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Poetry in motion: Increased chromosomal mobility after DNA damage

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Abstract

Double-strand breaks (DSBs) are among the most lethal DNA lesions, and a variety of pathways have evolved to manage their repair in a timely fashion. One such pathway is homologous recombination (HR), in which information from an undamaged donor site is used as a template for repair. Although many of the biochemical steps of HR are known, the physical movements of chromosomes that must underlie the pairing of homologous sequence during mitotic DSB repair have remained mysterious. Recently, several groups have begun to use a variety of genetic and cell biological tools to study this important question. These studies reveal that both damaged and undamaged loci increase the volume of the nuclear space that they explore after the formation of DSBs. This DSB-induced increase in chromosomal mobility is regulated by many of the same factors that are important during HR, such as ATR-dependent checkpoint activation and the recombinase Rad51, suggesting that this phenomenon may facilitate the search for homology. In this perspective, we review current research into the mobility of chromosomal loci during HR, as well as possible underlying mechanisms, and discuss the critical questions that remain to be answered. Although we focus primarily on recent studies in the budding yeast, *Saccharomyces cerevisiae*, examples of experiments performed in higher eukaryotes are also included, which reveal that increased mobility of damaged loci is a process conserved throughout evolution.

Keywords

Double-strand breaks; Homologous recombination; Homology search; Local chromosomal mobility; Global chromosomal mobility; Nuclear organization

Homologous recombination (HR) requires a multitude of carefully orchestrated steps to accomplish the repair of DNA double-strand breaks (DSBs) (Figure 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

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Conflict of Interest statement

The authors declare there are no conflicts of interest

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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. This increase in mobility is regulated by many of the same factors involved in DNA repair, thus showing that dynamic events in the nucleus are coupled to the process of recombination. By studying this process further, the means by which a cell detects changes in genome integrity and relays that information to the mechanical structures required to promote repair will be revealed. The budding yeast, *Saccharomyces cerevisiae*, a leading model organism in the genetic analyses of HR pathways, has been extensively used to explore these important questions. Thus, in this perspective, we focus mainly on studies of mitotic chromosome mobility in budding yeast that have helped to define this process and its genetic requirements.

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 and methylmethane sulfonate (MMS), as well as products of metabolism such as reactive oxygen species.^{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. 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, as in sister chromatid exchange (SCE) or the homologous chromosome in diploid cells (interhomolog repair).

Figure 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 NHEJ³. 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 kinases⁵. The activation of Tel1 is promoted by an interaction with Xrs2⁶, while the generation of RPA-coated ssDNA by the resection machinery leads to the recruitment of Ddc2-Mec1^{7,8} and subsequent phosphorylation of Rad9⁹. Rad9 promotes the activation of Rad53, a major checkpoint effector kinase, which then phosphorylates many downstream targets⁹. The PCNA-like sliding clamp complex 9-1-1 (Ddc1, Mec3, Rad17) also binds to newly generated ssDNA junctions and assists in checkpoint activation¹⁰.

The strand exchange reactions of HR are mediated by recombinase enzymes, frequently homologs of bacterial RecA¹¹. Yeast have two such enzymes—the mitotic recombinase Rad51 and the meiotic recombinase Dmc1¹². In mitotic cells, Rad51 forms oligomeric complexes on resected ssDNA called presynaptic filaments. These filaments bind dsDNA

complexes, assess homology and then promote strand invasion and repair. While binding of RPA to ssDNA protects it from additional lesions, RPA is inhibitory to nucleation of Rad51 filaments³. Therefore, mediators, such as RecFOR¹³ in bacteria and Rad52¹⁴ in yeast are needed to help overcome the inhibition of RPA and promote the binding and extension of recombinase filaments¹⁴. Once formed, presynaptic filaments seek out homology and perform strand exchange reactions. Rad54 interacts with Rad51 and facilitates displacement of strands within the target molecule, forming D-loops^{15,16}. 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 complex¹⁷, or by structure selective nucleases such as Mms4-Mus81¹⁸ and Yen1¹⁹.

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 allowed the exploration of 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 forms bright foci that can be easily distinguished by microscopy^{20,21}. In addition to tags on individual proteins, systems have been developed to permit the visualization of entire chromosomal regions²². 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 Rabl²³. Telomeric sequences form clusters at the nuclear periphery, with Yku70, Sir4 and nuclear pore components such as Mps3 participate in this tethering²⁴. 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 nuclei²⁵. 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 contradiction²⁶. 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 colleagues²⁷ 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^{28–31}, many studies have shown that nuclear loci are capable of large-scale reorganization following DSB formation^{32–37}. 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 foci³². Instead, multiple breaks coalesce to just a few repair centers in the cell. In 2004, Aten and colleagues³³ 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 machinery³⁴. Furthermore, Torres-Rosell and colleagues also reported that breaks that occur in the nucleolus migrate outside of this specific nuclear subdomain for repair³⁵. Later, similar observations were made in *Drosophila* heterochromatic regions (see section 4)³⁶. In mammalian cells, the Rad9 homolog 53BP1 has been implicated in the regulation of increased mobility of uncapped telomere ends, structures analogous to DSBs³⁷. Thus, damaged DNA appears highly dynamic and undergoes programmed movements throughout the nucleus during repair.

In 2012, two studies, one performed in haploid cells³⁸, and one in diploids³⁹, 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-*SceI* 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 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 mobility³⁸. 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 mobility³⁹. Interestingly, a later study in haploids reported that cut loci display transient confinement during the very early steps of resection at the *MAT* locus⁴⁰. 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 machinery¹, two pathways that coordinate the activation of mobility (Figure 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 haploids³⁸. However a subsequent study⁴¹ 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 Ddc2⁴². 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 DSBs³⁹. 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 eviction⁴³ (depicted in Figure 3). Intriguingly, the requirement for Rad51 in mobility is bypassed in haploids after zeocin treatment⁴¹. Chromatin remodeling following checkpoint activation is a persuasive mechanism for the control of global mobility⁴⁴, 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^{45,46} and the activity of many individual repair proteins, including Rad51⁴⁷, Rad55/57^{48,49} and Rdh54⁵⁰. In addition, mating-type heterozygosity leads to the production of the $\alpha 1$ - $\alpha 2$ transcriptional repressor complex, which strongly suppresses NHEJ in diploids⁵¹. 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^{34,52}. 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)⁵³. 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 envelope²⁴. In 2013, the Bloom group investigated the mobility of various loci along chromosome arms to evaluate what role centromeric tethering plays in mobility⁵⁴. 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^{54,55}. Studies by the Fabre group also raised the possibility that both nuclear and cytosolic actin contribute to chromosome mobility⁵⁶. 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.⁵⁷ 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 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 machinery³⁹, or chromatin remodelers⁴¹. Lee and colleagues⁵⁸ 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 *HTZI*, 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 mobility³⁹. 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 repair³⁶. Interestingly, this migration requires ATR checkpoint activation. Like in yeast, this compartment restriction is enforced by Smc5/6 complexes³⁵. In fission yeast, persistent DSBs recruit the SUN domain protein Sad1 and migrate to the nuclear periphery, where they become associated with microtubule linked KASH domain proteins⁵⁹. 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 exposure⁶⁰. 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^{37,53}. 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 yeast²⁶. However, like fission yeast⁵⁹, 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 DNA damage signaling induces microtubules in the cytoplasm to alter their contacts with DNA and 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 sites⁶¹. 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^{38,39,41,57}, however, work in mammalian cells suggests that mobility may also be required for NHEJ⁵³. 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 pairing⁶². Similarly, in *S. pombe* meiosis, the telomeres of chromosomes reorganize, and the entire nucleus moves dramatically to promote homologous pairing⁶³. 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 meiosis⁶⁴. 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 bacteria⁶⁵), changes to the physical characteristics of the DNA end or mechanically via the formation of RecA-like bundles as seen in bacteria⁶⁶? 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.

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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
KASH domain	Klarsicht, ANC-1, Syne Homology domain
ALT	alternative lengthening of telomeres
LINC complex	linker of nucleoskeleton and cytoskeleton

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Figure 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.

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Figure 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.

Figure 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 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.