How Broken DNA Finds Its Template for Repair: A Computational Approach

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Homologous recombination (HR) is the process by which a double-strand break in DNA is repaired using an identical donor template. Despite rapid progress in identifying the functions of the proteins that mediate HR, little is known about how broken DNA finds its homologous template. This process, coined homology search, has been difficult to monitor experimentally. Therefore, we present here a computational approach to model the effect of subnuclear positioning and chromatin dynamics on homology search. We found that, in our model, homology search occurs more efficiently if both the cut site and its template are at the nuclear periphery, whereas restricting the movement of the template or the break alone to the periphery markedly increases the time of the search. Immobilization of either component at any position slows down the search. Based on these results, we propose a new model for homology search, the facilitated random search model, which predicts that the search is random, but that nuclear organization and dynamics strongly influence its speed and efficiency.

§1. Double-strand breaks: their causes and consequences

DNA is under constant threat from both exogenous sources, such as ultraviolet light and ionizing radiation, and endogenous agents, including water and free radicals.[1)] These agents generate a number of different lesions to both the DNA bases and backbone, often leading to single- and double-strand breaks (DSBs).[2)] DSBs are particularly challenging to repair because the continuity of the genetic information at the break site is lost. Indeed a single irreparable DSB can lead to cell death.[3)] Aberrant repair can lead to carcinogenic mutations such as translocations.[4)–8)] Thus, the need to maintain genomic stability has pressured organisms to evolve robust processes to deal with this type of lesion.[9),10)]

Double-strand breaks can be repaired by either of two general pathways: non-homologous end joining (NHEJ) or homologous recombination (HR). During NHEJ the DNA ends are ligated back together. NHEJ often includes a processing step for the DNA ends, involving the addition and deletion of short DNA sequences. Therefore, NHEJ can be erroneous.[9)] HR, on the other hand, tends to be error-free as it uses a homologous (i.e., identical or nearly identical) template to restore the missing sequence.[10)] Species with smaller genomes such as bacteria and yeast tend to use HR more efficiently than species with larger genomes like mouse and human. In every case, however, HR requires the search for a template. This process, called

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homology search, is poorly understood.[11),12)] Here we focus on the mechanism of homology search in the baker's yeast *Saccharomyces cerevisiae* and compare the data with those obtained from other organisms. To simplify the discussion of homology search and focus on the biophysical concepts, we have omitted protein names from the text, but interested readers can find them in Fig. 1. Further details can be found in San Filippo et al.[10)]

Despite the passage of 47 years since Robin Holliday first proposed his model for HR,[13)] little has been done to address the mechanism of homology search.[11)] Part of the reason is that homology search is difficult to track experimentally. We therefore opted for a computational approach and simplified the problem to its core: how do two moving bodies find each other in a spherical confinement and how can this process be sped up? In this manuscript, we present our findings and discuss the biological implications of our results.

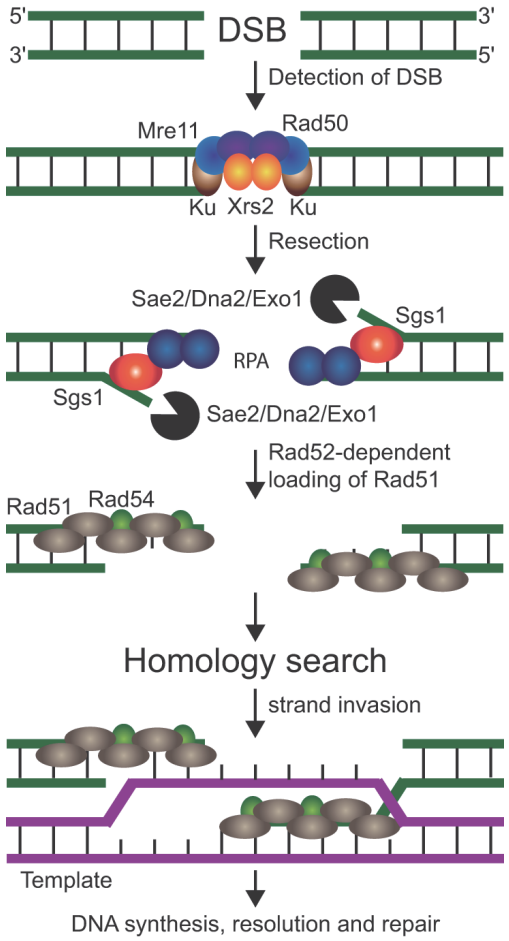

Fig. 1. Initial steps of homologous recombination in budding yeast. After the detection of a double-strand break (DSB), the Mre11-Rad50-Xrs2 complex is loaded onto the DNA ends. Along with Ku70 and Ku80, it holds the two ends of the break together. Then, nucleases and helicases, including Mre11, Sae2, Exo1, Dna2, and Sgs1 resect the DNA ends to create 3′ overhangs. RPA-coated single-stranded DNA overhangs bind Rad52 and drive Rad51 filament formation. The Rad51 filament is thought to stabilize the pairing of homologous sequences before mediating strand invasion. The cell can then resolve this structure in several different ways, leading to two intact DNA molecules.

§2. The initial steps of homologous recombination

The first step in homologous recombination is the detection of a DNA lesion. This step involves a number of signaling proteins that congregate to the broken DNA in an orderly fashion, ultimately forming foci called repair centers.[14)–16)] Then resection prepares the DNA ends for homology search. It involves digesting the 5′ strands on both sides of the break and leaving 3′ single-stranded DNA overhangs (Fig. 1). Throughout the process, the two DNA ends are held together by repair proteins.[17)–19)] The resulting complex of broken DNA and proteins can then conduct the search for a template. In yeast, as little as 20 base pairs can be used for homol-

ogous recombination, but longer stretches of homology recombine more efficiently. Once the template for repair has been found, the 3′ overhangs can invade the homologous template. New DNA synthesis begins to copy the lost information off the template (Fig. 1). At this point, the cell can resolve the structure into two intact DNA molecules using one of several pathways. A discussion of this process is beyond the scope of this manuscript, but details can be found in San Filippo et al.[10)]

§3. The problem of homology search

Homology search takes place between end resection and the invasion of the homologous partner. It involves the juxtaposition of the processed break (with the two ends held together) and its template site and a recognition of the shared homology. The search takes place in the complex environment of the cell nucleus, where large amounts of DNA are packaged into an extensive protein-DNA assembly called chromatin. Chromatin has the dual role of compacting DNA while allowing specific regions of the genome to be accessed as needed. Thus, chromatin is constantly being remodelled to allow different regions to be "read".[20)] It has been proposed that chromatin remodeling may drive chromatin movement.[21)]

In every species where it has been studied, chromatin moves with a diffusion coefficient of 10^{-4} to $10^{-3}\,\mu m^2/s$.[22)–26)] The movement characteristics are generally consistent with a constrained random walk model,[23)] although recent studies have documented exceptional cases of autocorrelation and directed movement.[22),27)] Another important aspect is that a single DNA locus on chromatin moves within a confined space smaller than the volume of the nucleus. In budding yeast, a genomic locus tagged for live imaging can roam around a volume with a radius of about 650 nm, or about 30% of the nuclear volume of a haploid yeast cell.[23),25)] This constraint is variable, however, as exemplified by a reduction of the radius of constraint as DNA replicates.[25)] In *Drosophila* spermatocytes[22)] and human fibrosarcoma cells[24)] the constraint is also variable, ranging from 0.2 to 3 μm, depending on the experimental conditions.

It is in the context of organized, densely packaged and moving chromatin that DNA is damaged and that its repair must occur. The extent to which broken DNA moves is unclear. The mobility of DSBs appears to range from just as much as intact chromatin[17),28),29)] to large-scale movement that allows clustering of distant breaks within minutes after induction.[30)] The source of this discrepancy is unclear but may include experimental setups, visualization methods, as well as the type and/or the amount of damage delivered.[17),31),32)] In any case, broken DNA has the potential to move over large distances, which would allow a global search for a homologous template.

Homologous recombination between sites on different chromosomes is less efficient in mammals than in yeast, presumably because the size of the nucleus and the complexity of the genome are significantly larger. Assuming a random process, homology search in a larger volume would take longer. Even so, in the larger genome of mammalian nuclei, both intra- and interchromosomal homologous recombination can happen.[33)–36)] Clearly, therefore, homology search can occur efficiently in mam-

malian cells. The question, in both complex and simple organisms, is how the search is conducted.

Budding yeast is easily manipulated genetically and has been extensively used to study homologous recombination. Homology search occurs quickly and efficiently in budding yeast. Once a DSB is generated, it takes 1 to 4 hours to find the homologous template and complete repair.[38)–42)] Astonishingly, the search occurs successfully in the majority of the cells. A DSB in the yeast nucleus, therefore, manages to rapidly, robustly and reproducibly sort through about 13 million base pairs to find the one stretch of homologous sequence. This is a remarkable accomplishment. Below we discuss the models that have been proposed thus far, although all fall short of explaining it.

3.1. *The simple diffusion model*

The simplest model, often referred to as the null hypothesis, as opposed to an active, directed search, stipulates that homology search is random and entirely dependent on the diffusion properties of broken and unbroken DNA. Others have largely discredited this model because DNA diffusion alone is thought to be too slow to account for the high efficiency of the search.[12)] In addition, this model may be an oversimplification since it does not take into account the possibility that diffusion properties may be altered in response to DNA damage or that the search could be facilitated by the organization of the genome.

Nonetheless, there is evidence that a random search does occur in budding yeast. If several templates are provided to repair a break, the efficiency of homologous recombination increases in proportion to the number of templates[43)] (and B. Towbin, H. van Attikum and S. M. Gasser, unpublished observations). In addition, several studies found that regardless of the location of the template, it is eventually found and used for repair.[43)–45)] The fact that templates located at different positions have an equal frequency of being found suggests that the search is random. There are, however, some "hot" and "cold" spots for homologous recombination,[46)–49)] an observation that is inconsistent with the simple diffusion model.

3.2. *Somatic pairing model*

At the other extreme of the spectrum lies the somatic pairing model, which proposes that homologous sequences are juxtaposed in the nucleus before there is any damage.[11)] This is clearly the case for sister chromatids after DNA replication, which are tethered together and, therefore, cannot diffuse very far apart.[50)] While pairing of homologous chromosomes has also been extensively documented in the interphase nucleus of Dipterans such as *Drosophila*,[51)] it is clear that in yeast and in other organisms somatic pairing occurs only in meiosis.[52)–55)] Thus, while proximity to a template would increase the efficiency of homology search, most loci are not paired in somatic cells.

3.3. *3D-1D random search*

To address the shortcomings of the simple diffusion model, one can propose that the broken piece of DNA could do a hybrid search that contains periods of

three-dimensional search interspersed by non-specific binding to other stretches of DNA. This type of facilitated diffusion has been studied extensively in the case of proteins searching for their DNA binding site, and it was shown that a combination of 3D jumps with 1D sliding on the length scale of 50 bp is the most efficient search method.[56] The case of homology search is closely related. It is therefore plausible that also in this case a combined 3D/1D search would be an improvement compared to a simple 3D search. However, it is unclear what the optimal sliding length would be and to what extent the search process could be sped up.

3.4. *The facilitated random search*

The models proposed thus far are, for the most part, unsatisfactory and cannot explain how homology search is so efficient. One obvious pitfall of the previously reported models is that they ignore the inherent organization found within the nuclear environment. In yeast, the spindle pole body sits on one end of the nucleus opposite to a large nucleolus. The centromeres are attached to the spindle pole body, while the telomeres are found at the nuclear periphery (henceforth referred to as anchored) typically away from the nuclear pores that transect the nuclear membrane.[57] Similarly, organization in more complex organisms includes specialized subnuclear compartments that favor specific processes such as transcription.[58]

We propose a facilitated random search model where the search for the homologous template is random, but it is influenced by the intrinsic nuclear and chromatin organization as well as being dependent on enzymatic hydrolysis of ATP. In addition to accommodating the organization of the nucleus, this would also be consistent with hot and cold recombination sites, thereby overcoming two major shortcomings of the simple diffusion model.

We tested here the predictions of the facilitated random search model *in silico*. We asked specifically whether peripheral anchoring of a break and/or a template site could contribute to the high efficiency of homology search observed *in vivo*.

§4. Random walk simulations to test the facilitated random search model

We used a simple random walk model to investigate some of the properties of diffusion-driven homology search. Our study focused on the effects of locus positioning. In budding yeast, the nuclear periphery is an important scaffold for nuclear organization[59] and there is evidence that it also plays a role in DNA damage repair.[60)−63] Therefore, we investigated how anchoring of break and/or template site to the nuclear periphery influences the duration of homology search. While a random walk model obviously neglects that chromosomes are possibly entangled polymers, which might further alter the search, it allows a clear view on the effects of different locus positions on the efficiency of the search. In our model, the two loci of interest are represented by two randomly moving spots that are either confined to the interior or the surface (see scenarios below) of the same sphere with a radius of 1000 nm — roughly the size of a haploid yeast nucleus. We compared the following scenarios (see also Fig. 2):

1. Both spots move inside the spherical confinement.
2. One spot moves inside the confinement, the other is fixed at the center.
3. One spot moves inside the confinement, the other on the surface of the sphere.
4. One spot moves inside the confinement, the other is fixed at a point on the surface.
5. Both spots move on the surface of the confinement.
6. One spot moves on the surface of the confinement, the other is fixed at a point on the surface.

An internal spot starts at a random position within the sphere and its movement is confined to the interior by reflective boundary conditions. For a peripheral spot, starting point and movement are restricted to the surface of the sphere. The spots exert independent isotropic random walks with a step size of 1 nm. Each simulation run ended when the distance between the two spots fell below a threshold of 10 nm as it is the width of the nucleosome, the DNA-protein complex that compacts DNA. It should be noted, however, that although the exact number of steps depends on this threshold, changing the threshold does not change the relative ranking of the different scenarios with regard to the time to collide (data not shown).

The results of our simulations are shown in Table I. The duration of the search is shortest by far in scenario 5, in which both loci move along the periphery. It is more than 5 times shorter than for two internal loci (scenario 1)

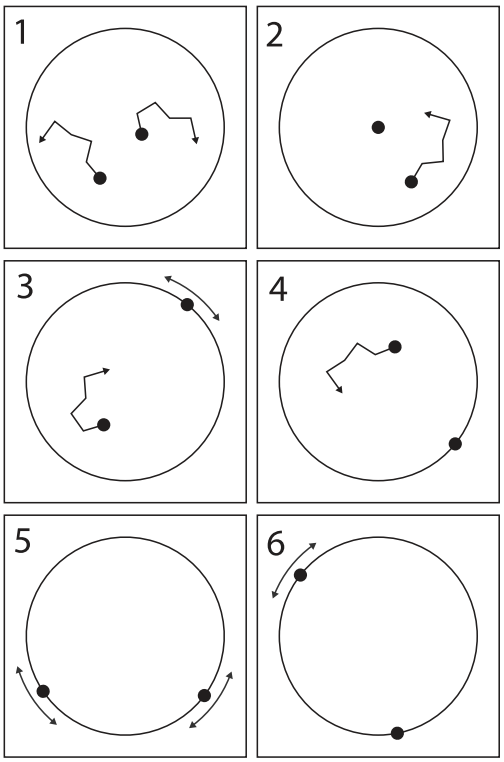

Fig. 2. Schematic representation of the scenarios simulated.

Table I. Time to collide (in million time steps) in different positioning scenarios (see text) inside a sphere of 1000 nm radius. The step size was 1 nm and homology search was considered complete when the spot to spot distance reached 10 nm or less. For each scenario, the results of 1000 simulations were averaged. The reported error is the standard deviation on the mean.

scenario	time to collide
1	112 ± 3
2	220 ± 7
3	236 ± 7
4	439 ± 1
5	21 ± 0.7
6	42 ± 1

and more than 20 times shorter than in the slowest scenario (scenario 4, one spot internal, one fixed at the periphery). It is important to note that the two fastest scenarios (5 and 6) both require the movement (or fixed position) of both loci to be restricted to the surface of the confinement. Moreover, the relocation of only the break site to the periphery (scenarios 3 and 4) would be most detrimental. At least in this simple diffusive model, it is not desirable with respect to the speed of homology search to relocate an internal site of damage to the nuclear periphery.

Our simulations lead to three conclusions:

1. Efficient homology search occurs at the periphery of the nucleus (scenarios 5 and 6).
2. Anchoring of the damaged site or the template alone slows down the search (scenarios 3 and 4).
3. Immobilization of the break or the template slows down the search (scenario 1 vs 2 and 5 vs 6).

§5. Improving the computational modeling

In this study, we used a random walk model to specifically investigate the influence of locus position on homology search. However, it must be emphasized that chromosomal loci do not move strictly according to a constrained random walk model[26] and that the possible entanglement of the chromosomes may further alter the duration of homology search. For a full understanding of the physical aspects of the search process, these effects have to be taken into account. This can be achieved by representing the chromosomes by a polymer chain model. One of the challenges of such an approach is computational efficiency, since the modeling of several sufficiently long polymer chains is much more time-consuming than a random walk simulation. An efficient way to model problems of random encounter is the so-called method of excess collisions (MEC), which has been successfully applied to the search of DNA binding proteins for their binding site.[64),65] Preliminary tests with our random walk model have demonstrated the applicability of the MEC approach to homology search simulations and suggest that a reduction of computation time by a factor of several hundreds could be possible.[66]

§6. *In vivo* evidence for a facilitated random search

The results presented here support a facilitated random search model, in which anchoring and immobilization impact search time. While our results may not be surprising, it is a highly non-trivial question whether and to what extent living cells use these basic principles to pair two homologous sequences. Our simple model clearly shows that anchoring only one of the two searching sequences involved would be least efficient. Thus, efficient homology search *in vivo* would have to avoid such a scenario. One way to do so would be to reorganize the nucleus such that both the potential template and the broken DNA lie in the same subnuclear region; for example at the periphery or in the nuclear lumen. Spontaneous and damage-induced repair foci are enriched in the nuclear interior, away from the periphery.[67] There are two possible

explanations for such a behavior. One is that damage primarily occurs on DNA found in the middle of the nucleus, away from the nuclear periphery; the other is that damage occurs with equal probability throughout the nucleus and the broken loci are transported (or retained after a random diffusion) to the nuclear interior. We believe that the latter applies since sequences damaged by two different agents show the same position preference. Zeocin, which causes single and double-strand breaks, and methyl methanesulfonate, which alkylates DNA and thus causes double-strand breaks only indirectly in a replication-dependent fashion, both cause repair foci that are enriched in the nuclear center.[67] These data, therefore, suggest that repair foci are relocalized to the nuclear interior where homologous recombination occurs.[67]

A curious phenomenon in yeast[14),68),69)] and possibly in mammals[30)] is that multiple DSBs converge to a single repair center, again arguing for a mechanism that recognizes DSBs and promotes their interaction. Confinement of a DSB to a specific region of the nucleus prior to the formation of a repair focus would be counterproductive according to the results of our modeling. Our simulations suggest that the reason for having a specialized region to which DSBs are confined is not to speed up homology search.

Reorganization of the genome may be part of the cell's response to DNA damage. One example is that upon DSB induction, proteins that anchor telomeres become uniformely distributed throughout the nucleus rather than forming foci that colocalize with telomeres.[70)−72)] Consequently, the movement of telomeres increases,[73)] although they appear to remain at the nuclear periphery.[70)] Such a situation corresponds to scenario 3 in our modeling. While broken DNA with a template at a telomere would avoid the slowest scenario (scenario 4) the search would not be as fast as if telomeres were released (scenario 1). The fact that telomeres are not released from the periphery may help explain why they are refractory to recombination. It is not clear whether other anchored loci such as centromeres and genes associated with the nuclear pores see their mobility increase upon damage. It is still unknown why centromeres have low recombination rates,[46)] but it likely reflects local chromatin structure or tethering to the spindle pole body. If centromeres are not released from the periphery upon damage, then our model predicts that they may fall into scenario 4 or 6 and therefore their low recombination frequencies could reflect inefficient homology search.

Several factors need to be characterized to decipher how such a search is organized *in vivo*. For instance, we need to define precisely the parameters and modifiers of chromatin and DSB movement and establish how fast and where the search occurs. We also need to know whether, and if so how, the genome is reorganized after DSB induction. Moreover, it is still unclear what the probability is that an encounter between the break and the template will lead to repair. Our understanding of homologous recombination and its role in genome stability requires that we solve the homology search enigma.

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