 1-2**
Schematic of mobility processes as observed in budding yeast. After DSB formation, both damaged and undamaged loci undergo an increase in mobility. Loci proximal to the site of the cut (red square) undergo the largest increase during *local mobility* (depicted as concentric red arcs), allowing a site previously confined to ~4% of the nucleus in undamaged cells to explore a tenfold increased volume. Undamaged loci undergo *global mobility* (depicted as a light green outline), and ultimately explore a volume of the nucleus around fivefold larger than in undamaged cells.

Centromere Detachment

P Cep3

Mec1
Rad53
Dun1

Chromatin Remodeling

Mec1
Rad53

Ino80
Complex

ATR Checkpoint Factors
(Ddc2-Mec1, 9-1-1)

HR Machinery
(Rad51/52/54)

Mobility

HR Machinery/Checkpoint Cooperation
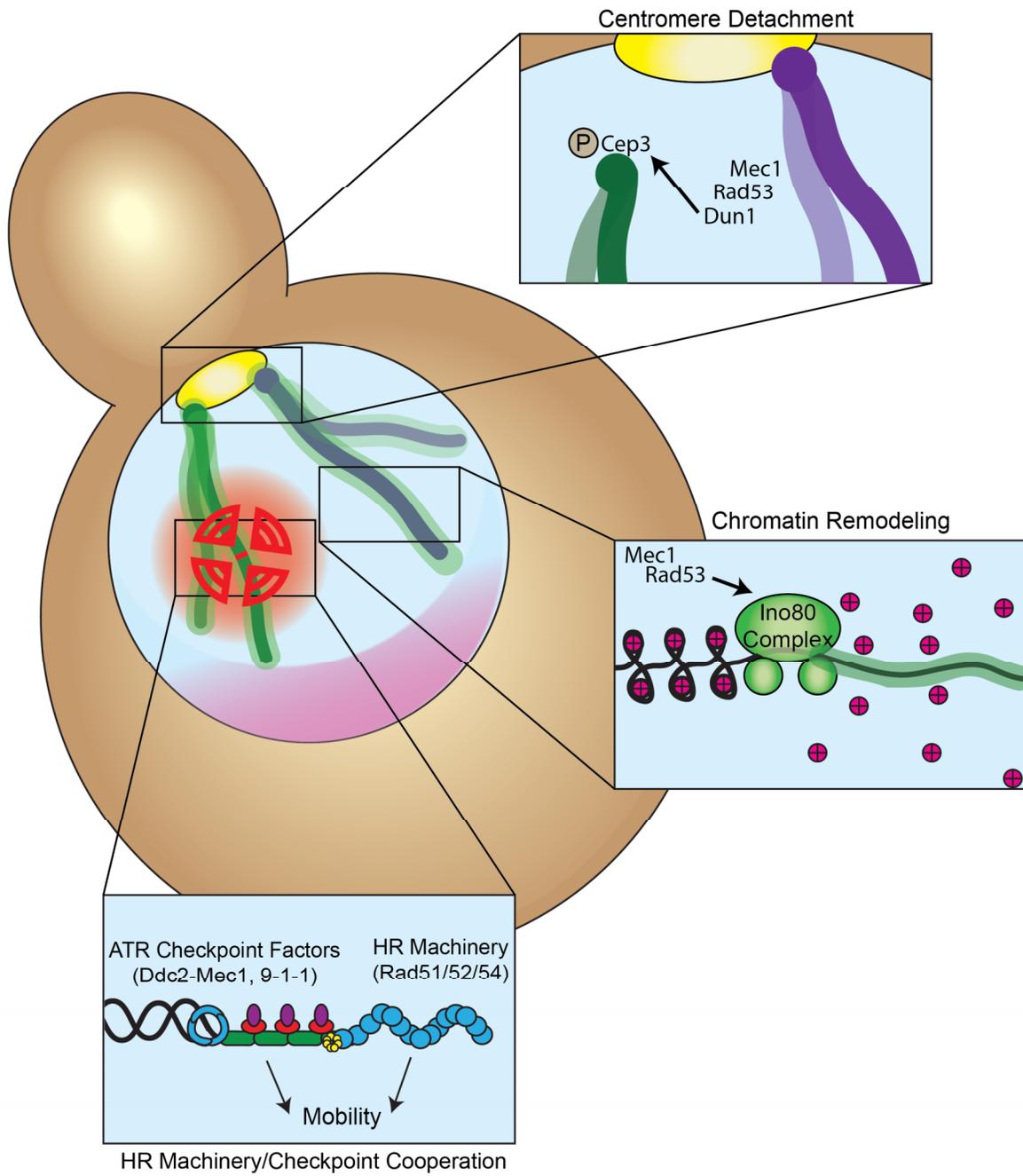
**Figure 1-3**

**Figure 1-3**

Schematic for the various models proposed for DNA damage-induced increased chromosomal mobility in budding yeast. As depicted in the bottom inset, resection after DSB formation generates ssDNA, which is bound by RPA to promote the loading of both checkpoint signaling factors and the recombination machinery. Mec1 checkpoint signaling is critical for both local and global mobility (see Figure 1-2). In addition, the HR machinery is also essential for mobility. We propose that components of the checkpoint apparatus and the HR machinery cooperate to promote mobility. Two putative downstream consequences of checkpoint activation on mobility have been identified. The Gasser group has proposed that mobility is promoted by the checkpoint activation of the INO80 chromatin remodeling complex, particularly its components Arp5 and Arp8 (middle inset). By reorganizing histones bound to chromatin, the physical characteristics of chromatin are altered to increase its mobility. The Durocher group has proposed that Mec1 activation leads ultimately to the phosphorylation of the kinetochore protein Cep3 through Dun1 (top inset). Cep3 phosphorylation modulates the kinetochore/centromere attachments and leads to increased dynamics of centromere proximal loci, thus generating mobility. The precise contributions of these three mechanisms along with other factors will be exciting avenues for future research.

**CHAPTER 2:**

**INCREASED CHROMOSOMAL MOBILITY AFTER DNA DAMAGE IS CONTROLLED BY**

**INTERACTIONS BETWEEN THE RECOMBINATION MACHINERY AND THE CHECKPOINT**

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# Summary

During homologous recombination, cells must coordinate repair, DNA damage checkpoint signaling, and movement of chromosomal loci to facilitate homology search. In *Saccharomyces cerevisiae*, increased movement of damaged loci (local mobility) and undamaged loci (global mobility) precedes homolog pairing in mitotic cells. Mechanisms responsible for changes in chromosome mobility remain unclear. Here, we elucidate a link between the DNA repair machinery and chromosomal mobility. Global mobility requires Rad51, but its function is distinct from its role in recombination, as only its ssDNA binding is essential. The importance of Rad52 is revealed in *rad51Δ rad52Δ* double mutants, which display constitutive global mobility driven by a hyperactive checkpoint. Rad52 mutant protein that fails to recruit Rad51 blocks increased mobility, indicating that Rad52 inhibits global mobility in the absence of Rad51. Thus, interplay between the recombination machinery and the checkpoint likely restricts activation of increased mobility until recombination proteins are assembled at damaged sites.

24

# Introduction

DNA damage-induced chromosomal mobility is an important facet of the cellular response to DNA damage that is positioned at the intersection of repair and checkpoint pathways [70,71]. In *S. cerevisiae*, both damaged and undamaged chromosomal loci expand their exploration of nuclear space in response to the formation of DNA double-strand breaks (DSBs) [43,44,46]. Sites close to the location of a DSB undergo a large increase in nuclear exploration termed local mobility, while undamaged sites undergo a smaller increase termed global mobility. It has been proposed that local mobility primarily drives homology search while global mobility is also induced to promote efficient pairing in the crowded nucleus [72].

Increased chromosomal mobility is conserved throughout evolution from yeast to human cells. During meiosis both in *S. cerevisiae* and *Schizosaccharomyces pombe*, dynamic telomere led movements may serve to promote stringent pairing of homologs [66,67]. Also, in *S. pombe*, persistent DSBs migrate to the nuclear periphery and become associated with microtubule linked KASH domain proteins [63]. In mammalian cells, expansions in chromosome mobility occur after the formation of double strand breaks [38] or the uncapping of telomeres [42]. 53BP1, the homolog of yeast Rad9, is a player in this response at telomeres, and mobility has been suggested to be important for both NHEJ and the avoidance of ectopic recombination during HR [57]. In addition, Rad51 has been implicated in the directional movement of telomeres during ALT in mammalian cells [65]. In *Drosophila*,

mobility is thought to be important for the migration of damaged heterochromatic sites into subdomains of the nucleus where recombination is permitted [41,73].

In budding yeast, a variety of factors have been implicated in the control of chromosome mobility. The checkpoint kinase Mec1, as well as its downstream interacting partners Rad9 and Rad53, is required for local mobility [43], and factors that generate ssDNA substrates, such as Sae2, contribute to the timing of mobility following a DSB [44]. Chromatin remodeling factors also play a role in global mobility [46,48,60], while another study reports that the kinetochore protein Cep3 modulates mobility after DNA damage by affecting centromeric attachments of chromosomes [61]. Telomeric attachments [74] as well as actin and actin-related proteins [60] have also been implicated in mobility. In addition to DNA damage checkpoint signaling, the recombination machinery also plays a role in regulating mobility as deletion of *RAD51* completely abrogates local mobility [43,44]. While the enzymatic and biochemical functions of both recombination machinery and the DNA damage checkpoint apparatus have been well studied, the synchronization of repair events with damage signaling pathways and the physical organization of chromosomes during the search for homology has not been elucidated.

Here, we find that the recombination machinery regulates global mobility in G1 and S phase diploid budding yeast. Rad51 is required for this mobility, and its association with ssDNA, but not its strand exchange and dsDNA-binding functions, is essential for this process. However, deletion of the recombination mediator Rad52 does not prevent global

mobility. Unexpectedly, a *rad51Δ rad52Δ* double mutant strain displays constitutively increased mobility as a result of constitutive checkpoint activation. Furthermore, elevated mobility does not occur in *rad52Δ409-412* mutant strains, in which Rad52 can associate with RPA-coated ssDNA but cannot recruit Rad51, indicating that Rad52 inhibits global mobility in the absence of Rad51. Thus, interactions between the recombination machinery and the DNA damage checkpoint ensure that globally increased chromosomal mobility occurs only after the proper assembly of recombination proteins at sites of DNA damage.

# Results

## Global mobility of chromosomes requires *RAD51*

To determine how the recombination machinery affects global mobility, we used a previously reported system for visualizing chromosome movements [44]. Briefly, this system includes a multiple tandem array of TetO sequences inserted at the *URA3* locus on Chromosome V, which is bound by RFP-tagged TetR protein. A YFP-tagged component of the spindle pole body, Spc110, acts as a reference for cellular motion. The Rad52 repair protein is tagged with CFP to detect DSBs [25]. We image early S phase cells every 10 seconds using 3D epifluorescence microscopy to measure the positions of the TetO array and the spindle pole body. As shown previously, the Mean Square Displacement (MSD) curves of the tracked chromosomal array plateau, which is indicative of confined Brownian diffusion [31,32]. The radius of that confinement  (Rc) can then be inferred from the plateau of the MSD curve.

To induce global mobility, we produced random DSBs by treating cells with ionizing radiation [44]. Approximately 4 DSBs per cell are created after 40 Gy of gamma radiation [75]. Because chromosome V is small (~2% of the genome), ionizing radiation-induced DSBs form there infrequently. Therefore, any observed increase in Rc reflects global mobility. As expected, 40 Gy of ionizing radiation produces a global mobility response at the *URA3* locus (Figure 2-1B, undamaged: 490 ± 20 nm, + 40 Gy: 570 ± 30 nm, p-value: 0.004). Previous studies have indicated that the *S. cerevisiae* RecA homolog, *RAD51*, is critical for local chromosome mobility after DNA damage [43,44,59]. To assess its contribution to global mobility, we subjected diploid *rad51Δ* cells to 40 Gy of radiation (Figure 2-1C). In contrast to wild type, *rad51Δ* cells do not display global mobility (undamaged: 490 ± 30 nm, + 40 Gy: 480 ± 50 nm, p-value: 0.5). Thus, Rad51 is required for mobility on sites that are *trans* to the damaged locus, suggesting that the recombination machinery controls damage signaling during global mobility.

**Rad51 is required for global mobility in the G1 phase of the cell cycle**

To determine how cell cycle phase affects global mobility, we turned our attention to G1 cells, since most chromosomal mobility studies have focused solely on S phase. First, we examined Rad51 focus formation, since earlier work in haploid yeast showed that the localization of another recombination protein, Rad52, is cell cycle restricted [76]. As expected, Rad52 foci do not form effectively in G1 cells (Figure 2-2A, B). However, Rad51 foci do form at high levels in damaged G1 and S phase cells in both haploids and diploids, (Figure

2-2A,B, Figure 2-S1). These results are surprising, since we assumed that Rad52 focus

formation was required for Rad51 foci. Therefore, either Rad52 focus formation is not

required for its mediator activity or other recombination mediators such as Rad55/57

assist in recombinase loading [77].

We next analyzed the mobility of the *URA3* locus in G1 wild-type and *rad51Δ* cells. Because

Rad52 foci do not form efficiently in G1, we identified damaged cells using CFP tagged Ddc1,

which can form foci in response to gamma irradiation throughout the cell cycle[26,76]. G1

diploids, like haploids [32], exhibit a higher baseline Rc than S phase cells, possibly due to

differences in cohesin loading between G1 and S [59]. Nevertheless, G1 diploid cells undergo

an additional increase in Rc after irradiation (Figure 2-2C, undamaged: 570 ± 70 nm, +40

Gy: 730 ± 100 nm, p-value: 0.02), indicating that, like in S phase, global mobility also

occurs during G1. In the absence of damage, G1 *rad51Δ* cells behave similarly to wild-type

cells, but, after irradiation, global mobility does not occur (Figure 2-2D, undamaged: 670 ±

70 nm, +40 Gy: 640 ± 50 nm, p-value: 0.7). Taken together with the S phase data (Figure 2-

1C), these results demonstrate that Rad51 plays an important role in global mobility in

both G1 and S phases of the cell cycle.

**Rad51 binding to ssDNA, but not its recombination functions, is required to promote
global mobility**

To gain biochemical insight into the requirement for Rad51 during global mobility, we

analyzed several *RAD51* separation-of-function mutants in S phase (Figure 2-3A, Figure 2-

S2). We first examined whether its recombination functions are required to promote global mobility. Rad51 has two DNA binding sites, sites I and II that are essential for recombination. Cloud and colleagues created an allele containing 3 alanine substitutions in site II at positions R188, K361, and K371[14]. Together, these 3 mutations prevent donor dsDNA from binding to ssDNA bound Rad51 within presynaptic filaments effectively blocking homology search and strand exchange while preserving filament formation [14]. The mobility of *URA3* in undamaged *rad51-II3A* cells is slightly elevated compared to wild type (although not statistically significant, p-value: 0.1, Table S2), which might reflect an effect of the mutant protein. However, after irradiation, mobility is significantly increased when compared to undamaged wild-type cells [Figure 2-3B, *rad51-II3A* undamaged: 530 ± 40 nm, *rad51-II3A* + 40 Gy: 590 ± 60 nm, p-value: 0.2; p-value for WT undamaged (data from Figure 2-1B) compared to *rad51-II3A* +40 Gy: 0.005]. Thus, *rad51-II3A* cells are competent for global mobility after DNA damage, indicating that global mobility is not a product of Rad51-catalyzed strand exchange.

We next asked whether Rad51 binding to ssDNA is necessary for global mobility. An alanine substitution of the invariant lysine 191 within the Walker A ATPase domain of Rad51[78,79] prevents ATP binding, and thus is defective for DNA binding  (Figure 2-3A, [16].
No increase in mobility is observed after irradiation in *rad51-K191A* mutant strains (Figure 2-3C, undamaged: 470 ± 60 nm, +40 Gy: 490 ± 30 nm, p-value: 0.6), demonstrating that Rad51 DNA binding is essential for global mobility. Since Rad51 protein can bind double- as well as single-stranded DNA [80], we took advantage of a lysine to glutamic acid

mutation at K342 isolated by Krejci and colleagues [81], which is defective for dsDNA, but not

ssDNA binding [15]. Notably, this mutant is proficient for global mobility (Figure 2-3D,

undamaged: 470 ± 40 nm, + 40 Gy: 610 ± 50 nm, p-value: 0.01), indicating that ssDNA

binding by Rad51, but not dsDNA binding, is essential for this process.


**Rad52 inhibits global mobility in the absence of Rad51**

Since ssDNA binding of Rad51 is essential for global mobility, we tested whether deletion of

*RAD52*, which is required for Rad51 focus formation (Figure 2-4A), affects this process. To

evaluate mobility, we constructed *rad52Δ* strains, and detected DSB formation with CFP-

tagged Ddc1 during S phase. Unexpectedly, global mobility is unaffected in the absence of

this mediator (Figure 2-4B, undamaged: 440 ± 20 nm, + 40 Gy: 580 ± 100 nm, p-value:

0.007). Next, to assay local mobility, we tracked an I-*Sce*I-induced site-specific DSB in a

*rad52Δ* strain. In wild-type cells, local mobility results in expansion of the Rc from ~450

nm to ~990 nm [44]. Interestingly, when a DSB is induced proximal to the tracked TetO array

in *rad52Δ* cells, the Rc only expands to 650 ± 40 nm (p-value: < 0.001), which is comparable

to the increase seen in wild-type cells undergoing global mobility (Figure 2-4C).


Since Rad52 is dispensable for global mobility (Figure 2-4B) and Rad51 ssDNA binding is

required (Figure 2-3), we reasoned that mobility in *rad52Δ* cells may be promoted by a

Rad52-independent association of Rad51 protein with ssDNA. To test this hypothesis, we

created a *rad51Δ rad52Δ* strain and assessed its mobility. If Rad52-independent association

of Rad51 with ssDNA solely drives global mobility, we expected that the double mutant

would not exhibit increased mobility. Surprisingly, we observe that the double mutant displays elevated mobility not only in damaged cells but also in undamaged cells (Figure 2-4D, undamaged: 660 ± 60 nm, damaged: 620 ± 100 nm, p-value: 0.2). Because previously published work indicated that the DNA damage checkpoint is important for local and global mobility in haploids [43,48,59,61], we suspected that the increase in mobility observed in *rad51Δ rad52Δ* cells might be due to increased checkpoint activation. First, we assessed the importance of the DNA damage checkpoint in diploids and found that, following treatment with the checkpoint kinase inhibitor caffeine, global mobility does not occur (Figure 2-S3A, B). Moreover, artificial checkpoint induction [47], is sufficient to trigger global mobility (Figure 2-S3C, D). Next, we treated *rad51Δ rad52Δ* cells with caffeine to determine whether the increase in mobility observed was due to aberrant checkpoint activation. Caffeine treatment completely blocks increased mobility in both undamaged and irradiated cells (Figure 2-4E, undamaged: 410 ± 40 nm, damaged: 420 ± 30 nm, p-value: 0.7). To detect DNA damage checkpoint activation, we examined Rad53 phosphorylation. We find that *rad51Δ rad52Δ* cells display elevated levels of phosphorylated Rad53, even in undamaged cells and these higher molecular weight species are eliminated following caffeine treatment (Figure 2-4F). These results show that, in the absence of these two recombination factors, checkpoint activation and global mobility is constitutive.


One simple explanation for our observations is that Rad52 prevents mobility in the absence of Rad51. To explore this possibility, we examined diploid, S phase cells bearing a deletion in a critical Rad51 interaction motif within Rad52 (*rad52Δ409-412*). This mutant protein

associates with ssDNA, but lacks mediator function and thus fails to recruit Rad51 [82].

Strikingly, although this *rad52Δ409-412* mutant displays normal mobility in undamaged cells, similar to a *rad51Δ* mutant, global mobility is blocked after irradiation (Figure 2-4G, undamaged: 480 ± 40 nm, damaged: 490 ± 30 nm, p-value: 1.0). Overall these observations indicate that the association of Rad52 with ssDNA in the absence of Rad51 is inhibitory to global mobility.

# Discussion

Our group and others have proposed that DNA damage-induced increased chromosomal mobility is a mechanism for homology search and assists broken DNA ends in efficiently navigating through the complexity of nuclear space to locate homology (reviewed in [71]). The results presented here demonstrate that a regulatory circuit between checkpoint and recombination factors governs the signaling events that produce global mobility. It is intriguing that these repair proteins not only affect mobility at the cut site[44], Figure 2-4C), but also at undamaged loci (Figure 2-1C, 2-4B). Thus, the cell uses crosstalk between the HR machinery and the DNA damage checkpoint to determine when it is appropriate to increase mobility throughout the nucleus.

Previous studies have implicated the checkpoint in the control of global mobility in haploid cells. Seeber and colleagues reported that checkpoint activation targets members of the INO80 chromatin-remodeling complex to produce global mobility [46], and a later study by

Hauer and colleagues reported that changes in histone occupancy on DNA following damage may be consequential for chromatin confinement and mobility [48]. Strecker and colleagues reported that the activation of the DNA damage checkpoint leads to phosphorylation of the kinetochore protein Cep3 and suggest that modulation of centromere to kinetochore attachments alters chromosome tethering to promote both local and global mobility [61]. In addition, DNA damage causes a release of telomeric sequences as well as expansion of pericentric chromatin domains [74]. Here, we find that the DNA damage checkpoint is necessary and sufficient for the induction of global mobility in diploids (Figure 2-S3), and that the activity of Rad51 and Rad52 profoundly affect checkpoint activation and downstream mobility (Fig. 4D, E, F). Our discovery of this relationship not only reinforces the critical importance of the DNA damage checkpoint, but also uncovers a novel role for the recombination machinery in regulating global mobility.


The observations described here support a model whereby the loading of the recombination machinery is coupled to DNA damage checkpoint activation and the initiation of global mobility (Figure 2-5). After a DSB, resection of 5' ends reveals stretches of ssDNA that rapidly accumulate RPA complexes [1]. The Ddc2-Mec1 complex as well as the 9-1-1 sliding clamp are then recruited to these resected ends, leading to the phosphorylation of downstream checkpoint factors [5]. While resection proceeds, and Rad51 is being recruited, Rad52 inhibits mobility until a proper presynaptic filament structure can be formed, after which RPA and Rad52 are displaced from ssDNA [83,84] and this inhibition is lost. The newly assembled Rad51 filament then promotes local mobility.

Mutant strains are deficient in various aspects of this pathway. In *rad51Δ* or in ssDNA-binding defective *rad51-K191A* strains, Rad52 is not removed from the RPA-coated ssDNA and thus global mobility is continuously inhibited (Figure 2-1C, 2-3C). The notion that Rad52 is an inhibitor is also supported by the fact that increased mobility does not occur in *rad52Δ409-412* cells, where Rad52 can bind ssDNA but not recruit Rad51 (Figure 2-4G). Inhibition by Rad52 does not occur in *rad52Δ* cells and therefore, the checkpoint directly promotes global mobility upon DNA damage at both damaged and undamaged loci (Figure 2-4B, C). Furthermore, in *rad51Δ rad52Δ* cells, inhibition of global mobility is also absent, and the lack of functional Rad51 leads to constitutive checkpoint activation of global mobility both before and after DNA damage, perhaps from Ddc2-Mec1 binding to stalled replication forks [7] or any other form of spontaneous DNA damage (Figure 2-4D, E, F).

This model raises several important questions on the interplay between mobility and the recombination and checkpoint machinery. How might Rad52 inhibit global mobility? It is possible that Rad52 directly influences checkpoint activation. For example, in *rad51Δ* cells, we observe less Rad53 hyperphosphorylation than in *rad51Δ rad52Δ* cells, suggesting that the presence of Rad52 reduces checkpoint signaling (Figure 2-4F). This reduction may occur by Rad52 sterically hindering the binding of checkpoint factors (e.g., Ddc2-Mec1 and the 9-1-1 complex) on the RPA-bound ssDNA substrate, or through a direct protein-protein interaction between Rad52 and checkpoint factors. Alternatively, Rad52 may interact with an as yet undiscovered downstream factor(s) required for mobility, thus bridging global mobility and the checkpoint.

What is the role of Rad51 in global and local mobility? RPA is removed from ssDNA following the formation of a tripartite complex between itself, Rad51, and Rad52 [84]. Therefore, the role of Rad51 in global mobility may simply be to remove the inhibitor, Rad52. However, two results suggest that the function of Rad51 in mobility is not limited to its displacement of Rad52. First, we observe no local mobility in *rad52Δ* cells, where Rad51 cannot be recruited to resected ends (Figure 2-4C). If checkpoint induction in the absence of Rad52 were able to directly induce local mobility at cut sites, we should have seen a large increase in Rc upon I-*Sce*I DSB induction [44]. However, we only observe global mobility at these sites (Rc of ~450 nm to ~600 nm), indicating that Rad51 is required at ends to promote local mobility. We suspect that the change in the local structure of DNA (e.g., stiffening), caused by loading of the Rad51 presynaptic filament at the cut site, facilitates enhanced exploration of the genome (Miné-Hattab et al., 2017, unpublished data). Second, in G1 cells, where Rad51 foci, but not Rad52 foci, form [76], Figure 2-2A, B), global mobility requires Rad51 (Figure 2-2D). Thus, the appearance of Rad52 foci is not essential for either Rad51 recruitment or global mobility in G1, suggesting that Rad51 may independently promote global mobility in this phase of the cell cycle. However, we cannot rule out the possibility that Rad52 retains mediator activity despite its inability to form foci effectively. Therefore, Rad51 might promote global mobility exclusively through Rad52 displacement in both G1 and in S.

There is also a connection between Rad51 and components of the DNA damage checkpoint that may be important for global mobility. Flott and colleagues have reported that Rad51 is phosphorylated by Mec1 within its Walker A ATPase domain [85], and we suspected that this interaction might affect mobility. To investigate this connection, we examined a phosphorylation-dead mutant at this site (*rad51-S192A*) as well as a phosphomimetic mutant (*rad51-S192E*) and found that both are defective for global mobility (Figure 2-S3E, S3F). However, since both mutations cause a strong ssDNA-binding defect [85] it is likely that the lack of mobility observed in these mutants is solely due to their inability to displace Rad52.

Why might the cell link the initiation of mobility to the recruitment of recombination factors? A simple explanation is that it is advantageous for the cell to restrict the activation of mobility only to those contexts where it has committed to HR (i.e., following resection) and has properly assembled the mechanical complexes such as the Rad51 presynaptic filament. Thus, tying mobility to HR progression and checkpoint activation adds a layer of stringency that prevents increased mobility at inappropriate times or after an inappropriate damage stimulus. For example, mistimed mobility could result in translocations or chromosome loss. The notion that mobility is restricted to contexts where HR is favorable may explain the observations that diploids, which can use the homolog for recombination, undergo mobility more readily than haploids, which are limited to sister chromatid exchange [43,44]. Alternatively, some have argued that one of the roles of increased mobility is to disrupt nonhomologous contacts through mechanical force

during the homology search [57]. However, no matter what purpose increased chromosomal dynamics serve, the linkage between recombination progression and checkpoint activation is undoubtedly critical for the control of mobility.

# Methods

**Strains**

Unless otherwise noted, all strains are *RAD5+* derivatives of W303 [86,87; 88] (See Table S1).

**Galactose induction**

For both I-*Sce*I cutting and DNA damage checkpoint induction experiments, cells were grown overnight in 3-5 mL cultures of SC + 2% raffinose at 23°C. In the morning, 2% galactose was added. I-*Sce*I experiments were induced for 150 minutes at 23°C, while DNA damage checkpoint induction strains were induced for 90 minutes. Following induction, I-*Sce*I cutting was halted by washing the cells in SC + 2% glucose. Both strains were then prepared for microscopy.

**Caffeine treatment**

Caffeine treatment was performed as previously described [89]. Briefly, fresh 100 mM stock solutions of caffeine were prepared each experimental day. Cells were treated with 20 mM caffeine for 30 minutes. Cells were irradiated and imaged in the presence of caffeine.

## γ-irradiation

Overnight cultures of strains designated for irradiation were diluted slightly in fresh media and allowed to grow for an hour at 23° C. Aliquots of these cultures were then exposed to defined doses of irradiation using a Nordion 220 ^{60}Co irradiator, and then were immediately prepared for imaging.


## Immunoblotting

Protein was harvested from cells via precipitation in 5% trichloroacetic acid followed by washing in acetone. Protein preparations were separated by SDS-PAGE [90] using Mini-TGX precast gels (Biorad) and transferred to nitrocellulose membranes with a Trans-Blot® SD Semi-Dry Transfer Cell (Biorad) at constant amperage for 1.5 hours. Membranes were blotted overnight with either α-Rad53 monoclonal antibody (1:1000, Abcam), or α-PGK1 22C5D8 monoclonal antibody (1:20000, Thermo-Fisher) as a loading control.


## Spot assays

Cells were diluted to 0.2 $OD_{600}$ and diluted serially tenfold before being plated. Irradiation was performed as described above.

**Microscopy**

Cells were pelleted from treated or untreated cultures and resuspended at higher density before being placed upon a 1.4% agarose slab for visualization. Images were acquired on a Leica DM5500B upright microscope (Leica Microsystems) illuminated with a 100W mercury arc lamp. High-efficiency filter cubes were used for fluorophore imaging (Chroma 41028, Chroma 31044v2 and Chroma 41002C, for YFP, CFP, and RFP, respectively). Images were captured with a Hamamatsu Orca AG cooled digital CCD (charged-coupled device), and analysis of image data was performed with Volocity software (Perkin-Elmer). We took 15 z-stacks spaced by 300 nm every 10 seconds for 70 timepoints. Exposure times were as follows: DIC (30ms), YFP (100ms), RFP (100ms), and CFP (800ms for Rad52-CFP, 2s for Ddc1-CFP). CFP images were taken as part of a complete stack of all colors performed before time-lapse imaging began.

**Image analysis**

Analyses were performed as described previously [44]. Briefly, positions of the TetO array and the SPB were measured every 10 seconds. The positions of the SPB were subtracted from the TetO array in order to correct for nuclear motion. Rc values were obtained by averaging individual MSD plots for each cell in an experiment into a mean MSD, fitting the resultant curve, and extracting the plateau value. It is worth noting that MSD plots would only pass through the origin in an ideal experiment in which there is no localization error. The Y intercept of these plots reflects the accuracy at which the position of the locus can be determined, and includes the error of this measurement as well as how much the tracked

locus moves during acquisition (reviewed in [91]). For these mean MSD curves, only the first 25-30% of the MSD values were used to generate a fit to avoid the imprecise MSD values at high $\Delta t$. Plateau values were extracted from the fit and used to determine Rc values, which we report to the nearest 10 nm. Error bars on MSD plots represent the 95% confidence interval (CI) at each $\Delta t$ value. In addition, Rc values were determined for individual cells and used to calculate 1 SEM for the mean Rc values displayed in Table 2-S2. Individual Rc values were also used to detect cells with Rc values of >1.5 times the interquartile range for a given experiment, which were excluded from analysis. Individual Rc values were also used for further statistical analysis.


## Statistics

We find that the distribution of Rc values for individual, undamaged cells is approximately normally distributed with a peak centered around 450 nm. However, damaged cells display a bimodal distribution, with one peak at 450 nm (i.e. no change in Rc) representing approximately 30% of cells and a second broad peak ranging from 600 to 800 nm (i.e. increased Rc) representing approximately 70% of cells (Figure 2-S4). Since this distribution does not meet assumptions of normality, we use the non-parametric two-sided Wilcoxon rank sum test [92] to estimate the shift in Rc distribution relative to control, and determine a p-value for each experiment. A table of all test results can be found in Table 2-S2. Statistical analyses were performed in R [93].

# Acknowledgments
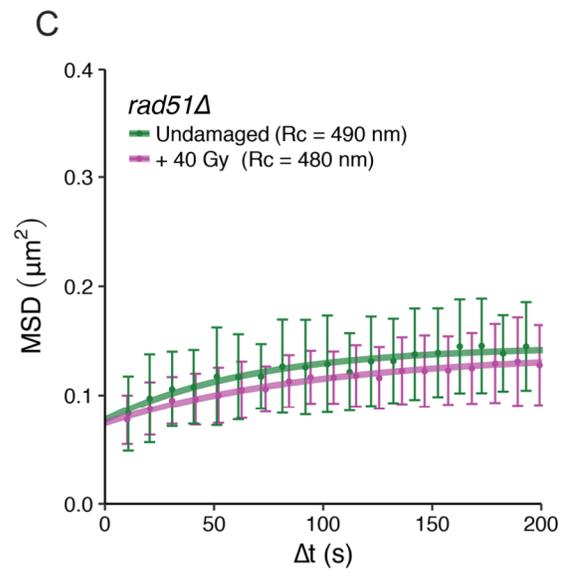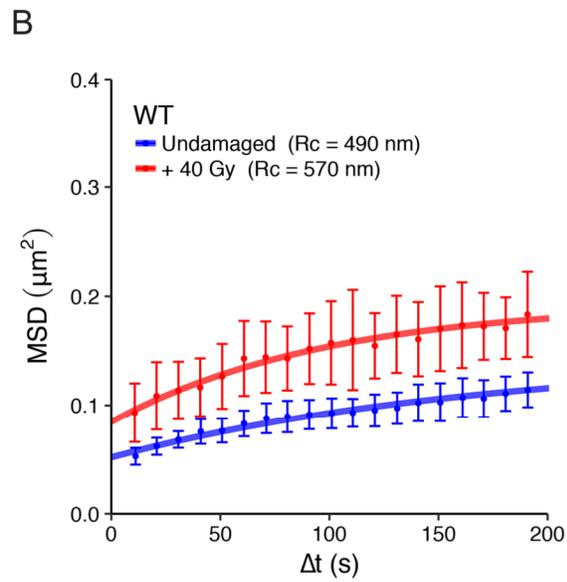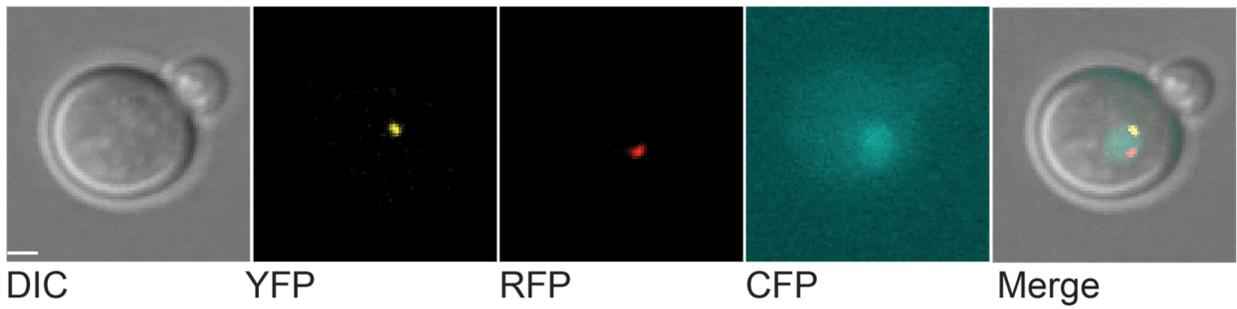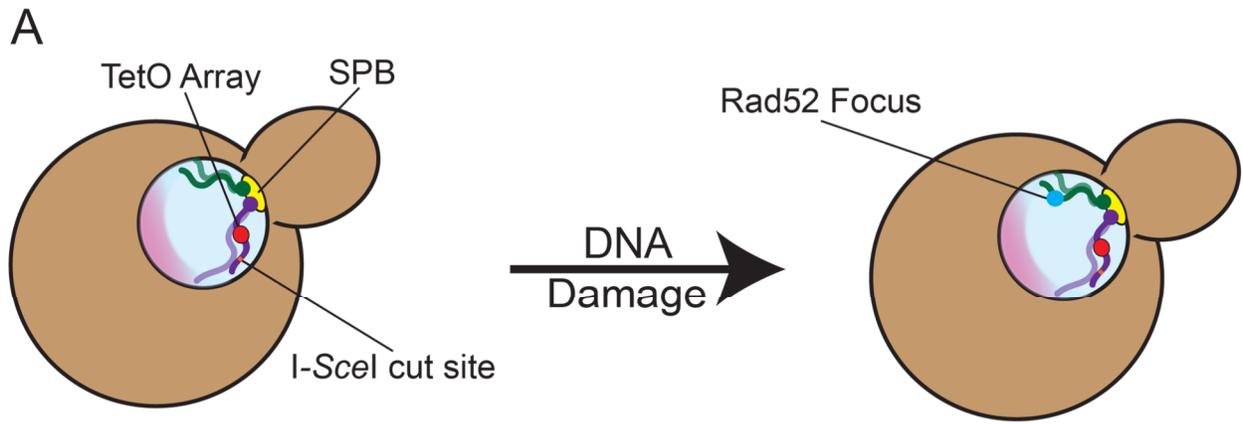
**Figure 2-1**: Rad51 is required for DNA damage-induced global mobility

**Figure 2-1**

**A**) Diagram of chromosomal locus tracking system. The TetO array is situated at the *URA3* locus on one copy of chromosome V in diploid yeast, 4 kb away from a telomere-proximal I-*Sce*I cut site. The other homolog is unlabeled. RFP-tagged TetR associates with the TetO array, forming foci. Spc110 is tagged with YFP as to serve as a reference point for cellular and nuclear motion, and Rad52 is tagged with CFP to assess whether DSBs have formed in a given cell. The nucleolus is shaded pink. Images show z-series projections of a representative cell of this system with each channel indicated, following deconvolution. (Scale bar = 1 micron) **B**) MSDs for the tagged *URA3* locus in WT strains, either undamaged (blue) or irradiated with 40 Gy of gamma radiation (red), show global mobility. **C**) As in B, in a *rad51Δ* strain, undamaged cells (green) are plotted alongside irradiated cells (magenta). Error bars of MSD plots represent the 95% CI.
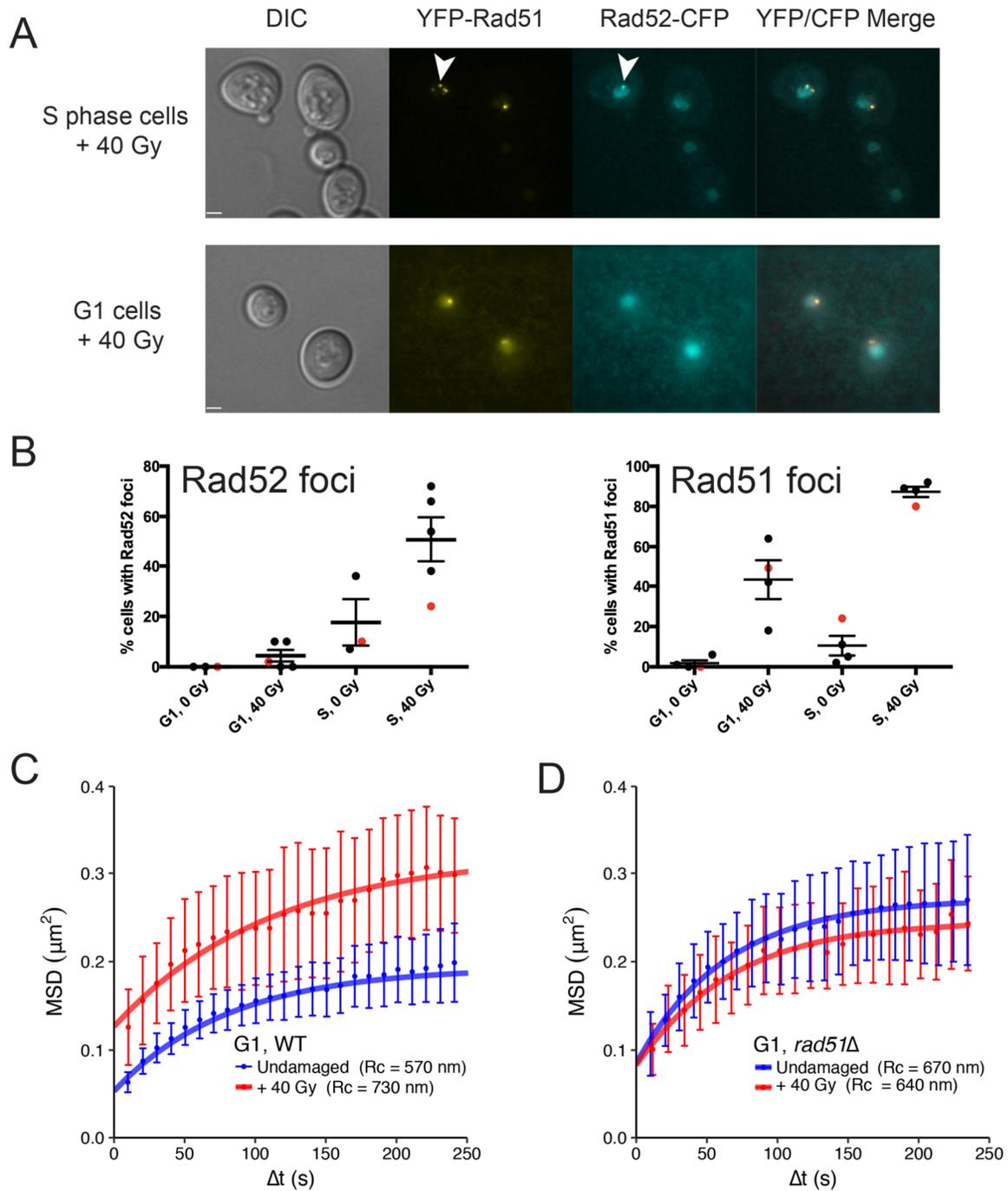
**Figure 2-2:** Rad51 forms repair foci and promotes global mobility in G1

**Figure 2-2**

**A)** Representative images of G1 and S phase cells depicting YFP-Rad51 and Rad52-CFP foci each tagged in the same strain. White arrowhead indicates a Rad52 focus colocalizing with a Rad51 focus. (Scale bar = 2 microns) **B)** Measurements of Rad52 (left) or Rad51 (right) focus formation in G1 and S phase cells, with and without treatment with 40 Gy of gamma radiation. Black points represent the percent foci for each tagged protein in independent experiments. Red points represent independent experiments in which Rad52 and Rad51 are both tagged in the same cells. For cells in the dual-tagged experiments, 59% of Rad52 foci colocalize with a Rad51 focus (n=17 cells). Error bars represent one SEM for each group of experiments. (See Table 2-S3 for results, Figure 2-S1 for haploid analysis) **C)** Analysis of Ddc1-CFP strain used for evaluating G1 mobility, showing higher baseline mobility when undamaged (blue), and further elevated mobility after irradiation (red). **D)** Analysis of G1, Ddc1-CFP tagged, *rad51Δ* cells without (blue) and with (red) irradiation shows a complete block in global mobility. Error bars of MSD plots represent the 95% CI.
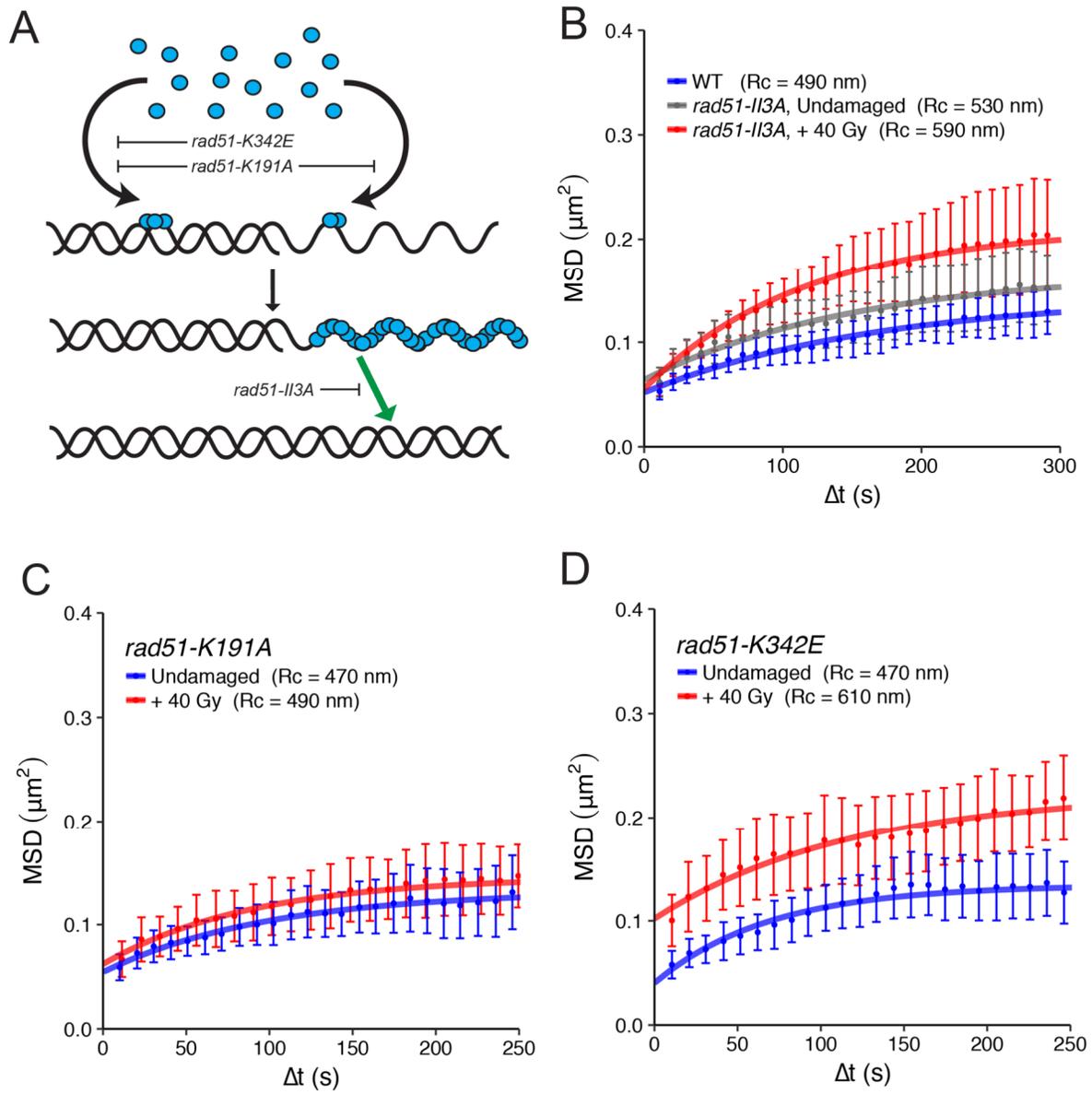
**Figure 2-3:** Analysis of Rad51 separation-of-function mutations

**Figure 2-3**

**A**) Diagram of Rad51 functions during repair (see Figure 2-S2). The Walker A ATPase domain mutant protein, Rad51-K191A, is unable to bind single- or double-stranded DNA. The DNA binding site II mutant protein, Rad51-II3A can bind ssDNA molecules, but is unable to accept donor dsDNA molecules (green arrow), and is thus defective for homology search and strand exchange. The Rad51-K342E mutant protein is defective in dsDNA binding. **B**) Strand exchange functions are dispensable for global mobility after DNA damage. *rad51-II3A* cells display slightly elevated mobility (gray) compared to WT (blue) and undergo an increase in mobility when damaged (red). **C**). Undamaged (blue) and irradiated (red) *rad51-K191A* cells display the same radius of confinement, and fail to undergo global mobility. **D**) *rad51-K342E* mutant strain shows global mobility, undamaged (blue) and irradiated (red). All MSD experiments were performed in Rad52-CFP tagged cells. Error bars represent the 95% CI.
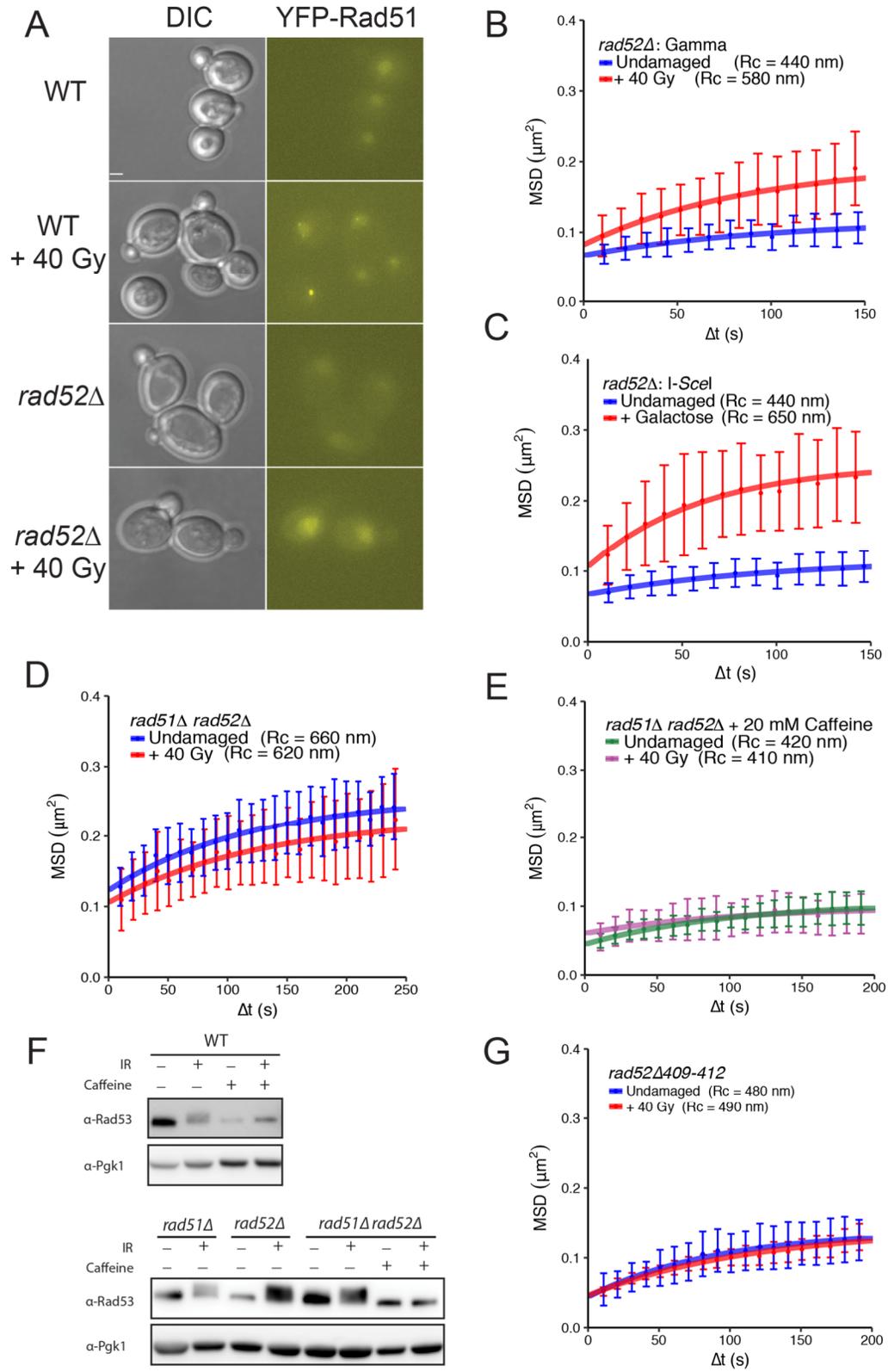
**Figure 2-4:** Rad52 negatively regulates global mobility in the absence of Rad51

**Figure 2-4**

**A**) *rad52Δ* cells do not form YFP-Rad51 foci effectively after irradiation. (Scale bar = 1.8 microns; rad52Δ, 0 Gy: 5.4% foci, 37 cells. rad52Δ + 40 Gy: 7.7% foci, 65 cells) **B**) Ddc1-CFP tagged rad52Δ cells display a global mobility response after gamma irradiation, compare undamaged (blue) to irradiated (red). **C**) Ddc1-CFP tagged rad52Δ cells were examined before and after a site-specific DSB was induced at *URA3* on Chr. V. The cut locus was tracked to evaluate mobility in the absence (blue) or presence (red) of galactose-induced I-SceI cutting. *rad52Δ* cells are capable of a global-like mobility response. **D**) *rad51Δ rad52Δ* cells, either undamaged (blue) or irradiated (red), show elevated mobility in both cases. **E**) Caffeine treatment blocks elevated mobility in both undamaged (magenta) and irradiated (green) *rad51Δ rad52Δ* cells (see Figure 2-S3). **F**) Protein blots of Rad53 protein in WT, *rad51Δ*, *rad52Δ*, and *rad51Δ rad52Δ* cells are shown before and after irradiation, as well as a Pgk1 loading control. In addition, caffeine-treated WT and *rad51Δ rad52Δ* cells are shown. All images are from the same blot, although the high abundance of Rad53 protein in mutant strains compared to WT necessitated separate contrast adjustments. Loading controls for WT and mutant strains were not adjusted. **G**) *rad52Δ409-412* cells either undamaged (blue) or irradiated (red), show no increase in mobility. All mobility experiments were performed in Ddc1-CFP tagged strains. Error bars for mobility experiments represent the 95% CI.
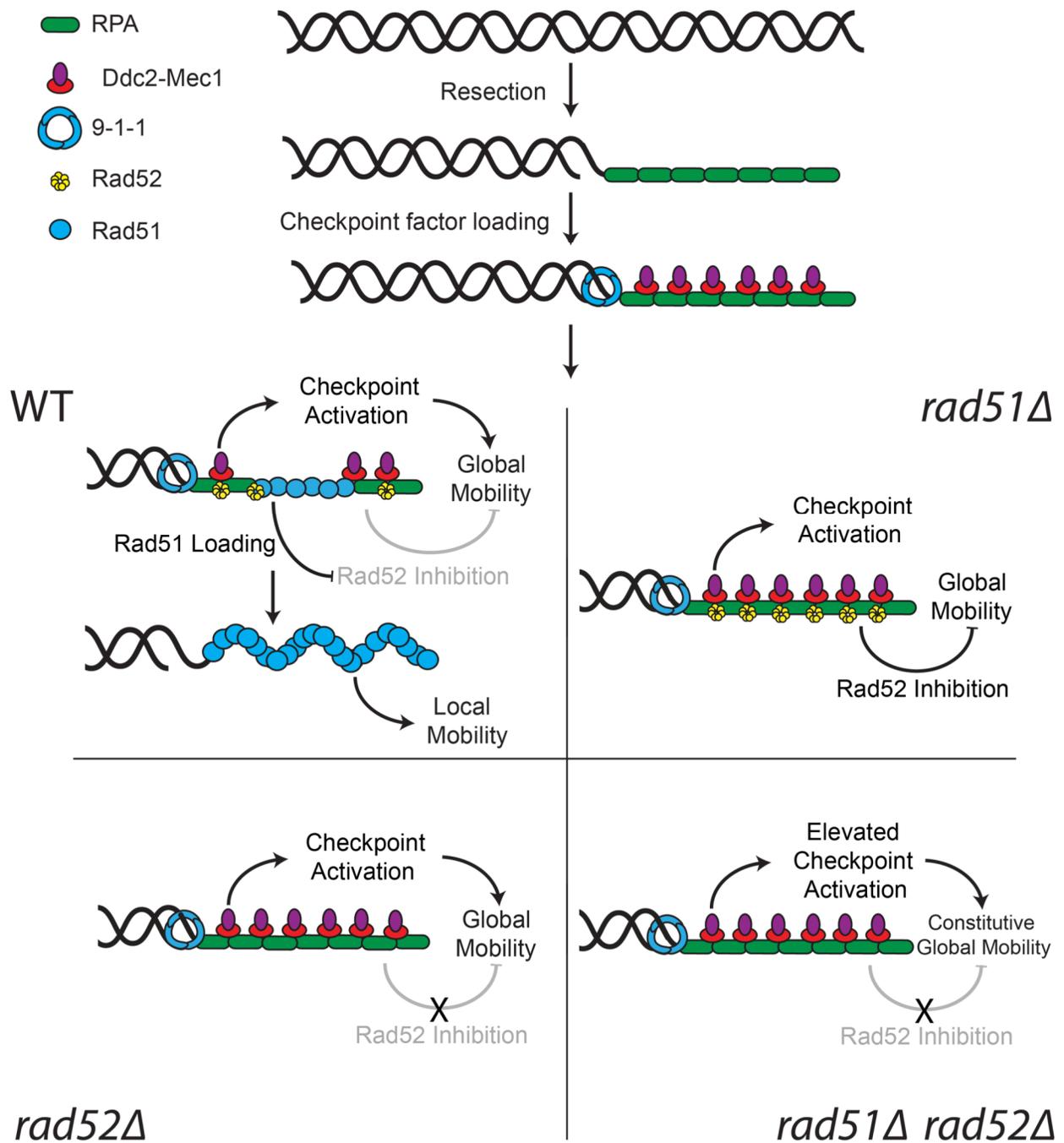
**Figure 2-5:** A model for the role of the recombination machinery in regulating global mobility

**Figure 2-5**

After DSB formation, end resection yields RPA-bound 3' ssDNA tracts that serve as platforms for both checkpoint signaling and repair factor loading. At this time, the DNA damage checkpoint becomes active, but the recruitment of Rad52 to the ssDNA inhibits the initiation of global mobility until Rad51 is sufficiently recruited. Rad51 loading removes Rad52 and alleviates this inhibition, allowing the checkpoint to promote global mobility. Following more extensive nucleation of Rad51, the recombinase may also participate in promoting local mobility. In *rad51Δ* cells, the association of Rad52 to RPA coated ssDNA prevents increased mobility. In *rad52Δ* cells, this inhibition is absent, and checkpoint activation drives global mobility directly upon DNA damage. In *rad51Δ rad52Δ* double mutant cells, irreparable DSBs become sites for constitutive checkpoint signaling and therefore induce constitutive global mobility, as Rad52 is also absent. Thus, proper assembly of the recombination machinery to sites of damage offers an additional layer of regulatory input to the checkpoint apparatus that restricts the activation of mobility to cells that are committed to HR.
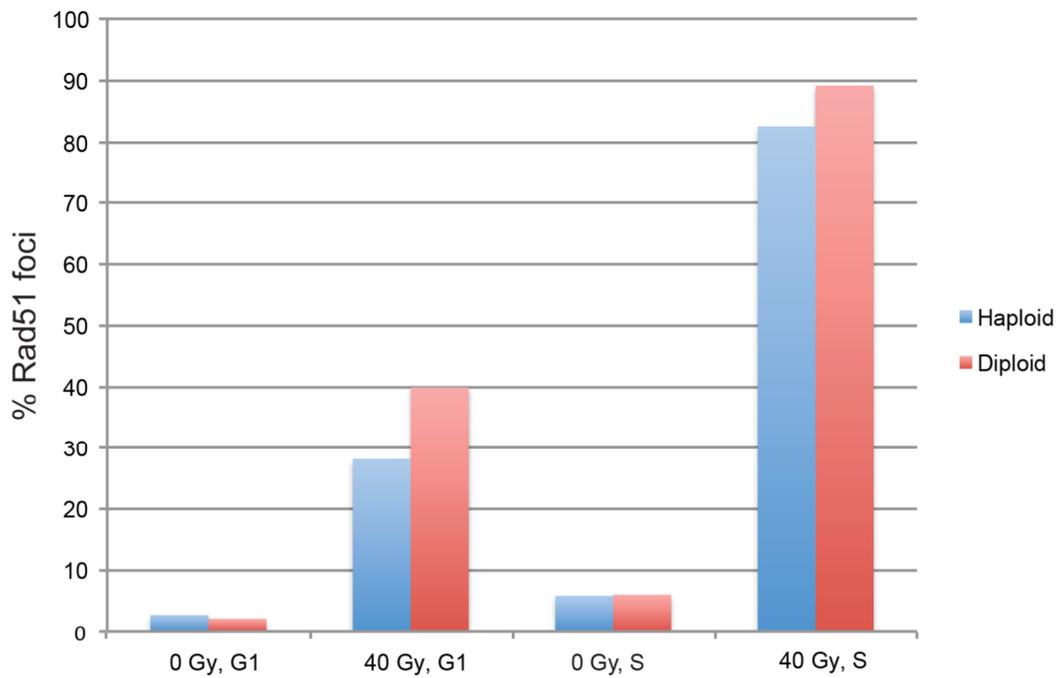
**Figure 2-S1:** Analysis of Rad51 foci in haploid cells, related to Figure 2-2. Percent of cells with one or more YFP-Rad51 foci before and after gamma irradiation in haploid versus diploid cells in G1 and S phase. (See Table 2-S2 for results).
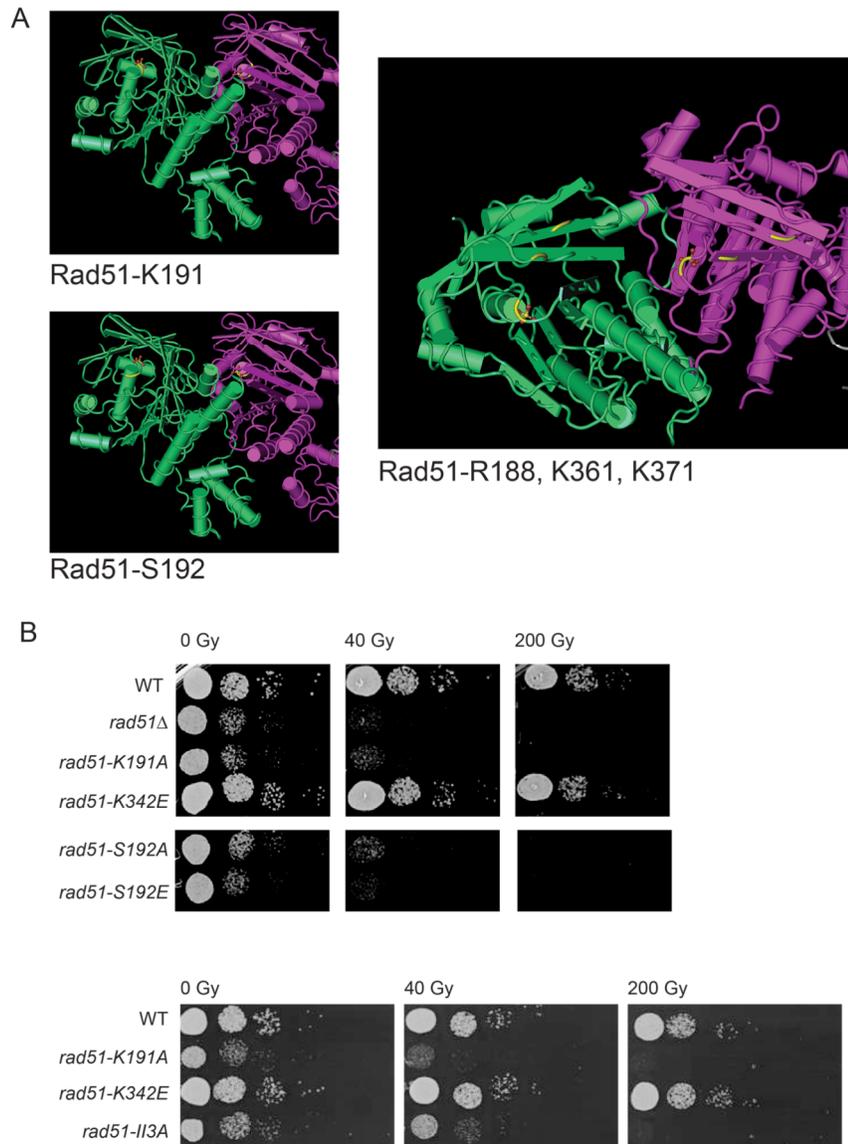
**Figure 2-S2:** Analysis of Rad51 separation-of-function mutants, related to Figure 2-3. **A**) Images of residues mutated in Rad51-K191A (top left), Rad51-S192A/E (bottom left), or Rad51-II3A (right) mapped on the crystal structure for a wild-type Rad51 dimer(Conway et al., 2004). Rad51-K342 is not shown as it is found on an unstructured loop that did not resolve in the crystal structure. **B**) Ten-fold serial dilution spot assays for RAD51 mutants at 0, 40, and 200 Gy.
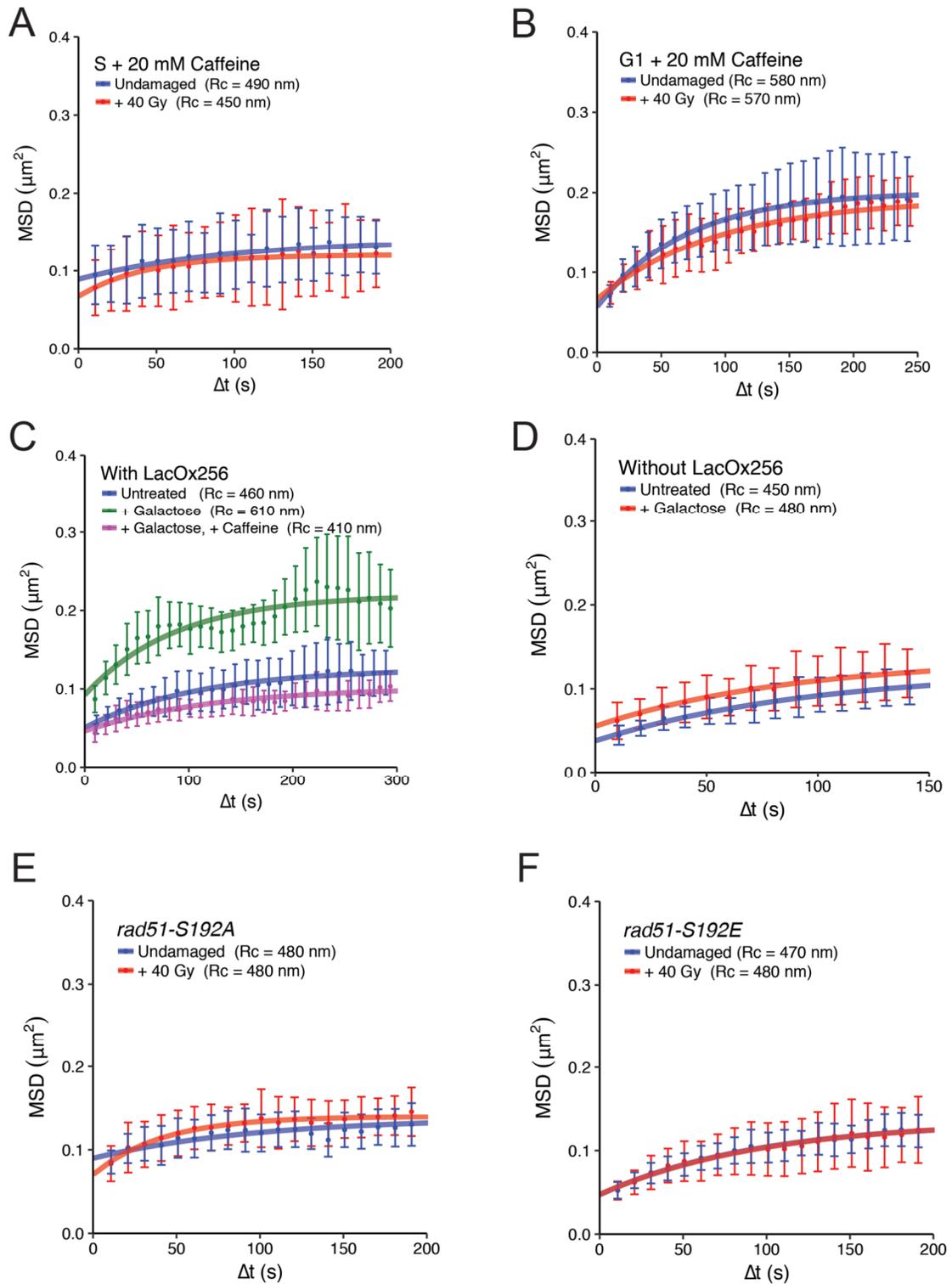
**Figure 2-S3:** DNA damage checkpoint activation is necessary and sufficient for global mobility, related to Figure 2-4.

**Figure 2-S3**

**A**) Rad52-CFP tagged, S phase cells treated with 20 mM caffeine. Undamaged (blue) and irradiated (red) cells show similar confinement radii. **B**) As in A, but performed in Ddc1-CFP tagged G1 cells, showing that the checkpoint also regulates global mobility in G1. **C**) Colocalization of Ddc1-LacI and Ddc2-LacI expressed after galactose induction leads to artificial checkpoint activation and global mobility in the absence of DNA damage. Strains containing the LacO array, either uninduced (magenta), induced (green), or induced in the presence of caffeine (blue) are shown. **D**) A checkpoint induction strain lacking the LacO array necessary for Ddc1 and Ddc2 colocalization shows no change in mobility either without (blue) or with (red) galactose induction. **E**) Mutations in a putative Mec1 phosphorylation site block global mobility. *rad51-S192A* mutant cells show no increase in mobility after irradiation, compare undamaged (blue) to irradiated (red). **F**) A *rad51-S192E* mutant strain, as in E. S phase experiments were performed in Rad52-CFP tagged strains and G1 experiments in Ddc1-CFP tagged strains. Error bars represent the 95% CI.
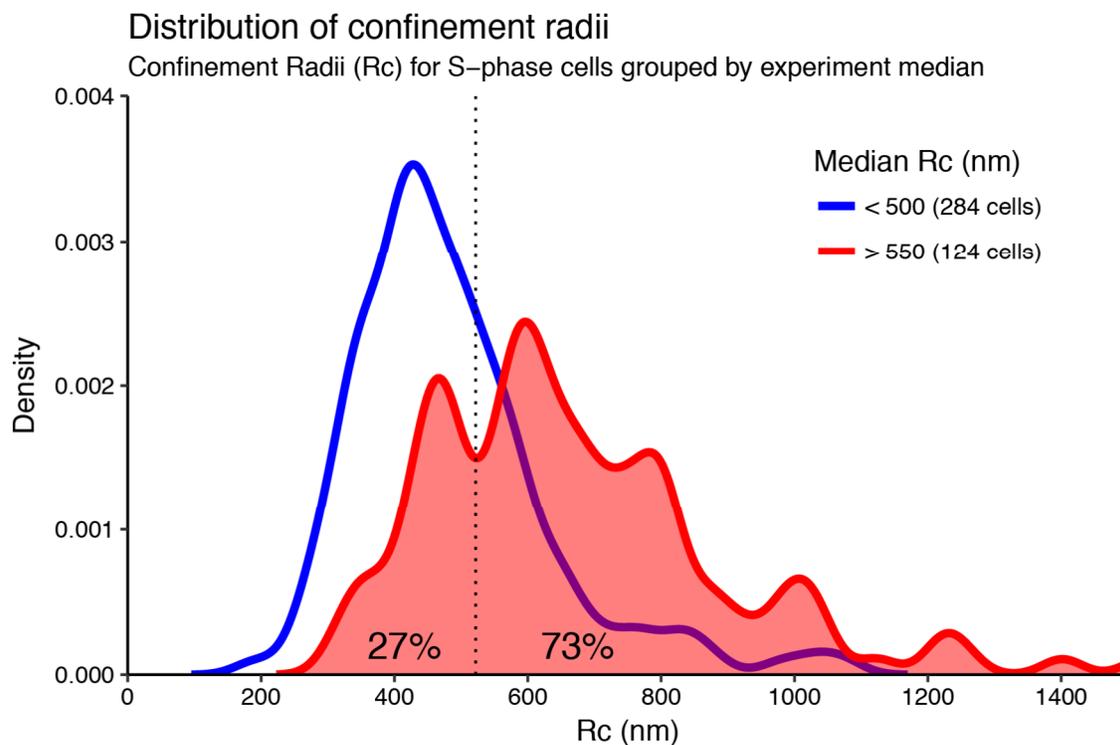
**Figure 2-S4:** Distribution of Rc values in all S phase experiments, related to Methods. Experiments were partitioned into two groups based on whether the median Rc was below 500 nm (blue) or above 550 nm (red). Cells in experiments with low median Rc display a distribution centered on 450 nm, while cells in experiments with elevated median Rc display a bimodal distribution with peaks at 450 nm and between 600 and 800 nm.

**Table 2-S1:** Strains used in this study

| Strain | Genotype | Background | Source |
|---|---|---|---|
| W9100-17D | MAT**a** ADE2 leu2-3,112 his3-11,15 ura3-1 can1-100 TRP1 lys2Δ RAD5 MET15 | W303 | Reid et al., 2016 |
| W9530-21C | MATα ura3::3xURA3-tetOx112 I-SceI(ura3-1) TetR-mRFP1(iYGL119W) lys2::GAL-I-SceI bar1::LEU2    RAD52-CFP | W303 | Mine-Hattab and Rothstein, 2012 |
| W11111-2C | MAT**a** ADE2 TRP1 RAD52-CFP SPC110-YFP::HIS3 | W303 | This Study |
| W11215 | W9530-21C X W11111-2C | W303 | This Study |
| W11016-24C | MAT**a** ADE2 rad51::KanMX ura3::3xURA3-TetOx112 I-SceIcs(ura3-1) TetR-mRFP1(iYGL119W) SPC110-YFP::HIS3 RAD52-CFP lys2 | W303 | This Study |
| W11018-12C | MATα rad51::KanMX GALS-DDC1-GFP-LACI::URA3 GAL-DDC2-GFP-LACI::HIS3 ddc1Δ 256xLACO::TRP1 | W303 | This Study |
| W11203 | W11016-24C X W11018-12C | W303 | This Study |
| W8702-13C | MAT**a** ADE2 YFP-RAD51 | W303 | This Study |
| W11202 | MAT**a**/MATα  YFP-RAD51/RAD51 ADE2/ADE2  trp1/TRP1 LYS2/lys2 | W303 | This Study |
| W8836-5A | MATα ura3::3xURA3-tetOx112 I-SceI (ura3-1) TetR-mRFP1(iYGL119W) | W303 | Mine-Hattab and Rothstein, 2012 |
| W9956-12C | MAT**a** ADE2 SPC110-YFP::HIS3 DDC1-CFP lys2 | W303 | This Study |
| W9998 | W8836-5A X W9956-12C | W303 | This Study |
| W10046-5B | MAT**a** ADE2 rad51::LEU2 SPC110-YFP::HIS3 DDC1-CFP | W303 | This Study |
| W10047-19D | MATα ura3::3xURA3-tetOx112 I-SceI (ura3-1) TetR-mRFP1(iYGL119W) rad51::LEU2 | W303 | This Study |

| W11204 | W10046-5B X W10047-19D | W303 | This Study |
|---|---|---|---|
| LSY1378-2A | *MATa rad51-K191A-URA3-rad51-K191A ade3::pGAL-HO* | W303 | Lorraine Symington |
| W11099-3D | *MATα ADE2 rad51-K191A SPC110-YFP::HIS3 Rad52-CFP* | W303 | This Study |
| W11101-13C | *MATa ADE2 rad51-K191A ura3::3xURA3-tetOx112 I-SceI (ura3-1) TetR-mRFP1(iYGL119W) lys2* | W303 | This Study |
| W11205 | W11099-3D X W11101-13C | W303 | This Study |
| W11133-9A | *MATa rad51-K342E ura3::3xURA3-tetOx112 I-SceI (ura3-1) TetR-mRFP1(iYGL119W) TRP1* | W303 | This Study |
| W11138-20D | *MATα ADE2 rad51-K342E SPC110-YFP::HIS3 Rad52-CFP lys2* | W303 | This Study |
| W11206 | W11133-9A X W11138-20D | W303 | This Study |
| DKB3630 | *MATα rad51-II3A::KanMX6* | SK-1 | Cloud et al., 2012 |
| W11142-4A | *MATa ADE2 rad51-II3A::KanMX ura3::3xURA3-tetOx112 I-SceI (ura3-1) TetR-mRFP1(iYGL119W)* | W303 | This Study |
| W11143-15D | *MATα rad51-II3A::KanMX SPC110-YFP::HIS3 RAD52-CFP lys2* | W303 | This Study |
| W11207 | W11142-4A X W11143-15D | W303 | This Study |
| W11174-27D | *MATα rad51-S192A ura3::3xURA3-tetOx112 I-SceI(ura3-1) TetR-RFP* | W303 | This Study |
| W11175-43D | *MATa rad51-S192A SPC110-YFP::HIS3 RAD52-CFP* | W303 | This Study |
| W11208 | W11174-27D X W11175-43D | W303 | This Study |
| W11197-21D | *MATα ADE2 rad51-S192E RAD52-CFP ura3::3xURA3-tetOx112 I-SceI(ura3-1) TetR-mRFP1(iYGL119W)* | W303 | This Study |
| W11198-24B | *MATa ADE2 rad51-S192E lys2 SPC110-YFP::HIS3 RAD52-CFP* | W303 | This Study |

| W11165-1C | *MATa rad52::KanMX* | W303 | This Study |
|---|---|---|---|
| W11154-2B | *MATα rad52::KanMX lys2::GAL-I-SceI ura3::3xURA3-tetOx112 I-SceI(ura3-1) TetR-mRFP1(iYGL119W) YFP-RAD51* | W303 | This Study |
| W11210 | W11165-1C X W11154-2B | W303 | This Study |
| W11000-6C | *MATa ADE2 SPC110-YFP::HIS3 DDC1-CFP rad52::KanMX* | W303 | This Study |
| W11006-4A | *MATα rad52::KanMX lys2::GAL-I-SceI ura3::3xURA3-tetOx112 I-SceI(ura3-1) TetR-mRFP1(iYGL119W)* | W303 | This Study |
| W11211 | W11000-6C X W11006-4A | W303 | This Study |
| W11147-6A | *MATa ADE2 SPC110-YFP::HIS3 DDC1-CFP rad52::KanMX rad51::HPH* | W303 | This Study |
| W11153-8B | *MATα rad52::KanMX rad51::HPH lys2::GAL-I-SceI ura3::3xURA3-tetOx112 I-SceI(ura3-1) TetR-mRFP1(iYGL119W)* | W303 | This Study |
| W11212 | W11147-6A X W11153-8B | W303 | This Study |
| CBY88 | *MATa RAD53-HA::LEU2 GALS-DDC1-GFP-LACI::URA3 GAL-DDC2-GFP-LACI::HIS3 ddc1Δ 256x LACO::TRP1 rad5* | W303 | Bonilla et al., 2008 |
| W10035-11D | *MATα RAD53-HA::LEU2 GALS-DDC1-GFP-LACI::URA3 GAL-DDC2-GFP-LACI::HIS3 ddc1Δ* | W303 | This Study |
| W10035-13D | *MATα RAD53-HA::LEU2 GALS-DDC1-GFP-LACI::URA3 GAL-DDC2-GFP-LACI::HIS3 ddc1Δ 256xLACO::TRP1* | W303 | This Study |
| W9913-42A | *MATa ADE2 ura3::3xURA3-TetOx112 I-SceIcs(ura3-1) TetR-mRFP1(iYGL119W) SPC110-YFP::HIS3 RAD52-CFP lys2* | W303 | This Study |
| W11213 | W10035-11D X W9913-42A | W303 | This Study |
| W11214 | W10035-13D X W9913-42A | W303 | This Study |

| | | | |
|---|---|---|---|
| W11147-6A | *MAT**a** ADE2 SPC110-YFP::HIS3 DDC1-CFP rad52::KanMX rad51::HPH* | W303 | This Study |
| W11153-8B | *MATα ADE2 rad52::KanMX rad51::HPH lys2::GAL-I-SceI ura3::3xURA3-tetOx112 I-SceI(ura3-1) TetR-mRFP1(iYGL119W)* | W303 | This Study |
| W11305 | W11147-6B X W11153-8B | W303 | This Study |
| W11301-8A | *MAT**a** rad52Δ409-412 SPC110-YFP::HIS3 DDC1-CFP* | W303 | This Study |
| W11302-25A | *MATα ADE2 rad52Δ409-412 ura3::3xURA3-tetOx112 I-SceI(ura3-1) TetR-mRFP1(iYGL119W) lys2::GAL-I-SceI* | W303 | This Study |
| W11306 | W11301-8A X W11302-25A | W303 | This Study |

All strains are *RAD5+* derivatives of W303[86] unless otherwise noted.

**Table 2-S2:** Summary of Results and Statistics, related to Methods.

Table S2: Summary of Results and Statistics, related to Methods

| Fig. | Experiment | Cells | Rc (nm) | ΔRc | 95% CI [low, high] | p-value |
|---|---|---|---|---|---|---|
| 1B | WT | 14 | 490 ± 20 | 150 | [50, 250] | 0.004** |
|  | WT +40Gy | 12 | 570 ± 30 | | | |
| 1C | *rad51Δ* | 10 | 490 ± 30 | -40 | [-110, 80] | 0.5 |
|  | *rad51Δ* +40Gy | 14 | 480 ± 50 | | | |
| 2C | G1 WT | 30 | 570 ± 70 | 220 | [10, 500] | 0.02* |
|  | G1 WT +40Gy | 23 | 730 ± 100 | | | |
| 2D | G1 *rad51Δ* | 18 | 670 ± 70 | -20 | [-170, 100] | 0.7 |
|  | G1 *rad51Δ* +40Gy | 16 | 640 ± 50 | | | |
| 3B | WT | 14 | 490 ± 20 | 150 | [50, 270] | 0.005** |
|  | *rad51-II3A* +40Gy | 16 | 590 ± 60 | | | |
| 3B | *rad51-II3A* | 15 | 530 ± 40 | 80 | [-40, 230] | 0.2 |
|  | *rad51-II3A* +40Gy | 16 | 590 ± 60 | | | |
| 3B | WT | 14 | 490 ± 20 | 70 | [-30, 180] | 0.1 |
|  | *rad51-II3A* | 15 | 530 ± 40 | | | |
| 3C | *rad51-K191A* | 15 | 470 ± 60 | 30 | [-80, 130] | 0.6 |
|  | *rad51-K191A* +40Gy | 13 | 490 ± 30 | | | |
| 3D | *rad51-K342E* | 14 | 470 ± 40 | 160 | [40, 290] | 0.01* |
|  | *rad51-K342E* +40Gy | 22 | 610 ± 50 | | | |
| 4B | *rad52Δ* | 15 | 440 ± 20 | 150 | [40, 300] | 0.007** |
|  | *rad52Δ* +40Gy | 14 | 580 ± 100 | | | |
| 4C | *rad52Δ* | 15 | 440 ± 20 | 250 | [140, 340] | < 0.001*** |
|  | *rad52Δ* +Gal | 10 | 650 ± 40 | | | |
| 4D | *rad51Δ rad52Δ* | 19 | 660 ± 60 | -100 | [-270, 80] | 0.2 |
|  | *rad51Δ rad52Δ* +40Gy | 12 | 620 ± 100 | | | |
| 4E | *rad51Δ rad52Δ* +Caffeine | 16 | 420 ± 40 | -20 | [-100, 80] | 0.7 |
|  | *rad51Δ rad52Δ* +Caffeine +40Gy | 11 | 410 ± 30 | | | |
| 4G | *rad52Δ409-412* | 17 | 480 ± 40 | 0 | [-100, 100] | 1.0 |
|  | *rad52Δ409-412* +40Gy | 32 | 490 ± 30 | | | |
| S3A | WT +Caffeine | 11 | 490 ± 40 | -30 | [-160, 110] | 0.8 |
|  | WT +40Gy +Caffeine | 6 | 450 ± 40 | | | |
| S3B | G1 WT +Caffeine | 16 | 580 ± 80 | -30 | [-230, 110] | 0.7 |
|  | G1 WT +40Gy +Caffeine | 15 | 570 ± 40 | | | |
| S3C | -LacOx256 +Gal | 12 | 480 ± 50 | 190 | [40, 330] | 0.01* |
|  | +LacOx256 +Gal | 11 | 610 ± 50 | | | |
| S3C | +LacOx256 | 10 | 460 ± 50 | 170 | [0, 320] | 0.06† |
|  | +LacOx256 +Gal | 11 | 610 ± 50 | | | |
| S3C | +LacOx256 +Gal | 11 | 610 ± 50 | -240 | [-330, -110] | 0.001** |
|  | +LacOx256 +Gal +Caffeine | 13 | 410 ± 30 | | | |
| S3D | -LacOx256 | 10 | 450 ± 20 | 10 | [-70, 140] | 0.7 |
|  | -LacOx256 +Gal | 12 | 480 ± 50 | | | |
| S3E | *rad51-S192A* | 15 | 480 ± 20 | 40 | [-40, 120] | 0.4 |
|  | *rad51-S192A* +40Gy | 10 | 480 ± 30 | | | |
| S3F | *rad51-S192E* | 14 | 470 ± 30 | -30 | [-120, 80] | 0.5 |
|  | *rad51-S192E* +40Gy | 11 | 480 ± 50 | | | |

Rc values include error ranges of 1 SEM. Daggers (†) indicate experiments with *p*-values less than 0.1. Single asterisks (*) represent p-values less than 0.05.

**Table 2-S3:** Summary of Rad51 and Rad52 focus counting results, related to Figure 2-2.

Table S3: Summary of Rad51 and Rad52 Focus Counting Results, Related to Figure 2

| Experiment | Total Cells Analyzed | Independent Experiments | Mean Percent Foci |
|---|---|---|---|
| Rad52 foci in diploid G1-0 Gy | 26 | 2 | 0% |
| Rad52 foci in diploid G1-40 Gy | 41 | 4 | 5% |
| Rad52 foci in diploid S-0 Gy | 97 | 2 | 16% |
| Rad52 foci in diploid S-40 Gy | 159 | 4 | 58% |
| Rad51 foci in diploid G1-0 Gy | 143 | 3 | 2% |
| Rad51 foci in diploid G1-40 Gy | 183 | 3 | 40% |
| Rad51 foci in diploid S-0 Gy | 115 | 3 | 6% |
| Rad51 foci in diploid S-40 Gy | 148 | 3 | 89% |
| Rad51 foci in haploid G1-0 Gy | 38 | 1 | 3% |
| Rad51 foci in haploid G1-40 Gy | 107 | 1 | 30% |
| Rad51 foci in haploid S-0 Gy | 52 | 1 | 6% |
| Rad51 foci in haploid S-40 Gy | 132 | 1 | 82.6% |
| Rad52 foci dual-tag strain G1-0 Gy | 32 | 1 | 3% |
| Rad52 foci dual-tag strain G1-40 Gy | 55 | 1 | 2% |
| Rad52 foci dual-tag strain S-0 Gy | 29 | 1 | 10% |
| Rad52 foci dual-tag strain S-40 Gy | 50 | 1 | 24% |
| Rad51 foci dual-tag strain G1-0 Gy | 32 | 1 | 0% |
| Rad51 foci dual-tag strain G1-40 Gy | 55 | 1 | 49% |
| Rad51 foci dual-tag strain S 0-Gy | 29 | 1 | 20% |
| Rad51 foci dual-tag strain S 40-Gy | 50 | 1 | 80% |

**CHAPTER 3:**

**MODULATION OF CHROMATIN STATE INFLUENCES THE MOBILITY OF THE *URA3***

**LOCUS**

# Abstract

It has been long appreciated that chromosomes are confined to particular territories of eukaryotic nuclei. In the budding yeast, *Saccharomyces cerevisiae*, these territories are in part defined by the attachment of centromeres and telomeres to tethers at the nuclear periphery. However, despite this apparently static configuration, changes in chromosome position and mobility must occur to promote various cellular processes, including homology search during homologous recombination. How the territories of individual loci are determined and regulated has not been well established. Here, using the *URA3* locus as a model site, I investigate factors that contribute to the baseline mobility of loci both in undamaged and damaged cells. I find that while the inhibition of both microtubule and actin polymerization does not affect the mobility of this locus, modulation of global chromosome state through HDAC inhibitor treatment or *HTZ1* deletion profoundly alters movement. In addition, I find that the chromatin remodeler and translocase Rdh54 is required for increased global mobility in response to damage, and that mobility is required for the global reorganization of repair centers following induction of multiple DSBs. Together, these results support the notion that regulation of chromatin state is critical for the mobility of loci in both damaged and undamaged nuclei, and that increased mobility is important for the clustering of multiple repair sites.

# Introduction

The nucleus is the defining feature of the eukaryotic cell, and its composition and contents are highly regulated in different cell types and species. The principal component of the nucleus is the chromatin, and the positions of the individual chromosomes that make

up the genome of the eukaryotic cells are also highly organized. It has become appreciated that chromosomes occupy distinct, partially heritable territories in the nucleus[30]. Despite this organization, various cellular processes require that the position of chromosomes be altered. For example, heterochromatic and euchromatic regions are positioned in different nuclear subdomains[94], as is the rDNA in the nucleolus[40]. An additional circumstance in which chromosome repositioning is critical is during the repair of DSBs by homologous recombination, as homologous sequences must physically interact to repair. A static conception of nuclear organization is thus at odds with the observed behavior of cells.

Seminal studies in budding yeast have indicated that although chromosomal loci are confined to particular domains when observed over long time intervals, MSD/MSCD analysis reveals that loci are highly dynamic over shorter intervals, and undergo confined Brownian diffusion[31,32]. Later studies in which loci were examined with millisecond time interval resolution have shown that loci undergo anomalous diffusion (subdiffusion), which indicates that motion is modulated spatiotemporally by scaling properties[95,96]. The driving forces behind this diffusive movement have not been fully explained. Marshall and colleagues reported that the diffusive movements observed were not sensitive to ATP depletion, and argued that collisions of the chromosomal loci with charged particles in the solvent (per classical Brownian diffusion) were the likely drivers of motion. However, Heun and colleagues found that larger movements were sensitive to ATP depletion and argued for some actively facilitated diffusion. Similar findings have been made in microrheology experiments in mammalian cells, where "cage-hopping" of confined beads is diminished in ATP depleted cells, but movements within nucleoplasmic "cages" are preserved[97]. A model that may account for these observations is reptation of DNA through a polymer melt[98].

In addition to the inherent mobility of a locus, diffusive behaviors may also be defined by the mechanisms by which loci are confined. Several mechanisms have been proposed for confinement, including tethering of chromosome arms to both centromeric and telomeric points of attachment[58,61,74], as well as the presence of chromatin remodelers[99], and the loading of cohesin[59]. Thus, while a chromosomal locus at short time scales may be induced to move through collisions with solvent, over longer periods of observation, actively regulated structures may corral the DNA segment. This model may explain the differing results obtained in the previously mentioned studies. It is also worth noting that the crowding of the nucleus itself can affect molecular diffusion[100], and the mobility of DNA is in part constricted by its own polymeric properties[101].

To clarify which factors contribute to the mobility and confinement of chromosomal loci, I assayed the contributions of several factors to the movement of the *URA3* locus. I find that the baseline confinement of this locus is not affected by microtubule or actin poisons, although treatment with DMSO or DMSO plus nocodazole prevents global mobility. I find that the confinement of *URA3* is increased by treating cells with the HDAC inhibitor valproic acid, which suggests that chromatin state is particularly important for the mobility of loci. To investigate how modulation of chromatin state affects increased mobility after DNA damage, I examined cells bearing deletions in *RDH54* and *HTZ1*, a chromatin remodeler[102] and a histone variant respectively. I find that the response to DSBs is prevented in these backgrounds. Last, I examined how mobility might contribute to the reorganization of the repair foci following formation of multiple DSBs. These results indicate that chromatin state, and not tether detachment, most noticeably affects the mobility of the *URA3* locus in both damaged and undamaged conditions.

# Results

**Nuclear Volume Does Not Increase Following DSB Induction**

First, I examined nuclear volumes in damaged and undamaged diploid cells to rule out the trivial explanation that confinement is defined and increased by changes in the size of the nucleus following DSB formation. In this view, an expansion in nuclear volume would lead to the expansion of an individual locus' radius of confinement. The status and plasticity of the nuclear envelope has been associated with many aspects of DNA metabolism, particularly DNA repair and replication[103]. For example, recent work by the Foiani group has shown that the ATR checkpoint kinase becomes associated with the nuclear envelope when mechanical stress is applied to the nucleus[104]. It has also been found that irreparable breaks in haploids are eventually redistributed to the nuclear periphery where alternative repair processes or *de novo* telomere addition may occur as salvage pathways[39].

To assess whether the nucleus changes in volume in response to DSBs, I tagged a component of the nuclear pore complex, Nic96 with GFP and observed wild-type cells either undamaged or following treatment with 40 Gy gamma radiation. I measured nuclear volume by assuming the shape of the nucleus to be spherical and measuring the diameter of the longest axis of the z-stack featuring the nuclear cross-section of the largest area (Figure 3-1, A). The thickness of the Nic96-GFP signal band was non-trivial, therefore, only the inner diameters of the object thus defined were measured.

When this analysis was applied to undamaged cells, the resultant volume measurements agreed well with previously published data[105,106]. We found that

undamaged cells had a median volume of 5.55 cubic microns (N = 59 cells). Irradiated cells

did not show a significant increase (Two-Sample t-test p value = .32) in volume with a

mean volume of 5.07 cubic microns (N = 94 cells) (Figure 3-1, B). Because nuclear volumes

can change throughout the cell cycle[106], I divided cells into groups based on bud-to-mother

ratio, a convenient proxy for progression through the cell cycle. No significant increase in

volume was observed between groups of undamaged or damaged cells of the same B/M

ratio (Figure 3-1, B). The nucleus remains largely spherical before and after irradiation, as

the average longest diameter was unchanged before and after irradiation (2.2 microns and

2.1 microns respectively).

Therefore, I conclude that the change in locus territories observed during local and

global mobility is not a result of a greater reorganization of the nucleus itself.


**Elimination of microtubule or actin polymerization does not affect the baseline**

**mobility of the *URA3* locus**

In both budding yeast and mammalian cells, the cytoskeleton has been implicated in

nuclear organization. For example, mammalian SUN and KASH proteins link the

microtubule network of the cytoplasm to the nuclear envelope, and it has been suggested

that direct contacts may occur between chromatin and these factors[57,107]. In budding yeast,

microtubules have a profound effect on chromosome organization throughout the cell

cycle[108], and the organization of chromosomes within the nucleus has been shown to be

consequential to recombination outcomes[109].

As such, cytoskeletal components such as microtubules and actin have been

investigated for their contributions to chromosome territories and mobility. Marshall and

colleagues found that the *LEU2* locus in diploid yeast expanded its radius of confinement after treatment with the microtubule poison nocodazole[31]. Others have reported that tethering of centromeres to the SPB influences mobility in undamaged[58] as well as damaged[61,74] cells. Therefore, I treated cells with 15 μg/mL nocodazole and measured the mobility of the *URA3* locus before and after treatment with 40 Gy of ionizing radiation by MSCD analysis. No change in mobility was observed in undamaged cells after nocodazole treatment and global mobility did not occur following irradiation (Figure 3-2, A, undamaged: 420 ± 20 nm, N = 8 cells, + 40 Gy: 460 ± 60 nm, N = 9 cells). The p value of undamaged, nocodazole treated cells compared to wild type is .6, and the p value of undamaged nocodazole treated cells compared to irradiated treated cells is .32. However, DMSO vehicle treated controls also failed to undergo global mobility (Figure 3-2, B, undamaged: 480 ± 30 nm, N = 16 cells, + 40 Gy: 450 ± 50 nm, N = 14 cells, p-value: .49), making interpretation of these results difficult. DMSO treatment may alter the characteristics of the nucleoplasm, as actin bundle formation has been observed following DMSO treatment in *Amoeba, Dictylostelium,* and human cells[110]. In either case, neither nocodazole nor DMSO affected the baseline mobility of the *URA3* locus in undamaged cells, suggesting that microtubule attachments to centromeres do not broadly influence the mobility of all loci along a chromosome.

In addition to microtubule networks, yeast and mammalian cells also feature actin networks, which serve many purposes. Budding yeast feature three main types of actin: cortical patches, implicated in endocytosis, actin cables, involved in cell polarity, and the actomyosin ring, critical for cytokinesis[111]. Cytoplasmic actin has been shown to transduce force through the nuclear membrane via Csm4 during meiotic prophase to facilitate a

reorganization of telomeres into a so-called "bouquet" structure[112,113], which may serve to discourage spurious attachments between chromosomes during homologous pairing[66]. Cells bearing mutations in actin-related proteins (which, notably share only approximately 17-60% identity with conventional actin[114]) such as Arp5 and Arp8, members of the INO80 chromatin remodeling complex, have defects in global mobility following DNA damage[46,48], while treatment of cells with the actin poison Latrunculin A(LatA)[115] reduced subdiffusive dynamics of chromatin loci[60]. The authors of the latter study argue that this reduction occurs through a combination of mechanisms including effects on forces applied to the nuclear envelope as well as on actin and actin-related proteins within chromatin remodeling complexes. In addition, actin is present within the nucleus, and has been implicated in several processes including transcription, maintenance of a nuclear endoskeleton, and chromatin remodeling[114]. While nuclear actin exists largely in a monomeric state, polymerization has been observed under several stress conditions in mammalian cells (including DMSO treatment, heat shock, and ATP depletion[116,117]), as well as in serum stimulated quiescent fibroblasts[118] and cells suffering DNA damage[119]. However, the precise contributions of nuclear actin to cellular processes has remained largely unclear.

To assess the contribution of actin to the mobility of the undamaged *URA3* locus, I treated cells with 200 µM LatA and measured MSCD values with 10 second time intervals. I observe no change in Rc between untreated and LatA treated cells (Figure 3-2, C, undamaged: 450 ± 10 nm, N = 5 cells, p-value compared to wild type: .62), suggesting that the activity of neither cytoplasmic or nuclear actin or actin-related species are required for the baseline mobility previously observed. One important caveat to these results concerns

the time scale of image acquisition. Spichal and colleagues[60] reported an effect of LatA when MSD values were determined with a 100ms time intervals. While I observed plateaued MSD plots, denoting confinement of the tracked particle, Spichal and colleagues report anomalous diffusion. The diffusive behavior of chromosomes changes based on the time interval of acquisition, and different effects are observable at different time scales (Mine-Hattab, unpublished data), therefore the influence of Lat A on *URA3* locus dynamics may not be observable on this scale. However, this result suggests that if actin, either within or outside of the nucleus, has any effect on the mobility of the *URA3* locus, it is modest, and most likely not contributory to the overall dynamics of this locus in the absence of damage.

**An Inhibitor of Histone Deacetylation Reduces *URA3* Locus Mobility**

Since altering chromosomal tethers did not appreciably alter steady-state *URA3* locus mobility, I next examined whether alterations in chromatin state affect mobility. Within the nucleus, DNA is packaged into a tight network by wrapping around histone octamers. Histone proteins can be modified in myriad ways, with various histone modifications implicated in a host of cellular processes from DNA repair, to transcriptional silencing. Given the impact of chromatin structure on diverse aspects of biology, it is reasonable to suspect that varying that structure may affect locus mobility.

Several studies have supported the hypothesis that chromatin remodeling is consequential for mobility. A 2012 study reported that targeting of the INO80 complex directly to a locus using a LexA fusion led to increased mobility at that site[99]. Seeber and colleagues[46] observed that members of the INO80 chromatin remodeling complex were

required for global mobility in haploid cells following zeocin treatment or induced activation of the checkpoint[47]. Hauer and colleagues reported in 2017 that histone eviction following DNA damage led to increased mobility of a site[48]. And recently, simulations have suggested that chromosome mobility may be achieved by local changes to the chromatin state around a break, leading to extrusion of damaged DNA from the compacted chromatin mass[95]. While INO80 and its putative remodeling effects on mobility have been recently well-studied, the influence of histone modifications has not been examined in great detail. To that end, in order to understand how histone modifications might affect mobility, we treated cells with an inhibitor of histone deacetylases, valproic acid (VPA)[120].

Histone acetyl transferases (HATs) and histone deacetylases (HDACs) act on both histone and non-histone proteins[121-123]. Histone acetylation has effects on transcription[124], and several studies suggest that histone acetylation occurs in response to DNA damage[123,125]. VPA is a drug used to treat epilepsy, which has recently shown promise alongside other HDAC inhibitors as a potential cancer therapeutic[126]. In yeast, VPA treatment has been shown to have broad effects on the DNA damage response, inhibits checkpoint activation and resection, and promotes autophagy of key resection factors[127]. Since histone acetylation is linked to various aspects of nuclear organization and metabolism, I reasoned that altering global acetylation levels through drug treatment might alter the mobility of chromosomal loci. Therefore, I treated yeast cells with 10mM VPA for 30 minutes and assessed the mobility of the *URA3* locus in undamaged yeast cells. VPA treatment profoundly alters *URA3* locus dynamics, reducing the Rc from 490 ± 20 nm in untreated WT cells to 300 ± 30 nm in VPA treated cells (N = 10 cells, p value compared to untreated, .01). Thus, alteration of global chromatin state is capable of changing the local

dynamics of loci, and, in the case of VPA seems to further constrain the movement of those loci and increase their overall confinement. I was not able to measure the mobility of damaged loci in the presence of VPA because treatment prevented the formation of Rad52 foci (data not shown), which are required to discriminate damaged from undamaged cells in our experimental system. This result may be due to reduced resection as shown by Robert and colleagues[127].

The cause of this phenomenon may be either direct or indirect. If direct, it is possible that over acetylation of histones brought on by VPA treatment alters the condensation of DNA and thus its physical polymeric characteristics. Alternatively, an indirect change in mobility may occur through altered transcription or protein metabolism. In either case, the balance of histone modifications appears to have a strong effect on the mobility of chromosomal loci.

**Rdh54 is required for global mobility**

Rad54 is a member of the Rad52 epistasis group (*RAD50, RAD51, RAD52, RAD54, RDH54/TID1, RAD55, RAD57, RAD59, MRE11,* and *XRS2*) and a member of the Swi1/Snf2 family of chromatin remodeling proteins. It is involved in both stabilization of the Rad51 filament *in vitro*[128,129] and facilitates D-loop formation during strand exchange[20,21,130]. Importantly, Rad54 has also been shown to be important for local mobility in haploids[43]. *RAD54* has a closely related homolog *RDH54/TID1*, which shares Rad54's translocase activity and Swi1/Snf2 family membership[131]. Interestingly, *rdh54* strains display a diploid-specific sensitivity to DNA damaging agents and are less able to perform interhomolog recombination[54]. Rdh54 interacts physically with Rad51[132] and removes Rad51 from

undamaged DNA to prevent the formation of toxic nonrecombinogenic complexes[133].

Rdh54 also plays a role in DNA damage checkpoint modulation, and is required for a cell to

adapt to a persistent DSB[134]. In addition, Rdh54 is phosphorylated in a Mec1 and Rad51

dependent manner[135]. Lastly, both Rad54[136] and Rdh54[102] are chromatin remodelers.

As Rdh54 is functionally similar to Rad54, and influences chromatin remodeling and

checkpoint adaptation, I assessed the contribution of Rdh54 to global mobility. In addition,

the importance of the chromatin state for baseline mobility suggested that repair genes

with chromatin remodeling activity would be good candidates for regulators of increased

movement. When *rdh54Δ* cells were examined following treatment with IR, I found that

global mobility did not occur (Figure 3-5, Undamaged: 480 ± 40 nm, N = 16 cells, +40 Gy:

460 ± 40 nm, N = 9 cells, p value =.18). This result raises several interesting possibilities.

First, if Rdh54 is required for mobility in a similar manner to Rad54, this result implies that,

in diploids, both genes are independently required, and, despite the closely shared roles of

both proteins, they cannot compensate for one another. Alternatively, as previous

experiments were tests of local mobility in haploids, it is possible that the requirement for

Rdh54 observed here is either diploid or global mobility specific. Thus, more tests of this

deletion, in background of both ploidies, will be required to fully understand the role of

these translocases (See Chapter 4 for additional discussion).


**Htz1 is required for local mobility**

In addition to modifications of histone tails (as discussed above), whole histone

proteins can be inserted in particular chromosomal regions to accomplish various

functions. The most well-known example of which is the H3 variant CENP-A (Cse4 in

budding yeast) which denotes centromeric regions of the genome[137]. One other variant of particular interest to the study of DNA repair is H2A.Z (Htz1). H2A.Z is a histone H2A variant distributed widely, but non-randomly throughout the genome, preferentially at inactive promoters as well as other intergenic regions[138]. It is inserted into DNA by the Swr1 complex, and deletions of this loading complex lead to sensitivity to DNA damaging agents[139-141]. Htz1 deposition may be required to prevent the spreading of silenced chromatin into certain domains of the genome, including telomere proximal genes and genes close to the silenced mating type loci[142]. Importantly, there is evidence that Htz1 is incorporated into the DNA surrounding a DSB in a manner that is antagonized by the Ino80 complex[143,144].

Since Swr1 and Htz1 have been implicated in DNA repair and nuclear organization, I made a preliminary analysis of the effects of Htz1 on the mobility of the *URA3* locus following the induction of a site-specific break at that locus. In undamaged cells, I observed that the mobility of the *URA3* locus was decreased significantly compared to wild-type cells (p value = .01), which indicates that the absence of this histone variant influences the overall mobility of the DNA, similarly to VPA treatment (see above), and when a DSB is induced at the *URA3* locus, I observe a small, but not statistically significant increase in mobility (Figure 3-6, undamaged: 370 ± 20 nm, N = 11 cells, + galactose: 460 ± 60 nm, N = 6 cells, p-value: .1). Despite the small number of cells analyzed, this result is reminiscent of the increase in mobility observed in *rad52Δ* cells (see Chapter 2) and suggests that *htz1Δ* cells undergo global mobility, but not local. A more robust analysis will be required to investigate this possibility. Local mobility is also blocked in *htz1Δ* haploid cells, although global mobility was not examined[145].

How does Htz1 deposition influence local mobility? A trivial explanation is that local mobility is delayed similarly to a *sae2Δ* mutant strain[44], as delays in resection have been observed in *htz1Δ* strains[146]. Extending the time of I-*Sce*I induction may eventually produce increased mobility in *htz1Δ* strains. Alternatively, Horigome and colleagues have argued that Htz1 deposition is required for a persistent DSB to relocate to the nuclear periphery[145]. It is unclear, however, why an S phase haploid, competent for sister chromatid exchange, or a diploid might relocate breaks to the nuclear periphery, seemingly distant from possible donor sequences. The Swr1 complex has also been reported to act in opposition to the Ino80 complex during early DSB processing, and this interplay appears to influence checkpoint activation[143]. Thus, the lack of mobility observed in *htz1Δ* strains may also reflect alterations in Ino80 recruitment and checkpoint activation. Additional experiments will be required to understand how to regulation of chromatin states after DSB formation contribute to the DNA damage checkpoint and increased mobility.

## Reorganization of repair foci is diminished in *rad51Δ* strains

Next, I examined whether defects in increased chromosome mobility affected the reorganization of repair foci following the appearance of multiple DSBs. The formation of a DSB initiates a complex cascade of reactions that eventually lead to HR. Various proteins load onto the damaged site, perform their function, and are displaced by subsequent factors that act upon the newly processed substrate. Because of the abundance of these proteins, the order of this cascade can be observed through the use of fluorescent protein tags, as many repair factors such as Mre11 and Rad52 form bright, visible foci after cells are treated with DNA damaging agents. Using this strategy, Lisby and colleagues[26]

elaborated the order in which factors load, as well as which factors were dependent upon the loading of others for focus formation.

Rad52 has been used as a general marker for DSB formation in various studies, including the present work. However, it is important to note that, the number of Rad52 foci does not necessarily correlate with the number of DSBs in the cell. In 2003, Lisby and colleagues reported that even after treatment with 800 Gy of gamma radiation, which should generate on average 80 DSBs per nucleus, cells only generated on average 2 or 3 Rad52 foci[37]. This result indicates that multiple damage sites migrate to a smaller number of repair centers. I reasoned that this migration may be influenced by DNA damage induced increased chromosomal mobility, and set out to test this notion by examining the number of Rad52 foci formed in both WT and mobility-defective *rad51Δ* cells after 40 Gy of gamma. I found that there was a statistically significant increase in the number of Rad52 foci per cell in the *rad51Δ* strain after radiation (WT median = 0 foci per cell, N = 87 cells, *rad51Δ* median = 2 foci per cell, N = 153 cells. Wilcoxon ranksum p value = $1.7 \times 10^{-12}$, Figure 3-4 A). In addition, since *rad51Δ* cells form spontaneous foci at a fairly high rate[147], I also restricted my analysis only to WT and *rad51Δ* cells which had at least one focus after DNA damage (WT median = 1 foci per cell, N = 37 cells, *rad51Δ* median = 2 foci per cell, N = 130 cells. Wilcoxon ranksum p value = .0041· Figure 3-4 B). Thus, in either case, the absence of Rad51 leads to a modest increase in the number of foci per cell. Although there are alternative explanations for these results, such as possible defects in end-processing and repair, this finding is consistent with the hypothesis that increased chromosomal mobility is important for the colocalization of multiple DSBs.

# Discussion

Developing a complete understanding of how chromosomal loci are confined in yeast has been difficult, owing to the wide differences between yeast strains, chromosomal loci, and imaging regimes used to measure diffusion. At present, multiple models for the establishment of confinement for individual loci have been proposed, which are largely centered on the notions of nuclear envelope attachment and chromatin remodeling[70,71]. Here, examining the *URA3* locus, I have found that the factors that most affect mobility are related to pathways involved in the regulation of chromatin state. The state of the chromatin is important not only for the determination of the baseline mobility of a locus, but also for increased mobility following DSB formation. Recent data and statistical simulations suggest that detachment from tether points alone is not sufficient to alter movement along the entire length of a chromosome arm[74,148]. The data presented here supports these simulations. Therefore, I propose that a second mechanism must account for the establishment and modulation of confinement at sites not proximal to tethers, such as the *URA3* locus[149]. Because alterations to global histone acetylation (Figure 3-3) or H2A variant loading (Figure 3-6) alter baseline mobility, it seems likely that the relative compaction of the chromatin fiber may be the key determinant of medial locus mobility. This notion is supported by a recent study which reports that chromatin stiffening is a major contributor to modulated mobility after DNA damage[148].

How might these alterations affect baseline mobility and how might chromatin remodeling induce mobility? Loci may become more confined in VPA treated cells because more loosely packed chromatin bound by acetylated histones transmits force along its length less effectively than a tightly packed fiber would. The reduction observed in cells

lacking Htz1 may reflect improper spreading of histone marks into inappropriate domains, or defects in the initiation of chromatin stiffening after DNA damage[148]. It is also possible that these modifications have direct effects on the ability of the DNA polymer to diffuse or reptate[98]. In physiological settings, alteration of chromatin modifications either by remodeling complexes such as Ino80, Swr1, or Rad54/Rdh54 may induce these changes in response to DNA damage in order to accomplish increased mobility. Additional work will be required to understand the precise nature of chromatin modifications induced after DSBs and how those changes physically alter exploration of chromosomal loci.

## Methods

### Strains

All strains are *RAD5*+ derivatives of W303 [86,87],[88] (See Table 3-1).

### Galactose induction

Galactose induction was performed as in Chapter 2. I-*Sce*I was induced in *htz1Δ* strains for 120 minutes.

### Inhibitor treatment

For nocodazole studies, cultures were made 1% DMSO before 15 μg per mL nocodazole was added. Latrunculin A was used at 200 μM and dissolved in ethanol as a vehicle.

Valproic acid was used at a final concentration of 10 mM. In all cases, cells were incubated

with drug at room temperature for 15 to 30 minutes before being prepared for microscopy.


## γ-irradiation

Irradiation was performed as in Chapter 2


## Microscopy

Microscopy was performed as in Chapter 2. Twenty second time intervals were used for

nocodazole and DMSO treated cells in order to better capture the plateau of the MSCD plot.


## Image analysis

MSD analysis was performed as in Chapter 2. For MSCD analysis, the distance between

TetO:TetR-RFP and LacO:LacI-YFP labeled *URA3* homologs was taken at each time point as

in[31]. MSCD and MSD analysis are essentially interchangeable methods of determining

confinement provided that the two homologs tracked have similar confinement[44]. Error

bars on MSD and MSCD plots represent one standard error of the mean (SEM) at each $\Delta t$

value. Measures of nuclear volume were obtained by imaging cells in which the nuclear

pore component Nic96 had been tagged with GFP (see Figure 3-1A). The nucleus was

assumed to be spherical, and the longest cross section was used to manually measure a

diameter using Volocity (Perkin-Elmer). Each measurement was made three times and

averaged to account for imprecision in the method. These average diameters were used to calculate a volume for each individual cell. Bud-to-mother ratios were determined similarly by assuming mother and buds to be spherical and taking a single measurement along the longest diameter for each cell. These values were then divided to calculate the final ratio. Focus counts were performed as in Chapter 2.

**Statistics**

Statistics for mobility experiments were performed as in Chapter 2. Nuclear volume analysis was performed in Prism (GraphPad), and Student's T test was used to determine significance. Foci per cell analyses were performed in Microsoft Excel and MATLAB (Mathworks). The distribution of foci per cell did not meet assumptions of normality, therefore the Wilcoxon ranksum test was used in order to determine significance.
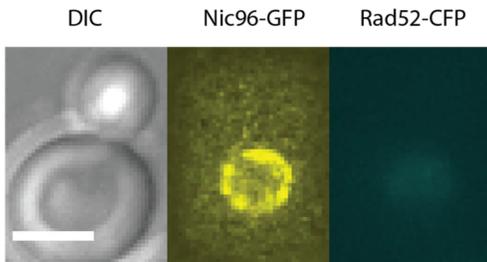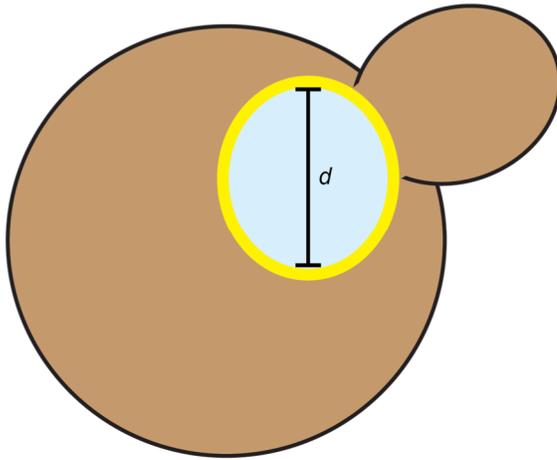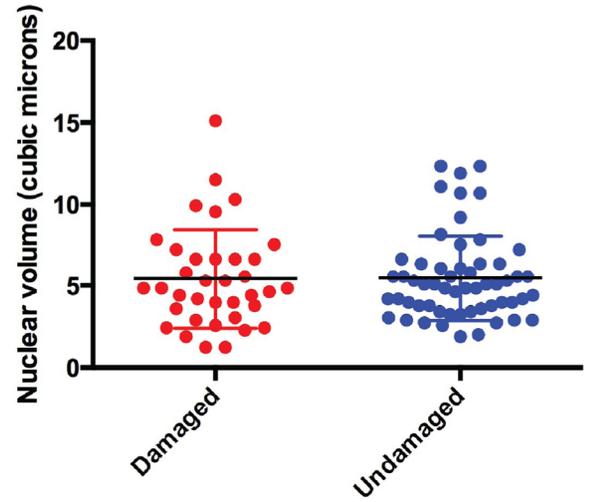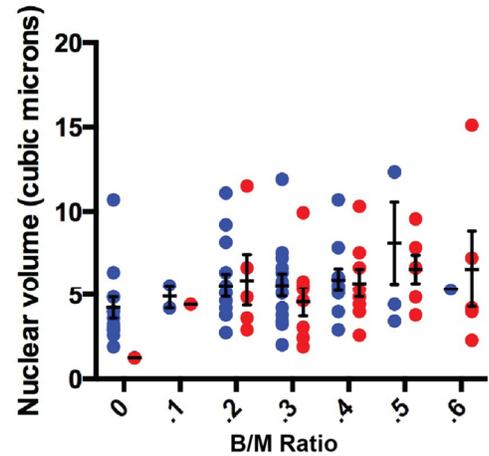
**Figure 3-1:** Nuclear volume in diploids is not changed after irradiation. **A**) Schematic of system and measurement methodology, top, and image of representative cell. Scale bar = 2.8 microns. **B**) Nuclear volumes of damaged (roughly 15 minutes after treatment with 40 Gy) and undamaged cells. The results do not differ significantly. **C**) As in B, with cells separated according to bud-to-mother ratio. Again, results are not significantly different.
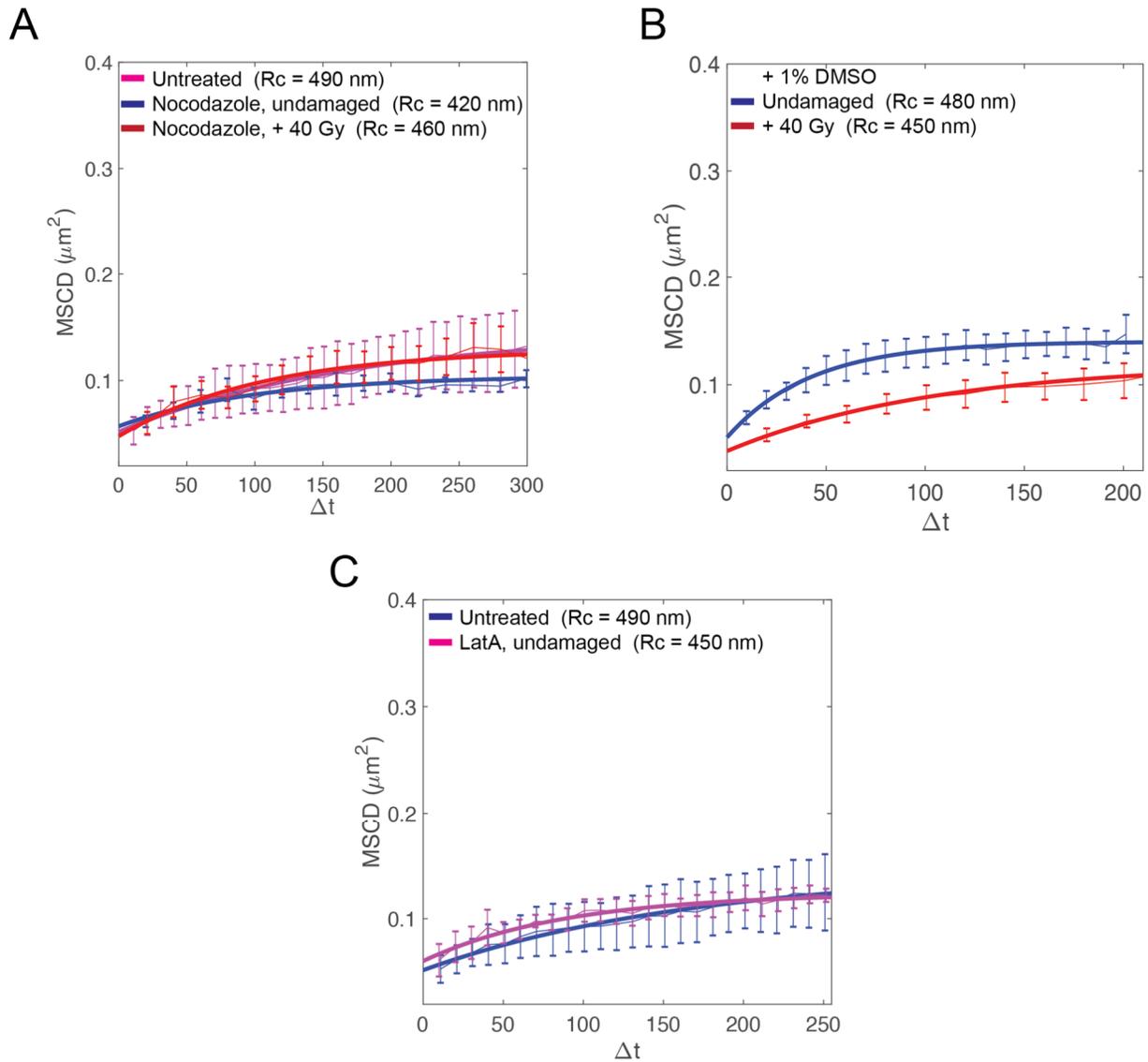
**Figure 3-2:** The confinement of the *URA3* locus is not altered by the loss of microtubules or actin. **A**) MSCD plots for undamaged (blue) and damaged (red) cells treated with 15 µg/mL nocodazole in 1% DMSO compared to MSD plot of untreated WT cells (magenta). No change in mobility is observed. **B**) Vehicle control of A, showing MSCD plots of 1% DMSO treated undamaged (blue) or damaged (red) cells. DMSO treatment alone blocks increased mobility. **C**) MSD of untreated cells (blue) compared to MSCD plot of cells treated with 200 µM latrunculin A. No change in mobility is observed. Error bars are ± 1 SEM. Nocodazole and DMSO treated cells were imaged using a 20 second time interval in order to capture a plateau in MSCD.
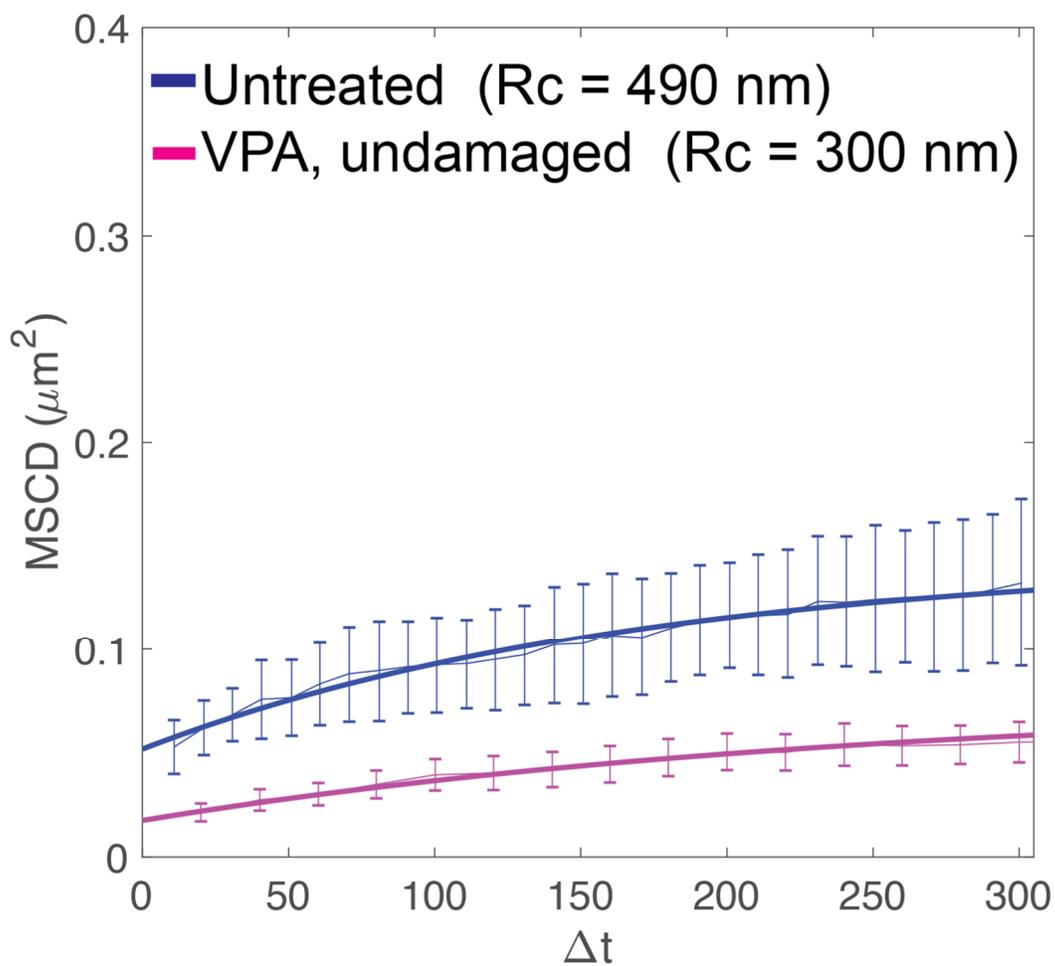
**Figure 3-3:** Valproic acid treatment increases the confinement of the *URA3* locus. MSD plot of untreated cells (blue) compared to cells treated with 10mM VPA. VPA treated cells were imaged with a 20 second time interval in order to capture a plateau in MSCD (see Methods).
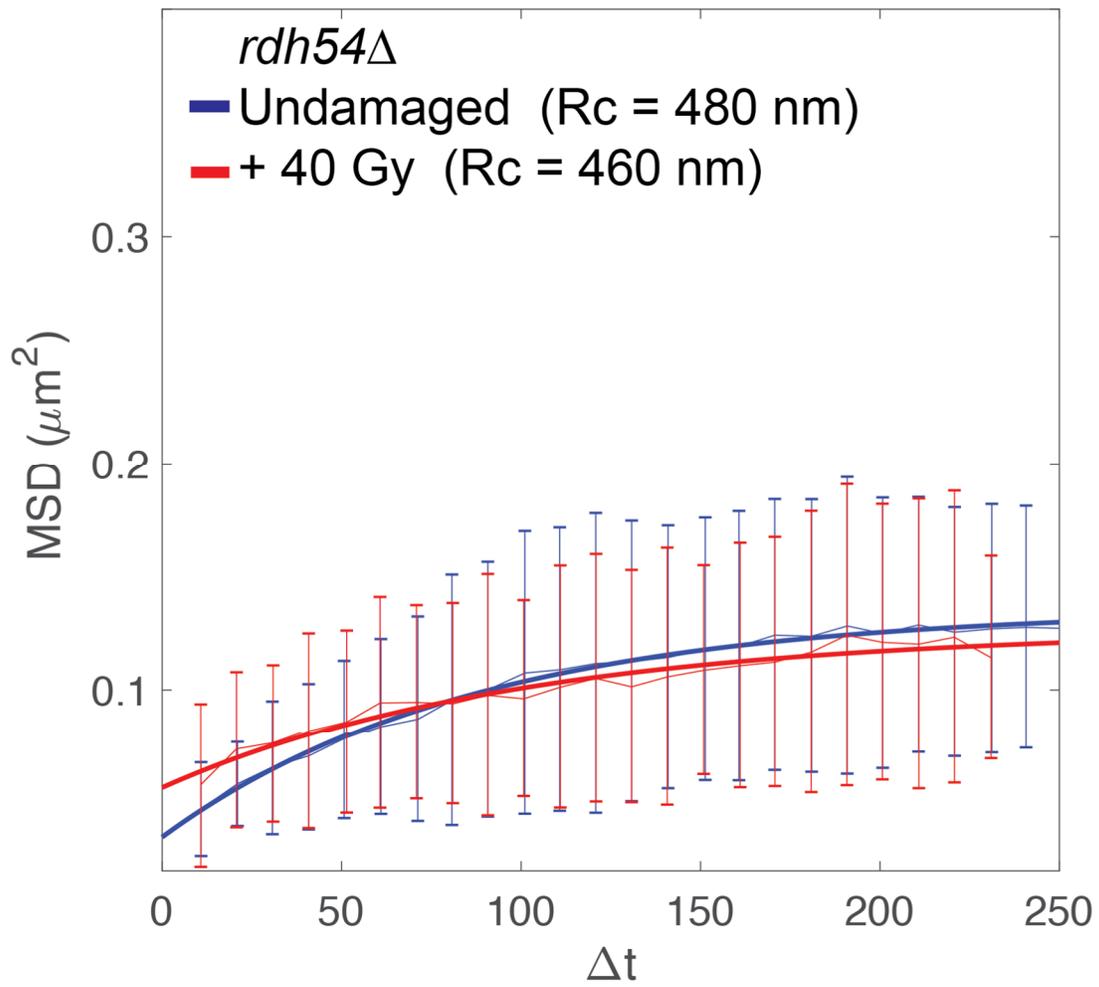
**Figure 3-4:** Rdh54 is required for global mobility. Undamaged (blue) and damaged (red) *rdh54Δ* cells are shown.
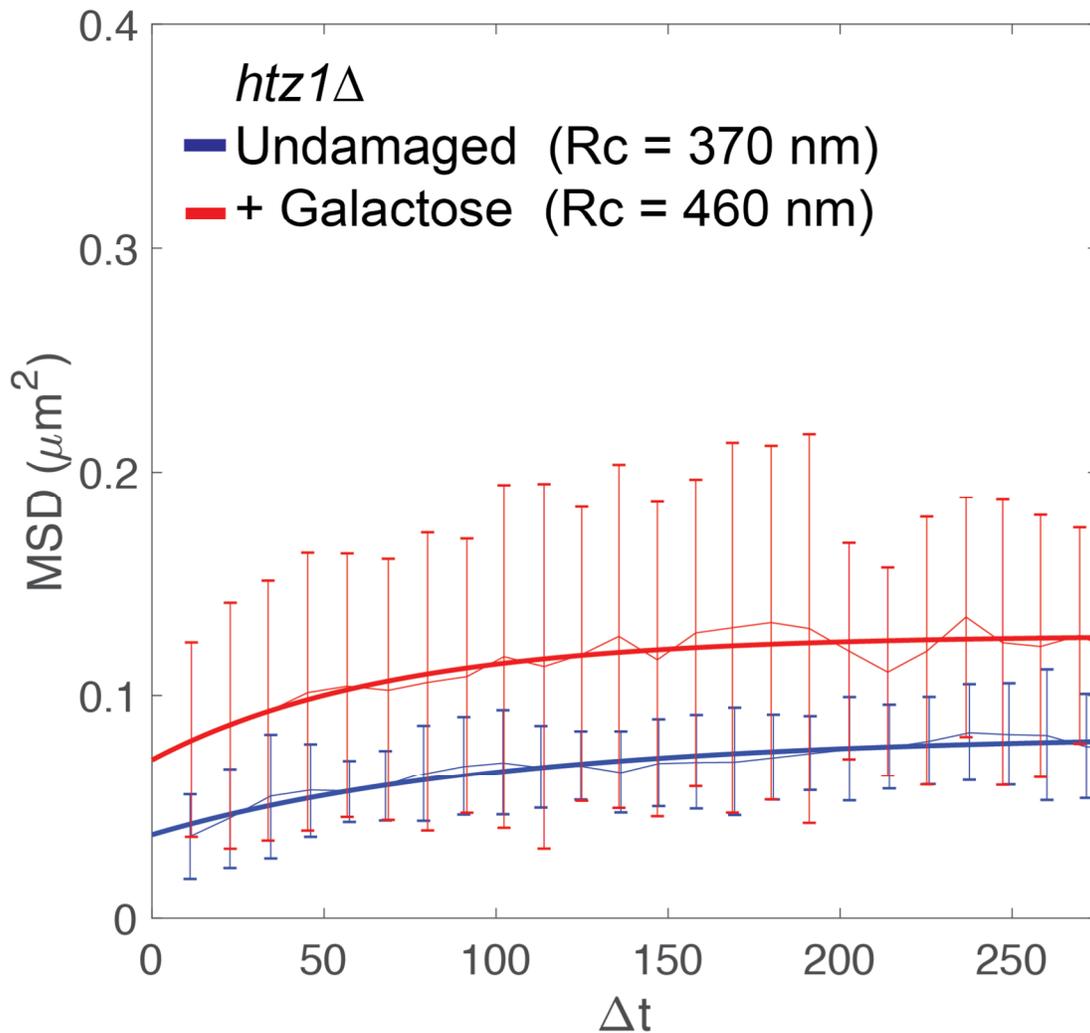
**Figure 3-5:** Local mobility is blocked in *htz1Δ* cells. Undamaged (blue) and cells cut with I-*Sce*I following 120 minutes of galactose induction (red) are shown. In damaged cells, the confinement of the *URA3* locus is largely unaffected by this endonuclease induction regime.
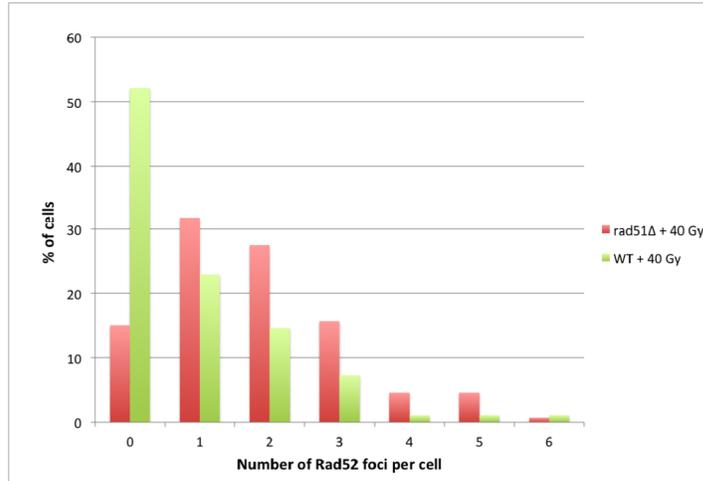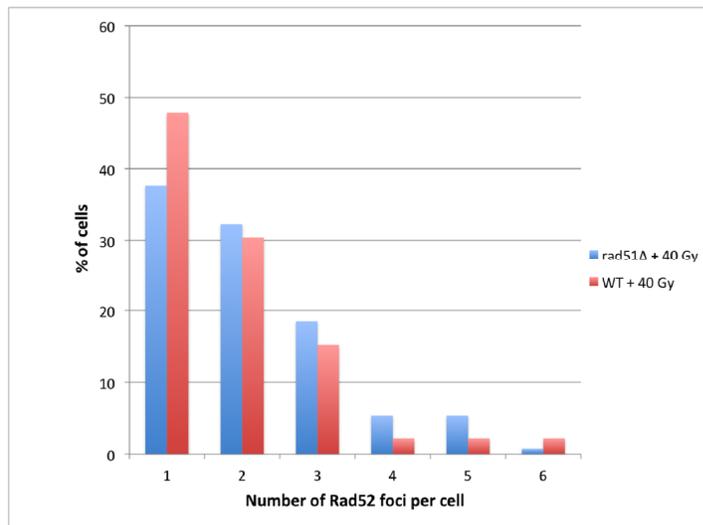
**Figure 3-6:** *rad51Δ* cells show defects in repair focus migration. The percent of WT and *rad51Δ* diploid cells that show 0 through 6 Rad52 foci per nucleus following treatment with 40 Gy of gamma radiation is shown. In **A**, cells without foci are included in the analysis, while the analysis in **B** is restricted to cells that have at least one focus.

**Table 3-1:** Strains used in this study

| Strain | Genotype | Source |
|---|---|---|
| W9957-17D | *MATα ADE2 GFP-NIC96::HIS3 LYS2 RAD52-CFP* | This study |
| W9100-17D | *MATa ADE2 leu2-3,112 his3-11,15 ura3-1 TRP1 lys2Δ RAD5 MET15* | Reid, et al., 2016 |
| W11311 | W9957-17D X W9100-17D | This study |
| W9593 | *MATα/MATa ade2-1/ADE2 ura3-1::URA3-LacOx256/ura3-1::3xURA3-TetOx112 I-SceIcs(ura3-1) lys2D/lys2::GAL-I-SceI RAD52-CFP his3-11,15::YFP-LacI-197K-HIS3/his3-11,15 TetR-mRFP1(iYGL119W)* | Mine-Hattab and Rothstein, 2012 |
| W11215 | W9530-21C X W11111-2C | This study |
| W11203 | W11016-24C X W11018-12C | This study |
| W11219-2C | *MATa ADE2 rdh54::KanMX SPC110-YFP::HIS3 lys2 trp1 TetR-mRFP* | This study |
| W11220-9B | *MATα ADE2 rdh54::KanMX ura3::3xURA3-tetOx112 I-SceI(ura3-1) TRP1 RAD52-CFP* | This study |
| W11312 | W11219-2C X W11220-9B | This study |
| W11045-17C | *MATα ADE2 htz1::KanMX SPC110-YFP::HIS3 lys2 RAD52-CFP* | This study |
| W11046-8A | *MATa ADE2 htz1::KanMX lys2::GAL-I-SceI ura3::3xURA3-tetOx112 I-SceI(ura3-1) TetR-mRFP1(iYGL119W)* | This study |
| W11313 | W11045-17C X W11046-8A | This study |

**CHAPTER 4:**

**DISCUSSION**

**Introduction**

The development of cell biological techniques including fluorescent protein tags and advanced imaging platforms have allowed an unprecedented view into the inner workings of cellular life, from bacteria to human cells. I have used these techniques, alongside classical genetic analysis to explore a fundamental aspect of biology, the repair of DSBs and the search for homology. In so doing, new insight has been gained into the precise behavior of both damaged and undamaged loci during the response to damage, and the genes and signaling pathways involved in its control. I have also analyzed and explored the contribution of the cytoskeleton to the mobility of chromosomes, as well as histone tail modifications and histone variants, finding that the movement of the *URA3* locus is most influenced by the status of the chromatin. Taken together with results in the literature, it is possible to construct models for the control of chromosome confinement and the induction of both local and global mobility.

**Mobility of loci in the undamaged nucleus**

The yeast nucleus is highly organized, but in this seemingly static context, dramatic changes in chromosome position must be achieved to accomplish various cellular processes, including the search for homology. How is the mobility of specific loci in yeast defined and controlled? Because yeast chromosomes are oriented with centromeres tethered to the spindle pole body and telomeres clustered and tethered to the nuclear periphery[56], it seems reasonable to suspect that the points of attachment largely define the mobility of individual loci. Indeed, in a 2013 study, Verdaasdonk and colleagues[58] found that those loci closest to the centromere were the most tightly confined. This tight

confinement could be relaxed by releasing centromeric tethering by driving transcription through the centromere. Strecker and colleagues reported a similar finding for centromeric tethering, and also reported that confinement of telomere proximal sequences could be reduced by deleting *sir4Δ*[61]. However, sites not proximal to either centromeres or telomeres are not tightly held to points of attachment[58], and I do not find that inhibiting microtubules (and thus centromeric attachment) via nocodazole treatment affects the mobility of the *URA3* locus. In addition, recent mathematical simulations support the notion that relaxation of tethering is insufficient for increased mobility along the entire length of the chromosome arm[74,148].

Since the baseline mobility of the *URA3* locus is altered by drugs and mutations that affect chromatin state, it is possible that the mobility of those loci distant from either tethered end of a chromosome is most affected by changes in chromatin packaging. Supporting this view, I have observed alterations in the baseline mobility of the *URA3* locus only under four circumstances: after VPA HDAC inhibitor treatment, after Htz1 deletion, in G1 cells (in which cohesin is not loaded) and in a *rad51Δ rad52Δ* mutant strain (which can be explained by checkpoint activation, see Chapter 2). Both the influence of cohesin[32,58,59] and the occupancy of histones[48,58] have been observed by other groups to influence the mobility of yeast chromosomes. Because these structures are amenable to rapid alteration in response to various stimuli, they are an attractive mechanism for the management of the movement of chromosomal loci. Thus, I propose that while tethering of chromosomes to the nuclear periphery does define the overall position and territory of a given arm, the mobility at the scale of an individual locus is largely defined by local compaction of the DNA

polymer, whether that be by the immediate chromatin state, or the action of cohesin or replicative complexes.

**Mechanisms for increased mobility after DNA damage**

How is the increased exploration of chromosomal loci during both local and global mobility ultimately manifested, and how do these two modes of mobility differ? Local mobility differs from global mobility in that there are particular factors loaded and reactions performed only at the site of the cut, which include recombination factor loading, checkpoint activation, and chromatin remodeling. Global mobility ultimately occurs as a result of a nucleus-wide damage-signaling event, and the specific mechanistic control of global mobility most likely differs from that of local mobility. Below, I discuss possible mechanisms for both forms of mobility.

### I. Local mobility

Previous work in yeast has determined that increased mobility is tied to HR progression. Both 2012 studies performed in yeast[43,44] found that Rad51 is essential for local mobility. Mine-Hattab and Rothstein additionally found that the resection factor Sae2 is required for timely initiation of mobility, while Dion and colleagues identified Rad54 as critical. I have found that the deletion of Rad52 restricts cut loci to global-like mobility. Thus, it is clear that the generation of ssDNA and the recruitment of proteins involved in the mechanics of recombination are key to increased mobility of cut loci. Because Rad51 is required in order to displace inhibitory Rad52 (Chapter 2) and for the recruitment of Rad54/Rdh54, the recombinase is key regulator of local mobility. In addition, because *rad52* mutant cells display only global mobility at the site of a break (Figure 2-4), it appears

that checkpoint activation alone is not sufficient for local mobility. Thus, Rad51 binding to ssDNA is specifically required for enhanced exploration at a cut locus. How is this effect manifested?

Rad51 may directly promote local mobility due to its structural influence on the DNA. According to a recent study by Mine-Hattab and colleagues (Mine-Hattab et al., 2017, in press), Rad51 filament formation may promote decreased exploration at short timescales to facilitate more extensive exploration over longer time intervals. This may occur through a stiffening of the local DNA upon oligomerization of Rad51, allowing the filament to pierce through the complex polymer networks found in the nucleus and efficiently locate homologous targets. However, this model cannot fully account for the observed requirement for Rad54 during local mobility[43]. An alternative possibility is that Rad51's role may include the recruitment of Rad54. As the events of local mobility are likely to be presynaptic, it is unlikely that Rad54 promotes mobility during strand invasion or D-loop formation. Both Rad54 and Rdh54 are members of the Swi1/Snf2 chromatin remodeling complex family and possess the ability to shift nucleosomes bound to DNA[102,136]. It is possible that mobility is achieved through local displacement of nucleosomes catalyzed by these recombination factors at the site of the break. Amitai and colleagues have recently modeled that displacement of histones from the region of a densely packed network of DNA can lead to extrusion of that region from the network[95]. Thus, the recruitment of Rad54/Rdh54 may ultimately lead to local mobility caused by chromatin remodeling. Alternatively, as Rad54/Rdh54 stabilize Rad51 filaments *in vitro*[128,131], these proteins may be required in order to ensure that Rad51 filaments are stable and prevent the inhibitory re-association of Rad52. This notion is contradicted by

the observation that *rad54-K341A* ATPase deficient protein, which is sufficient to stabilize Rad51 filaments in vitro[128], does not support increased mobility, as well as the observation that Rad51 foci are more frequent in *rad54Δ* cells[26]. The contribution of these two genes to chromosomal mobility has not been extensively studied, and more work will be required to clarify their roles during this process.

Although local mobility requires the loading of recombination factors, evidence from Dion and colleagues suggest that formation of repair complexes may not itself be sufficient for increased mobility[43]. They observed that local mobility was blocked in *mec1Δ sml1Δ, rad9Δ*, and *rad53Δ sml1Δ* strains, demonstrating that the ATR arm of the checkpoint must also contribute to increased mobility at cut sites. Based on my results from Chapter 2, it is reasonable to suggest that the checkpoint and recombination factors cooperate to induce local as well as global mobility. For example, the Mec1 checkpoint, from its vantage on the ssDNA generated by resection, may specifically modify recombination factors to induce a more highly mobile state, perhaps by altering the binding of various oligomeric complexes. Alternatively, the checkpoint-induced global effects on the mobility of chromatin (chromatin remodeling[46,48], detachment of tethering[61], etc) may be required in addition to recombination factor loading for local mobility. It is important to note that if DNA damage sensors can be attracted to unbroken dsDNA[47] without concomitant damage, checkpoint activation and mobility are observed (Figure 2-S3). This suggests that the recombination machinery's role in global mobility is limited to the modulation of binding or activation of checkpoint components, as bypassing end processing steps through artificial checkpoint activation is sufficient for mobility.

It is also possible that effects on the immediate chromatin surrounding the DSB contribute to the induction of local mobility, as the Swr1 and Ino80[145] complexes have been implicated in this process. These chromatin-remodeling complexes may, as previously stated, lead to increased mobility through shifting, replacement, or eviction of histones following cutting, and thus extrusion or altered behavior of the damaged site[95]. However, the action of these complexes is unlikely to be blocked in *rad51Δ* or *rad54Δ* mutant strains, and so this model does not account for the requirement for the recombination machinery.

Lastly, the untethering of chromosomes from centromeric and telomeric points of attachment has also been proposed as the motive force behind both local and global mobility[61,74]. In this model, local mobility is caused by the checkpoint signaling-induced modulation of centromeric attachment, whereas global mobility is caused by detachment of nearby undamaged chromosomes. While it is possible that increased kinetochore-centromere dynamics after DSB formation may contribute to increased movement, the fact that nocodazole treatment does not induce increased exploration at the *URA3* locus suggests that this phenomenon is not the sole contributor to mobility, although telomeric detachment may also be required. Moreover, two recent reports[74,148] have called the phenomenology of centromeric detachment as proposed by Strecker and colleagues[61] in question. If tether detachment is in fact important for local mobility, relaxation of tethering may be required to prime chromosomes to undergo mobility through a second mechanism, such as chromatin remodeling. The relative contributions of these factors remain to be determined.

## II. Global mobility

The induction of global mobility is rapid and general, and thus any putative mechanism by which it is achieved must function as such. For this reason, chromatin remodeling has become an attractive mechanism for this process. In a 2017 study[48], the Gasser group has suggested that, following DSB formation and checkpoint activation, the Ino80 complex evicts histones from DNA genome wide. In fact, blocking the expression of histone transcripts is sufficient to induce increased movement of a locus. Such eviction could cause subtle effects on the behavior of the compacted DNA polymer, increasing its flexibility and its capacity to explore space. My results (Chapters 2 and 3) support a model in which the interplay between Rad51, Rad52 and the checkpoint leads to alterations in chromatin state. However, Hauer and colleagues do not observe that *rad51Δ* prevents histone eviction. In addition, in a 2013 study, Seeber and colleagues[46] found that global mobility could occur in *rad51Δ* haploid strains following zeocin treatment. These discrepancies may be due to the high levels of zeocin used in these studies (250 μg/mL and up to 750 μg/mL in the 2013 and 2017 studies respectively). Thus, there may be a threshold of DNA damage beyond which inhibition by Rad52 is not sufficient to block mobility. In addition, recent work by Herbert and colleagues[148] has suggested that chromatin stiffening, and not relaxation as would be expected by the eviction model, promotes increased movement of loci after DNA damage. Thus, the precise nature of the chromatin changes that drive mobility remain unclear. It is possible that "loosening" and "stiffening" of differing regions of a chromosome arm may need to be accomplished in parallel in order to drive increased mobility.

Strecker and colleagues[61] have proposed that global mobility, as they argued for local mobility, may be induced by centromeric uncoupling from the kinetochore. They

observe that only those breaks created nearest to the centromere cause an undamaged chromosome to become mobile. Thus, checkpoint signaling proximal to a centromere sequence facilitates centromeric uncoupling in not only damaged but also undamaged chromosomes. This may explain the discrepancy observed for the 2012 Mine-Hattab[44] and Dion[43] studies, as the loci that, after cutting, caused global mobility at the *URA3* locus (such as the *URA3* homolog and *MAT*) in the Mine-Hattab study are nearer to the centromere than the *ZWF1* locus cut by the Gasser group. Global mobility is also induced in diploids following random DSBs produced by gamma radiation. Might this increase be a result of a random break occurring at a centromeric proximal site? Strecker and colleagues observe that cutting 30 kB away from the centromere induces mobility at an undamaged chromosome. If we assume that cutting within that range (30 kB on either side of the centromere, so 60 kB per chromosome in total) produces global mobility, then we would expect that a random DSB in a diploid cell would hit that range 8% of the time on average. Assuming that 4 DSBs per cell occur after 40 Gy (as used in this study) would yield a prediction (via a binomial probability calculation) that around 28% of cells in a given experiment would become mobile via this mechanism. Since we observe that 70% of cells become mobile following irradiation (see Chapter 2, Methods), the centromere proximal region would have to be 100 kB to totally account for our results. However, Strecker and colleagues report that cutting at this distance from the centromere did not yield increased mobility at a tracked undamaged site in their system. It is therefore likely that this mechanism does not completely explain our observations of global mobility.


**The function of DNA damage-induced chromosomal mobility**

The mechanisms for increased mobility after DNA damage are being studied in several organisms, but the precise purpose of this movement is not a settled matter. While the observation that members of the *RAD52* epistasis group directly regulate both local and global mobility (Chapter 2 and [43,44]) strongly indicates that increased exploration of chromosomal loci is important for homologous recombination, the precise nature of this importance is unclear. As a side effect of this tight association between recombination proteins and movement, it has been difficult to assess how the loss of mobility impacts recombination and its outcomes without totally disrupting HR. Below, I discuss three possible functions for DNA damage-induced increased chromosomal mobility.

### I. Increased chromosomal mobility facilitates homology search

The most obvious explanation for the increase in mobility observed at damaged and undamaged sites is that mobility is important for allowing the cut chromosome to navigate the nucleus and pair with its homolog. In S phase diploid cells, the homologs of *URA3* are distant from one another, and pair only rarely[44]. Thus, some form of movement is required in order for the donor and acceptor DNAs to come into contact. Local mobility is specifically induced at the cut site by the loading of checkpoint and recombination machinery, and the checkpoint response mobilizes a global response to the damage. In this model, the greatly expanded radius of exploration that the cut site undergoes allows rapid sampling of many possible homologous targets. Nonhomologous pairing is energetically unfavorable[150], and only pairing events with good sequence similarity are stabilized. Global mobility is likely necessary to increase the efficiency of this search process. For example, if the cut site explores 30% of the nuclear volume, but the selection of templates in that physical region are statically positioned and incompatible, then it will be difficult to locate a homologous

site outside of this range. Global mobility may allow a wider range of chromosomal regions to enter the search radius of the cut chromosome.

There are several weaknesses to this model. First, haploids undergo local, and in some circumstances, global mobility, but are unable to perform interhomolog repair[43,46]. Therefore, either the mechanistic underpinnings of mobility remain atavistically in the haploid state, or mobility is used for a different, possibly haploid-specific purpose. Second, a mutation has been identified in *CEP3*, a kinetochore protein, that blocks mobility but is not sensitive to DNA damaging agents[61]. In addition, this mutation does not affect rates of ectopic recombination in haploids, which suggests that increased mobility does not influence the ability to locate homologous sequences. However, if mobility is most critical for interhomolog repair events, then ectopic recombination assays in haploids are a poor test of its function. It will be critical in the future to test how mutations that affect mobility affect HR in diploid and meiotic cells, where interhomolog recombination becomes more important. If a mutation outside of canonical repair pathways can be identified that blocks mobility and also greatly reduces the efficiency of interhomolog recombination, increased mobility will be more clearly demonstrated to be important for homology search.

## II. Increased mobility is a stringency mechanism

The association of mobility with genes required for HR does not necessarily indicate that mobility is required for homology search *per se*. Data from meiotic yeast cells suggests that formation of a telomere bouquet and the rapid mobility that follows serves to promote stringent pairing (reviewed in[66]). A similar model has been proposed for the 53BP1 dependent mobility of uncapped telomeres in mammalian cells[57]. In this view, damaged and undamaged loci become mobile in order to discourage incompletely homologous

contacts from stabilizing. Thus, the physical shaking of chromosomes applies forces that more rapidly dissociate energetically unfavorable, low-homology interactions. It is important to note that this conception of mobility is not mutually exclusive with the homology search model discussed above. However, if the movements observed after DSB induction in yeast function solely to ensure accurate pairing, then the mechanism for homology search has not yet been identified. This model would also predict an increase in ectopic recombination events in backgrounds deficient for mobility, but, at present, this notion has not been extensively tested.

### III. Increased mobility is important for nuclear migration

Persistent DSBs have been observed to migrate to the nuclear periphery in haploid budding yeast and in *S. pombe*. Some groups have argued that the role of this process is to direct damaged DNA to specific subdomains where repair is favorable[63]. In yeast[40] and flies[73], DSBs that form in specialized nuclear subdomains (the nucleolus and the heterochromatin respectively) cause the damaged region to migrate out of those domains in order to recruit HR proteins. Thus, mobility may be important for moving damaged DNA to certain regions of the nucleus in order for the broken chromosome to undergo repair. Haploid cells may undergo increased mobility for precisely this reason, as persistent breaks must be adapted to if the cell is to survive the cell cycle. Again, this conception of mobility is not mutually exclusive with the other models discussed here. The mobility phenomena that I and others observe may be part of a ubiquitous pathway used to reorganize the nucleus in response to various stress stimuli and physiological needs.


**Interactions between recombination and checkpoint**

In this study, I have uncovered a novel regulatory circuit between the recombination machinery and DNA damage checkpoint that governs the activation of global mobility. This network raises exciting and intriguing questions about the cooperation of these two pathways. As discussed in Chapter 2, it is reasonable to suggest that mobility, and possibly other checkpoint dependent activities, might be restricted to certain damage contexts by association of checkpoint components with the mechanistic proteins required for the repair of that damage. For example, mobility is restricted to HR progression through a requirement for Rad52 displacement by Rad51.

The ATR/Mec1 arm of the checkpoint and the recombination machinery act on the shared substrate of the ssDNA[5]. Thus, components of the two pathways are in close proximity during DSB processing. The association of recombination factors may impinge upon the binding or activation of checkpoint factors and thus specify the checkpoint's influence. There may be other damage-stimulus specific effects of the DNA damage checkpoint, and the components of other repair pathways may signal to checkpoint pathways in a similar manner to the regulatory network described in Chapter 2. In the future, it will be important to identify the nature of these interactions on a genetic and physical level.

One previously reported system in which observations similar to those reported here have been observed is checkpoint adaptation[151]. Briefly, following the continuous induction of a single DSB, the checkpoint becomes active and damaged cells arrest. Some cells, however, downregulate the checkpoint and resume division. Many proteins involved in recombination impinge upon this process. In fact, there is an interesting correlation between the capacity for a mutant strain to undergo increased mobility in diploids and the

ability to adapt in haploid cells. For example, *rad51Δ, rad54Δ,* and *rad52Δ409-412* mutant strains all show defects in both adaptation and mobility after damage[134]. It appears that strains that fail to undergo mobility show defects in adaptation. This observation is somewhat puzzling, given that mobility arises from checkpoint activation and that adaptation is blocked by excessive checkpoint activation. A simple explanation might be that the interactions between recombination factors and checkpoint machinery allow the cell to properly detect that a DSB has occurred. Following that, a response to the damage is mobilized and then resolved, either in repair, or in the adaptation to that break. If the break cannot be properly detected, the cell may not be able to be properly resolve checkpoint signaling.

**Future directions and challenges**

Advances made in the study of cell biology and repair processes have permitted the study of how the biochemical reactions of DNA repair are coordinated with larger scale events in the life of the cell. The study of chromosome mobility in the context of DSBs is one such field that has come into being as a result of these strides in technology and research. Although at present, little is known about the physical behavior of genetic loci during damage stimuli in various organisms, progress will likely rapidly advance as researchers turn their efforts towards these exciting problems. In the future, in yeast and in other organisms, many interesting questions remain to be investigated.

In the yeast models and elsewhere, it will be important to define precisely which genes are involved in increased chromosomal mobility following DNA damage. This includes assessing the role of known recombination and signaling genes alongside the

discovery of new genes important for movement. It will be useful to apply a systematic approach and test the contributions of nearly all genes most critical for recombination in order to obtain a full picture of how the recombination machinery participates in mobility. In order to identity yet unknown genes involved in damage-induced mobility, a screening approach might be most useful. However, owing to the time intensive microscopy methodologies typically employed in these studies, it will be necessary to use a candidate gene approach or develop a more rapid method of visualizing cells. It may be necessary to employ automated microscopy platforms for data acquisition and machine learning approaches to analysis in order to greatly accelerate the discovery pipeline for this and other novel cell biological pathways.

A limitation of present studies of damage-induced mobility is the relatively low temporal resolution enforced by photobleaching of fluorophores and phototoxicity of image acquisition. As such, it has been difficult to observe the behavior of damaged loci throughout the repair process. Most studies have been restricted to the seconds and minutes following DSB induction, and more extensive time-lapse approaches have been limited. Thus, it will be critical to expand the view of how mobility occurs as breaks form, are processed, and are resolved, which may require the development of more photostable fluorophores or less demanding imaging regimes. In addition, the widespread use of galactose-inducible rare-cutting endonucleases for DSB induction is inherently confounding as it is difficult to identify when precisely a break formed, and how far along it is in its repair. Thus, more precise mechanisms of cutting should be employed in order to improve the resolution of the timing of mobility. This could be achieved through anchors-

away[152] like strategies, coupling endonucleases to cytoplasmic receptors[153], or employing optogenetic systems[154].

In addition to the basic temporal order of events during increased mobility, it will be useful to apply the methodologies used in studies of mitotic HR to meiosis. If damaged-induced increased mobility is important for homology search, then it should also be critical for meiosis. It will be interesting to determine how similar mitotic and meiotic mobility phenomena are, and analyze the capacity of Dmc1, the meiotic RecA homolog, to promote mobility as Rad51 does in S phase. As Rad51 serves as a cofactor during homology search in meiosis, it will be interesting to explore how the two recombinases cooperate to regulate mobility if such events also occur in meiosis[14].

The specific interactions between the recombination machinery and checkpoint apparatus that I have identified are an exciting step forward in understanding how repair is coupled to signaling. Going forward, it will be critical to understand precisely how Rad52 negatively regulates mobility. Numerous mutants of this recombination mediator have been developed, and so a focused screen of these strains during global mobility could provide useful information. In addition, it will be important to study the effects of Rad54/Rdh54 on mobility, and determine if these proteins play a role in the regulatory circuit described in Chapter 2. Other important questions that remain to be resolved include the influence of recombination and checkpoint on chromatin remodeling, the mobility of various damage types (nicks, single-ended breaks, etc.), the influence of ploidy on global mobility, and the determination of the relative influence of remodeling and tether detachment in both global and local mobility. Furthermore, it will be necessary to identify

additional targets of the checkpoint that are important for mobility in order to understand how the signaling of damage ultimately leads to the physical movements of chromosomes.

While researchers are still at relatively early steps in understanding chromosome mobility during DNA repair, the rapid pace of this newly developing field ensures that the level of knowledge will only increase. In the future, damage-induced chromosomal mobility will doubtless one day become well appreciated and its role alongside other processes essential for recombination will be clearly elucidated.

# References

1       Symington, L. S., Rothstein, R. & Lisby, M. Mechanisms and regulation of mitotic recombination in Saccharomyces cerevisiae. *Genetics* **198**, 795-835, doi:10.1534/genetics.114.166140 (2014).

2       Renkawitz, J., Lademann, C. A. & Jentsch, S. Mechanisms and principles of homology search during recombination. *Nat Rev Mol Cell Biol* **15**, 369-383, doi:10.1038/nrm3805 (2014).

3       Symington, L. S. & Gautier, J. Double-strand break end resection and repair pathway choice. *Annu Rev Genet* **45**, 247-271, doi:10.1146/annurev-genet-110410-132435 (2011).

4       Brill, S. J. & Stillman, B. Replication factor-A from Saccharomyces cerevisiae is encoded by three essential genes coordinately expressed at S phase. *Genes Dev* **5**, 1589-1600 (1991).

5       Gobbini, E., Cesena, D., Galbiati, A., Lockhart, A. & Longhese, M. P. Interplays between ATM/Tel1 and ATR/Mec1 in sensing and signaling DNA double-strand breaks. *DNA Repair (Amst)* **12**, 791-799, doi:10.1016/j.dnarep.2013.07.009 (2013).

6       Nakada, D., Matsumoto, K. & Sugimoto, K. ATM-related Tel1 associates with double-strand breaks through an Xrs2-dependent mechanism. *Genes Dev* **17**, 1957-1962, doi:10.1101/gad.1099003 (2003).

7       Zou, L. & Elledge, S. J. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* **300**, 1542-1548, doi:10.1126/science.1083430 (2003).

8       Paciotti, V., Clerici, M., Lucchini, G. & Longhese, M. P. The checkpoint protein Ddc2, functionally related to S. pombe Rad26, interacts with Mec1 and is regulated by Mec1-dependent phosphorylation in budding yeast. *Genes Dev* **14**, 2046-2059 (2000).

9       Sweeney, F. D., Yang, F., Chi, A., Shabanowitz, J., Hunt, D. F. & Durocher, D. Saccharomyces cerevisiae Rad9 acts as a Mec1 adaptor to allow Rad53 activation. *Curr Biol* **15**, 1364-1375, doi:10.1016/j.cub.2005.06.063 (2005).

10      Majka, J., Niedziela-Majka, A. & Burgers, P. M. The checkpoint clamp activates Mec1 kinase during initiation of the DNA damage checkpoint. *Mol Cell* **24**, 891-901, doi:10.1016/j.molcel.2006.11.027 (2006).

11      Brown, M. S. & Bishop, D. K. DNA strand exchange and RecA homologs in meiosis. *Cold Spring Harb Perspect Biol* **7**, a016659, doi:10.1101/cshperspect.a016659 (2014).

12      Roeder, G. S. Meiotic chromosomes: it takes two to tango. *Genes Dev* **11**, 2600-2621 (1997).

13      Morgan, E. A., Shah, N. & Symington, L. S. The requirement for ATP hydrolysis by Saccharomyces cerevisiae Rad51 is bypassed by mating-type heterozygosity or RAD54 in high copy. *Mol Cell Biol* **22**, 6336-6343 (2002).

14      Cloud, V., Chan, Y. L., Grubb, J., Budke, B. & Bishop, D. K. Rad51 is an accessory factor for Dmc1-mediated joint molecule formation during meiosis. *Science* **337**, 1222-1225, doi:10.1126/science.1219379 (2012).

15      Zhang, X. P., Galkin, V. E., Yu, X., Egelman, E. H. & Heyer, W. D. Loop 2 in Saccharomyces cerevisiae Rad51 protein regulates filament formation and ATPase activity. *Nucleic Acids Res* **37**, 158-171, doi:10.1093/nar/gkn914 (2009).

16      Bell, J. C., Plank, J. L., Dombrowski, C. C. & Kowalczykowski, S. C. Direct imaging of RecA nucleation and growth on single molecules of SSB-coated ssDNA. *Nature* **491**, 274-278, doi:10.1038/nature11598 (2012).

17      Krejci, L., Altmannova, V., Spirek, M. & Zhao, X. Homologous recombination and its regulation. *Nucleic Acids Res* **40**, 5795-5818, doi:10.1093/nar/gks270 (2012).

18      Martino, J. & Bernstein, K. A. The Shu complex is a conserved regulator of homologous recombination. *FEMS Yeast Res* **16**, doi:10.1093/femsyr/fow073 (2016).

19      Liu, J. & Heyer, W. D. Who's who in human recombination: BRCA2 and RAD52. *Proc Natl Acad Sci U S A* **108**, 441-442, doi:10.1073/pnas.1016614108 (2011).

20      Petukhova, G., Stratton, S. & Sung, P. Catalysis of homologous DNA pairing by yeast Rad51 and Rad54 proteins. *Nature* **393**, 91-94, doi:10.1038/30037 (1998).

21      Wright, W. D. & Heyer, W. D. Rad54 functions as a heteroduplex DNA pump modulated by its DNA substrates and Rad51 during D loop formation. *Mol Cell* **53**, 420-432, doi:10.1016/j.molcel.2013.12.027 (2014).

22      Cejka, P., Plank, J. L., Bachrati, C. Z., Hickson, I. D. & Kowalczykowski, S. C. Rmi1 stimulates decatenation of double Holliday junctions during dissolution by Sgs1-Top3. *Nat Struct Mol Biol* **17**, 1377-1382, doi:10.1038/nsmb.1919 (2010).

23      Boddy, M. N., Gaillard, P. H., McDonald, W. H., Shanahan, P., Yates, J. R., 3rd & Russell, P. Mus81-Eme1 are essential components of a Holliday junction resolvase. *Cell* **107**, 537-548 (2001).

24      Ip, S. C., Rass, U., Blanco, M. G., Flynn, H. R., Skehel, J. M. & West, S. C. Identification of Holliday junction resolvases from humans and yeast. *Nature* **456**, 357-361, doi:10.1038/nature07470 (2008).

25      Lisby, M., Rothstein, R. & Mortensen, U. H. Rad52 forms DNA repair and recombination centers during S phase. *Proc Natl Acad Sci U S A* **98**, 8276-8282, doi:10.1073/pnas.121006298 (2001).

26      Lisby, M., Barlow, J. H., Burgess, R. C. & Rothstein, R. Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. *Cell* **118**, 699-713, doi:10.1016/j.cell.2004.08.015 (2004).

27      Robinett, C. C., Straight, A., Li, G., Willhelm, C., Sudlow, G., Murray, A. & Belmont, A. S. In vivo localization of DNA sequences and visualization of large-scale chromatin organization using lac operator/repressor recognition. *J Cell Biol* **135**, 1685-1700 (1996).

28      Rabl, C. Uber Zelltheilung. *Morphologisches Jahrbuch* **10**, 214-330 (1885).

29      Taddei, A. & Gasser, S. M. Structure and function in the budding yeast nucleus. *Genetics* **192**, 107-129, doi:10.1534/genetics.112.140608 (2012).

30      Cremer, T. & Cremer, C. Chromosome territories, nuclear architecture and gene regulation in mammalian cells. *Nat Rev Genet* **2**, 292-301, doi:10.1038/35066075 (2001).

31      Marshall, W. F., Straight, A., Marko, J. F., Swedlow, J., Dernburg, A., Belmont, A., Murray, A. W., Agard, D. A. & Sedat, J. W. Interphase chromosomes undergo constrained diffusional motion in living cells. *Curr Biol* **7**, 930-939 (1997).

32      Heun, P., Laroche, T., Shimada, K., Furrer, P. & Gasser, S. M. Chromosome dynamics in the yeast interphase nucleus. *Science* **294**, 2181-2186, doi:10.1126/science.1065366 (2001).

33      Soutoglou, E., Dorn, J. F., Sengupta, K., Jasin, M., Nussenzweig, A., Ried, T., Danuser, G. & Misteli, T. Positional stability of single double-strand breaks in mammalian cells. *Nat Cell Biol* **9**, 675-682, doi:10.1038/ncb1591 (2007).

34      Jakob, B., Splinter, J., Durante, M. & Taucher-Scholz, G. Live cell microscopy analysis of radiation-induced DNA double-strand break motion. *Proc Natl Acad Sci U S A* **106**, 3172-3177, doi:10.1073/pnas.0810987106 (2009).

35      Nelms, B. E., Maser, R. S., MacKay, J. F., Lagally, M. G. & Petrini, J. H. In situ visualization of DNA double-strand break repair in human fibroblasts. *Science* **280**, 590-592 (1998).

36      Kruhlak, M. J., Celeste, A., Dellaire, G., Fernandez-Capetillo, O., Muller, W. G., McNally, J. G., Bazett-Jones, D. P. & Nussenzweig, A. Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks. *J Cell Biol* **172**, 823-834, doi:10.1083/jcb.200510015 (2006).

37      Lisby, M., Mortensen, U. H. & Rothstein, R. Colocalization of multiple DNA double-strand breaks at a single Rad52 repair centre. *Nat Cell Biol* **5**, 572-577, doi:10.1038/ncb997 (2003).

38    Aten, J. A., Stap, J., Krawczyk, P. M., van Oven, C. H., Hoebe, R. A., Essers, J. & Kanaar, R. Dynamics of DNA double-strand breaks revealed by clustering of damaged chromosome domains. *Science* **303**, 92-95, doi:10.1126/science.1088845 (2004).

39    Oza, P., Jaspersen, S. L., Miele, A., Dekker, J. & Peterson, C. L. Mechanisms that regulate localization of a DNA double-strand break to the nuclear periphery. *Genes Dev* **23**, 912-927, doi:10.1101/gad.1782209 (2009).

40    Torres-Rosell, J., Sunjevaric, I., De Piccoli, G., Sacher, M., Eckert-Boulet, N., Reid, R., Jentsch, S., Rothstein, R., Aragon, L. & Lisby, M. The Smc5-Smc6 complex and SUMO modification of Rad52 regulates recombinational repair at the ribosomal gene locus. *Nat Cell Biol* **9**, 923-931, doi:10.1038/ncb1619 (2007).

41    Chiolo, I., Minoda, A., Colmenares, S. U., Polyzos, A., Costes, S. V. & Karpen, G. H. Double-strand breaks in heterochromatin move outside of a dynamic HP1a domain to complete recombinational repair. *Cell* **144**, 732-744, doi:10.1016/j.cell.2011.02.012 (2011).

42    Dimitrova, N., Chen, Y. C., Spector, D. L. & de Lange, T. 53BP1 promotes non-homologous end joining of telomeres by increasing chromatin mobility. *Nature* **456**, 524-528, doi:10.1038/nature07433 (2008).

43    Dion, V., Kalck, V., Horigome, C., Towbin, B. D. & Gasser, S. M. Increased mobility of double-strand breaks requires Mec1, Rad9 and the homologous recombination machinery. *Nat Cell Biol* **14**, 502-509, doi:10.1038/ncb2465 (2012).

44    Mine-Hattab, J. & Rothstein, R. Increased chromosome mobility facilitates homology search during recombination. *Nat Cell Biol* **14**, 510-517, doi:10.1038/ncb2472 (2012).

45    Saad, H., Gallardo, F., Dalvai, M., Tanguy-le-Gac, N., Lane, D. & Bystricky, K. DNA dynamics during early double-strand break processing revealed by non-intrusive imaging of living cells. *PLoS Genet* **10**, e1004187, doi:10.1371/journal.pgen.1004187 (2014).

46    Seeber, A., Dion, V. & Gasser, S. M. Checkpoint kinases and the INO80 nucleosome remodeling complex enhance global chromatin mobility in response to DNA damage. *Genes Dev* **27**, 1999-2008, doi:10.1101/gad.222992.113 (2013).

47    Bonilla, C. Y., Melo, J. A. & Toczyski, D. P. Colocalization of sensors is sufficient to activate the DNA damage checkpoint in the absence of damage. *Mol Cell* **30**, 267-276, doi:10.1016/j.molcel.2008.03.023 (2008).

48    Hauer, M. H., Seeber, A., Singh, V., Thierry, R., Sack, R., Amitai, A., Kryzhanovska, M., Eglinger, J., Holcman, D., Owen-Hughes, T. & Gasser, S. M. Histone degradation in response to DNA damage enhances chromatin dynamics and recombination rates. *Nat Struct Mol Biol*, doi:10.1038/nsmb.3347 (2017).

49    Seeber, A. & Gasser, S. M. Chromatin organization and dynamics in double-strand break repair. *Curr Opin Genet Dev* **43**, 9-16, doi:10.1016/j.gde.2016.10.005 (2016).

50    Friis, J. & Roman, H. The effect of the mating-type alleles on intragenic recombination in yeast. *Genetics* **59**, 33-36 (1968).

51    Heude, M. & Fabre, F. a/alpha-control of DNA repair in the yeast Saccharomyces cerevisiae: genetic and physiological aspects. *Genetics* **133**, 489-498 (1993).

52    Lovett, S. T. & Mortimer, R. K. Characterization of null mutants of the RAD55 gene of Saccharomyces cerevisiae: effects of temperature, osmotic strength and mating type. *Genetics* **116**, 547-553 (1987).

53    Fung, C. W., Mozlin, A. M. & Symington, L. S. Suppression of the double-strand-break-repair defect of the Saccharomyces cerevisiae rad57 mutant. *Genetics* **181**, 1195-1206, doi:10.1534/genetics.109.100842 (2009).

54    Klein, H. L. RDH54, a RAD54 homologue in Saccharomyces cerevisiae, is required for mitotic diploid-specific recombination and repair and for meiosis. *Genetics* **147**, 1533-1543 (1997).

55    Kegel, A., Sjostrand, J. O. & Astrom, S. U. Nej1p, a cell type-specific regulator of nonhomologous end joining in yeast. *Curr Biol* **11**, 1611-1617 (2001).

56    Therizols, P., Fairhead, C., Cabal, G. G., Genovesio, A., Olivo-Marin, J. C., Dujon, B. & Fabre, E. Telomere tethering at the nuclear periphery is essential for efficient DNA double strand break repair in subtelomeric region. *J Cell Biol* **172**, 189-199, doi:10.1083/jcb.200505159 (2006).

57    Lottersberger, F., Karssemeijer, R. A., Dimitrova, N. & de Lange, T. 53BP1 and the LINC Complex Promote Microtubule-Dependent DSB Mobility and DNA Repair. *Cell* **163**, 880-893, doi:10.1016/j.cell.2015.09.057 (2015).

58    Verdaasdonk, J. S., Vasquez, P. A., Barry, R. M., Barry, T., Goodwin, S., Forest, M. G. & Bloom, K. Centromere tethering confines chromosome domains. *Mol Cell* **52**, 819-831, doi:10.1016/j.molcel.2013.10.021 (2013).

59    Dion, V., Kalck, V., Seeber, A., Schleker, T. & Gasser, S. M. Cohesin and the nucleolus constrain the mobility of spontaneous repair foci. *EMBO Rep* **14**, 984-991, doi:10.1038/embor.2013.142 (2013).

60    Spichal, M., Brion, A., Herbert, S., Cournac, A., Marbouty, M., Zimmer, C., Koszul, R. & Fabre, E. Evidence for a dual role of actin in regulating chromosome organization and dynamics in yeast. *J Cell Sci* **129**, 681-692, doi:10.1242/jcs.175745 (2016).

61    Strecker, J., Gupta, G. D., Zhang, W., Bashkurov, M., Landry, M. C., Pelletier, L. & Durocher, D. DNA damage signalling targets the kinetochore to promote chromatin mobility. *Nat Cell Biol* **18**, 281-290, doi:10.1038/ncb3308 (2016).

62    Lee, C. S., Wang, R. W., Chang, H. H., Capurso, D., Segal, M. R. & Haber, J. E. Chromosome position determines the success of double-strand break repair. *Proc Natl Acad Sci U S A* **113**, E146-154, doi:10.1073/pnas.1523660113 (2016).

63    Swartz, R. K., Rodriguez, E. C. & King, M. C. A role for nuclear envelope-bridging complexes in homology-directed repair. *Mol Biol Cell* **25**, 2461-2471, doi:10.1091/mbc.E13-10-0569 (2014).

64    Krawczyk, P. M., Borovski, T., Stap, J., Cijsouw, T., ten Cate, R., Medema, J. P., Kanaar, R., Franken, N. A. & Aten, J. A. Chromatin mobility is increased at sites of DNA double-strand breaks. *J Cell Sci* **125**, 2127-2133, doi:10.1242/jcs.089847 (2012).

65    Cho, N. W., Dilley, R. L., Lampson, M. A. & Greenberg, R. A. Interchromosomal homology searches drive directional ALT telomere movement and synapsis. *Cell* **159**, 108-121, doi:10.1016/j.cell.2014.08.030 (2014).

66    Koszul, R. & Kleckner, N. Dynamic chromosome movements during meiosis: a way to eliminate unwanted connections? *Trends Cell Biol* **19**, 716-724, doi:10.1016/j.tcb.2009.09.007 (2009).

67    Yamamoto, A. & Hiraoka, Y. How do meiotic chromosomes meet their homologous partners?: lessons from fission yeast. *Bioessays* **23**, 526-533, doi:10.1002/bies.1072 (2001).

68    Courcelle, J., Khodursky, A., Peter, B., Brown, P. O. & Hanawalt, P. C. Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient Escherichia coli. *Genetics* **158**, 41-64 (2001).

69    Lesterlin, C., Ball, G., Schermelleh, L. & Sherratt, D. J. RecA bundles mediate homology pairing between distant sisters during DNA break repair. *Nature* **506**, 249-253, doi:10.1038/nature12868 (2014).

70    Seeber, A. & Gasser, S. M. Chromatin organization and dynamics in double-strand break repair. *Curr Opin Genet Dev* **43**, 9-16, doi:10.1016/j.gde.2016.10.005 (2017).

71    Smith, M. J. & Rothstein, R. Poetry in motion: Increased chromosomal mobility after DNA damage. *DNA Repair (Amst)*, doi:10.1016/j.dnarep.2017.06.012 (2017).

72    Mine-Hattab, J. & Rothstein, R. DNA in motion during double-strand break repair. *Trends Cell Biol* **23**, 529-536, doi:10.1016/j.tcb.2013.05.006 (2013).

73    Amaral, N., Ryu, T., Li, X. & Chiolo, I. Nuclear Dynamics of Heterochromatin Repair. *Trends Genet* **33**, 86-100, doi:10.1016/j.tig.2016.12.004 (2017).

74    Lawrimore, J., Barry, T. M., Barry, R. M., York, A. C., Cook, D. M., Akialis, K., Tyler, J., Vasquez, P., Yeh, E. & Bloom, K. Microtubule dynamics drive enhanced chromatin motion and mobilize telomeres in response to DNA damage. *Mol Biol Cell*, doi:10.1091/mbc.E16-12-0846 (2017).

75    Ma, W., Resnick, M. A. & Gordenin, D. A. Apn1 and Apn2 endonucleases prevent accumulation of repair-associated DNA breaks in budding yeast as revealed by direct chromosomal analysis. *Nucleic Acids Res* **36**, 1836-1846, doi:10.1093/nar/gkm1148 (2008).

76    Barlow, J. H., Lisby, M. & Rothstein, R. Differential regulation of the cellular response to DNA double-strand breaks in G1. *Mol Cell* **30**, 73-85, doi:10.1016/j.molcel.2008.01.016 (2008).

77    Sung, P. Yeast Rad55 and Rad57 proteins form a heterodimer that functions with replication protein A to promote DNA strand exchange by Rad51 recombinase. *Genes Dev* **11**, 1111-1121 (1997).

78    Sung, P. & Stratton, S. A. Yeast Rad51 recombinase mediates polar DNA strand exchange in the absence of ATP hydrolysis. *J Biol Chem* **271**, 27983-27986 (1996).

79    Fung, C. W., Fortin, G. S., Peterson, S. E. & Symington, L. S. The rad51-K191R ATPase-defective mutant is impaired for presynaptic filament formation. *Mol Cell Biol* **26**, 9544-9554, doi:10.1128/MCB.00599-06 (2006).

80    Zaitseva, E. M., Zaitsev, E. N. & Kowalczykowski, S. C. The DNA binding properties of Saccharomyces cerevisiae Rad51 protein. *J Biol Chem* **274**, 2907-2915 (1999).

81    Krejci, L., Damborsky, J., Thomsen, B., Duno, M. & Bendixen, C. Molecular dissection of interactions between Rad51 and members of the recombination-repair group. *Mol Cell Biol* **21**, 966-976, doi:10.1128/MCB.21.3.966-976.2001 (2001).

82    Krejci, L., Song, B., Bussen, W., Rothstein, R., Mortensen, U. H. & Sung, P. Interaction with Rad51 is indispensable for recombination mediator function of Rad52. *J Biol Chem* **277**, 40132-40141, doi:10.1074/jbc.M206511200 (2002).

83    Gibb, B., Ye, L. F., Kwon, Y., Niu, H., Sung, P. & Greene, E. C. Protein dynamics during presynaptic-complex assembly on individual single-stranded DNA molecules. *Nat Struct Mol Biol* **21**, 893-900, doi:10.1038/nsmb.2886 (2014).

84    Sugiyama, T. & Kowalczykowski, S. C. Rad52 protein associates with replication protein A (RPA)-single-stranded DNA to accelerate Rad51-mediated displacement of RPA and presynaptic complex formation. *J Biol Chem* **277**, 31663-31672, doi:10.1074/jbc.M203494200 (2002).

85    Flott, S., Kwon, Y., Pigli, Y. Z., Rice, P. A., Sung, P. & Jackson, S. P. Regulation of Rad51 function by phosphorylation. *EMBO Rep* **12**, 833-839, doi:10.1038/embor.2011.127 (2011).

86    Thomas, B. J. & Rothstein, R. The genetic control of direct-repeat recombination in Saccharomyces: the effect of rad52 and rad1 on mitotic recombination at GAL10, a transcriptionally regulated gene. *Genetics* **123**, 725-738 (1989).

87    Reid, R. J., Du, X., Sunjevaric, I., Rayannavar, V., Dittmar, J., Bryant, E., Maurer, M. & Rothstein, R. A Synthetic Dosage Lethal Genetic Interaction Between CKS1B and PLK1 Is Conserved in Yeast and Human Cancer Cells. *Genetics* **204**, 807-819, doi:10.1534/genetics.116.190231 (2016).

88    Zhao, X., Muller, E. G. & Rothstein, R. A suppressor of two essential checkpoint genes identifies a novel protein that negatively affects dNTP pools. *Mol Cell* **2**, 329-340 (1998).

89    Barlow, J. H. & Rothstein, R. Rad52 recruitment is DNA replication independent and regulated by Cdc28 and the Mec1 kinase. *EMBO J* **28**, 1121-1130, doi:10.1038/emboj.2009.43 (2009).

90    Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685 (1970).

91 Michalet, X. Mean square displacement analysis of single-particle trajectories with localization error: Brownian motion in an isotropic medium. *Phys Rev E Stat Nonlin Soft Matter Phys* **82**, 041914, doi:10.1103/PhysRevE.82.041914 (2010).

92 Whitney, H. B. M. a. D. R. On a Test of Whether one of Two Random Variables is Stochastically Larger than the Other. *The Annals of Mathematical Statistics* **18**, 50-60 (1947).

93 RCoreTeam. *R: A language and environment for statistical computing.* , < https://www.R-project.org/.> (2016).

94 Lammerding, J. Mechanics of the nucleus. *Compr Physiol* **1**, 783-807, doi:10.1002/cphy.c100038 (2011).

95 Amitai, A., Seeber, A., Gasser, S. M. & Holcman, D. Visualization of Chromatin Decompaction and Break Site Extrusion as Predicted by Statistical Polymer Modeling of Single-Locus Trajectories. *Cell Rep* **18**, 1200-1214, doi:10.1016/j.celrep.2017.01.018 (2017).

96 Barkai, E., Garini, Y. & Metzler, R. STRANGE KINETICS of single molecules in living cells. *Phys Today* **65**, 29-35 (2012).

97 Hameed, F. M., Rao, M. & Shivashankar, G. V. Dynamics of passive and active particles in the cell nucleus. *PLoS One* **7**, e45843, doi:10.1371/journal.pone.0045843 (2012).

98 McLeish, T. A tangled tale of topological fluids. *Phys Today* **61**, 40-45, doi:Doi 10.1063/1.2970211 (2008).

99 Neumann, F. R., Dion, V., Gehlen, L. R., Tsai-Pflugfelder, M., Schmid, R., Taddei, A. & Gasser, S. M. Targeted INO80 enhances subnuclear chromatin movement and ectopic homologous recombination. *Genes Dev* **26**, 369-383, doi:10.1101/gad.176156.111 (2012).

100 Muramatsu, N. & Minton, A. P. Tracer diffusion of globular proteins in concentrated protein solutions. *Proc Natl Acad Sci U S A* **85**, 2984-2988 (1988).

101 Lieberman-Aiden, E., van Berkum, N. L., Williams, L., Imakaev, M., Ragoczy, T., Telling, A., Amit, I., Lajoie, B. R., Sabo, P. J., Dorschner, M. O., Sandstrom, R., Bernstein, B., Bender, M. A., Groudine, M., Gnirke, A., Stamatoyannopoulos, J., Mirny, L. A., Lander, E. S. & Dekker, J. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* **326**, 289-293, doi:10.1126/science.1181369 (2009).

102 Kwon, Y., Seong, C., Chi, P., Greene, E. C., Klein, H. & Sung, P. ATP-dependent chromatin remodeling by the Saccharomyces cerevisiae homologous recombination factor Rdh54. *J Biol Chem* **283**, 10445-10452, doi:10.1074/jbc.M800082200 (2008).

103 Kidiyoor, G. R., Kumar, A. & Foiani, M. ATR-mediated regulation of nuclear and cellular plasticity. *DNA Repair (Amst)* **44**, 143-150, doi:10.1016/j.dnarep.2016.05.020 (2016).

104 Kumar, A., Mazzanti, M., Mistrik, M., Kosar, M., Beznoussenko, G. V., Mironov, A. A., Garre, M., Parazzoli, D., Shivashankar, G. V., Scita, G., Bartek, J. & Foiani, M. ATR mediates a checkpoint at the nuclear envelope in response to mechanical stress. *Cell* **158**, 633-646, doi:10.1016/j.cell.2014.05.046 (2014).

105 Winey, M., Yarar, D., Giddings, T. H., Jr. & Mastronarde, D. N. Nuclear pore complex number and distribution throughout the Saccharomyces cerevisiae cell cycle by three-dimensional reconstruction from electron micrographs of nuclear envelopes. *Mol Biol Cell* **8**, 2119-2132 (1997).

106 Jorgensen, P., Edgington, N. P., Schneider, B. L., Rupes, I., Tyers, M. & Futcher, B. The size of the nucleus increases as yeast cells grow. *Mol Biol Cell* **18**, 3523-3532, doi:10.1091/mbc.E06-10-0973 (2007).

107 Starr, D. A. & Fridolfsson, H. N. Interactions between nuclei and the cytoskeleton are mediated by SUN-KASH nuclear-envelope bridges. *Annu Rev Cell Dev Biol* **26**, 421-444, doi:10.1146/annurev-cellbio-100109-104037 (2010).

108   Jin, Q. W., Fuchs, J. & Loidl, J. Centromere clustering is a major determinant of yeast interphase nuclear organization. *J Cell Sci* **113 ( Pt 11)**, 1903-1912 (2000).

109   Agmon, N., Liefshitz, B., Zimmer, C., Fabre, E. & Kupiec, M. Effect of nuclear architecture on the efficiency of double-strand break repair. *Nat Cell Biol* **15**, 694-699, doi:10.1038/ncb2745 (2013).

110   Fukui, Y. & Katsumaru, H. Nuclear actin bundles in Amoeba, Dictyostelium and human HeLa cells induced by dimethyl sulfoxide. *Exp Cell Res* **120**, 451-455 (1979).

111   Mishra, M., Huang, J. & Balasubramanian, M. K. The yeast actin cytoskeleton. *FEMS Microbiol Rev* **38**, 213-227, doi:10.1111/1574-6976.12064 (2014).

112   Conrad, M. N., Lee, C. Y., Chao, G., Shinohara, M., Kosaka, H., Shinohara, A., Conchello, J. A. & Dresser, M. E. Rapid telomere movement in meiotic prophase is promoted by NDJ1, MPS3, and CSM4 and is modulated by recombination. *Cell* **133**, 1175-1187, doi:10.1016/j.cell.2008.04.047 (2008).

113   Koszul, R., Kim, K. P., Prentiss, M., Kleckner, N. & Kameoka, S. Meiotic chromosomes move by linkage to dynamic actin cables with transduction of force through the nuclear envelope. *Cell* **133**, 1188-1201, doi:10.1016/j.cell.2008.04.050 (2008).

114   Bettinger, B. T., Gilbert, D. M. & Amberg, D. C. Actin up in the nucleus. *Nat Rev Mol Cell Biol* **5**, 410-415, doi:10.1038/nrm1370 (2004).

115   Spector, I., Shochet, N. R., Kashman, Y. & Groweiss, A. Latrunculins: novel marine toxins that disrupt microfilament organization in cultured cells. *Science* **219**, 493-495 (1983).

116   de Lanerolle, P. Nuclear actin and myosins at a glance. *J Cell Sci* **125**, 4945-4949, doi:10.1242/jcs.099754 (2012).

117   Grosse, R. & Vartiainen, M. K. To be or not to be assembled: progressing into nuclear actin filaments. *Nat Rev Mol Cell Biol* **14**, 693-697, doi:10.1038/nrm3681 (2013).

118   Baarlink, C., Wang, H. & Grosse, R. Nuclear actin network assembly by formins regulates the SRF coactivator MAL. *Science* **340**, 864-867, doi:10.1126/science.1235038 (2013).

119   Belin, B. J., Lee, T. & Mullins, R. D. DNA damage induces nuclear actin filament assembly by Formin -2 and Spire-(1/2) that promotes efficient DNA repair. [corrected]. *Elife* **4**, e07735, doi:10.7554/eLife.07735 (2015).

120   Gottlicher, M., Minucci, S., Zhu, P., Kramer, O. H., Schimpf, A., Giavara, S., Sleeman, J. P., Lo Coco, F., Nervi, C., Pelicci, P. G. & Heinzel, T. Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. *EMBO J* **20**, 6969-6978, doi:10.1093/emboj/20.24.6969 (2001).

121   Bird, A. W., Yu, D. Y., Pray-Grant, M. G., Qiu, Q., Harmon, K. E., Megee, P. C., Grant, P. A., Smith, M. M. & Christman, M. F. Acetylation of histone H4 by Esa1 is required for DNA double-strand break repair. *Nature* **419**, 411-415, doi:10.1038/nature01035 (2002).

122   Choudhary, C., Kumar, C., Gnad, F., Nielsen, M. L., Rehman, M., Walther, T. C., Olsen, J. V. & Mann, M. Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* **325**, 834-840, doi:10.1126/science.1175371 (2009).

123   Peterson, C. L. & Cote, J. Cellular machineries for chromosomal DNA repair. *Genes Dev* **18**, 602-616, doi:10.1101/gad.1182704 (2004).

124   Rando, O. J. & Winston, F. Chromatin and transcription in yeast. *Genetics* **190**, 351-387, doi:10.1534/genetics.111.132266 (2012).

125   Hunt, C. R., Ramnarain, D., Horikoshi, N., Iyengar, P., Pandita, R. K., Shay, J. W. & Pandita, T. K. Histone modifications and DNA double-strand break repair after exposure to ionizing radiations. *Radiat Res* **179**, 383-392, doi:10.1667/RR3308.2 (2013).

126   Bolden, J. E., Peart, M. J. & Johnstone, R. W. Anticancer activities of histone deacetylase inhibitors. *Nat Rev Drug Discov* **5**, 769-784, doi:10.1038/nrd2133 (2006).

127    Robert, T., Vanoli, F., Chiolo, I., Shubassi, G., Bernstein, K. A., Rothstein, R., Botrugno, O. A., Parazzoli, D., Oldani, A., Minucci, S. & Foiani, M. HDACs link the DNA damage response, processing of double-strand breaks and autophagy. *Nature* **471**, 74-79, doi:10.1038/nature09803 (2011).

128    Mazin, A. V., Alexeev, A. A. & Kowalczykowski, S. C. A novel function of Rad54 protein. Stabilization of the Rad51 nucleoprotein filament. *J Biol Chem* **278**, 14029-14036, doi:10.1074/jbc.M212779200 (2003).

129    Wolner, B., van Komen, S., Sung, P. & Peterson, C. L. Recruitment of the recombinational repair machinery to a DNA double-strand break in yeast. *Mol Cell* **12**, 221-232 (2003).

130    Mazin, A. V., Bornarth, C. J., Solinger, J. A., Heyer, W. D. & Kowalczykowski, S. C. Rad54 protein is targeted to pairing loci by the Rad51 nucleoprotein filament. *Mol Cell* **6**, 583-592 (2000).

131    Petukhova, G., Sung, P. & Klein, H. Promotion of Rad51-dependent D-loop formation by yeast recombination factor Rdh54/Tid1. *Genes Dev* **14**, 2206-2215 (2000).

132    Santa Maria, S. R., Kwon, Y., Sung, P. & Klein, H. L. Characterization of the interaction between the Saccharomyces cerevisiae Rad51 recombinase and the DNA translocase Rdh54. *J Biol Chem* **288**, 21999-22005, doi:10.1074/jbc.M113.480475 (2013).

133    Chi, P., Kwon, Y., Seong, C., Epshtein, A., Lam, I., Sung, P. & Klein, H. L. Yeast recombination factor Rdh54 functionally interacts with the Rad51 recombinase and catalyzes Rad51 removal from DNA. *J Biol Chem* **281**, 26268-26279, doi:10.1074/jbc.M602983200 (2006).

134    Lee, S. E., Pellicioli, A., Vaze, M. B., Sugawara, N., Malkova, A., Foiani, M. & Haber, J. E. Yeast Rad52 and Rad51 recombination proteins define a second pathway of DNA damage assessment in response to a single double-strand break. *Mol Cell Biol* **23**, 8913-8923 (2003).

135    Ferrari, M., Nachimuthu, B. T., Donnianni, R. A., Klein, H. & Pellicioli, A. Tid1/Rdh54 translocase is phosphorylated through a Mec1- and Rad53-dependent manner in the presence of DSB lesions in budding yeast. *DNA Repair (Amst)* **12**, 347-355, doi:10.1016/j.dnarep.2013.02.004 (2013).

136    Jaskelioff, M., Van Komen, S., Krebs, J. E., Sung, P. & Peterson, C. L. Rad54p is a chromatin remodeling enzyme required for heteroduplex DNA joint formation with chromatin. *J Biol Chem* **278**, 9212-9218, doi:10.1074/jbc.M211545200 (2003).

137    McKinley, K. L. & Cheeseman, I. M. The molecular basis for centromere identity and function. *Nat Rev Mol Cell Biol* **17**, 16-29, doi:10.1038/nrm.2015.5 (2016).

138    Li, B., Pattenden, S. G., Lee, D., Gutierrez, J., Chen, J., Seidel, C., Gerton, J. & Workman, J. L. Preferential occupancy of histone variant H2AZ at inactive promoters influences local histone modifications and chromatin remodeling. *Proc Natl Acad Sci U S A* **102**, 18385-18390, doi:10.1073/pnas.0507975102 (2005).

139    Mizuguchi, G., Shen, X., Landry, J., Wu, W. H., Sen, S. & Wu, C. ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science* **303**, 343-348, doi:10.1126/science.1090701 (2004).

140    Kobor, M. S., Venkatasubrahmanyam, S., Meneghini, M. D., Gin, J. W., Jennings, J. L., Link, A. J., Madhani, H. D. & Rine, J. A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. *PLoS Biol* **2**, E131, doi:10.1371/journal.pbio.0020131 (2004).

141    Krogan, N. J., Keogh, M. C., Datta, N., Sawa, C., Ryan, O. W., Ding, H., Haw, R. A., Pootoolal, J., Tong, A., Canadien, V., Richards, D. P., Wu, X., Emili, A., Hughes, T. R., Buratowski, S. & Greenblatt, J. F. A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. *Mol Cell* **12**, 1565-1576 (2003).

142    Meneghini, M. D., Wu, M. & Madhani, H. D. Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. *Cell* **112**, 725-736 (2003).

143    Papamichos-Chronakis, M., Krebs, J. E. & Peterson, C. L. Interplay between Ino80 and Swr1 chromatin remodeling enzymes regulates cell cycle checkpoint adaptation in response to DNA damage. *Genes Dev* **20**, 2437-2449, doi:10.1101/gad.1440206 (2006).

144    van Attikum, H., Fritsch, O. & Gasser, S. M. Distinct roles for SWR1 and INO80 chromatin remodeling complexes at chromosomal double-strand breaks. *EMBO J* **26**, 4113-4125, doi:10.1038/sj.emboj.7601835 (2007).

145    Horigome, C., Oma, Y., Konishi, T., Schmid, R., Marcomini, I., Hauer, M. H., Dion, V., Harata, M. & Gasser, S. M. SWR1 and INO80 chromatin remodelers contribute to DNA double-strand break perinuclear anchorage site choice. *Mol Cell* **55**, 626-639, doi:10.1016/j.molcel.2014.06.027 (2014).

146    Kalocsay, M., Hiller, N. J. & Jentsch, S. Chromosome-wide Rad51 spreading and SUMO-H2A.Z-dependent chromosome fixation in response to a persistent DNA double-strand break. *Mol Cell* **33**, 335-343, doi:10.1016/j.molcel.2009.01.016 (2009).

147    Alvaro, D., Lisby, M. & Rothstein, R. Genome-wide analysis of Rad52 foci reveals diverse mechanisms impacting recombination. *PLoS Genet* **3**, e228, doi:10.1371/journal.pgen.0030228 (2007).

148    Herbert, S., Brion, A., Arbona, J. M., Lelek, M., Veillet, A., Lelandais, B., Parmar, J., Fernandez, F. G., Almayrac, E., Khalil, Y., Birgy, E., Fabre, E. & Zimmer, C. Chromatin stiffening underlies enhanced locus mobility after DNA damage in budding yeast. *EMBO J*, doi:10.15252/embj.201695842 (2017).

149    Berger, A. B., Cabal, G. G., Fabre, E., Duong, T., Buc, H., Nehrbass, U., Olivo-Marin, J. C., Gadal, O. & Zimmer, C. High-resolution statistical mapping reveals gene territories in live yeast. *Nat Methods* **5**, 1031-1037, doi:10.1038/nmeth.1266 (2008).

150    Qi, Z., Redding, S., Lee, J. Y., Gibb, B., Kwon, Y., Niu, H., Gaines, W. A., Sung, P. & Greene, E. C. DNA sequence alignment by microhomology sampling during homologous recombination. *Cell* **160**, 856-869, doi:10.1016/j.cell.2015.01.029 (2015).

151    Harrison, J. C. & Haber, J. E. Surviving the breakup: the DNA damage checkpoint. *Annu Rev Genet* **40**, 209-235, doi:10.1146/annurev.genet.40.051206.105231 (2006).

152    Haruki, H., Nishikawa, J. & Laemmli, U. K. The anchor-away technique: rapid, conditional establishment of yeast mutant phenotypes. *Mol Cell* **31**, 925-932, doi:10.1016/j.molcel.2008.07.020 (2008).

153    Hartlerode, A., Odate, S., Shim, I., Brown, J. & Scully, R. Cell cycle-dependent induction of homologous recombination by a tightly regulated I-SceI fusion protein. *PLoS One* **6**, e16501, doi:10.1371/journal.pone.0016501 (2011).

154    Yang, X., Jost, A. P., Weiner, O. D. & Tang, C. A light-inducible organelle-targeting system for dynamically activating and inactivating signaling in budding yeast. *Mol Biol Cell* **24**, 2419-2430, doi:10.1091/mbc.E13-03-0126 (2013).