DNA DAMAGE SERIES

Mechanisms and principles of homology search during recombination

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Abstract | Homologous recombination is crucial for genome stability and for genetic exchange. Although our knowledge of the principle steps in recombination and its machinery is well advanced, homology search, the critical step of exploring the genome for homologous sequences to enable recombination, has remained mostly enigmatic. However, recent methodological advances have provided considerable new insights into this fundamental step in recombination that can be integrated into a mechanistic model. These advances emphasize the importance of genomic proximity and nuclear organization for homology search and the critical role of homology search mediators in this process. They also aid our understanding of how homology search might lead to unwanted and potentially disease-promoting recombination events.

Mating-type switching
A process by which yeast cells switch their mating-type through programmed homologous recombination.

Homologous recombination is a central cellular pathway for the repair of DNA double-strand breaks (DSBs), and it also promotes genetic exchange[1], for example during meiotic recombination[2] or the process of mating-type switching in yeast[3]. The elegance of homologous recombination is that it uses undamaged homologous sequences as templates for repair[1]. Repair by this pathway therefore requires direct physical proximity between the homologous sequences to enable base pairing of a single DNA strand of one end of the broken chromosome with complementary DNA of an undamaged homologous donor sequence.

Although the necessity of direct spatial proximity between the complementary DNA strands was emphasized in Robin Holliday's genetic exchange model that was formulated 50 years ago[4], the question of how homologous DNA comes together, or more specifically, how homology search is carried out in the context of a crowded nuclear environment, has remained an intriguing and intensely debated issue. Indeed, this lack of knowledge, which is caused by the absence of appropriate experimental data, is exemplified by several partially opposing models that have been entertained during the past years.

Homology search could be highly spatially restricted, as homologous recombination usually targets homologous sequences that innately reside in close vicinity, such as the homologous DNA of sister chromatids (which are tethered by cohesin) in the G2 phase of the cell cycle or newly replicated sister DNA during DSB repair in S phase[5]. However, homologous recombination does also occur between ectopic (non-allelic) DNA and even between DNA elements that are located on different chromosomes[6]. The surprisingly high recombination efficiency between sequences that are located on different chromosomes of the yeast *Saccharomyces cerevisiae* has even led to the proposal that an efficient homology search is carried out by comprehensively scanning the entire genome[7]. However, this model has been challenged by estimates of the scanning time that would be required to achieve this, which suggests that it is nearly completely impossible for a genome-wide homology search to occur *in vivo* within a realistic time frame[5,6]. To explain this apparent paradox between observed and estimated homology search scanning times it was proposed that a pre-alignment of every homologous sequence in the nucleus may take place before the occurrence of a DSB[6]. However, this model does also not completely agree with the observed data[6]. Thus, how homologous sequences find each other, especially in the context of chromosomes and the nuclear environment, remains an interesting and unsolved scientific issue. Moreover, a detailed understanding of homology search is expected to provide important insights into disease-related processes, such as unwanted chromosomal rearrangements, and also into the evolution of genomes.

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doi:10.1038/nrm3805
Published online 14 May 2014

Several recent methodological advances, in particular single-molecule studies and genome-wide protein profiling approaches, have led to several independent but complementary new insights into the mechanism of homology search. Single aspects of these findings have recently been separately discussed [8–10]. This Review attempts to integrate existing *in vitro* and *in vivo* data from bacteria, yeast and higher eukaryotes into a mechanistic model of homology search. We explain that different methodologies analyse distinct steps of homology search and emphasize the importance of nuclear organization and territories for recombination in homology search. Finally, we discuss the proteins that mediate cellular homology search before proposing a model for the underlying mechanism of this process.

Local homology search

The process of homologous recombination is typically divided into three different phases[1] (BOX 1). In the presynaptic phase, recombinase proteins of the recombinase A (RecA) superfamily (including RecA (bacteria), RadA (archaea), RAD51 and DMC1 (eukaryotes)) form right-handed helical nucleoprotein filaments on single-stranded DNA (ssDNA)[11] that is generated during DNA end-resection[12]. In the synaptic phase, these nucleoprotein filaments stably pair with homologous DNA sequences. In the postsynaptic phase, the recombinase proteins are disassembled from the stably paired duplex DNA, and the repair process is completed by new DNA synthesis[1]. Homology search thereby defines the crucial event that takes place directly before the synaptic phase. It mediates the establishment of spatial proximity between the presynaptic filament and the target chromosomal segments, the probing of the DNA in these segments for homology and finally the recognition of homology through base pairing. In this section, we first discuss how homology probing and recognition is achieved when homologous DNA is present in close proximity (to which we refer to as 'local homology search'). In subsequent sections, we discuss how the juxtaposition of homologous regions might be achieved in the context of chromosomes and the nuclear environment (to which we refer to as 'cellular homology search').

Homology recognition. The active element for homology probing and recognition is the presynaptic nucleoprotein filament. This structure identifies homologous DNA sequences to drive DNA strand exchange, while generating a novel heteroduplex between the complementary donor strand and itself[11] (FIG. 1). The design of specific *in vitro* assays that use RecA–ssDNA (or RAD51 in eukaryotes) filaments and double-stranded DNA (dsDNA) as substrates has been important for our current understanding of presynaptic nucleoprotein filament functions. In such experiments, multiple diffusible DNA molecules or single molecules that are accessible to micromanipulation were used, which enabled homology probing and recognition to be investigated in isolation, disregarding genomic distance, multiple chromosomes, chromatin and the nuclear environment[8] (BOX 2).

Recombination requires homology, but how is homologous DNA recognized? Both RecA and its eukaryotic homologue RAD51 harbour two DNA-binding sites. Whereas the primary binding site is required and sufficient to bind resected ssDNA, the secondary DNA-binding site is needed for homology probing[13,14]. On contact of the presynaptic nucleoprotein filament with a target dsDNA the RecA secondary binding site stochastically interacts with a short stretch of one of the two target strands[15,16] (FIG. 1). Although it was proposed that RecA might actively facilitate this early interaction[17], the affinity of the secondary binding site for ssDNA was shown to be too weak to enable the generation of stable ssDNA bubbles in dsDNA[16]. Thus, the initiation of homology probing is probably due to intrinsic DNA breathing, which is a spontaneous and transient opening of one or a few nucleic acid base pairs that generates short bubbles containing ssDNA. This idea is supported by the fact that homology recognition proceeds faster in thermally more unstable AT-rich regions[18,19] and with DNA substrates that have been partially unwound[16]. In contrast to the target strand of DNA that binds to the secondary binding site of RecA, the other strand of the target DNA makes almost no physical contact with the recombinase. Interaction between the DNA strand that has almost no contact with the recombinase and the presynaptic nucleoprotein filament is therefore dependent on base pairing and is consequently limited to cases in which homologous DNA is present; this minimizes the amount of undesired stable interactions with non-homologous sequences[20]. Equally crucial for successful homology probing is the ability of RecA family proteins to stretch the filament DNA to a length that is one-and-a-half times that of the original dsDNA in B-DNA form[11]. However, the stretching is not uniform, as DNA segments of 3 bp in length always remain in B-DNA-like conformations with long internucleotide stretches that are located between them[20,21] (FIG. 1). This DNA conformation still enables proper Watson–Crick base pairing[20], but the deformation of the target DNA that is necessary to achieve this is only energetically favoured if new base pairing occurs between the complementary strands[22] (a process known as 'conformational proof-reading'). This finding is further supported by the fact that applying force to the opposite termini of dsDNA stabilizes its otherwise unstable interaction with a non-homologous presynaptic nucleoprotein filament[23,24]. The physical distance between the two DNA-binding sites in RecA dictates that a minimal homologous region of approximately 8 bp in length is required to initiate strand exchange[16], which provides the advantage that recombination between frequently occurring microhomologies is inefficient[25].

As soon as a sufficient amount of homology is captured by Watson–Crick base pairing, the presynaptic nucleoprotein filament and the target DNA form a metastable structure that is known as the paranaemic joint, the stability of which strongly depends on the presence of the recombinase itself[26]. Only when strand exchange proceeds until the free end of the broken chromosome is engaged in pairing, the new heteroduplex becomes topologically intertwined in a stable and protein-independent plectonaemic joint[26]. Interestingly, homology recognition

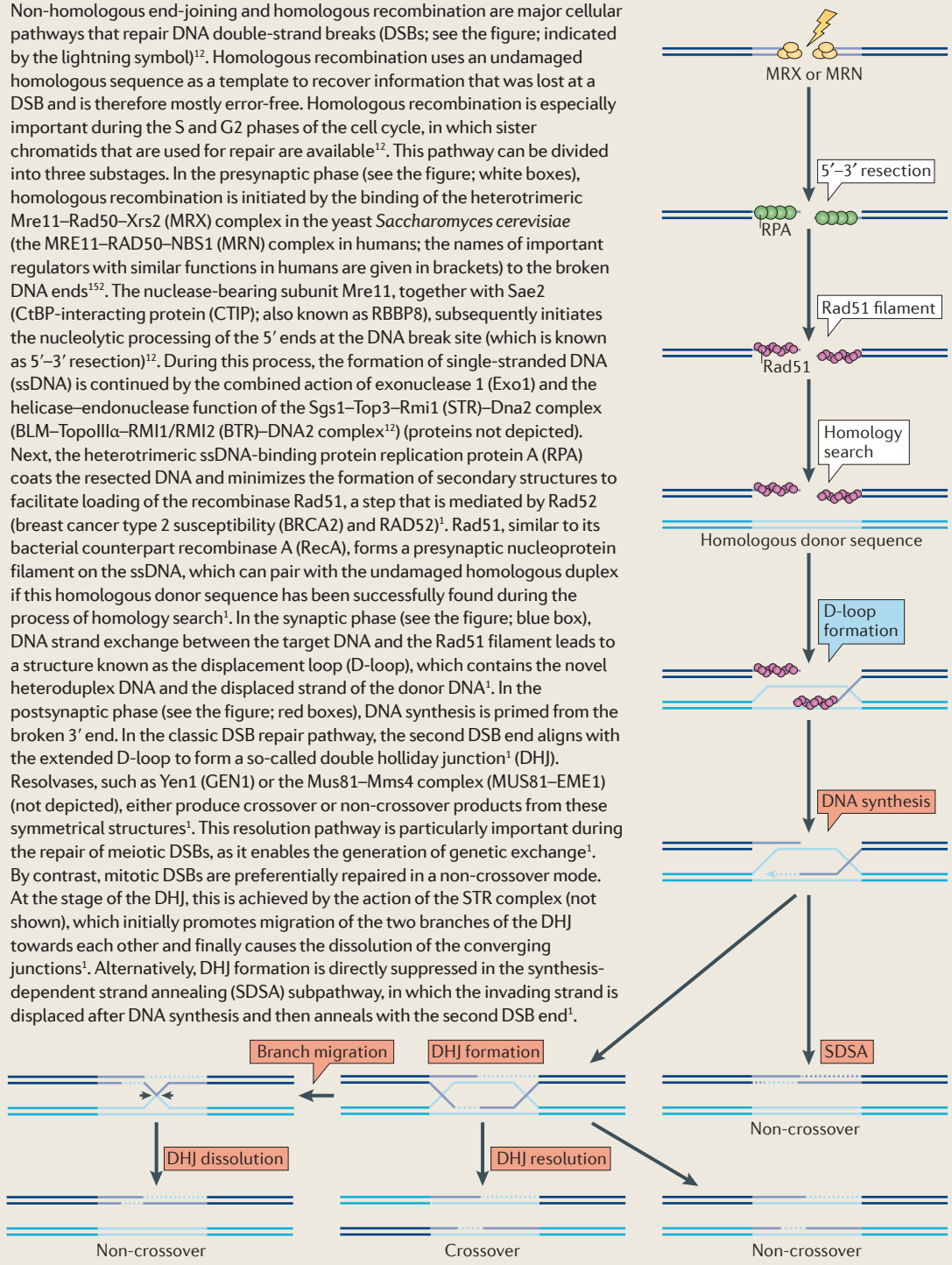
Strand exchange
A continuous establishment of base pairing between the recombinase-coated single-stranded DNA (ssDNA), at the DNA double-strand break (DSB), and the complementary strand of the homologous DNA.

Heteroduplex
Double-stranded DNA that consists of two single DNA strands of different origin.

B-DNA
Standard conformation of DNA as it exists in most functional organisms.

Box 1 | **The homologous recombination pathway**

Non-homologous end-joining and homologous recombination are major cellular pathways that repair DNA double-strand breaks (DSBs; see the figure; indicated by the lightning symbol)[12]. Homologous recombination uses an undamaged homologous sequence as a template to recover information that was lost at a DSB and is therefore mostly error-free. Homologous recombination is especially important during the S and G2 phases of the cell cycle, in which sister chromatids that are used for repair are available[12]. This pathway can be divided into three substages. In the presynaptic phase (see the figure; white boxes), homologous recombination is initiated by the binding of the heterotrimeric Mre11–Rad50–Xrs2 (MRX) complex in the yeast *Saccharomyces cerevisiae* (the MRE11–RAD50–NBS1 (MRN) complex in humans; the names of important regulators with similar functions in humans are given in brackets) to the broken DNA ends[152]. The nuclease-bearing subunit Mre11, together with Sae2 (CtBP-interacting protein (CTIP); also known as RBBP8), subsequently initiates the nucleolytic processing of the 5′ ends at the DNA break site (which is known as 5′–3′ resection)[12]. During this process, the formation of single-stranded DNA (ssDNA) is continued by the combined action of exonuclease 1 (Exo1) and the helicase–endonuclease function of the Sgs1–Top3–Rmi1 (STR)–Dna2 complex (BLM–TopoIIIα–RMI1/RMI2 (BTR)–DNA2 complex[12]) (proteins not depicted). Next, the heterotrimeric ssDNA-binding protein replication protein A (RPA) coats the resected DNA and minimizes the formation of secondary structures to facilitate loading of the recombinase Rad51, a step that is mediated by Rad52 (breast cancer type 2 susceptibility (BRCA2) and RAD52)[1]. Rad51, similar to its bacterial counterpart recombinase A (RecA), forms a presynaptic nucleoprotein filament on the ssDNA, which can pair with the undamaged homologous duplex if this homologous donor sequence has been successfully found during the process of homology search[1]. In the synaptic phase (see the figure; blue box), DNA strand exchange between the target DNA and the Rad51 filament leads to a structure known as the displacement loop (D-loop), which contains the novel heteroduplex DNA and the displaced strand of the donor DNA[1]. In the postsynaptic phase (see the figure; red boxes), DNA synthesis is primed from the broken 3′ end. In the classic DSB repair pathway, the second DSB end aligns with the extended D-loop to form a so-called double holliday junction[1] (DHJ). Resolvases, such as Yen1 (GEN1) or the Mus81–Mms4 complex (MUS81–EME1) (not depicted), either produce crossover or non-crossover products from these symmetrical structures[1]. This resolution pathway is particularly important during the repair of meiotic DSBs, as it enables the generation of genetic exchange[1]. By contrast, mitotic DSBs are preferentially repaired in a non-crossover mode. At the stage of the DHJ, this is achieved by the action of the STR complex (not shown), which initially promotes migration of the two branches of the DHJ towards each other and finally causes the dissolution of the converging junctions[1]. Alternatively, DHJ formation is directly suppressed in the synthesis-dependent strand annealing (SDSA) subpathway, in which the invading strand is displaced after DNA synthesis and then anneals with the second DSB end[1].



and strand exchange that are mediated by RecA and eukaryotic RAD51 are independent of ATP hydrolysis *in vitro*[27–29].

The distance of the two DNA-binding sites within the recombinase proteins and the increased entropic freedom of the displaced strand when it is bound to the secondary DNA-binding site seem to be crucial factors for the promotion of the otherwise isoenergetic strand exchange reaction[23,30,31]. By contrast, ATP hydrolysis is crucial for nucleoprotein filament disassembly, which is needed for downstream DNA transactions to occur[32].

Accelerated homology probing. RecA family proteins mediate the recognition between perfectly aligned homologous sequences. However, even in the case of sister chromatids, homologous sequences are unlikely to be

positioned in perfect alignment (see below). Early studies investigating whether the correct alignment is solely established through random collisions or by facilitated diffusion had contradictory results[33–35], probably because studies were limited by the difficulty in directly observing short-lived non-homologous contacts. Nonetheless, recent evidence has shed light on this important issue and revealed two key aspects of efficient homology recognition.

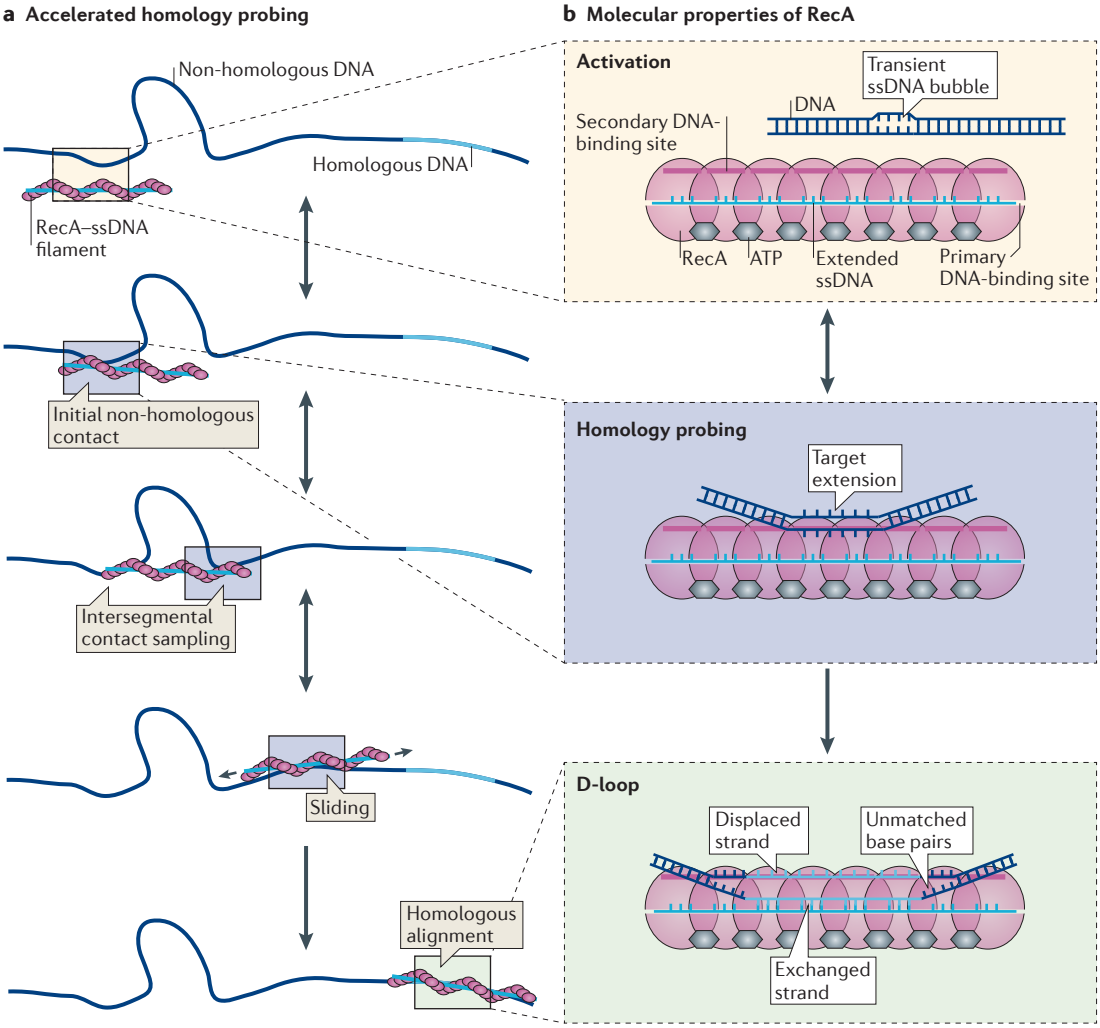
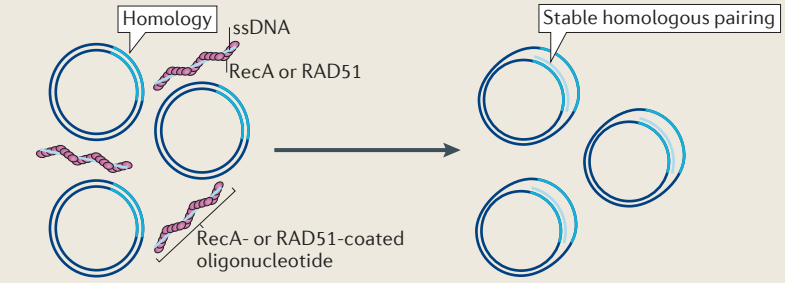


Figure 1 | **Homology probing *in vitro*.** The active element for homology probing and recognition is the presynaptic nucleoprotein filament, which is formed between recombinase A (RecA) and single-stranded DNA (ssDNA). Insight has been gained into how this structure can identify homologous DNA sequences *in vitro* and drive DNA strand exchange. **a** | A RecA–ssDNA filament can diffuse in three dimensions and will make a non-homologous contact with DNA when it first encounters it. Another segment of the same filament can encounter a different stretch of DNA, thereby accelerating the sampling reaction (known as 'intersegmental contact sampling'). The filament also can randomly slide along DNA (indicated by small arrows), a process that considerably accelerates homology detection. Homology probing at non-homologous sites is highly transient and the filament can easily switch between the different search modes (indicated by double-headed arrows between panels). After alignment with the homologous region, homology is recognized by base pairing, strands are exchanged and a rather stable structure is formed. **b** | Magnification of the process of homology probing shown in part **a**. During presynaptic nucleoprotein filament activation, ssDNA (light blue) that is bound to the primary binding site of RecA is extended by 50%. Local stretches of 3 bp in length remain in a configuration similar to B-DNA (beige box). The secondary DNA-binding site of RecA initiates homology probing at transient ssDNA bubbles by binding to one of the two strands of the target DNA, while the other strand does not form relevant contacts with RecA. Similar to DNA that is held in the primary binding site, the target DNA becomes extended after RecA binding (purple box). Without the possibility of base pairing between the free strand of the target DNA and the strand that is held in the primary binding site of RecA, this state is highly unstable and thus non-homologous synapses during homology probing are limited to a few base pairs. As soon as homologous DNA comes into reach, base pairing between the complementary strand and the strand in the primary binding site can occur (green box). The displaced strand is kept bound to the secondary binding site of RecA, which results in the formation of a structure known as the displacement loop (D-loop). The distance between the two DNA-binding sites disfavours base pairing at the edge of homology (unmatched base pairs), with the result that homology must be at least 8 bp in length to form energetically stable heteroduplexes.

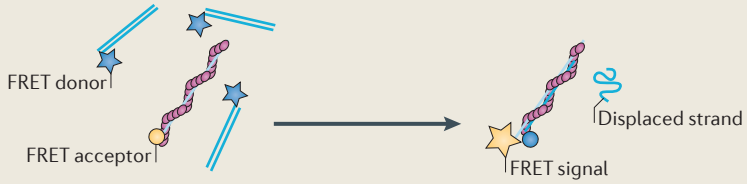
Box 2 | **Methods to study homology search *in vitro***

Classic *in vitro* approaches to study homology search concern the formation of stable displacement loops (D-loops) (see the figure, part **a**) and DNA strand exchange assays (not shown), in which recombinase A (RecA) family proteins are either assembled on circular or linear single-stranded DNA (ssDNA)[153]. Pairing of RecA-coated ssDNA (or RAD51-coated ssDNA) with a linear or circular homologous double-stranded DNA (dsDNA) is detected by gel electrophoresis and southern blotting. Similarly, homologous pairing can be observed by fluorescence resonance energy transfer (FRET) ensemble studies in solution (see the figure, part **b**). To this end, fluorescent probes are coupled to two of the three participating linear strands so that the FRET signal is either increased (as depicted in part **b**) or decreased when pairing occurs[154]. Recently, FRET has been combined with single-molecule analyses[31,39,155], which also enable the detection of unstable non-homologous contacts. FRET experiments usually involve very short oligomers. However, longer molecules of several kilobases in length can be manipulated through optical and/or magnetic tweezers[16,23,40,41,156] (see the figure, part **c**). In this way, effects on the recognition process, such as conformation or supercoiling of the dsDNA target, can be studied. Recognition is either observed directly by fluorescence microscopy of the tagged molecules[41], or indirectly through changes in target length or force transmissions[16,23,40].

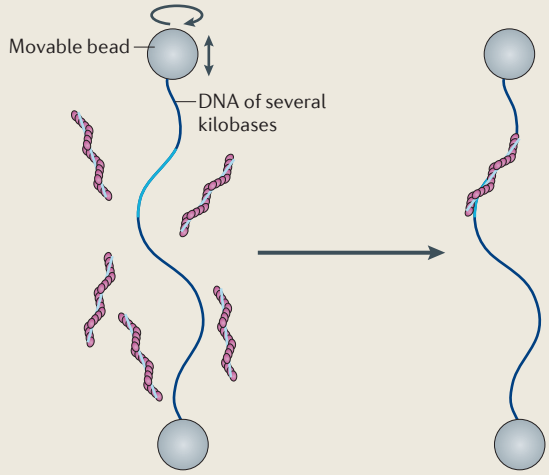
a D-loop assay



b FRET-based assay



c Optical or magnetic tweezer assay



One mechanism to achieve efficient homology recognition is by random sliding of the presynaptic nucleoprotein filament along the donor DNA (FIG. 1); this is analogous to several DNA sequence-specific binding proteins that use short-range sliding to locate their target site[36,37]. Studies suggested that RecA-coated DNA is unable to undergo long-range sliding in the range of kilobases *in vitro*[38]. By contrast, a recent study showed that RecA-coated DNA can undergo limited sliding within a range of 60–300 bp during recombination-based sequence probing and that this is primarily mediated by electrostatic interactions[39]. It is important to mention that the entire recombinase-coated presynaptic nucleoprotein filament does not slide along target dsDNA, as probing synapses seem to be limited to less than 16 bp in length[31,40]. Nevertheless, the observed short-range sliding accelerates homology recognition by 200-fold, as multiple possible alignments can be tested without a need for reoccurring dissociation and reassociation[39]. However, studies of sliding have been exclusively carried out with isolated DNA *in vitro*; thus, it is unclear whether sliding exists or whether it is even more pronounced *in vivo* in the presence of chromatin, as accessory proteins might assist in this hypothetical sliding process (see below).

A second mechanism that ensures a processive homology search is the ability of the presynaptic nucleoprotein filament to probe multiple DNA regions at the same time, a model that is in line with the observation that the association of a presynaptic nucleoprotein filament with dsDNA does generally not require a free DNA end[16]. Indeed, a recent *in vitro* study showed that a single searching filament can bridge two non-contiguous DNA segments[41]. Furthermore, efficient homology recognition strongly depended on a flexible target DNA conformation that enabled multiple simultaneous interactions with the fairly rigid presynaptic nucleoprotein filament to occur[41]. As is the case for filament sliding, this mechanism, which is known as 'intersegmental contact sampling', thus accelerates homology recognition *in vitro*[41] (FIG. 1). Remarkably, *in vivo* studies indicated that presynaptic nucleoprotein filaments can be up to several kilobases in size[42–44], which makes an individual presynaptic nucleoprotein filament ideally suited to form multiple contacts with different genomic sequences in living cells. However, an alignment through partial homologies that are distant from the DSB have the potential to cause undesired recombination events, as only sequences homologous to the end of the broken chromosome are compatible with correct DSB repair. In conclusion, RecA family recombinases are well-tailored for efficient and accurate homology recognition, and sliding of the presynaptic nucleoprotein filament, together with intersegmental contact sampling, might considerably accelerate the pre-alignment of homologous DNA segments.

Cellular homology search

Although crucial insights into the mechanism of homology recognition have been gained by studying the behaviour of presynaptic nucleoprotein filaments *in vitro*, homology search faces additional challenges *in vivo*. The main obstacles are that the filament has to probe DNA sequences that are embedded in chromatin and that the bulk of all chromosomal DNA is not homologous to

the sequences of the DSBs. Furthermore, several DSBs may have to be repaired at the same time, and undesired recombination, also through short repetitive sequences, should be avoided.

Thus, several key questions remain. How does the presynaptic nucleoprotein filament find homologous DNA in the nuclear context? Does homology search efficiently function in a genome-wide manner or is it restricted? What are the parameters and cellular mediators that enable homology search in the complex nuclear environment, and how does homology search cope with chromatin rather than with 'naked' DNA?

Scenarios of cellular homology search. The main events in which homologous recombination is used for DSB repair are recombination at the replication fork in S phase, recombination between sister chromatids in G2 and recombination between homologous parental chromosomes during meiosis[45]. In these cases, DSBs and donor homology sequences are already in fairly close spatial proximity owing to the architecture of the replication fork, cohesin-mediated sister-chromatid cohesion or homologous chromosome alignment, respectively[46–48]. Hence, it was argued that cells might not need sophisticated mechanisms for homology search[5]. However, even the replication fork and in particular cohesin, which is usually present at ~10-kb intervals along the DNA[49,50], does not guarantee that homologies will be perfectly juxtaposed. Indeed, freshly replicated sister loci in mammalian cells are frequently more than 1 μm (approximately 50% the length of an *Escherichia coli* bacterium) apart[51]. Moreover, homologous recombination is not restricted to recombination between homologous DNA of homologous sister chromatids or chromosomes, as exemplified by recombination that occurs between repetitive elements that are dispersed within the genome[52,53]. This phenomenon probably reflects the fact that homology search can continue for hours if homology is not found immediately[44], thereby promoting ectopic homologous recombination between, for example, a repetitive element that is close to a DSB and a similar repetitive element located elsewhere in the genome[52,53]. Although this mechanism ultimately repairs the broken chromosome, it might cause toxic genomic rearrangements[54,55].

Further robust evidence that homology search is not restricted to highly spatially confined regions is that recombination efficiency is generally high in yeast even if the DSB and the donor homology sequence are located on different chromosomes[44,56–62]. Indeed, the comparably high efficiency of interchromosomal recombination was the basis for the hypothesis that homology search can efficiently scan the entire genome[7], a model that has only been sporadically questioned[5,6].

Genome-wide visualization of homology search. Research on homology search *in vivo* was mostly hampered by the lack of experimental tools. However, a recent study successfully addressed the different opposing models of homology search *in vivo* by using chromatin immunoprecipitation (ChIP) to detect cellular homology search by precipitating recombination proteins[44]. ChIP analysis of Rad51 at various time points after the induction of a single DSB visualized 'snapshots' of ongoing homology search[44] (BOX 3). Surprisingly, Rad51 signals were not restricted to DNA that surrounds the DSB as had been previously reported[63] but were distributed over much of the broken chromosome. As these signals were also observed outside of the area of DNA end-resection, these DSB-distant signals must derive from homology probing rather than from the extension of a presynaptic nucleoprotein filament along the chromosomal axis. Furthermore, ChIP experiments using a mutant Rad51 protein that lacks the secondary DNA-binding site needed for homology probing[13] failed to produce broad ChIP signals outside of the area of DNA resection[44]. Therefore, the contacts of the presynaptic nucleoprotein filament with the target DNA that give rise to Rad51 ChIP signals at a distance from the DSB seem to require Rad51 to probe DNA with its secondary DNA-binding site, which suggests that this probing involves strand invasion. As the Rad51 ChIP signals accumulated mainly on the chromosome that carries the DSB, it seems that homology search functions more efficiently in *cis* on the broken chromosome than on other chromosomes in *trans*. This would rule out a model in which the entire genome is probed with the same efficiency or the view that homology search does not occur in cells. This finding is further supported by previous data that show differences in the generally high recombination efficiencies between homologous sequences at different genomic locations in yeast, which also suggests that intrachromosomal recombination events are favoured over interchromosomal recombination events[58–62].

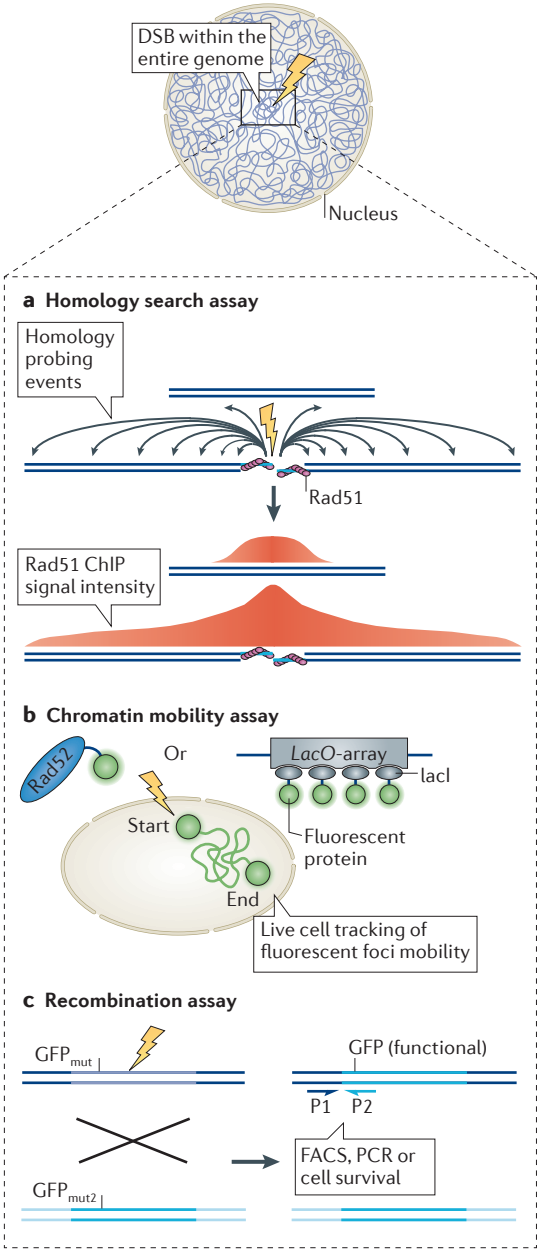
Nuclear organization influences homology search. The nonrandom organization of chromosomes in the nucleus affects numerous nuclear processes (BOX 4) and genome stability[64]. Notably, chromosomal translocations through non-homologous end-joining seem to be mostly dictated by the spatial proximity of chromosomes[65–73]. Evidence that the spatial proximity of DNA and nuclear organization affect homologous recombination and homology search in *S. cerevisiae* was recently presented. As mentioned above, Rad51 ChIP data revealed that homology search mainly functions intrachromosomally and that recombination efficiency decreases with increasing distance from the DSB[44]. This finding is in line with observations that in cases in which multiple homologous sequences are dispersed in the genome, DSB repair is generally more efficient between homologies on the same chromosome[52,59,74]. However, it is likely that recombination efficiency between alleles is not simply a consequence of the linear distance of homologies along the chromosomal axis but that it is mainly due to the proximity of DNA in a three-dimensional setting. Indeed, chromosome proximity data from cells that were not challenged with DNA damaging agents indicate that three-dimensional interactions between DNA segments *in vivo* preferentially occur between elements of the same chromosome rather than in *trans* between DNA that is located on different chromosomes[75].

Repetitive elements
Genetic elements, such as satellite DNA or retrotransposons, that are present in multiple copies in a genome and/or consist of small repetitive building blocks.

Ectopic homologous recombination
Recombination with a homologous sequence that is located in a genomic location other than the corresponding allele on the sister chromatid or homologous chromosome.

Box 3 | **Methods to study homology search *in vivo***

In vivo approaches to study homology search use site-specific DNA double-strand breaks (DSBs) (see the figure; indicated by lightning symbols). In yeast and mammalian cells, these can be generated by the controlled expression of specific nucleases that induce DNA breaks at pre-defined loci[77,157,158]. Ongoing homology search can be monitored through chromatin immunoprecipitation (ChIP) of recombinase proteins, such as Rad51 (REF. 44) (see the figure, part **a**), at fixed time points after DSB induction. This is possible because each homology probing event represents a transient interaction of the Rad51-coated presynaptic nucleoprotein filament with its target DNA, and this state can be captured by crosslinking proteins to DNA. Chromatin mobility can be observed through live cell imaging of DSB-binding proteins that are tagged with fluorescent proteins, such as Rad52–YFP (see the figure, part **b**). Alternatively, specific DNA sequences of DNA-binding proteins are integrated next to a DSB, and their mobility is observed by the binding of fluorescently tagged proteins. An example for this is the *lacO* operator, a genetic element that is bound by lacI (also known as lactose operon repressor), which can be fused to GFP[85,86] (see the figure, part **b**). As a third possibility, discrete homologies that are located at defined chromosomal positions are used to measure recombination efficiencies[56] (see the figure, part **c**), which can provide values for the efficiency of the homology search process. However, in principle, this approach only monitors the end-point of the recombination reaction. Successful recombination can be, for instance, monitored using fluorescence-activated cell sorting (FACS) by observing the restoration of a functional GFP open reading frame. For example, a point mutation in one of the two GFP open reading frames that creates a premature stop codon (GFP_{mut}) is rescued by recombination with a second copy that does not contain this mutation but lacks a functional promotor (GFP_{mut2}). Alternatively, homologies are designed to enable the formation of a specific PCR product after successful recombination (this means that they contain short stretches of heterologous sequences, where specific primers P1 and P2 can bind to) or cell survival is analysed. This approach uses the fact that the presented homology contains a mutation in the recognition sequence for the endonuclease and that successful recombination will mediate resistance to expression of the endonuclease.



The concept that homology search is mostly driven by spatial proximity is further supported by studies of the yeast mating-type system. Yeast mating-type switching involves homologous recombination-mediated genetic exchange between the mating-type locus *MAT* and alternative mating-type specifying DNA elements, which are known as *HML* and *HMR*[76,77]. The key events are a programmed DSB at *MAT*, which is located at an internal position of chromosome III, and alternative repair through *HML* or *HMR*, which are located on opposite arms of the same chromosome. Intriguingly, in yeast cells of the a mating-type (*MATa*), which preferentially repair the DSB at *MAT* through *HML*, a large chromosomal loop of roughly 170 kb in length is formed between the *MAT* locus and a recombination enhancer element, which is located right next to *HML*[57,78–80]. Experiments have shown that the recombination enhancer element and hence loop formation are crucial for the efficient use of *HML* to repair the DSB at *MAT*, as recombination enhancer element deletions led to repair through the alternative *HMR* element[57]. Importantly, this finding is supported by ChIP data showing that Rad51 is also immediately found at *MAT* and around the recombination enhancer element in *MATa* cells after DSB induction at *MAT* but only modestly at the DNA that forms the loop[44]. These two lines of evidence thus suggest that homology search probes the DNA of the loop that is formed between *MAT* and the recombination enhancer element less efficiently.

Recombination enhancer
A specific sequence that is present in the genome of the yeast *Saccharomyces cerevisiae*, which enables the establishment of a large chromosomal loop to facilitate recombination in cells of mating-type a (*MATa*).

Box 4 | **Three-dimensional nuclear organization**

Genomes are nonrandomly organized not only during mitosis and meiosis but also during interphase. Modern techniques revealed how genomes from different organisms are generally organized or positioned in territories[129,159–165]. In particular, high-resolution microscopy[166], chromosome conformation capture[167] and DNA adenine methyltransferase identification (DamID)[168] have contributed to our current view of the nucleus. In mammalian cells, current models envision a hierarchy of nuclear organization, including territories of individual chromosomes, chromosomal domains and chromosomal loops[129,159,164], that is linked to processes, such as transcriptional regulation and genome stability[169–172]. Notably, in higher eukaryotes, interchromosomal interactions are mostly restricted to the periphery of chromosomal territories[129,159,169], whereas in *Saccharomyces cerevisiae* these interactions occur much more frequently owing to a reduced spatial organization[75,81]. However, intrachromosomal interactions are still more frequent than interchromosomal interactions in yeast[75,173], and nuclear landmarks or territories also exist in yeast[81,162]. First, approximately one-third of the yeast nuclear volume is occupied by the nucleolus, which appears microscopically as a crescent-shaped structure that is adjacent to the nuclear periphery[173]. Second, opposite the nucleolus, the centromeres of all chromosomes localize within one cluster even during interphase[82]. Third, telomeres localize to the nuclear periphery in yeast[81,174]. These few landmarks seem to be sufficient to predict the overall large-scale nuclear organization of *S. cerevisiae*[175,176].

Another intriguing example of how nuclear organization influences homology search is with respect to centromeres, which cluster in the nucleus[81,82] (BOX 4). Genetic data showed that recombination between homologous sequences that are located proximal to centromeres is more efficient than between elements that are located on chromosomal arms[44,56,59]. Indeed, Rad51 ChIP data revealed that induction of a DSB close to a centromere gives rise to small Rad51 signals that surround the centromeres of all other yeast chromosomes[44]. This means that homology search probes the broken chromosome, but due to centromere clustering, probing can also efficiently occur on DNA that is located proximal to the centromere of other chromosomes. In addition, recombination between alleles that are found at subtelomeric locations is higher between chromosome arms that are known to reside in closer spatial proximity[56]. In general, recombination efficiencies between alleles or other DNA elements seem to mostly inversely correlate with the distance that separates them in the three-dimensional space. This is further indicated by a comparison of either recombination efficiencies with computational predictions of three-dimensional distances[83] or of three-dimensional distances that are measured by chromosome conformation capture approaches[75] with genome-wide Rad51 ChIP signals (J.R., C.A.L. and S.J., unpublished observations).

Chromatin mobility induced by DSBs. As the spatial proximity of DNA elements seems to influence homology search, cohesion, DNA looping and tethered genomic elements (such as centromeres and telomeres) are expected to direct homology probing to a certain nuclear territory and may hinder efficient homology search within a wider area in the nucleus. Accordingly, one would predict that the likelihood of a search that finds distantly located homologies rather than local homologies (for example, in the case of sister chromatids) is increased if the DSB and/or the homology is mobile[84]. Indeed, recent studies discovered that a DSB can induce chromatin mobility at the damaged locus in *S. cerevisiae* (BOX 3), which possibly enables cells to probe for homology within a larger nuclear volume[85,86]. Notably, chromatin mobility that is induced by DSBs in *S. cerevisiae* does not only affect the damaged chromosomal locus but also global chromatin[86,87]. This global enhancement of chromatin mobility seems to result from the activation of a strong DNA damage signalling response, which explains why it requires a higher load of DNA damage to be detectable[87]. However, the existence and significance of DSB-induced chromatin mobility remains uncertain at least for mammalian cells[9].

Increased chromosomal mobility may be unnecessary, if not even counterproductive, for events such as recombination at the replication fork or between cohesin-tethered DNA elements, and for the desired recombination for mating-type switching. However, it is likely that it helps to repair a broken chromosome if successful recombination is not immediately possible, for example if the donor homology sequence is located on another spatially distant chromosome in the nucleus[86]. In support of this idea, deletion of the yeast Rad9 checkpoint protein (the homologue of mammalian p53-binding protein 1 (53BP1)), which is required for global[87] and DSB-specific[85] increased chromatin mobility, delays slow interchromosomal recombination but not fast intrachromosomal recombination at *MAT*[85]. Moreover, spontaneously occurring DNA lesions during S phase, which can be repaired using the sister chromatid, do not exhibit induced chromatin mobility as long as cohesin is present[88]. Finally, targeting a subunit of the INO80 chromatin remodelling complex to a DNA locus not only increased its chromatin mobility but also the spontaneous recombination of this locus with an ectopic homologous sequence[89].

Although chromosomal mobility might help searching for distant homologous DNA to enable DSB repair, it is important to note that it could still reflect an indirect consequence of, for example, chromatin remodelling around the DSB[90] or a prolonged activation of the DNA damage response. To link chromosomal mobility to homology search directly, it will be crucial to monitor homology search *in vivo* (for example, by probing for chromatin-bound Rad51) in mutants in which DSB-induced chromatin mobility is defective.

Chromosome conformation capture
A method to analyze the spatial interaction frequency of either selected genomic loci or of loci on a genome-wide level. It is based on the chemical crosslinking of samples, which is followed by their restriction digest and a subsequent ligation procedure.

Mediators of cellular homology search

When the first proteins of the RecA family were discovered more than 30 years ago, they were considered to be the 'wizards' of recombination that have the 'magic' ability to search and recognize any homologous sequence in a test tube or inside a cell. Indeed, a recent *in vivo*

study suggests that bundles of RecA protein form and assist cellular homology search in bacteria[91]. However, given the enormous complexity of eukaryotic cells, including parameters such as genome size, chromatin and nuclear organization, it seems likely that additional protein mediators assist the important functions of the RecA family proteins during homology search. In the following paragraphs, we review the accumulating evidence for the requirement of homology search mediators and focus on data that have been derived from eukaryotic cells.

Rad54 and Rdh54. A key mediator of homologous recombination is Rad54, a conserved ATPase that is found in all eukaryotes and that is a member of the Snf2/Swi2 family of superfamily 2 (Sf2) helicases[92]. *In vitro*, Rad51–ssDNA filaments stimulate the dsDNA-dependent ATPase activity of Rad54, which in turn greatly increases the ability of Rad51–ssDNA filaments to form stable pairing products with homologous DNA[93,94]. In addition, Rad54 is a processive DNA translocase[95], which is also able to remodel nucleosomes on *in vitro* substrates[96–98]. *In vivo*, Rad54 seems to use these activities to function at several stages during the recombination process. Although a minor, ATP-independent role for Rad54 in stabilizing Rad51 filaments has been reported, the crucial functions of Rad54 in homologous recombination seem to lie downstream of this stage[92].

ChIP experiments revealed that one of these functions is in the homology search step. Whereas the absence of Rad54 alone only modestly decreases the efficiency of homology search[44], additional deficiency in the Rad54 homologue Rdh54, which shares all of the *in vitro* activities of Rad54 mentioned above[99–102], substantiates the importance of the two proteins during this process. Indeed, a double deficiency in Rad54 and Rdh54 causes a marked reduction of Rad51 at the *HML* donor locus[63], whereas a genome-wide analysis of Rad51 reveals that homology search is nearly completely absent in these cells[44]. Along this line, live cell imaging revealed a strong requirement for Rad54 in the increased mobility of DSBs[85], which also underscores the importance of this protein in the homology search step.

There are several models for how Rad54 and Rdh54 might support homology search. One possibility is that their translocation on dsDNA increases the transient opening of DNA strands, thus enabling Rad51–ssDNA to initiate homology probing more efficiently[93,103]. This model is in line with the observed requirement for the negative supercoiling of target DNA[16,40,104] and the proposed importance of DNA breathing during *in vitro* recombination experiments[18]. In addition to this possible mechanism, the Rad54- and Rdh54-mediated remodelling of nucleosomes might increase the efficiency of Rad51-mediated homology probing. A speculative but intriguing possibility is that Rad54 and Rdh54 proteins function as motor proteins that promote more processive and directional sliding of Rad51–ssDNA filaments along target DNA, an activity that could be mediated by the direct interaction of Rad51 with Rad54 and Rdh54 (REFS 94,99).

In addition to a role during homology search that is shared with Rdh54, Rad54 alone is required during the subsequent synaptic phase of homologous recombination. There is substantial *in vitro* and *in vivo* evidence that Rad54 is required for the conversion of unstable paranaemic joints to stable plectonaemic joints[63,105]. Some data indicate that Rad54-mediated nucleosome remodelling might be important during this joint conversion[106,107]. So far, the most important function of Rad54 in this regard seems to be its ability to coordinate the ordered removal of Rad51 molecules from the paranaemic joint while extending a stable displacement loop (D-loop) to generate a template for subsequent homologous recombination-associated DNA synthesis[108].

Chromatin modifiers. As the donor DNA that is needed for homologous recombination is embedded in chromatin, an important question is whether nucleosomes or other chromatin-associated proteins hinder homology search. Although Rad51–ssDNA can capture homologies on nucleosomal DNA *in vitro*[105], it is not sufficient when homologous DNA is present in heterochromatinized nucleosomal DNA[109]. *In vivo*, several ATP-dependent chromatin remodelling complexes have been implicated in homologous recombination[90,110], and preliminary evidence suggests that the INO80 and SWI/SNF complexes might indeed remodel nucleosomes at the site of the homologous donor sequence directly before or during the synaptic phase[111,112]. Furthermore, the INO80 subunit Arp8 is required for efficient recombination[56] and, as mentioned above, INO80 also increases local and global chromatin mobility[87], as well as spontaneous recombination when targeted to one of two homologous loci through Arp8 (REF. 89).

Several mediators of the DNA damage response cascade, including yeast Mec1, Rad53 and Rad9 (which are homologues of mammalian ATR, CHK2 and 53BP1, respectively), were also shown to be important for increased chromatin mobility after DSB induction[85,87]. The DNA damage response also induces phosphorylation of histone H2A (H2A.X in mammals; referred to as γH2A in this Review) initially close to the DSB, although this chromatin modification can expand on chromosomes for hundreds of kilobases in yeast and up to megabases in mammals[44,113,114]. However, the chromosomal distribution of γH2A does not simply derive from a linear spreading of the modification from the break along the chromosomal axis but rather parallels the pattern of homology search that is monitored by Rad51 ChIP signals[44,115], and it is even detected on different, unbroken chromosomes in spatial proximity[44,116]. As various proteins are known to be recruited to DSBs by γH2A, γH2A-modified chromatin might also assist in this way during homology search[115], although the importance of γH2A at least for the recruitment of chromatin remodelling complexes has been recently questioned[117].

Cohesin. As homology search is strongly influenced by nuclear organization, protein complexes that affect the structure and organization of chromosomes are likely to have a decisive impact on recombination.

Displacement loop
(D-loop). A structure in which the two strands of a DNA duplex are separated by the binding of a third strand to one of these strands.

ATP-dependent chromatin remodelling complexes
Protein complexes that use the energy of ATP to reposition nucleosomes, evict histones or incorporate new histone variants.

Cohesin, a member of the structural maintenance of chromosome proteins (SMC proteins)[118], controls sister-chromatid cohesion throughout the cell cycle and long-range chromosomal interactions during interphase[119]. Moreover, cohesion between sister chromatids facilitates post-replicative DSB repair[120], and it is also actively established throughout the genome after the induction of DSBs in *S. cerevisiae*[121,122]. Establishment of cohesion is important for locating homologies on sister chromatids[123] but apparently not for recombination between homologous sequences that are located on different chromosomal regions[124]. A function similar to cohesin has also been attributed to the related Smc5–Smc6 complex[125,126], which suggests that proteins of the SMC family might be generally involved in suppressing unwanted recombination events between distant homologies[127] and in constraining the mobility of broken chromosomes[88].

In short, unlike homology recognition *in vitro*, homology search *in vivo* requires assistance from proteins that function at several levels to cope with the complex nuclear organization and the size of the genome. Whereas recombinase proteins such as Rad51 mainly drive core repair activities, other proteins enable homology probing in the context of chromatin, guide and facilitate homology alignment, or restrict or broaden the nuclear territory in which homology search is desired.

A model for cellular homology search

Previous techniques and experimental approaches revealed numerous important mechanistic insights into homology search but proved unsuccessful for providing a unifying model *in vivo*. However, with the advances of single-molecule studies *in vitro*, fluorescent particle tracking inside single cells and modern genome-wide approaches, our knowledge of key aspects and parameters that are relevant for homology search *in vivo* has recently been vastly expanded. By integrating these new findings, we propose a mechanistic model that we term 'accelerated random search model' (FIG. 2). This model covers steps of homologous recombination that range from homology search in the genomic context to homology recognition at the site of homology.

Accelerated random search model. Homology search immediately starts with the formation of a presynaptic nucleoprotein filament that is composed of resected ssDNA that is coated with recombinase proteins of the RecA family (FIG. 2a); it probably also contains DNA stretches that harbour the ssDNA-binding proteins replication protein A (RPA; known as ssDNA-binding protein (SSB) in bacteria) and mediators, such as RAD52 or RAD54. This would enable the filament to locally probe the DNA of the broken chromosome, and, if no homologous DNA was immediately detected, the short-lived nature of non-homologous probing events[41] would enable the filament to probe other chromosome segments that are in close spatial proximity. Probing efficiency decreases with distance in three dimensions[44,56], which reflects the probability and frequency with which regions of differential three-dimensional distance are encountered[128]. Increased chromatin movements might help to find more distantly located homologies[9,10] (FIG. 2b).

In addition to a search by diffusion in three dimensions, probing might be considerably accelerated by sliding of the presynaptic nucleoprotein filament along DNA. Sliding enables multiple DNA sequences to be tested without the reoccurring dissociation and reassociation of the filament and occurs at a short range *in vitro*[39]. However, the extent of sliding *in vivo* and whether it is facilitated by additional protein factors, such as Rad54 and Rdh54, is unclear. Intersegmental contact sampling (that is, the property of a single filament to make multiple contacts between non-contiguous DNA segments) may further accelerate the search[41]. An interesting hypothesis is that sliding and intersegmental contact sampling might function cooperatively, which means that at every contact of the presynaptic nucleoprotein filament with DNA simultaneous sliding of the filament also assists in finding homology (FIG. 2c). As cellular homology search has to cope with chromatin rather than with naked DNA, protein mediators that transiently open chromatin, perhaps by shifting or evicting nucleosomes, are expected to also strongly accelerate homology probing (FIG. 2d). However, it seems likely that certain chromatin structures might also pose a strong barrier to homology search; for example, homology within heterochromatin is perhaps not as efficiently detected compared with homology that is located outside of these areas.

All of the processes that have been discussed will enable the presynaptic nucleoprotein filament to search the bulk of DNA that is found in a certain nuclear volume and, when homology has been found, will lead to the initiation of strand exchange that is executed by the activity of RecA family members. Notably, this model predicts that in organisms with small genomes and DNA-containing volumes, such as bacteria or yeast, accelerated random search during homologous recombination will eventually result in homology probing of a considerable portion of the genome (FIG. 2e), which is consistent with observed ectopic recombination events[7]. Although these events generally occur with a high frequency, studies in yeast showed that they rarely reach 100% efficiency, because homology search is still faster and more efficient if the homologous DNA is located in spatial proximity[44,56]. As the nuclear volume of mammalian cells is approximately 1,000-fold larger than in *S. cerevisiae*, but chromatin mobility in these organisms is comparable[64], homology search is expected to be generally restricted to small volumes or territories in mammals. Of note, mammalian nuclear territories are fairly stable inside a single cell, but their locations differ between individual cells[129], which suggests that spatial proximity is a feature of the individual cell and hence recombination is probably variable in a cell population. Therefore, although certain ectopic recombination events, such as interchromosomal recombination, can occur in higher eukaryotes[130], ectopic recombination is probably very low for an entire cell population or tissue.

Structural maintenance of chromosome proteins (SMC proteins). A conserved ATPase protein family that coordinates many aspects of chromosome organization, for example sister-chromatid cohesion and chromosome condensation in mitosis.

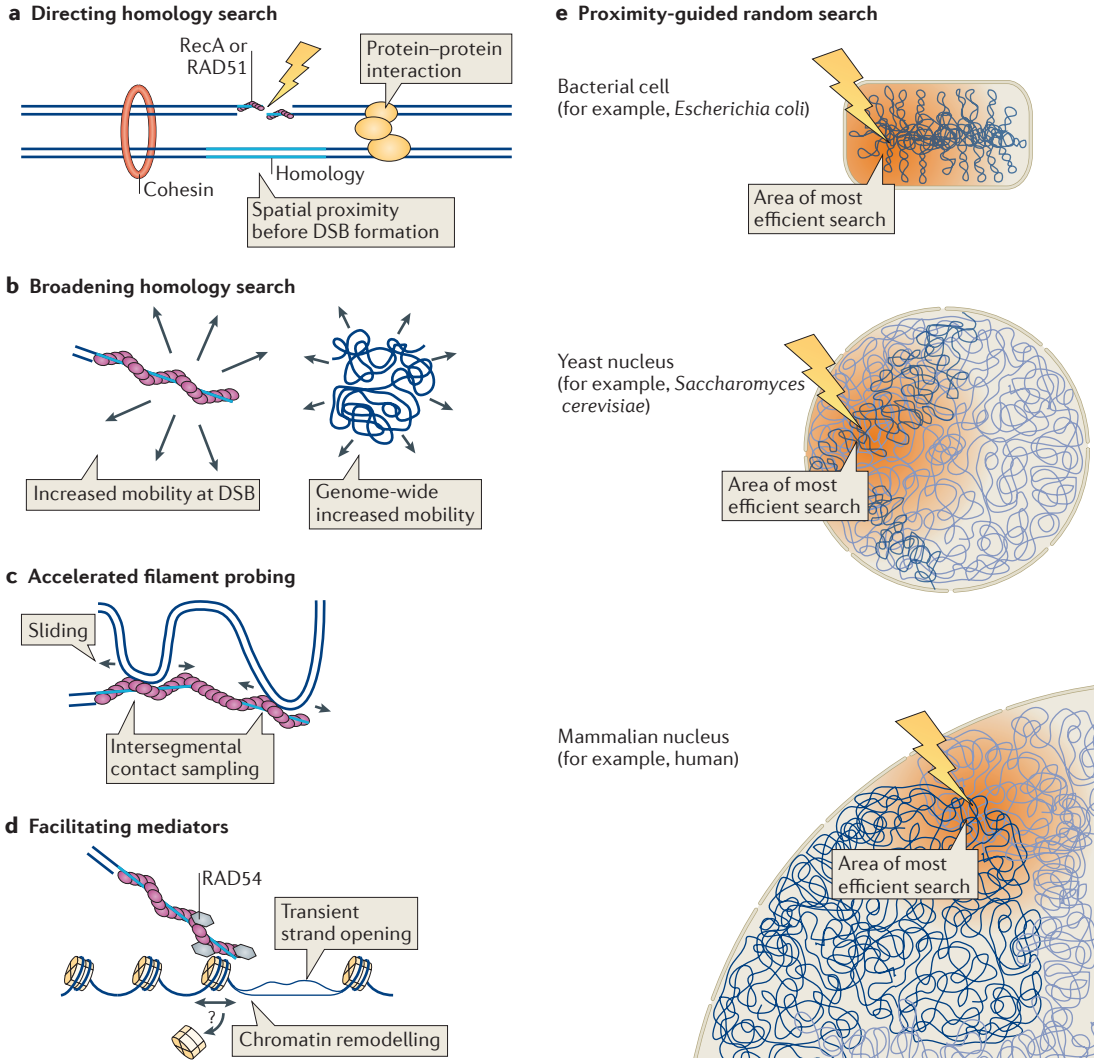


Figure 2 | **Accelerated random search model.** Homology search is principally based on random probing events within an area of spatial proximity. However, dependent on the context in which it occurs, homology search is also influenced by additional parameters. **a** | As spatial proximity is a crucial factor for recombination efficiency, homologous recombination efficiency is increased if the DNA double-strand break (DSB) and the respective donor homology sequence are already juxtaposed before DSB formation. An archetypical mediator for this function is cohesin. **b** | Conversely, if probing in a larger volume is desired (for example, to enable ectopic (non-allelic) recombination), increased mobility of the DSB and/or the entire genome might be beneficial. **c** | Homology probing might be accelerated by intersegmental contact sampling or sliding, or it might even occur simultaneously through these processes. **d** | Finally, homology probing is actively facilitated by protein mediators that increase DNA accessibility, such as the conserved RAD54 protein, potentially through the remodelling of nucleosomes (indicated by arrows and the question mark). **e** | As the mobility of chromatin is approximately the same in different species, including bacteria, yeast and mammals, the percentage of the genome that is effectively probed by random homology search in each species is mainly dictated by the size of the respective genomes and their nuclear volumes. DSBs are indicated by lightning symbols.

Guiding and safeguarding homology search. A homology search mechanism that primarily functions by random principles might require special measures to restrict areas of homology search even further or to guide it to distant locations if desired. As mentioned above, DSB repair by homologous recombination regularly occurs between homologous DNA found either on the sister chromatid or on homologous parental chromosomes in meiotic cells. In all these cases, spatial proximity between homologous DNA is provided by tight structural constraints (such as the replication fork, cohesin and homologue pairing) so that desired recombination results can be achieved. Another interesting example is the yeast mating-type system, in which several features of this chromosome apparently have evolved to guide homology search from the DSB at the *MAT* locus towards one of the two donor loci (*HML* or *HMR*), which is dependent on the initial mating-type[44,76] (as mentioned above). In addition to elements such as cohesin, centromeres, telomeres and

V(D)J recombination
Combination of variable (V), diverse (D) and joining(J) gene segments during a programmed recombination event in lymphoid cells to form diverse immunoglobulins and T cell receptors.

Class switch recombination
Programmed recombination in B lymphocytes to generate different antibody subtypes with the same antigen specificity.

Surface protein gene arrangements
A mechanism that enables some pathogens to evade the host adaptive immune system by altering their immunogenic epitopes (antigenic variation) through recombination-mediated switching between several different surface protein variants.

recombination enhancer element-induced loops, any factor that causes compact chromosomes or mediates DNA spatial proximity, for example enhancer loops, has the potential to increase recombination efficiency between DNA in the affected areas (FIG. 2a). An undesired side-reaction of this principle is that ectopic recombination between repetitive elements that are located within this particular cellular volume could also be increased, as exemplified by increased ectopic homologous recombination between paired homologous chromosomes during meiosis[131]. Thus, chromosome compaction might cause undesired recombination events between repetitive sequences. Along this line, in various organisms, DNA that is linked to DSBs moves from more compact heterochromatic regions to euchromatic regions for repair[132,133], perhaps to reduce unwanted recombination events. Similarly, heterochromatin is refractory to γH2A modification in yeast (or γH2A.X in mammals)[134], and Rad51 filament formation in yeast is suppressed at DSBs that occur at the highly repetitive locus that encodes ribosomal RNA (rDNA locus); this only happens when DSBs relocate away from the nucleolus to other nuclear areas[135].

It is of note that different organisms seem to require different minimal homology lengths for successful recombination. Whereas in *E. coli* this minimal segment of homology (also known as minimal efficient processing segment (MEPS)) is only 23–27 bp long[136], organisms with larger genomes seem to require larger tracts of homology for successful recombination (in yeast, a minimum of 100 bp[137] is required and in mammalian cells approximately 500–1000 bp are required[138–140]). These differences in homology length requirements might reflect a direct safeguarding mechanism in organisms with larger genome sizes, which might involve, for example, helicases such as Sgs1 that can dismantle short Rad51 strand invasion products[141]. Alternatively, and in line with the accelerated random search model, this difference in homology length requirement could be an indirect effect of the likelihood of finding sequences in the differently sized search compartments.

Finally, in yeast, unrepairable or slowly repairable DSBs relocate to the nuclear periphery after some time of unsuccessful homology search[142–144]. An interesting hypothesis is that relocation of the DSB to the nuclear periphery might temporarily protect the ends of broken chromosomes as a last resort when homology search and DSB repair were unsuccessful. Hence, intranuclear repositioning of a DSB relative to nuclear structures or landmarks is also a crucial parameter for repair efficiencies.

Perspective

Although it is considered to be one of the most enigmatic processes in DNA repair, we are now beginning to understand the mechanism of homology search. Current data suggest that homology search functions by a random probing mechanism that is carried out by the RecA or RAD51 presynaptic nucleoprotein filament within the three-dimensional nuclear space. Random probing may be accelerated by the possibility that a filament makes multiple contacts with different DNA segments at the same time by sliding of the filament along the DNA and by induced chromatin mobility. Currently, the most important open questions regard the identity and function of the players that affect or even guide the search, or that make it possible in the context of chromatin. It will also be interesting to address whether homology search influences, or is influenced by, other nuclear activities, such as transcription, replication, chromatin dynamics and epigenetic modifications. Notably, as current experimental approaches to visualize homology search and to measure recombination efficiency are limited to cell populations[44,56], future analyses need to be combined with novel experimental approaches for monitoring homology search in individual cells.

Cells use DSB repair not only for spontaneously occurring DSBs but also for desired genetic exchange through programmed DSBs. For biological systems in which genetic exchange is induced by programmed DSBs, for example in meiosis or for yeast mating-type switching, the two-faced nature of recombination requires safeguarding mechanisms that maintain a balance between desired and erroneous, possibly toxic, genomic rearrangements. Interestingly, spatial DNA proximity is also important for homologous recombination-independent rearrangements, such as immunoglobulin V(D)J recombination and class switch recombination[145,146]. Thus, it will be interesting to investigate whether controlled spatial proximity of DNA is a general stimulus for genetic exchange, including surface protein gene arrangements in certain pathogenic bacteria as well as in *Trypanosoma* and *Plasmodium* spp.[147–150]. Notably, as homology search can continue for several hours if no homology is found[44], undesired recombination, in particular between interspersed repeats, may accumulate in some cells over time. Given the high number of repetitive elements in the genomes of higher eukaryotes[151], misguided homology search that is influenced by the parameters discussed in this Review are likely to contribute to disease-related genomic rearrangements; however, on the more optimistic side, misguided homology search contributes to the evolution of genomes.

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Acknowledgements
S.J. is supported by the Max Planck Society, Deutsche Forschungsgemeinschaft, Center for Integrated Protein Science Munich, RUBICON EU Network of Excellence, European Research Council (ERC) Advanced Grant and the Louis-Jeantet Foundation. J.R. was supported by a Boehringer Ingelheim Fonds PhD stipend.

Competing interests statement
The authors declare no competing interests.