DNA Repair 56 (2017) 102–108

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Review

Poetry in motion: Increased chromosomal mobility after DNA damage

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ARTICLE INFO

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

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.

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

Fig. 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

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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http://dx.doi.org/10.1016/j.dnarep.2017.06.012

Available online 09 June 2017

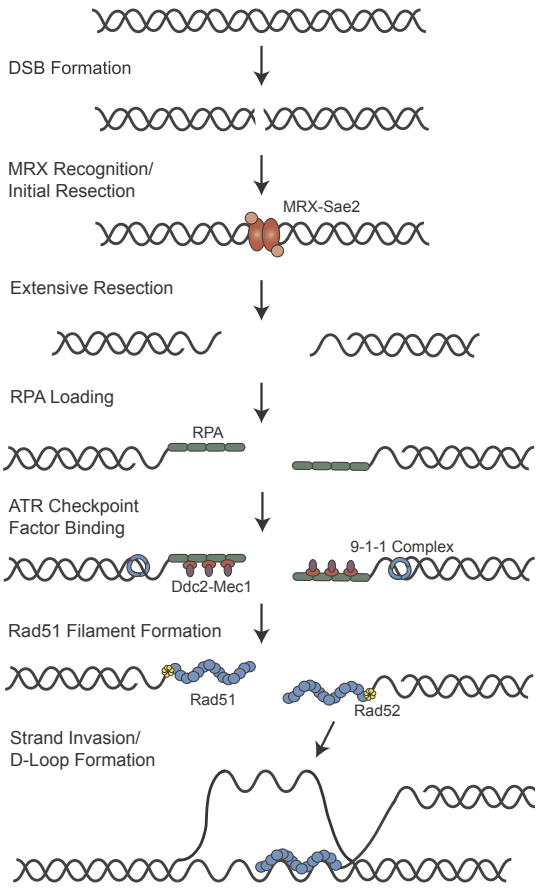

Fig. 1. Major steps in the HR pathway in budding yeast. After DSB formation, the break is recognized by the MRX (Mre11, Rad50, Xrs2) complex along with the cofactor Sae2 to initiate initial 5′ resection. Then, Sgs1, Dna2 and Exo1 (not shown) cooperate to catalyze more extensive resection, revealing 3′ 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.

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—the mitotic recombinase Rad51 and the meiotic recombinase Dmc1 [12]. 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 filaments [3]. Therefore, mediators, such as RecFOR [13] in bacteria and Rad52 [14] in yeast are needed to help overcome the inhibition of RPA and promote the binding and extension of recombinase filaments [14]. 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 complex [17], or by structure selective nucleases such as Mms4-Mus81 [18] and Yen1 [19].

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 regions [22]. 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 [23]. Telomeric sequences form clusters at the nuclear periphery, with Yku70, Sir4 and nuclear pore components such as Mps3 participate in this tethering [24]. 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 [25]. 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 [26]. 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 [27] 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