Finding a match: how do homologous sequences get together for recombination?

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Abstract | Decades of research into homologous recombination have unravelled many of the details concerning the transfer of information between two homologous sequences. By contrast, the processes by which the interacting molecules initially colocalize are largely unknown. How can two homologous needles find each other in the genomic haystack? Is homologous pairing the result of a damage-induced homology search, or is it an enduring and general feature of the genomic architecture that facilitates homologous recombination whenever and wherever damage occurs? This Review presents the homologous-pairing enigma, delineates our current understanding of the process and offers guidelines for future research.

Homing endonucleases
A large and universal class of nucleases, usually encoded by mobile genetic elements such as group I introns and inteins, that promote their own dissemination by homologous recombination.

Integrative transformation
A process by which a linear molecule of DNA is introduced into a cell and is incorporated into its genome.

High-frequency recombination conjugation
A mechanism by which bacteria can exchange large chromosomal fragments.

General transduction
A process in which bacterial viruses transfer chromosomal regions between bacteria.

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Homologous recombination (HR) is a universal DNA repair mechanism that faithfully restores genomic integrity following DNA double-strand breaks (DSBs)[1]. DSBs are common and potentially devastating lesions that result from external insults, toxic metabolic by-products, or the stalling and collapsing of replication forks. In addition to HR, DSBs can be repaired by non-homologous end joining (NHEJ), which entails the direct ligation of the broken ends[1]. The relative use of the two pathways varies between species: HR predominates in yeast, whereas NHEJ contributes significantly to DSB repair in vertebrates.

In some settings, programmed DSBs are specifically induced to initiate recombination. These include diverse biological processes such as meiosis (in yeast, plants and mammals)[2], the yeast mating-type switch[3] and the lateral transfer of homing endonucleases[4]. HR, as well as NHEJ, also serves to integrate exogenous linear DNA fragments with exposed ends that are identified as DSBs (for example in integrative transformation, high-frequency recombination conjugation and viral general transduction)[5]. Therefore, HR promotes genome stability in mitosis and genome variability in meiosis and in lateral gene transfer.

The elaborate process of HR is initiated by the detection of a DSB[1] (BOX 1). First, the exposed ends at each side of the break are processed. A 5′ to 3′ resection leaves a 3′ protruding single strand, which invades a homologous double strand, thus forming a joint-molecule intermediate. The product of the joint-molecule resolution can involve reciprocal relocations (crossing over), non-reciprocal copying (gene conversion), or both. These alternative products often lead to differentiable phenotypes, the relative frequency of which provides insight into the mechanism of the recombination process. Indeed, most consensual models of HR concentrate on the joint-molecule intermediate and its resolution[6–9]. Conversely, all models either ignore or take for granted the manner by which the two loci initially get together to engage in recombination.

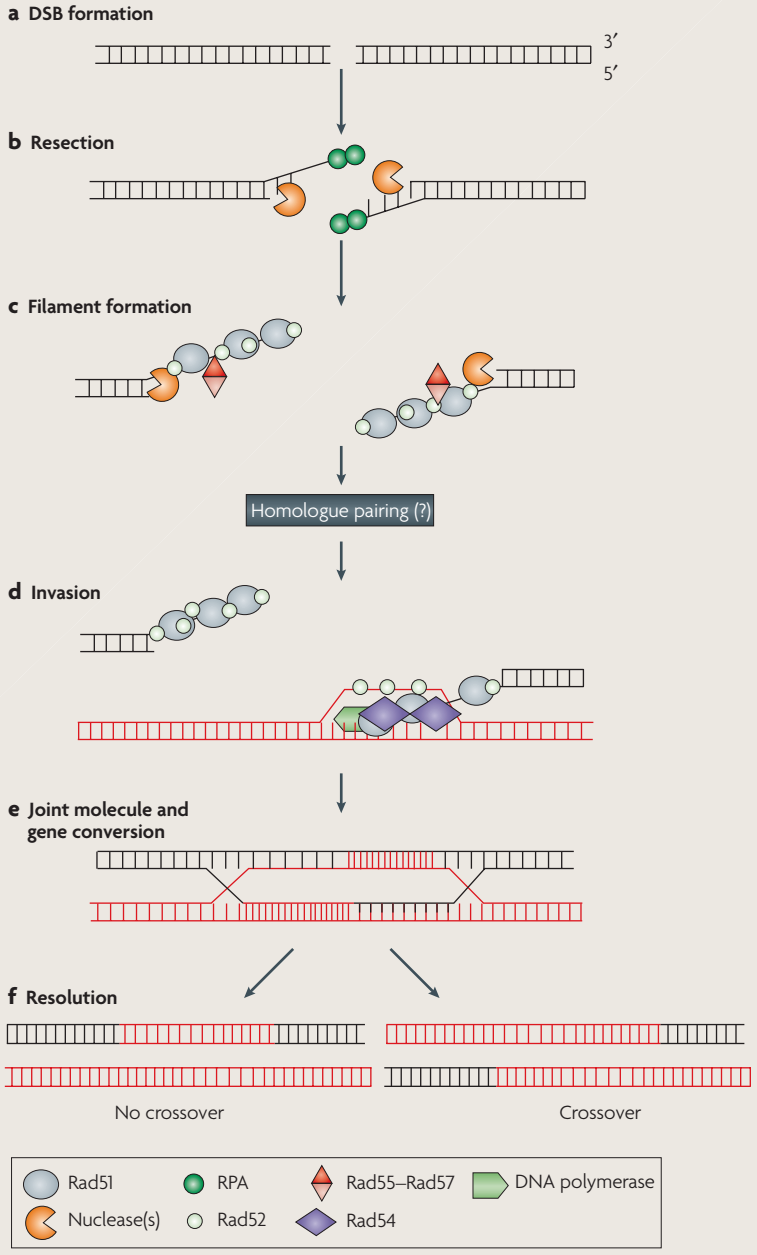
Homologous pairing is perhaps the most enigmatic stage of HR, with implications reaching beyond the range of DNA repair alone. *A priori*, the colocalization of the homologous sequences may precede the recombination-inducing lesion. This is often (although not always[10]) the case in sister chromatid recombination (SCR), which, in many organisms, is the preferable DSB repair mechanism during the G2 cell-cycle phase[11]. However, in many different settings recombination occurs at high rates between homologous chromosomes and even between dispersed non-allelic homologous sequences (ectopic homology)[12]. Could these types of homologous sequences also be paired before damage? Under this premise — and as the break point is typically not known in advance — we would have to assume that homologous pairing is an innate general characteristic of the spatial organization of the genome. By contrast, if we assume no prior pairing, the broken molecule must somehow swiftly scan the immense and condensed genome to find a homologous sequence. Does homologous pairing precede the damage or does it follow it? Is pairing the result of diffusion and chance encounters, or is there a search apparatus dedicated to

Box 1 | **Double-strand break (DSB) repair pathways**

In recent years, an understanding of the basic steps in homologous recombination (HR) has been achieved by a combination of sophisticated genetic experiments and *in vitro* work (reviewed in REFS 16,27). Upon the detection of a DSB (part **a**), the broken ends are resected by an as yet unidentified exonuclease, exposing 3′ ssDNA. Resection is promoted by meiotic recombination defects (Mre11) and exonuclease 1 (Exo1). The protruding strands are covered by replication protein A (RPA) to preclude secondary structure formation (part **b**). RPA is then replaced by Rad51 in a process that is enhanced by the presence of mediator proteins such as Rad52, Rad55 and Rad57. The resulting Rad51 nucleofilament (part **c**) serves to invade the homologous double-stranded counterpart, thus initiating the information transfer reactions[1] (part **d**). In meiosis, disrupted meiosis cDNA 1 (Dmc1) has a similar mode of action to that of Rad51. They have both overlapping and non-overlapping functions[8,9] (BOX 3). Note that this description avoids accounting for the colocalization of the two homologous sequences; this topic is the focus of the main text.

Similarly to the recombination-deficient A (RecA) filament, the Rad51 nucleofilament catalyses a strand-transfer reaction in which ssDNA strands exchange partners. This process is ATP-dependent. At this stage the Rad54 protein has an important but ill-defined function. Rad54, a member of the SWI–SNF family of helicases, might be involved in opening the chromatin configuration to allow invasion and in dissociating Rad51 from dsDNA, the product of the strand-transfer reaction.

Invasion results in the formation of a D-loop, a joint molecule intermediate that entails heteroduplex DNA (part **d**, bottom). The invading 3′ ends prime DNA synthesis. In some forms of the model (for example, synthesis-dependent strand-annealing[6]), the invading strand can disengage and re-anneal with the other broken arm, resulting in gene-conversion events. Alternatively, it can reattach to the other side of the DSB, thus forming a double Holliday junction intermediate (part **e**) Mismatch repair and DNA polymerase proofreading acting on the heteroduplex will result in a gene-conversion event, a copying of information from the intact molecule to the broken molecule. The Holliday junctions can relocate along the joint molecule in a Rad54-promoted process known as branch migration, which extends the stretch of heteroduplex DNA (not shown). Finally, alternative cutting and re-ligation of the double Holliday junction intermediate can lead to a crossover — an arms swapping between the interacting chromatids. Therefore, HR can result in gene conversion that may or may not be associated with a crossover (part **f**).



bringing the homologous sequences together? If so, what are the constituents of that apparatus? According to what logic is the search conducted? When and how is it set into action? And finally, what does the possibility of homologous pairing tell us about the spatial structure of the genome and how might it relate to the global regulation of transcription?

These questions are the focus of this Review, although we make no pretence of fully resolving the enigma; our approach is that of comparative analysis. Using the budding yeast as our reference point, we integrate data from various phyla such as plants, insects and mammals. We then discuss the pattern of homologous pairing in the different phyla and its implications.

Heteroduplex DNA
A DNA molecule generated by annealing of complementary single strands derived from different parental duplex molecules. Heteroduplex DNA often contains mismatches.

The budding yeast paradigm

The most comprehensive genetic studies of HR have been conducted in the budding yeast, *Saccharomyces cerevisiae*. Almost all mechanistic models of HR were derived from studies in this organism, which still serves as the arena in which these models are contested[13].

HR in yeast can take place between sister chromatids, between alleles on homologous chromosomes of a diploid cell or between ectopic (non-allelic) homologous sequences. Ectopic homologous sequences can occur on plasmids or on exogenous linear DNA fragments; however, they can also be present as chromosomal repeats in the tandem or inverted orientation, or as dispersed copies at unrelated genomic locations. Remarkably, when a

DSB is introduced in a locus of a vegetative haploid yeast cell carrying a dispersed ectopic homologous sequence, the efficiency of recombinational repair can be as high as 100%[12,14,15]. The high efficiency of this interaction indicates the existence of an effective homology-search mechanism.

Many different gene products operate in the yeast recombination process; most are encoded by members of the radiation sensitive 52 (*RAD52*) epistasis group[16]. The pivotal role is attributed to Rad51, a homologue of the bacterial recombination-deficient A (RecA) protein, which is essential for nearly every type of recombination in *Escherichia coli*[17]. Both the bacterial protein and its eukaryotic homologue polymerize around ssDNA to promote a strand-exchange reaction with a homologous dsDNA molecule (BOX 1). The strand-exchange reaction is highly sensitive to the degree of homology between the interacting molecules[18,19]. This has led many authors to suggest that RecA and Rad51 are responsible for conducting the homology search. However, it is important to make a distinction between local homology recognition and global homology search. The homology recognition that is facilitated by these recombinases can occur only when the interacting molecules are already colocalized. The question of how the broken molecule arrives at its homologous counterpart remains. Does it randomly scan the entire genome with the Rad51 filament assessing homology with every candidate sequence that it encounters? Conversely, could information about the positions of homologous sequences be stored in the spatial organization of the genome, to be used in the event of DNA damage?

In the following sections we explore the null hypothesis, which maintains that homologous pairing can be accomplished by random diffusion alone. We assess whether the high efficiency of yeast ectopic recombination can be explained by such a model. We then discuss alternative hypotheses that entail various degrees of constitutive homologous associations. We do so by reviewing data on somatic and meiotic pairing in yeast and other organisms.

A null model of homology search

Interchromosomal ectopic recombination. The most instructive instance of recombination, from the homology-search perspective, is interchromosomal ectopic recombination, wherein two short and dispersed homologous sequences find each other in the genomic wilderness. This process occurs naturally in the yeast genome, which contains dispersed repeated sequences[20]. However, the full capacity of the budding yeast's recombination apparatus is revealed by induced ectopic recombination assays[12,21–23], in which a DSB is induced at a pre-specified locus in virtually all of the cells in the population (FIG. 1). It was so found that a single recipient locus and a single donor locus that share as little as 1.2 kb of homology will find each other in the 15,000 kb of condensed *S. cerevisiae* genome and engage in repair with >90% efficiency, by no more than 2 hours after the DSB formation[12,22,24]. Furthermore, the interacting sequences need not be identical; induced ectopic recombination takes place, albeit at lower rates, in the presence of non-negligible internal heterology and even gaps[22,25,26].

Two short segments of DNA in two unrelated areas of the genome colocalize efficiently based purely on their mutual similarity. How is this accomplished? *A priori*, each of the 3×10^7 bp of a G2 haploid *S. cerevisiae* genome might mark the beginning of the desired homologous segment. If we take the homology assessments of the candidates to be sequential, equal and independent, then it would take 3×10^7 trials on average for a recipient to find its appropriate donor. As a homology search in each cell takes between 1 hour and 2 hours[12], the time from the beginning of one trial to the beginning of the next should be approximately 2.5×10^{-4} seconds to allow for the null model ($2.5\times10^{-4} \cdot 3\times10^7 = 7{,}500$ seconds = 125 minutes). For the sake of reference, it takes more than 40 times as long ($>10^{-2}$ seconds) for a DNA polymerase to add a single nucleotide to a growing DNA chain.

Limitations of the null model. The null model is an oversimplification in many respects. In induced recombination assays (FIG. 1), the recognition site for the endonuclease is present on both chromatids, so that when ectopic recombination takes place (in the G2 phase[21,27]) there are actually two DSBs defining two sister recipients (and also two sister ectopic donors). Because the DSB in each of the sister recipients divides the chromatid in two, the induction of an endonuclease in a single cell can, in theory, lead to four independent homology searches. However, this does not seem to be the case. Using fluorescent markers at each side of the break, Lisby and Rothstein have shown that the two chromatids and the two ends of a DSB tend to stay attached during the search[28].

Even so, several homology assessments can be conducted simultaneously if different parts of the recipient 'tetramer' can explore several adjacent candidates. This consideration is important because the null model falsely takes each of the base pairs in the genome to stand for a single candidate homologous sequence (hence there are 3×10^7 candidates). In fact, the possibility of recombination with degenerate homologous sequences, which might contain mismatches and gaps, is suggestive of a much more flexible and inclusive definition of candidate homologous sequences[22,25,26]. The number of candidates so defined is higher by orders of magnitude.

This complication can be circumvented by assessing longer DNA segments at each trial and being satisfied with finding partial homology. Indeed, the Rad51 nucleofilament can be several kilobases long[12,21] and its different parts might perform simultaneous homology assessments. Nevertheless, this type of 'block' search will not reduce the number of candidates to be tested below the 3×10^7 threshold, as any stretch of homology is uniquely defined by its beginning and each nucleotide in the genome can *a priori* serve as the beginning of the stretch. For example, if the search block is 100 bp long, it still may have to carry out ~3×10^7 100-bp alignment assessments to find a match. Even if multiple blocks along the Rad51 nucleofilament can search simultaneously,

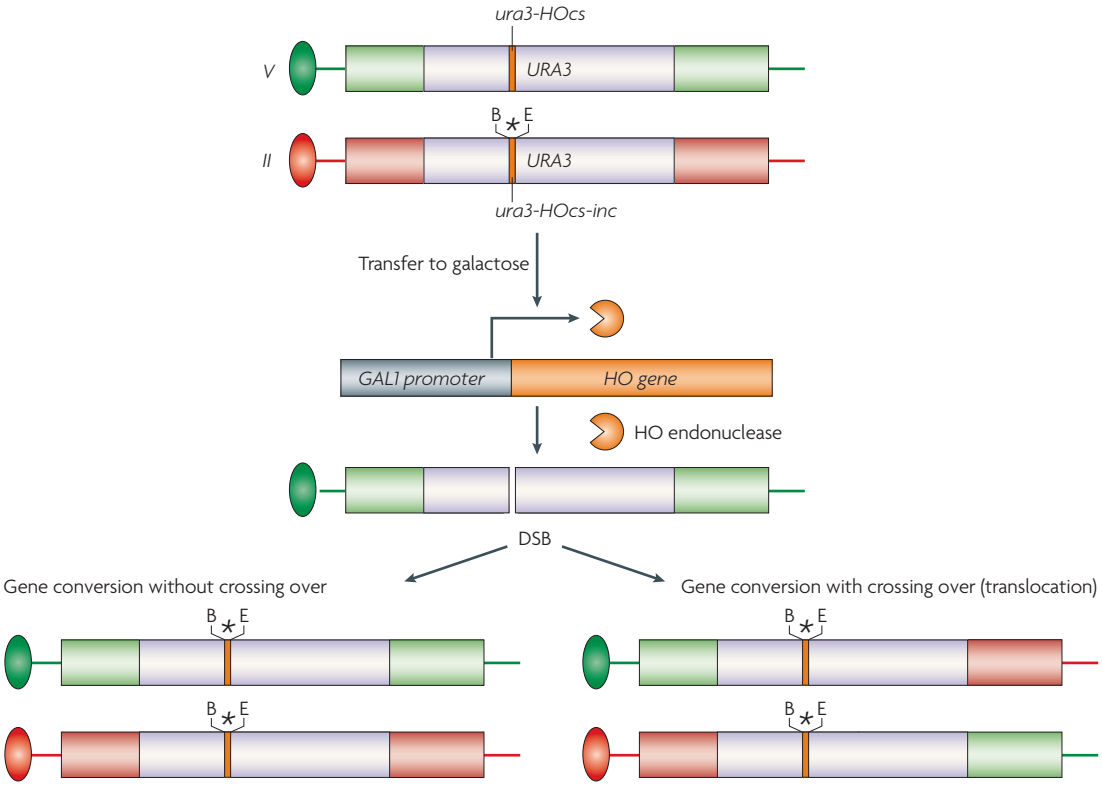


Figure 1 | **Ectopic recombination assay induced by a double-strand break (DSB).** Induced recombination assays[12] allow the monitoring of DSB repair without imposing bias in the form of selection. Here a haploid yeast strain, which bears two copies of the gene of interest (*URA3*), is used. One copy, located on chromosome *V*, carries the recognition site for the yeast HO (homing) site-specific endonuclease inserted as a short oligonucleotide (*ura3-HOcs*). The second copy, located on chromosome *II*, carries a similar site containing a single-base-pair mutation (*ura3-HOcs-inc*) that prevents recognition by the endonuclease. In addition, the *ura3* alleles differ at two restriction sites, located to the left (*Bam*HI; B) and to the right (*Eco*RI; E) of the *HOcs-inc* insertion. These polymorphisms are used to follow the transfer of information between the chromosomes. In these strains, the *HO* gene is under the transcriptional control of the *GAL1* promoter. Upon transfer of the cells to galactose-containing medium, the HO endonuclease is produced at high levels. The enzyme creates a single DSB in each cell of the population. The broken chromosomes are then repaired by a mechanism that copies the *HOcs-inc* information together with the flanking markers, resulting in a gene-conversion event. Similar genetic systems use the I-*Sce*I endonuclease[13]. These enzymes usually cut with high efficiency, and at the G2 cell-cycle phase they usually cut both sister chromatids. Therefore, the sister chromatids cannot serve as donors and ectopic recombination becomes a necessity.

they cannot do so independently because of spatial constraints and so the magnitude of the challenge is not significantly reduced.

The diameter of a DNA helix is approximately 2 nm, whereas the diameter of the *S. cerevisiae* nucleus is approximately 2–5 μm, corresponding to a nuclear volume of 3–50 μm^3. Could a broken chromosome travel such long distances in such a short time? Chromosomes are large molecules that diffuse slowly. Although ATP-dependent 'jumps' have been detected in the trajectory of an EGFP-tagged chromosome, even with no DSB induction, the chromosome does not cover more than 150 nm in 0.3–2 seconds[29]. Moreover, simply getting near a candidate sequence is probably insufficient to assess homology. Eukaryotic DNA is tightly packaged with histones to form chromatin and higher-order structures, most of which are inaccessible to the transcription machinery; why should it be accessible to a wandering recipient? Nevertheless, when recombination is artificially induced in heterochromatic regions, ectopic gene conversions occur at considerable rates[23]. Could the whole genome be included in every search? Although, obviously, the experiment has not been carried out at every possible position of the genome, there is evidence of efficient ectopic HR at all positions tested. A caveat to this assertion is that, for practical reasons, both the broken and the intact loci were usually euchromatic.

Finally, a null model of homology search would have the chromosomes distributed randomly in the nucleus, with the recipient moving between them. This might create a topological chaos in the nucleus, with the recipient chromosome ending up intertwined with other chromosomes.

Even if we correct the null model to include ATP-dependent motion, a negative correlation with the level of chromatin condensation and a compartmentalization of the chromosomes within the nucleus, the model still would not account for the high speed and efficiency of yeast recombinational repair. We therefore have to look for a fundamentally different explanation.

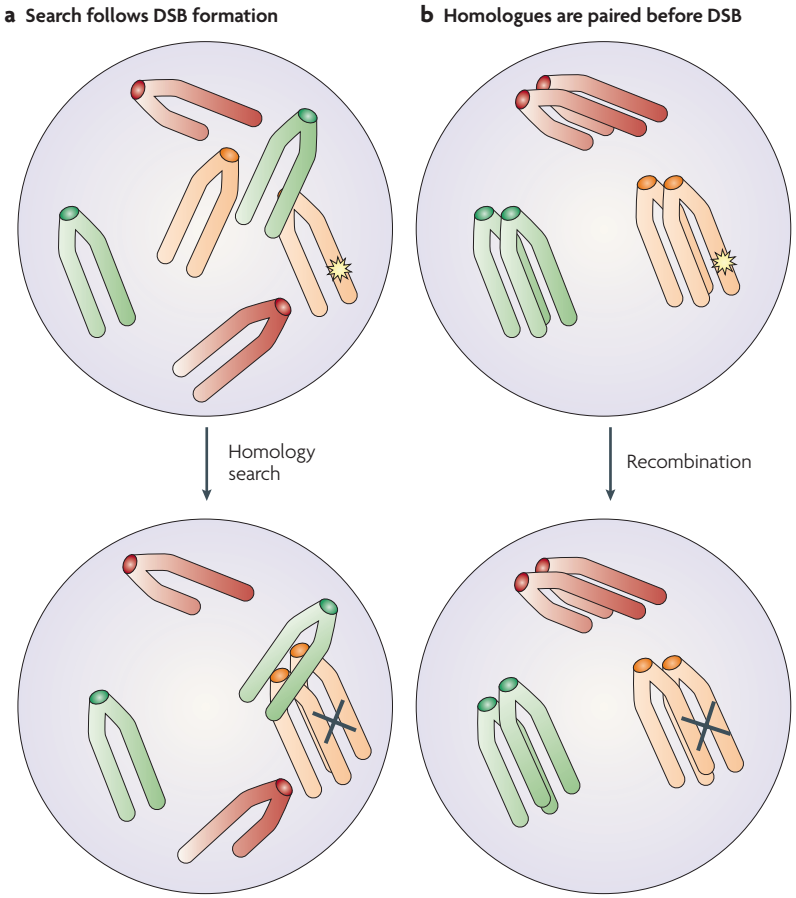


Figure 2 | **Homologous pairing: damage-induced or constitutive?** Double-strand break (DSB) repair by homologous recombination requires the colocalization of the homologous sequences. This Review presents two alternative models to account for homologous pairing. **a** | The detection of a DSB sets a homology search into action. During the search, the broken molecule moves around the nucleus and assesses its homology with sequences that it encounters until it finds a match. **b** | Homologous pairing is an innate and constitutive feature of the genomic architecture. Whenever and wherever damage occurs, the homologues are already aligned and ready to recombine. There is, of course, a full range of possibilities between these two extremes (see the main text).

Alternative models of homology-driven pairing

At the opposite extreme to the null model stands the hypothesis that homologous sequences, whether allelic or ectopic, are already paired at the time of the recombination-inducing break. However, as we assume no premonition with regards to the location of the break, the alternative to be explored is that global homologous pairing is a constitutive feature of the genomic architecture (FIG. 2). We refer to this hypothesis as somatic (and pre-meiotic) homologous pairing. Note that it is possible to imagine a situation in which homologous sequences are pre-aligned, even when the homology is ectopic; triple alignments are also plausible.

Somatic homologous pairing. The importance of somatic and pre-meiotic homologous pairing has been investigated extensively in the budding yeast and in various other species including flowering plants, insects and mammals. Most of these studies have examined the spatial interactions between homologous chromosomes or allelic loci thereof. Conversely, evidence for ectopic interactions was taken to represent a background level — a negative control for investigations of allelic pairings. For example, similar rates of ectopic and allelic spontaneous recombination were taken to imply that homologous chromosomes do not colocalize during interphase[30,31]. However, the same results are seen in a different light if we assume that ectopic homologous sequences can themselves colocalize before recombination initiation. An equal frequency of ectopic and allelic recombination can simply suggest that allelic and ectopic homologous sequences have a similar tendency to pair. Indeed, in induced recombination assays, a broken locus that has both an allelic homologue and an ectopic homologous sequence of 12 kb in length has an equal propensity to use either as the donor for repair[14].

Can homologous pairing precede damage? The existence of allelic homologous pairing (let alone ectopic pairing) in vegetative and pre-meiotic yeasts is highly controversial. The pattern of meiotic recombination (discussed in detail in the later section on meiotic homologue pairing) has been interpreted as evidence of pre-meiotic pairings. In meiosis there is a bias favouring allelic over ectopic recombinations, with the frequency of the former being as much as 100-fold higher than the frequency of the latter[20,32]. This could indicate that allelic loci are in proximity before the meiosis-inducing DSB. Indeed, the rate of meiotic ectopic recombination between loci on homologous chromosomes is negatively correlated with their distance from the allelic position. Moreover, the rate of meiotic recombination between two similar sequences at non-allelic loci on homologous chromosomes is seven to eight times higher than the rate of recombination between loci on heterologous chromosomes[30,33,34]. This was taken to suggest that homologous chromosomes are not only close together but are aligned end to end before the induction of a DSB.

However, there is an alternative explanation for the proximity of the marked alleles at the time when they are induced to recombine. A prior DSB occurring at an unmarked locus elsewhere along the chromosome might have elicited a global homologue alignment, which in turn led to the colocalization of the marked alleles[35].

Conflicting evidence for somatic homologous pairing. The Kleckner laboratory has pioneered the use of fluorescence *in situ* hybridization (FISH) analysis of spread chromosomes to characterize somatic pairing of homologous chromosomes[36,37] (FIG. 3a). The conclusion from these studies was that homologous chromosomes in interphase and pre-meiotic cells are paired by multiple transient interstitial interactions. Weak somatic associations were also found in Cre–*LoxP* recombination assays[38] and chromosome conformation capture (3C) assays [39] (FIG. 3b,c).

Critics of the somatic-pairing hypothesis claim that the minor tendency of allelic loci to be found closer together need not imply homologous pairing. It can simply be explained by the arrangement of interphase

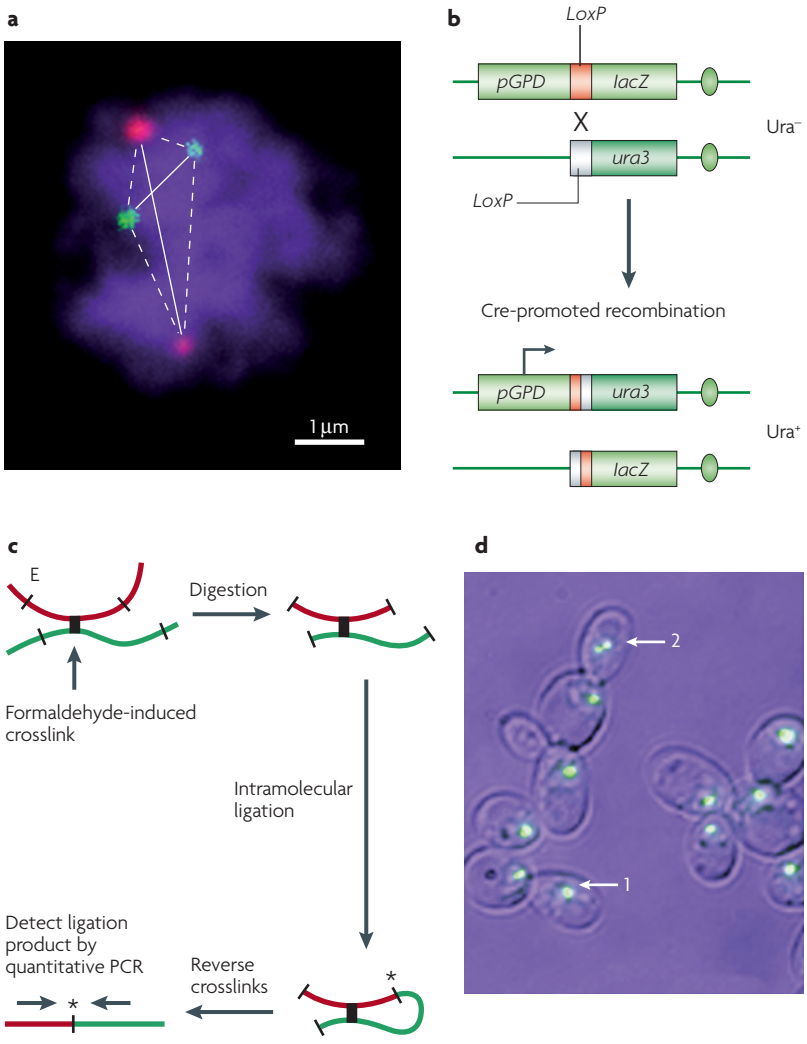


Figure 3 | **Assays for somatic pairing.** The idea of somatic homologous pairing has been explored by diverse experimental methodologies. **a** | Florescence *in situ* hybridization (FISH) analysis of spread nuclei. Two differentially labelled probes, red and green, are each targeted at a different pair of allelic loci. The distance between foci of the same colour reflects the distance between homologous chromosomes, whereas the distance between foci of different colours is taken as a negative control[36,37,41]. **b** | Cre–*LoxP* site-specific recombination system. *LoxP* sites are inserted at different relative positions (allelic, near-allelic, interchromosomal ectopic and inverted repeats) and recombinants are selected for. All other factors being constant, the rate of Cre-mediated recombination in this system reflects the *in vivo* collision probability of the two *LoxP* sites and hence their spatial proximity[38]. The figure shows Cre-induced recombination leading to the rescue of uracil auxotrophy, as recombination places the *URA3* gene under the control of an active promoter (*pGPD*). **c** | Chromosome conformation capture (3C). This technique entails the fixation of the chromosomal conformation by formaldehyde and the subsequent detection of the crosslinked molecules by digestion (E, *Eco*RI restriction enzyme site), intramolecular ligation, reversal of crosslinking and PCR[39]. Only paired chromosomes should give a signal. **d** | *In vivo* fluorescent tagging. A series of isogenic strains is constructed, each carrying either *LacO* or *TetO* arrays (or both) in either allelic or ectopic loci. The strains also express either LacI–GFP or TetR–GFP fusions (or both), respectively. The distances between allelic and ectopic arrays are measured and compared (seen as coloured dots; 1 marks an overlapping association and 2 marks a non-overlapping association between two GFP-fusion proteins and their cognate arrays). In addition, the impact of the array itself can be assessed by positioning identical arrays at ectopic loci or different arrays at allelic loci[42]. Panel **a** reproduced with permission from REF 41 © American Society for Microbiology Panel **d** reproduced with permission from *Nature Cell Biology* REF 42 © Macmillan Publishers Ltd.

centromeres, which are clustered in a rosette known as the Rabl configuration (FIG. 4). Because of this clustering, loci with similar distances from their respective centromeres (allelic loci, for example) are expected to be found closer to one another. Moreover, the very idea of somatic pairing is challenged by FISH results showing that homologous centromeres are randomly distributed within the centromeric rosette[40]. This claim is supported by Lorenz *et al.*[41], who critically revisited and expanded former FISH studies to determine that there is no evidence for somatic homologous pairing in budding yeast[41].

Perhaps the most direct approach to the matter is *in vivo* fluorescent tagging, which allows the monitoring of interchromosomal interactions in living cells (FIG. 3d). Aragón-Alcaide *et al.*[42] have used GFP fusions to follow pairing in both vegetative and meiotic yeast cells. Strikingly, high rates of mitotic associations were found regardless of whether the tags were in allelic or ectopic positions. The rates were high even between a proximal tagged locus and a distal tagged locus, thus avoiding nonspecific Rabl effects.

This is therefore evidence for constitutive ectopic homologous pairing. The discrepancy between these results and the FISH results of Lorenz *et al.* is perplexing[41]. On the one hand, spreading of the nuclei for FISH analysis may disrupt the spatial organization of the genome and prevent the detection of significant associations. On the other hand, *in vivo* fluorescent tagging makes use of repetitive arrays that might have different pairing properties from endogenous loci[42].

Interestingly, damage-induced chromosome relocation can involve more than just homologous pairing. Lisby and co-workers monitored the localization of Rad52–GFP foci as markers for recombinational repair. In addition, they fluorescently tagged the induced-DSB sites. They found that multiple DSBs tend to cluster in a single Rad52 focus. This led these authors to put forward the 'repair centre' hypothesis which proposes that the repair of broken DNA molecules necessitates their transport to specialized nuclear repair centres[43]. Note that the idea of repair centres does not entail or preclude the idea of homologous pairing. Repair centres cluster broken molecules for concomitant repair, whereas homologous pairing requires the colocalization of the broken molecule with an intact counterpart.

Somatic homologous pairing in higher eukaryotes. Somatic homologous pairing in *Drosophila melanogaster* is an established phenomenon[44]. Importantly, pairing takes place not only between homologous chromosomes but also between ectopic homologous sequences[45]. Somatic homologous pairing in *D. melanogaster* can result in transvection. Additional pairing-dependent effects on gene expression occur in several fungi and plant taxa (BOX 2).

The pre-meiotic pairing of homologues in *D. melanogaster* may have far reaching implications for DSB repair in somatic and pre-meiotic cells. Rong *et al.* found that the homologous chromosome was used as a donor for the repair of up to 65% of DSBs induced by the

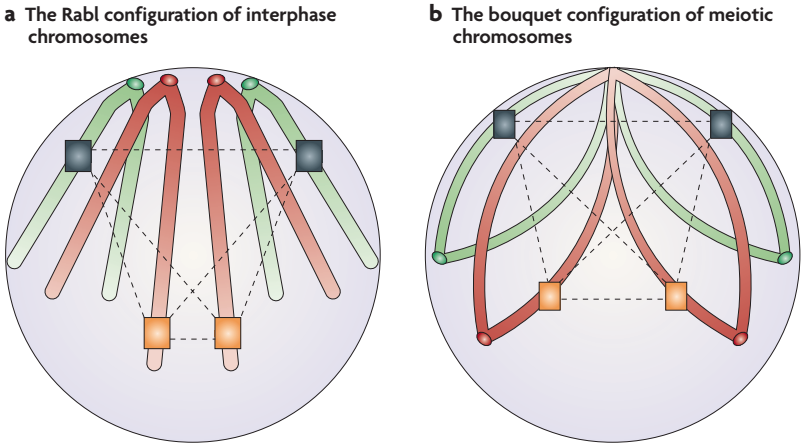


Figure 4 | **Rabl and bouquet conformations.** The centromeres of interphase chromosomes in *Saccharomyces cerevisiae* cluster in the Rabl conformation[40] (shown in part **a**). Conversely, telomere clustering, known as the bouquet, is seen in meiotic cells[74] (shown in part **b**). In both conformations, allelic loci that have the same nuclear altitude (distance from the closest spindle pole body) are found to be closer together on average than random ectopic loci (black and orange boxes are closer to a box of the same colour than they are to a box of a different colour)[41]. In addition, centromeres and telomeres can act as pairing centres where homologue alignment is initiated[72,75].

*I-Sce*I endonuclease in the *D. melanogaster* germ line[44]. Homologous pairing in *D. melanogaster* might therefore serve not only in transcriptional regulation but also to facilitate HR.

The question of somatic pairing as a general phenomenon is most adequately addressed by cytological investigations of spread nuclei and live cells. FISH studies in angiosperm interphase nuclei showed that the relative nuclear positioning of the homologous chromosomes is random, with the exception of the nucleolar organizing region (NOR)-bearing chromosomes[10]. By contrast, significant pre-meiotic homologous pairing was detected in two rice species: *Oryza sativa* and *Oryza punctata*[46]. In addition, in a population of diploid fission yeasts, homologous chromosomes shared a common nuclear domain and their centromeres were found to pair in 80% of the cells[47].

Thus, evidence exists in several organisms for the existence of somatic pairing. Importantly, where it has been investigated, the mechanisms for allelic and ectopic homologous sequences are similar, suggesting that the homology search is position-independent.

Homologous pairing and genome structure in mammals. Evidence is accumulating for the importance of somatic homologous pairing in mammalian cells, although its relevance seems to be restricted to a few specialized processes. Homologous pairing functions in X inactivation[48], and is dependent on the *Xite* and *Tsix* sites, which were previously attributed only to X inactivation *in cis*. Interestingly, an ectopic insertion of *Xite* and *Tsix* leads to *de novo* X–autosome interactions at the expense of the native X–X interaction[48]. Another recently discovered manifestation of pairing-dependent gene expression is the epigenetic dysregulation of γ-aminobutyric acid A (GABAA) receptor genes in autism-spectrum disorders[49]. A disruption of homologous pairing prevents the biallelic expression of the 15q11–13 GABAA receptor genes in neurons.

The spatial organization of the mammalian genome has attracted growing attention in recent years[50]. The emerging picture is that of a structured nucleus in which the folding and relative positioning of the chromosomes constitutes a high-order regulatory mechanism of gene expression, superimposed over the local control that is exerted by transcription factors and chromatin modifiers. Mammalian chromosomes are organized in discrete, non-overlapping chromosome territories (CTs), with the CTs of homologues usually not adjacent[51]. Recent studies show an equal distribution of transcribed, non-transcribed and non-coding sequences within the CTs[52], and electron spectroscopic imaging (ESI) has revealed a highly porous global chromatin texture that is permeable to the transcription apparatus[53]. It is now believed that the network of fibres that makes up one chromosome territory intermingles with the networks of adjacent chromosomes[53,54].

Chromosomal segments can relocate substantial distances from their respective CT[50]. Human α-globin and β-globin genes colocalized in speckles outside their CTs in correlation with their co-transcription[55]. Human ribosomal RNA (rRNA) genes, which are encoded on five different chromosomes, colocalize to the nucleolus[56]. The T_H2-cytokine locus on chromosome 11 interacts with the interferon-γ (*Ifng*) gene on chromosome 10 of naive mouse T cells[57]. Perhaps most remarkably, 3C experiments with mouse sensory neurons revealed that a single enhancer element H on chromosome 14 can interact with any one and only one of the ~1,300 odorant receptor genes that are distributed on different chromosomes[58]. These findings demonstrate that CT restriction might be less stringent than formerly speculated[50].

Mammalian homologous pairing and DNA repair. Could the reduced stringency of nuclear compartmentalization allow for homologous pairing to facilitate recombination in the event of DNA damage? The evidence is conflicting. FISH technology detected immediate pairing of homologous heterochromatin regions in response to treatments such as ionizing radiation, exposure to mitomycin C, UV irradiation and heat shock[59]. As the damage induced by these treatments differs considerably, it was proposed that the heterochromatin pairing is a general stress response. Importantly, no damage-induced colocalization was ever detected for euchromatic regions[59].

By contrast, several lines of evidence suggest that mammalian DSB repair usually does not entail extensive relocations[60]. Live cell imaging of a single broken locus has established that both sides of the break exhibit only small-scale local motion and interact preferentially with chromosomes in their spatial proximity. Interestingly, significant mobility and separation of broken ends were visible in Ku80 mutant mammalian cells, which are defective in NHEJ repair. This result implies that DSB

Isogenic strains
Strains that are genetically identical, except for a single, or a few, specific trait(s).

Transvection
A *trans* effect on gene expression that is conveyed between homologous regulatory regions, such as enhancers or silencers.

Nucleolar organizing region
A chromosomal segment, rich in ribosomal DNA (rDNA), that has the ability to organize the nucleolus around it.

X inactivation
The process in which one X chromosome in each cell of the female embryo is inactivated.

Cis-acting pairing centres
Chromosomal regions that are important for pairing of homologues during meiosis.

Distributive disjunction
The meiotic segregation of chromosomes that did not engage in recombination.

repair by HR, which involves extensive motion, might take place when the NHEJ pathway is downregulated[60].

NHEJ is the predominant DSB repair pathway in mammalian cells[61]; however, HR can also be important. Notably, HR seems to be crucial in early development, as *Rad51*$^{-/-}$ mice are embryonic lethal[62]. The rates of spontaneous HR between tandem repeats in mammalian chromosomes can be as high as 7×10^{-3}, indicating that the recombination machinery can be active in mammalian cells[63]. This level can reach 50% when a single DSB is introduced between the repeats[64]. Finally, upon DSB formation, murine dispersed repetitive sequences show low but significant levels of gene conversion[65]. This finding suggests that the mammalian genome retains a sufficient degree of flexibility to allow for homologous sequences to colocalize. However, it remains to be determined whether mammalian ectopic recombination is dependent on a regulated genome-wide homology search, or whether its low rates are the result of chance diffusion.

Meiotic homologous pairing

Despite the conflicting interpretations of homologous pairing in somatic and pre-meiotic cells of yeast and higher eukaryotes, one might have hoped that a clear picture would be available for the analogous process in meiosis, for which homologous pairing is a necessity. In fact, many questions concerning meiotic homologous pairing are still open. Insights from meiotic research can nevertheless be useful in accounting for the pairing efficiency of mitotic cells, and vice versa.

DSB-dependent meiotic pairing. Homologous pairing is a universal feature of meiosis, ensuring the proper segregation of the homologues to the daughter cells[2]. Formation of a nucleoprotein structure, called the synaptonemal complex (SC) is often correlated with meiotic homologous pairing. However, the interdependency between DSB formation, homologous pairing and SC formation (synapsis) varies among organisms.

Meiotic pairing in budding yeasts, plants and mammals depends on HR, which is initiated by sporulation 11 (Spo11)-induced DSBs. It requires the action of disrupted meiosis cDNA 1 (Dmc1), a second-strand exchange protein, in addition to Rad51, which has both distinct and overlapping roles[66] (BOX 3). In these species, SC formation depends on recombination. This led to a model in which DSBs induce recombination, which in turn allows SC formation. However, this theme cannot be generalized; synapsis is recombination-independent in worms and flies, and no synapsis is evident in fission yeast and male *D. melanogaster*[2]. Moreover, the canonical view of meiosis as progressing from DSB to pairing to synapsis[67] might be oversimplified even for *S. cerevisiae*. Tsubouchi and co-workers have found that in budding yeast a non-homologous pairing of centromeres precedes DSB formation. This pairing is dependent on zipper 1 (Zip1), an element of the SC[68]. Therefore, even in DSB-dependent meiosis, genomic reorganization may commence before DSB formation and may require SC components. However, homologous-centromere pairing is established only after a series of partner-switching, which is itself Spo11-dependent[68]. Therefore, yeast meiotic homologous pairing is truly DSB-dependent.

DSB-independent meiotic pairing. DSB-independent homologous pairing is found in the meiosis of *Schizosaccharomyces pombe*, *D. melanogaster* and *Caenorhabditis elegans*[2]. Several different mechanisms may underlie DSB-independent meiotic pairing and synapsis. Some organisms use specialized *cis*-acting pairing centres (PCs)[69]. For example, the meiotic PCs on the X chromosomes of *C. elegans* bind a zinc-finger protein, high incidence of males 8 (Him-8) , that facilitates their colocalization at the nuclear envelope and their subsequent pairing[69]. Interestingly, PC-initiated synapsis in *C. elegans* can join non-homologous chromosomes into which the PCs are inserted[70]. Concordantly, the ribosomal DNA (rDNA) region of *D. melanogaster* sex chromosomes allows the pairing of the near-heterologous X and Y chromosomes in male meiosis[71]. The centromeres too can be seen as important PCs: distributive disjunction in *S. cerevisiae* is mediated by centromere pairing[72]. Similarly, the pairing of pericentric heterochromatic regions allows non-exchange chromosomes of female *D. melanogaster* to be segregated properly[73].

Meiotic telomere clustering, also known as the meiotic bouquet[74], may have a supportive function in both DSB-dependent and DSB-independent homologous pairing (FIG. 4). In yeast, the chromosome ends associate with the nuclear envelope in a DSB-independent and SC-independent manner. However, bouquet formation

Box 2 | Genetic and epigenetic effects of homologous pairing

Transvection

Transvection is a pairing-mediated *trans*-effect on gene expression that is usually conveyed by enhancers and silencers[90]. First reported as the underlying mechanism of complementation in the bithorax complex locus (*BX-C*) of *Drosophila melanogaster*[91], transvection has since been genetically demonstrated at various other loci. The fact that transvection is often disrupted by proximal chromosomal rearrangements has led some authors to conclude that somatic pairing in *D. melanogaster* initiates at the centromeres and propagates distally. However, this zipper model was later disputed[90]. Most interestingly, Gemkow *et al.* used fluorescence *in situ* hybridization (FISH) technology to show that a translocation of one *BX-C* allele from the right to the left chromosomal arm retains high pairing frequency with the second endogenously located allele (in up to 30% of the examined cells)[45]. This implies that a somatic pairing of ectopic homologous sequences could also be highly efficient in *D. melanogaster*.

Repeat-induced point mutation and methylation induced pre-meiotically

Pre-meiotic pairing is also prevalent in several fungal taxa. Repeat-induced point mutation (RIP) involves the pairing-dependent methylation of homologous sequences, which leads to a high rate of G→C to A→T transitions and subsequent gene silencing. It is a highly efficient defence mechanism against transposons and foreign DNA[92]. RIP was first identified in *Neurospora crassa* but has since been reported in other species including, recently, *Aspergillus oryzae*, *Microbotryum violaceum* and *Leptosphaeria maculans*[92].

A similar but reversible silencing process, called MIP (methylation induced pre-meiotically), was found in *Ascobolus immersus*[93]. Although repeat and transgene silencing in plants was attributed to RNAi[94], the RIP mechanism in *N. crassa* was proved to be RNAi-independent[95]. An interesting exception to the RNAi dogma in plants is presented by Skarn *et al.*, who describe the homology-dependent silencing of the T-DNA-born neomycin phosphotransferase II gene (*nptII*) in *Arabidopsis thaliana* by a second truncated allele in a relative orientation that precludes dsRNA formation[96].

Box 3 | **Double-strand break (DSB) dependent meiotic pairing**

What is the link between homologous recombination (HR) and meiotic pairing[2]? Meiotic recombination in *Saccharomyces cerevisiae* depends on Rad51 and disrupted meiosis cDNA 1 (Dmc1), two homologues of the bacterial recombination-deficient A (RecA) ATPase. These proteins form nucleoprotein filaments at the resected DSB sites that promote strand-exchange reactions[66]. They are assisted in this process by a heterodimer composed of the homologous pairing 2 (Hop2) and meiotic nuclear division 1 (Mnd1) proteins[97,98]. Homologous pairing and synapsis are significantly delayed in both *rad51* and *dmc1* mutants of *S. cerevisiae*[66] and rice[99]. The Dmc1-dependent pathway favours recombination between homologous chromosomes over recombination between sister chromatids[100]. In doing so it relies on the synaptonemal complex (SC) elements homologous pairing 1 (Hop1) and reductional division 1 (Red1), and on the meiotic kinase 1 (Mek1)[101,102].

Although all the aforementioned proteins may function in the elusive search apparatus, the picture is far from complete. Most remarkably, *rad51 dmc1* double mutants, although being more defective than each single mutant, still show a level of crossover intermediates that is only 20-fold lower than that of the wild type[89]. This residual but non-negligible rate implies that other alternative and accessory factors of the DSB-dependent homologous-pairing pathway await discovery.

in yeast is dependent on non-disjunction 1 (Ndj1), and *ndj1* null mutants are characterized by delayed SC formation[75]. Interestingly, early recombination intermediates appear with wild-type kinetics in these mutants, implying that Ndj1 functions to stabilize the SC at a later stage[76]. Defects in homologous pairing and SC formation were also found in *S. pombe taz1* mutants, which are defective in telomere replication[77], and in mice telomerase mutants[78]. Meiotic homologous pairing in fission yeast depends on a combination of telomere clustering and programmed nuclear oscillations[79]. By contrast, meiotic homologous pairing precedes telomere grouping in both *Sordaria macrospora*[80] and rye[81].

Genomic reorganization preceding DSB creation. We have mentioned two examples of genomic reorganization in budding yeast meiosis that occurs independently of DSB formation: a Zip1-dependent non-homologous centromere clustering[68] and an Ndj1-dependent telomere clustering[75,82]. This may imply that the DSB-dependent homologous-pairing pathway makes use of some of the attributes of the DSB-independent pathway. Moreover, it has been suggested that *S. cerevisiae* homologous chromosomes are already aligned end to end when they commit to recombine (by DSB formation), presumably by telomere clustering[33,34]. Indeed, the efficiency of ectopic recombination between loci on heterologous chromosomes declines as the sequences are inserted farther away from their respective telomere[33]. On a related note, models for homologous pairing in plants have been proposed that begin with rough homologous alignment using the colocalization of allelic transcription units in the same transcription centre[83]. This idea is reminiscent of transcription-induced pairing in male *Drosophila melanogaster* (DSB-independent meiosis)[84]. Thus, DSB-independent clustering of specific chromosomal features (centromeres, telomeres, transcription centres or specific pairing sites) could pre-align chromosomes in a way that would facilitate the homology search following DSB formation.

Transcription factory
A nuclear subcompartment that is rich in RNA polymerases and transcription factors where dispersed genes gather to become active.

Concluding remarks

HR is a universal biological process that has been the focus of extensive research for many decades. Nevertheless, one of the earliest and most pivotal stages of recombination, the colocalization of the homologous counterparts, has yet to receive a comprehensive account. In this Review, we have explored two main alternative explanations for the high efficiency of homologous pairing. We initially discussed the feasibility of a null model, which assumes random assortment of the homologous sequences before damage and a diffusion-driven homology search after the induction of recombination. We concluded that the null model is unlikely to account for the high efficiency of ectopic recombination in budding yeast[12,14,15] as it would require the assessment of numerous candidate homologous sequences in a short time. The null model is also incompatible with the limited chromosomal motion in mammalian cells that is imposed by the functional compartmentalization of the nucleus[51,60].

We then explored the opposite extreme — the evidence for global homologous pairing as an innate constitutive characteristic of genome organization. Although somatic pairing is well established in *D. melanogaster* and *Neurospora crassa*, its significance is equivocal in *S. cerevisiae*[36–38,40–42], questionable in *Arabidopsis thaliana*[10,85] and limited to a few special cases in mammalian cells[48,49]. It is likely that the truth lies somewhere in between the two extremes; a lack of homologous pairing does not necessarily mean random nuclear assortment of homologous sequences. Homologous sequences could be confined to a joint nuclear subdomain, thus reducing the search space for the homologous-pairing apparatus.

There are several ways of explaining the confinement of these joint homologous sequences. Allelic loci map at equal distances from their respective centromeres and at equal distances from their respective telomeres. They therefore share a common 'nuclear altitude' (distance from the nearest spindle pole body) both in the mitotic Rabl configuration[40] and in the meiotic bouquet configuration[74] (FIG. 4). In addition, homologous chromosomes that are not fully paired could still be linked by a few specialized pairing centres[48]. By analogy to the initiation of meiosis, the centromeres could serve as pairing centres where homologous pairing is initiated and then propagates distally[68]. Finally, even if homologous chromosomes do not share the same chromosomal territory, the existence of CTs reduces the problem of homology search by dividing it into two simpler tasks: first finding the CT of a homologue (for example, using pairing centres or centromeres) and then aligning with it.

The shortcoming of the above reasoning is that its applicability is limited to the pairing of allelic loci. However, we began our detailed discussion by presenting the overwhelming efficiency of ectopic recombination. How should ectopic homologous pairing be accounted for? Ectopic markers that have similar promoters might colocalize to the same 'transcription factory'[55,56,86]. Heterochromatic regions might also tend to cluster[59]. However, we have found high rates of ectopic

recombination in yeast even between euchromatic regions — between a transcribed gene and a short, promoterless ectopic homologous sequence (B. Lifshitz and M.K., unpublished observations).

Some claims have been made regarding a possible role for reverse transcription (RT) in the recombinational repair process[13,87]. However, even RNA involvement does not mitigate the problem. Although a DSB might induce local transcription, it is the transcript of the intact donor locus that is needed for repair by RT. Moreover, RT cannot account for crossover products. Finally, several articles have demonstrated the damage-induced establishment of nuclear repair centres where several lesions can be simultaneously handled[43,88]. However, this only adds to the mystery — two chromosome relocation mechanisms must now be accounted for: one that joins the ectopic homologous sequences and the other that brings them to the repair centre (not necessarily in that order).

After a long journey we are back at the starting position. The mechanism of homologous pairing has so far resisted our survey of possible explanations. However, this Review has given us new perspectives on the global structure and dynamics of the genome.

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DATABASES

Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
Him-8 | *Ifng* | RAD52 | Rad55 | Rad57 | RecA
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
autism-spectrum disorders
UniProtKB: http://ca.expasy.org/sprot
Exo1 | Dmc1 | Hop1 | Hop2 | Ndj1 | Mek1 | Mnd1 | Mre11 | Rad51 | Rad54 | Red1 | Spo11 | Zip1

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Martin Kupiec's homepage:
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