Genome destabilization by homologous recombination in the germ line

Mariko Sasaki[*‡||], *Julian Lange*[*||] *and Scott Keeney*[*‡§]

Abstract | Meiotic recombination, which promotes proper homologous chromosome segregation at the first meiotic division, normally occurs between allelic sequences on homologues. However, recombination can also take place between non-allelic DNA segments that share high sequence identity. Such non-allelic homologous recombination (NAHR) can markedly alter genome architecture during gametogenesis by generating chromosomal rearrangements. Indeed, NAHR-mediated deletions, duplications, inversions and other alterations have been implicated in numerous human genetic disorders. Studies in yeast have provided insights into the molecular mechanisms of meiotic NAHR as well as the cellular strategies that limit it.

Homologous chromosomes
Chromosome pairs in a diploid individual that share the same genes, but not necessarily the same alleles, at loci along their lengths.

Aneuploidy
The condition of a cell or organism that is associated with having an extra or a missing chromosome, caused by inaccurate chromosome segregation during cell division.

*Molecular Biology Program, Memorial Sloan–Kettering Cancer Center, ‡Weill Graduate School of Medical Sciences, Cornell University, and §Howard Hughes Medical Institute, 1275 York Avenue, New York, 10065, USA. ||These authors contributed equally to this work. Correspondence to S.K. e-mail: s-keeney@ski.mskcc.org
doi:10.1038/nrm2849
Published online 18 February 2010

Gametes are the products of a meiotic programme in which diploid germ cells undergo one round of DNA replication followed by two successive rounds of cell division. During this process, gametes acquire chromosomes comprising new assortments of parental alleles. Subsequent fusion of two gametes during sexual reproduction results in the reconstitution of a diploid genome, yielding offspring that are genetically distinct from their parents.

In most sexually reproducing organisms, homologous recombination lies at the heart of meiosis by promoting the proper segregation of homologous chromosomes (also referred to as homologues) (reviewed in REF. 1). Prior to the first meiotic division, recombination begins when DNA double-strand breaks (DSBs) are deliberately introduced into each chromosome. The DSBs are then repaired by genetic exchange with allelic sequences, resulting in physical linkages between pairs of homologues. These connections ensure that homologues orient correctly on the meiotic spindle and migrate to opposite spindle poles. Meiotic recombination therefore promotes genetic stability and faithful transmission of the genome by limiting the repair of each DSB primarily to allelic DNA sequences and by ensuring the accurate distribution of chromosomes to gametes.

Errors in meiotic recombination can affect genome stability during gametogenesis. Research spanning several decades has shown that chromosome non-disjunction (that is, missegregation) in meiosis results in constitutive aneuploidy, which can lead to spontaneous abortion or congenital birth defects (reviewed in REF. 2). More recent work has uncovered a second process that influences genome stability in the germ line: aberrant meiotic recombination between non-allelic DNA segments that share high sequence similarity. This process is known as non-allelic homologous recombination (NAHR; the synonymous term 'ectopic recombination' is more prevalent in older literature and in yeast studies — NAHR is a more recent, mechanistically evocative term and is used in this Review). Because eukaryotic genomes from yeasts to humans harbour repeated blocks of DNA[3,4], a meiotic DSB formed within a repeat has the potential to cause genomic rearrangement through repair with non-allelic sequences. In humans, NAHR-mediated events leading to chromosomal alterations have been implicated in numerous disorders. It is therefore important to understand the mechanisms behind NAHR in the germ line.

This Review focuses on genome instability induced by meiotic NAHR. We begin with an overview of the mechanism of meiotic recombination and then discuss the types of chromosomal rearrangements and human disorders that are associated with NAHR between duplicated regions of the genome. We next examine insights into NAHR mechanisms that have been obtained from studies in other organisms. Finally, we review cellular strategies that prevent meiotic NAHR and favour recombination between allelic sequences, and consider some of the future challenges in the field.

Meiotic recombination

Meiotic recombination is inherently hazardous because it is initiated by developmentally programmed DSBs at multiple sites in the genome (reviewed in REF. 5) (FIG. 1). DSBs are repaired by a cascade of homologous recombination events that normally use DNA at allelic positions

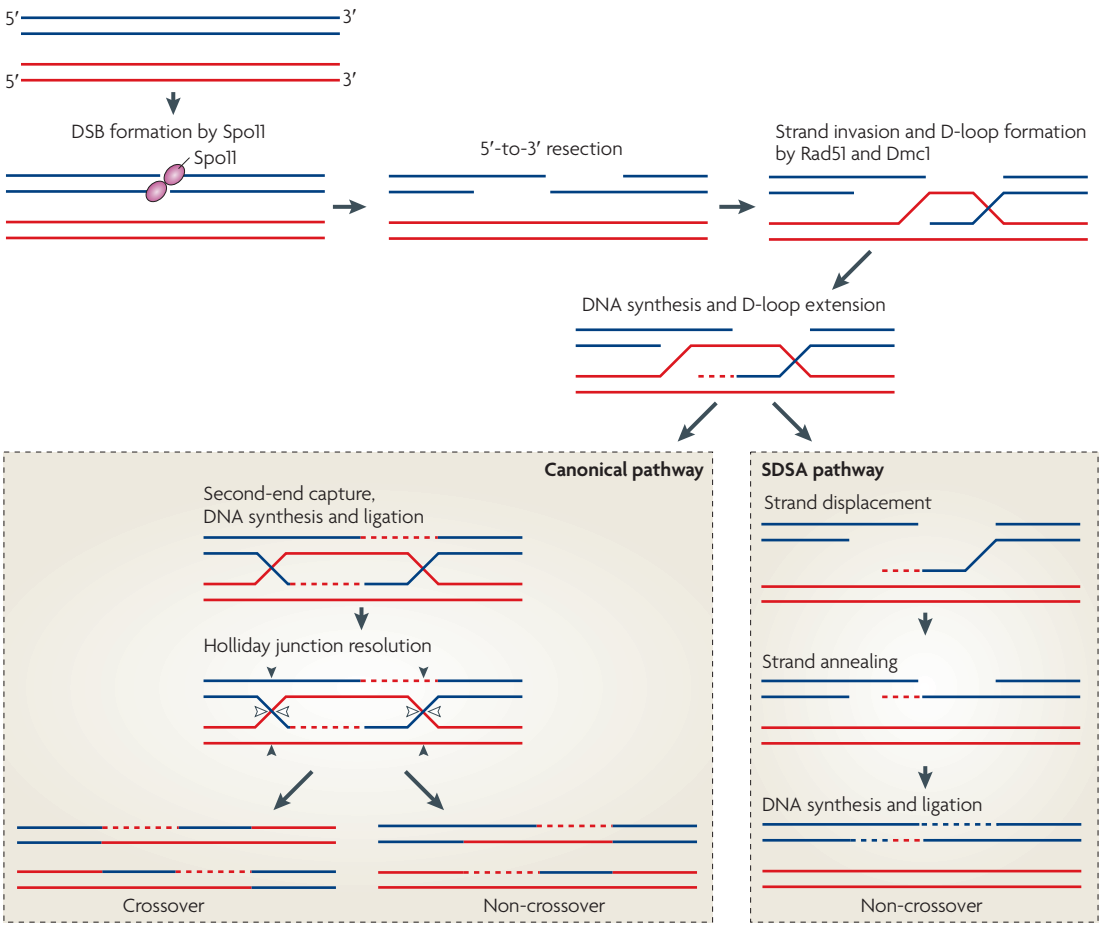

Figure 1 | **Meiotic recombination pathway.** A double-strand break (DSB) introduced by sporulation-specific protein 11 (Spo11) is resected to expose 3′ single-stranded DNA (ssDNA) tails. Mediated by Rad51 and Dmc1, one of the 3′ ssDNA tails first invades the homologous chromosome to form a displacement loop (D-loop) and heteroduplex DNA, and then initiates DNA synthesis. In the canonical pathway, capture of the second end, DNA synthesis and ligation generate Holliday junctions that flank the DSB site. The Holliday junctions can be resolved by cutting the outside strands (filled arrowheads) or crossed strands (open arrowheads) of each junction (two of the four possible resolution configurations are shown here). In principle, resolution can lead to either a crossover or a non-crossover configuration of the DNA flanking the recombination site. *In vivo*, however, most resolution products are crossovers. An alternative recombination pathway, depicted on the right (known as synthesis-dependent strand annealing (SDSA)), leads primarily to non-crossovers. In both types of pathway, gene conversion can occur by mismatch repair if DNA sequence polymorphisms are incorporated into the heteroduplex DNA that forms as part of the recombination process.

Heteroduplex DNA
Hybrid, double-stranded DNA that consists of one strand from each homologue. It is formed by strand invasion of one DSB end into a homologous DNA duplex during recombination.

D-loop
A single DNA strand that is displaced when strand invasion induces the dissociation of a DNA duplex.

Holliday junction
A four-stranded, branched DNA structure that is formed as an intermediate in homologous recombination.

as templates. The repair process occurs either with reciprocal exchange of chromosome arms that flank the DSB site (a crossover) or without exchange of flanking arms (a non-crossover).

Mechanisms of meiotic recombination. Much of what is known about the molecular events of meiotic recombination comes from studies in the budding yeast *Saccharomyces cerevisiae* (reviewed in REFS 5,6) (FIG. 1). Each initiating DSB is formed by a homodimer of the enzyme sporulation-specific protein 11 (Spo11), which mediates a topoisomerase-like transesterification reaction. Each Spo11 monomer remains covalently attached to the 5′ terminus of a DNA strand at each DSB and is released following endonucleolytic cleavage of each strand at sites 3′ to the DSB. The two 5′ ends of the DSB are further resected to expose 3′ single-stranded DNA (ssDNA) tails.

The strand exchange proteins Rad51 and Dmc1 bind these tails, search for homologous DNA sequences and then catalyse invasion by one of the two 3′ ssDNA tails into the homologous sequence of an intact, double-stranded homologue (that is, not the sister chromatid), thereby generating heteroduplex DNA. This results in separation of the strands of the invaded DNA duplex and the formation of an intermediate known as a displacement loop (D-loop). Re-synthesis of DNA strands destroyed by resection is primed by each of the 3′ ssDNA tails, using the strands of the invaded homologue as templates. For some recombination events, branched DNA intermediates containing pairs of Holliday junctions are formed and subsequently resolved, yielding mature recombinant DNA molecules that are primarily in a crossover configuration. Other recombination events are thought to proceed through a mechanism termed synthesis-dependent strand

Box 1 | **Determinants of meiotic break formation and recombination**

Budding yeast

- Meiotic double-strand breaks (DSBs) occur preferentially in intergenic promoter-containing regions rather than in genes[112].
- An open chromatin environment, as assayed by hypersensitivity to nucleases, correlates with an increased frequency of recombination, and, at some sites, DSB formation depends on the binding of specific transcription factors[112]. Transcription does not seem to be required for hotspot activity, but there is a weak correlation between the expression level of promoters and their activity as recombination hotspots[20].
- Although crossovers are suppressed near centromeres, DSBs do form there, indicating that pericentric DSBs may be resolved preferentially by inter-sister chromatid recombination[20,23].
- DSBs occur infrequently in telomeric regions and retrotransposons[112].
- Trimethylation of Lys4 of histone H3 is constitutively higher in regions close to DSB sites, and deletion of the Set1 methyltransferase (which generates this methyl mark) dramatically reduces DSB frequency in most hotspots[113].

Larger eukaryotes

- In humans, crossover rates are lower in genes than in flanking sequences, based on analysis of a genome-wide catalogue of crossover hotspots that were inferred from population genetic data[17].
- The degenerate 13-nucleotide motif CCNCCNTNNCCNC accounts for > 40% of human hotspots[25]. This finding was supported by detailed sperm typing studies at one hotspot that contains the motif. A polymorphism that disrupts the consensus sequence was associated with decreased crossover frequencies[114].
- In the genomes of flies, birds and mammals, there are fewer interspersed repetitive elements in regions of higher recombination[115,116]. This may reflect the effects of selection against initiating recombination in such elements because of the potential of non-allelic homologous recombination.
- A study of recombination across mouse chromosome 1 identified a positive but non-uniform correlation between gene density and crossover rate[117].
- Several histone modifications are enriched at the known mouse hotspot *Psmb9*: dimethylation and trimethylation of Lys4 of H3 and acetylation of Lys9 of H3 are thought to be part of the substrate for the initiation of recombination, and H4 hyperacetylation increases after DSB formation by SPO11 (the mouse homologue of yeast sporulation-specific protein 11)[118].
- PR domain-containing protein 9 (PRDM9) has recently been identified as an important determinant of meiotic hotspots in humans and mice[119,120]. In mice, PRDM9 has been shown to trimethylate Lys 4 of H3 and is expressed specifically by meiotic germ cells[121]. Both human and mouse PRDM9 proteins have an array of zinc fingers, and human PRDM9 binds specifically to the 13-nucleotide consensus motif *in vitro*. In addition, allelic variation in human *PRDM9* correlates with differences in hotspot usage. These findings suggest that the binding of PRDM9 to specific DNA sequences could target recombination to those sites and that variation in *PRDM9* could account for variability in hotspot usage between and within species.

annealing (SDSA) (FIG. 1), in which the invading DNA strand is displaced after DNA synthesis and then anneals to the complementary ssDNA tail on the other side of the DSB. This mechanism leads primarily to a non-crossover outcome (reviewed in REFS 5,6). The choice between crossover and non-crossover outcomes is highly regulated (reviewed in REF. 7).

In both recombination pathways, heteroduplex DNA forms between strands of the two parental alleles, and, importantly, gene conversion can occur when heterologies that are caused by sequence differences between the two strands are corrected by mismatch repair. Gene conversion can occur with both crossover and non-crossover modes of recombination. The direction of genetic information transfer caused by gene conversion is most often such that the site where the DSB was originally formed is converted to a copy of the sequence of the unbroken homologue. As a consequence of this genetic information transfer, recombination results in sequence homogenization between the participating alleles (reviewed in REFS 5,6) (FIG. 1).

Many of the molecular players involved in meiotic recombination in yeast are conserved in larger eukaryotes (reviewed in REFS 1,6). For example, disruption of Spo11 homologues in *Caenorhabditis elegans*[8], *Drosophila melanogaster*[9] and *Mus musculus*[10,11] eliminates meiotic recombination. Furthermore, orthologues of Dmc1 and Rad51, as well as proteins acting later in the crossover and non-crossover pathways (such as mismatch repair proteins), have roles in recombination in larger eukaryotes[12,13]. The high degree of conservation means that studies in experimentally tractable organisms can provide insight into mechanisms of allelic recombination, and thus NAHR, in humans.

Meiotic recombination hotspots. The distribution of meiotic recombination in genomes from yeasts to mammals is not random: most recombination occurs in highly localized 1–2-kb genomic domains termed hotspots, which are thought to be preferential sites of DSB formation by Spo11 (reviewed in REF. 14). Humans and mice display sex-specific variation of recombination[15,16]. Individual human crossover hotspots were first identified at high resolution by sperm typing, a PCR-based approach for recovering crossover DNA molecules directly from sperm DNA[14]. Subsequently, a genome-wide map of crossover hotspots inferred from population genetic methods was generated, leading to an estimate of 25,000–50,000 crossover hotspots in the human genome[17]. Genome-wide meiotic DSB and/or recombination maps are also available in budding and fission yeasts[18–24]. However, despite the hotspot catalogues available, little is known about the factors that govern where DSBs are formed (BOX 1). In perhaps the most significant association in humans to date, a single sequence feature — the degenerate 13-nucleotide motif CCNCCNTNNCCNC — is present in > 40% of the meiotic crossover hotspots inferred from population genetic analyses[17,25]. The fact that recombination is not distributed randomly across the genome is important for understanding the mechanisms behind NAHR because NAHR events are, by definition, highly dependent on the sequence context in which DSBs form (that is, whether DSBs occur in unique sequences or in repeated DNA).

NAHR in the human genome

The human genome is replete with sequences that are present in a high number of copies, such as transposable elements (for example, Alu elements and long interspersed repetitive elements (LINEs)), or in a low number of copies[26]. Approximately 5% of the human genome comprises low-copy repeats (LCRs; also known as segmental duplications), which are blocks of DNA that share ≥ 90% sequence identity over ≥ 1 kb (REF. 4). Interest in LCRs has intensified as their roles in human disease, genome architecture and genome evolution have emerged (reviewed in REF. 27).

Gene conversion
The transfer of DNA sequence information that 'overwrites' the sequence of one allele with the sequence of the other allele. It is carried out by mismatch repair on heteroduplex DNA.

Mismatch repair
The repair system that recognizes and corrects mismatches that form during DNA replication and recombination.

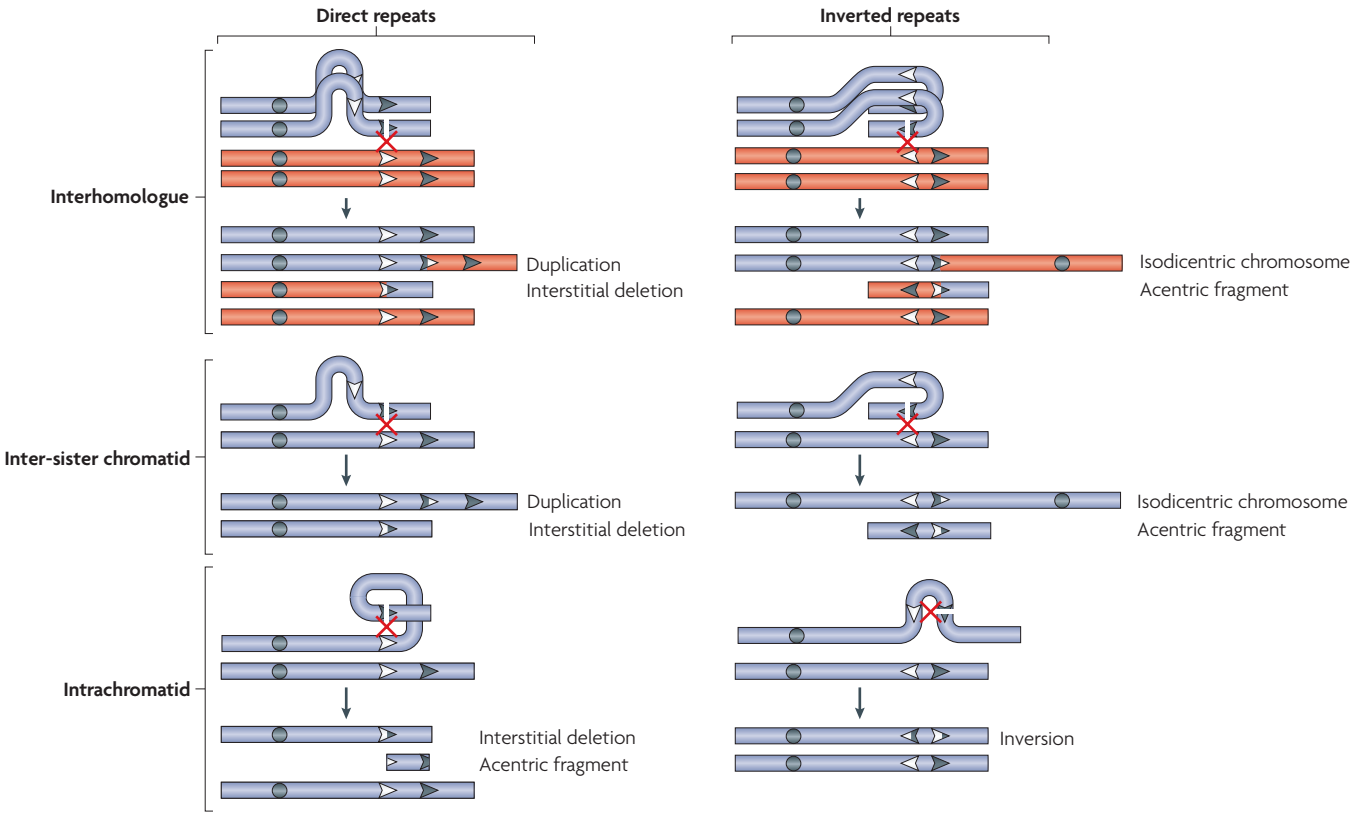

Figure 2 | **Genome rearrangement by non-allelic homologous recombination.** Crossover recombination between repeated DNA sequences at non-allelic positions can generate a deletion, a duplication, an inversion or an isodicentric chromosome. Depicted here are six chromosomal outcomes of non-allelic homologous recombination (NAHR) between repeats located on the same chromosome, with two orientations of repeats relative to one another (direct or inverted) for each of three types of interactions (between homologues, between sister chromatids or in the same chromatid). Homologous chromosomes are shown in blue and red, and sister chromatids are depicted in the same colour (homologous chromosomes are not shown in the schematics depicting inter-sister chromatid or intrachromatid exchanges for simplicity). Low-copy repeats (LCRs) are shown as white and black arrowheads. Figure is modified, with permission, from REF. 55 © (2009) Elsevier.

Genomic disorders: outcomes of NAHR. The high sequence identity over large stretches of DNA renders LCRs susceptible to germline NAHR events that result in deletion, duplication, inversion or isodicentric chromosome formation (FIG. 2). Structural alterations that change the constitutional copy number of dosage-sensitive genes, disrupt genes or generate gene chimeras result in conditions collectively termed genomic disorders (reviewed in REF. 28). Much of the work on these disorders was pioneered by Lupski and colleagues, who first recognized the role of LCRs in the aetiology of two inherited neurological disorders: Charcot–Marie–Tooth disease type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP)[29,30]. Unequal crossing over between two LCRs on chromosome 17p results in the duplication (causing CMT1A) or deletion (causing HNPP) of genes located in the intervening 1.4-megabase (Mb) region. NAHR-mediated events occurring in the germ line are now known to account for over 30 genomic disorders, with phenotypes ranging from mental retardation to blood diseases or male infertility (TABLE 1). NAHR also contributes to copy number and structural variation in the human genome through LCR-mediated duplications, deletions and inversions that are not pathogenic[31–33].

The study of genomic disorders has uncovered several parallels between meiotic allelic homologous recombination and NAHR, suggesting that they share common mechanisms and that, by inference, NAHR events occur during prophase of the first meiotic division. First, for some rearrangements caused by NAHR, recombination breakpoints cluster in narrow, 1–2-kb hotspots in LCRs, which are reminiscent of allelic recombination hotspots[34–40]. Interestingly, the allelic recombination hotspot motif CCNCCNTNNCCNC is found in many of these NAHR hotspots[25].

Second, allelic crossover hotspots identified by population genetic analysis in the LCRs on chromosome 17p coincide with the hotspots targeted in duplications and deletions that give rise to CMT1A and HNPP, consistent with the hypothesis that allelic homologous recombination and NAHR at these hotspots are both initiated by similarly programmed DSBs[41]. Hotspots of both allelic and non-allelic recombination also coincide within a set of LCRs on chromosome 17q that undergo recurrent NAHR associated with neurofibromatosis 1, a pleiotropic disorder that includes the development of cutaneous neurofibromas[42]. However, more general tests of whether

Isodicentric chromosome
A mirror-image duplication of part of a chromosome, including the centromere.

Genomic disorder
A group of conditions that arise in the germ line by submicroscopic, regional chromosomal rearrangements, which alter the copy number of dosage-sensitive genes, disrupt genes or generate fusion genes.

Recombination breakpoint
The place in the product of a recombination reaction at which the DNA molecule switches from being of one parental origin to the other.

Table 1 | **Recurrent human genomic disorders that are probably due to meiotic non-allelic homologous recombination**

Disorder	NAHR event	Causal genotypes	Phenotypes	Refs
Neurological disorders				
Charcot–Marie–Tooth disease type 1A*	1.4-Mb duplication on 17p	Duplication of *PMP22*	Weakness and atrophy of muscles of the lower legs and hands	29
Hereditary neuropathy with liability to pressure palsies*	1.4-Mb deletion on 17p	Deletion of *PMP22*	Episodes of numbness, tingling and/or weakening and atrophy of muscles, most commonly in wrists, elbows and knees	30
Developmental disorders				
1q21 deletion syndrome*	1.35-Mb deletion on 1q	Deletion of many genes	Moderate mental retardation, facial features and cardiac abnormalities	122
Sotos syndrome*	1.9-Mb deletion on 5q	Deletion of *NSD1*	Facial dysmorphism, mental retardation and childhood overgrowth	40
Congenital adrenal hyperplasia‡	30-kb deletion or gene conversion on 6p	Deletion of *CYP21* by unequal crossing over between *CYP21* and pseudogene *CYP21P*, or gene conversion of *CYP21P* to non-functional *CYP21*	Abnormal sexual development and growth defects; severe form can include dehydration	123, 124
Williams–Beuren syndrome*	1.5-Mb deletion on 7q	Deletion of many genes	Developmental abnormalities, including cardiac, neurodevelopmental and facial phenotypes	125
7q11.23 duplication syndrome*	1.5-Mb duplication on 7q	Duplication of Williams–Beuren syndrome region	Delay in expressive speech	126
Prader–Willi syndrome*	Paternal 4-Mb deletion on 15q	Deletion of paternally imprinted genes	Neurobehavioural disorder with some congenital abnormalities	127
Angelman syndrome*	Maternal 4-Mb deletion on 15q	Deletion of maternally imprinted genes	Neurobehavioural disorder with some congenital abnormalities	127
15q13.3 deletion syndrome*	680-kb, 1.5-Mb or 3.95-Mb deletion on 15q	Deletion of *CHRNA7*	Developmental delay, mental retardation and seizures	128, 129
15q24 deletion syndrome*	3.9-Mb deletion on 15q	Deletion of many genes	Developmental abnormalities, including mental retardation, growth retardation and facial features	130
Smith–Magenis syndrome*	3.7-Mb deletion on 17p	Deletion of many genes	Congenital anomalies, neurodevelopmental and behavioural phenotypes	131
Potocki–Lupski syndrome*	3.7-Mb duplication on 17p	Duplication of many genes	Congenital anomalies, neurodevelopmental and behavioural phenotypes	47
17q21.31 deletion syndrome*	500–650-kb deletion on 17q	Deletion of *MAPT*	Severe hypotonia, moderate mental retardation and facial features	132–134
Familial isolated growth hormone deficiency type 1A‡	6.7-kb deletion on 17q	Deletion of *GH1*	Severe growth retardation	135
DiGeorge's syndrome (also known as velocardiofacial syndrome)*	1.5-Mb or 3-Mb deletion on 22q	Deletion of many genes	Hypocalcaemia, cardiac malformations and facial features	136
Xp11.22–p11.23 duplication syndrome*	Variable-sized duplications on Xp	Duplication of many genes	Mental retardation and speech delay	137
Hunter's syndrome§	20-kb inversion or 30-kb deletion on Xq	Disruption of *IDS* by inversion of a portion of *IDS* by intra-chromosomal crossing over with the *IDS* pseudogene or by a deletion that includes *IDS*	Lysosomal storage disease that results in progressive damage of various tissues and organs, leading to numerous phenotypes, including distinct facial features, enlarged organs, cardiovascular disorders and deafness	138, 139

allelic recombination and NAHR share crossover hotspots in LCRs are hampered by the difficulty in identifying crossover hotspots in LCRs by population genetic methods because scoring of allelic polymorphisms in one LCR can be confounded by identical sequences in its non-allelic counterpart[43].

Third, several studies directly tested the rates of recurrent, reciprocal NAHR-mediated duplications and deletions in DNA isolated from sperm and blood of healthy males (for example, reciprocal duplication and deletion associated with CMT1A and HNPP). One study found that each of the events tested was specific to the germ

Table 1 (cont.) | **Recurrent human genomic disorders that are probably due to meiotic non-allelic homologous recombination**

Disorder	NAHR event	Causal genotypes	Phenotypes	Refs
Metabolic and tissue function disorders				
Bartter's syndrome type 3*	11-kb deletion on 1p	Deletion of *CLCNKB*	Defective renal reabsorption of sodium chloride and abnormal kidney function, leading to polyuria, polydipsia and a tendency to dehydration	140
Gaucher's disease‡	16-kb deletion on 1q	Deletion of *GBA* by unequal crossing over between *GBA* and *GBA* pseudogene	Can affect many organs (such as spleen, liver, kidney and bone marrow) and can include neurological phenotypes (such as muscle twitches and dementia)	141
Familial juvenile nephronophthisis 1‡	290-kb deletion on 2q	Deletion of *NPHP1*	Juvenile kidney disorder with polyuria and polydipsia	142
Facioscapulohumeral muscular dystrophy type 1A*	Variable-sized deletions on 4q	Causal gene deletion not known	Progressive skeletal muscle weakness of facial and shoulder muscles, followed by other muscles and arms	143
Glucocorticoid-remediable aldosteronism*	45-kb duplication on 8q	Fusion gene comprising the 5′ regulatory region of the 11β-hydroxlase gene and the coding sequence of the aldosterone synthase gene	Hypertension, variable hyperaldosteronism and abnormal adrenal steroid production	144
Polycystic kidney disease 1*	Gene conversion on 16p	Non-conservative amino acid substitutions in *PKD1* by gene conversion with nearby duplicated copies	Bilateral renal cyst formation resulting in enlargement and impairment of renal functions	145
Neurofibromatosis type 1*	1.5-Mb deletion on 17q	Deletion of *NF1*	Cafe-au-lait spots and fibromatous tumours of the skin	146
17q12 deletion syndrome*	1.5-Mb deletion on 17q	Deletion of *TCF2*	Congenital renal abnormalities and/or maturity-onset diabetes	147
Ichthyosis§	1.5-Mb deletion on Xp	Deletion of *STS*	Dry, scaly skin	148
Incontinentia pigmenti§	10-kb deletion on Xq	Deletion of *NEMO*	Highly variable abnormalities of the skin, hair, nails, teeth, eyes and central nervous system	149,150
Red–green colour blindness§	Variable-sized deletions and duplications or gene conversion on Xq	Deletion in the array of red and green pigment genes, duplication that creates a hybrid gene or gene conversion between genes	Inability to discriminate between red and green	151
Blood diseases				
β-Thalassaemia‡	7-kb deletion on 11p	Deletion of the β-globin gene by unequal crossing over between repeated sequences in the δ- and β-globin gene cluster	Anaemia	152
α-Thalassaemia‖	3.7-kb or 4.2-kb deletion on 16p	Deletion of the α-globin gene or genes by unequal crossing over between repeated sequences in the α-globin locus	Anaemia	153
Haemophilia A§	500-kb or 600-kb inversion on Xq	Disruption of factor VIII gene by intrachromosomal inversion between inverted repeats within and outside of the gene	Impaired blood coagulation	154
Sex disorders				
Isodicentric Y chromosome formation¶	Variable-sized isodicentric Y chromosomes	Inter-sister chromatid exchange at inverted repeats	Infertility, sex reversal or Turner syndrome owing to the loss of spermatogenesis genes and/or to the instability of dicentric chromosomes	55
Male infertility or subfertility¶	Variable-sized deletions on Yq	Deletion of genes crucial for spermatogenesis by unequal exchange between direct repeats	Loss of, or severe reduction in, sperm production	56–61

CHRNA7, neuronal nicotinic acetylcholine receptor subunit α7; *CLCNKB*, chloride channel Kb; *CYP21*, cytochrome P450 21; *GBA*, glucocerebrosidase; *GH1*, growth hormone 1; *IDS*, iduronate 2-sulfatase; *MAPT*, microtubule-associated protein tau; *NEMO*, NF-κB essential modifier; *NF1*, neurofibromin 1; *NPHP1*, nephronophthisis 1; *NSD1*, nuclear receptor-binding SET domain-containing protein 1; *PKD1*, polycystic kidney disease 1; *PMP22*, peripheral myelin protein 22; *STS*, steroid sulfatase; *TCF2*, transcription factor 2. Mode of inheritance is *autosomal dominant, ‡autosomal recessive, §X-linked or ¶Y-linked. ‖There are two α-globin genes at the α-globin locus. The severity of the thalassaemia is correlated with the number of functional α-globin genes: –/α, –/α or –/–, α/α causes mild anaemia, –/–, –/α causes severe anaemia and –/–, –/– leads to death *in utero* or shortly after birth .

line and that sperm-based frequency estimates of *de novo* rearrangements encompassed the disease-based estimates. For example, for the CMT1A duplication, a frequency of 1/23,000–1/79,000 was estimated from sperm analysis and a frequency of 1/23,000–1/41,000 was estimated in the population[44]. Further studies showed that LCR-mediated deletions and duplications at the α-globin gene locus are significantly more common in sperm than in blood[45,46]. Together, these findings establish that the NAHR events under study are detected primarily in cells that have gone through meiosis, which is consistent with the hypothesis that meiotic allelic homologous recombination and NAHR share common mechanisms.

Fourth, for many disorders, the causal NAHR events are predominantly either paternal or maternal. This aspect is congruent with the fact that sex-specific differences in allelic recombination rates at loci throughout the genome are a known feature of allelic meiotic recombination (for example, see REFS 36,47) (BOX 1). Fifth, statistical analysis of the sequence divergence between the LCRs targeted in CMT1A and HNPP uncovered evidence that the repeat sequences have been homogenized by gene conversion. This would be predicted if the repeats were undergoing repeated non-crossover recombination[48]. Last, in several HNPP patients, the breakpoint junction in the recombinant LCR remaining after deletion of the intervening sequence displays interspersed patches of sequences from each of the two LCRs. This finding provides evidence of crossover-associated gene conversion, similar to what would occur during allelic recombination[35].

Taken together, these results build a strong case that pathological NAHR events in humans are initiated during meiosis by the same type of SPO11-dependent DSBs that initiate normal allelic recombination, although it is possible that at least some NAHR events occur in mitotically dividing cells in the germ line. Studies in mice, in which meiotic recombination mutants such as *Spo11*$^{-/-}$ knockouts are available[10,11], may help resolve this issue.

The Y chromosome: stability and instability by NAHR. Ninety-five percent of the sequence of the human Y chromosome is restricted to males[49]. The remaining 5% of its sequence participates in recombination with the X chromosome and is therefore shared with females. The male-specific region of the Y chromosome (MSY) is unique compared with all other nuclear DNA in that it is haploid and passed on clonally. Until recently, the MSY was thought to not recombine. Because meiotic recombination can purge the genome of deleterious mutations by breaking up linkage groups and segregating otherwise linked loci away from one another, this assumption led some to speculate that, in the absence of recombination, the Y chromosome will disappear in 15 million years[50,51].

At the expense of this 'imminent demise' hypothesis, recent sequencing of the human Y chromosome and comparative analyses with primate Y chromosomes have overturned the notion that the MSY does not participate in recombination[49,52,53]. Approximately one-third of the MSY is composed of LCRs in direct or inverted orientation[49]. The most conspicuous of these LCRs are eight palindromes (inverted repeat pairs) that harbour mirror imaged gene pairs with crucial roles in spermatogenesis[49]. Ranging in size from 30 kb to 2.9 Mb, the palindromes show > 99.9% arm-to-arm sequence identity. Such high similarity would normally suggest that they arose on the MSY ~ 200,000 years ago[54]; however, Y-linked orthologues of these palindromes are found (with equally high arm-to-arm sequence identity) in chimpanzees, indicating that the palindromes predate the human–chimp divergence over 6 million years ago. Because the arm-to-arm identity of these palindromes in each species is higher than the identity of the palindromes between species (98.6%), it can be inferred that the palindrome arms have been undergoing sequence homogenization since the human–chimp divergence, which would be expected if intrapalindrome NAHR occurred repeatedly[52]. Such concerted evolution, along with evidence in modern human lineages of recent gene conversion in MSY palindromes, led to the hypothesis that the palindromes have been maintained by intrapalindrome arm-to-arm recombination and that such recombination provides a mechanism to preserve the testis-specific genes located there[52].

One group[55] proposed that this mechanism would come at a cost. They predicted that, if non-crossover resolution of recombination arising from a DSB in a palindrome accounts for intrapalindrome sequence homogenization, then crossover resolution of such a DSB by unequal sister-chromatid exchange should generate an isodicentric Y chromosome (FIG. 2). Indeed, from a group of > 2,300 patients, they identified 51 individuals with isodicentric Y chromosomes that had evidently formed by this mechanism. These chromosomes were associated with a wide range of sex-linked reproductive disorders, including failure to produce sperm, anatomic feminization of individuals bearing Y chromosomes and Turner syndrome in women[55]. Sex reversal is probably caused by the loss of the mitotically unstable isodicentric Y chromosome in the cells of the fetal gonad that determine sex. This model could also account for the features of Turner syndrome seen in some of the individuals.

NAHR in the human Y chromosome is not limited to LCRs in inverted orientation. Several recurrent interstitial deletions ranging in size from 0.8–7.7 Mb and associated with reduced spermatogenesis arise by NAHR between LCRs in direct orientation[56–61]. As predicted by the model of meiotic recombination, reciprocal duplications of these deletions[33,62], evidence of gene conversion[63] and hotspots of NAHR[56–59] are all observed for Y chromosome-linked LCRs in direct orientation. For at least one pair of LCRs, recurrent reciprocal deletion and duplication events have been shown to be specific to the germ line[44].

The results of studies of recurrent NAHR events in the human Y chromosome are consistent with the hypothesis that these events are meiotic in origin and are initiated by SPO11-generated DSBs. Furthermore, chromosome-associated complexes of MSH4 (a protein that is required for meiotic recombination in mice and yeast) have been observed on the MSY in cytological studies of spread human spermatocytes[64]. These findings support the conclusion that meiotic DSBs form on the MSY, although it is not possible from available studies to determine whether DSBs localize to LCRs.

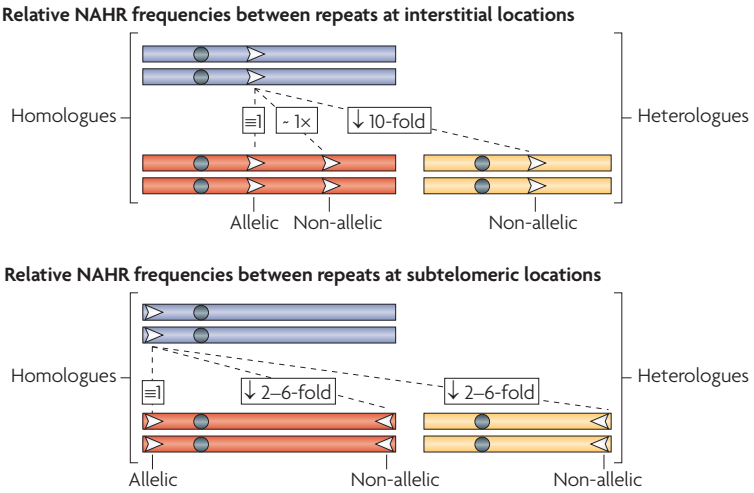

Figure 3 | **Non-allelic homologous recombination between artificially duplicated repeats in yeast. a** | Non-allelic homologous recombination (NAHR) between interstitial repeats. Artificial repeats (arrowheads) located interstitially in allelic positions and artificial repeats in non-allelic positions on homologous chromosomes (blue and red) recombine with similar efficiency. By contrast, repeats located on heterologous chromosomes (blue and yellow) recombine approximately tenfold less efficiently than allelic sequences. These findings suggest that, compared with repeats on heterologues, repeats on homologues are in a closer proximity, probably through recombination-dependent homologue pairing. **b** | NAHR between subtelomeric repeats. An artificial repeat located in a subtelomeric region recombines only slightly less efficiently with another subtelomeric repeat (twofold–sixfold less than between allelic repeats on homologues), whether on the opposite chromosome arm of the homologue or on a heterologue. The dynamic organization of telomeres during prophase of the first meiotic division (that is, attachment to the nuclear envelope and bouquet formation) might increase the proximity of telomeric regions of different chromosomes and thus influence the frequency of NAHR. The efficiencies with which repeats in different configurations recombine with one another are indicated by the numbers, with the allelic efficiency set to 1 (≡1).

Tetrad analysis
The analysis of all four products (spores in yeast) of a single meiosis. Spores in a tetrad are grouped together in an ascus and can be individually isolated by micromanipulation and grown in culture for further analysis.

Heteroallele
One of two different alleles of a gene, each of which carries a mutation or mutations at a different position in the coding sequence.

Telomere
A segment at the end of each chromosome arm that consists of repetitive sequences and that prevents the normal chromosome ends from being recognized as DSBs.

Heterologous chromosomes
Chromosomes that are neither homologues nor sister chromatids.

Meiotic NAHR in yeast

The consequences of NAHR in humans emphasize the importance of understanding the molecular mechanisms of this process. Studies in experimentally tractable organisms such as *S. cerevisiae* have played a key part in addressing this issue. The ease of genetic manipulation of this organism enables facile analysis of genes that are involved in NAHR. Moreover, the ability to recover all four meiotic products (spores) through tetrad analysis enables the comprehensive investigation of NAHR-mediated products. Finally, the availability of physical assays to detect directly the DNA intermediates of recombination provides highly detailed views of the molecular steps in this pathway.

Mechanistic parallels to allelic recombination. Meiotic NAHR has been studied by artificially dispersing heteroalleles of a gene in various non-allelic positions in the *S. cerevisiae* genome. Recombination between mutant heteroalleles can restore a wild-type sequence in one of the gene copies, allowing rapid, quantitative analysis of even infrequent recombination events. NAHR between artificially dispersed genes occurs frequently during meiosis (often at 10–100% of allelic frequencies)[65–67]. Moreover, NAHR in yeast is mechanistically similar to allelic recombination by several criteria. First, NAHR is initiated by Spo11-dependent DSBs[68]. Second, heteroduplex DNA is formed as an intermediate in NAHR, as assessed by post-meiotic segregation patterns in tetrad analysis[69,70] and by physical assays that directly detect heteroduplexes in genomic DNA extracted from meiotic cells[70]. Last, the ratio of crossover- to non-crossover-associated gene conversions is similar for allelic recombination and NAHR in at least some instances[66].

Nuclear organization of repeat sequences. The frequency of NAHR between artificial repeats is strongly influenced by the spatial organization of the repeated loci in the nucleus[65,67]. For example, if repeats are at interstitial locations (≥ 60 kb away from the closest telomere) on heterologuous chromosomes (heterologues) they recombine approximately tenfold less efficiently than the same sequences in allelic positions (FIG. 3a). By contrast, repeats dispersed in non-allelic positions on homologues recombine essentially as efficiently as allelic sequences[65–67]. One interpretation of these findings is that the progressive pairing of homologues during meiosis (discussed below) favours recombination between repeated sequences on homologues by increasing their proximity in the nucleus[65,71]. If so, repeats on heterologues would not benefit from this proximity advantage.

However, the frequency of NAHR is complicated further by the proximity of the artificial repeats to telomeres[65,67]. Heteroallelic markers integrated near chromosome ends (< 17 kb from each telomere) recombine only twofold–sixfold less efficiently than allelic sequences, regardless of whether they are on homologues (at opposite chromosome ends) or on heterologues[65,67] (FIG. 3b). During early meiotic prophase, telomeres become physically attached to the inner surface of the nuclear envelope, which is a conserved phenomenon in many organisms, including mammals[1,72]. Telomeres then begin rapid, irregular movements and undergo clustering in a small region of the nuclear envelope, in a process known as bouquet formation (reviewed in REFS 1,72). Increased proximity driven by this clustering may underlie the increased recombination frequency between non-allelic sequences located near telomeres.

NAHR between natural repeats. The frequency of NAHR between natural repetitive elements is generally lower than NAHR between artificial repeats. The *S. cerevisiae* genome contains dispersed repetitive elements such as Ty elements, which are present in ~ 30–40 copies per haploid genome[73]. If Ty elements behave in the same way as artificial repeats, they could have a marked impact on cell viability. NAHR has been studied between Ty elements engineered to contain different selectable markers and located in allelic or non-allelic positions, as well as between marked Ty elements and endogenous ones[74,75]. Allelic Ty elements recombine less frequently than allelic non-Ty (and non-repeat) sequences, and the frequency of NAHR is dramatically reduced — about 100-fold — compared with allelic recombination[73–75]. Moreover, recombination products are predominantly non-crossover gene conversions; crossover-associated gene conversions are extremely rare (< 1% of recombinants)[74].

Similar observations have been made in the fission yeast *Schizosaccharomyces pombe*. tRNA genes, which are another example of dispersed repeat sequences, recombine infrequently, and gene conversions are rarely associated with crossovers[66]. The reasons for the reduced probability of a crossover outcome for NAHR in these two different systems remain to be determined, but possible mechanisms are discussed below.

Insights into meiotic NAHR in mammals. Studies in yeast have shown that meiotic NAHR is mechanistically similar to allelic recombination. The recombination pathways and the players involved are conserved in mammals, reinforcing the view that human germline NAHR is an outcome of aberrant meiotic recombination.

Yeast studies have also shown that the spatial organization of repeats in a nucleus influences NAHR. Non-random nuclear organization of chromosomes has been observed in both somatic and germ cells of mammals, and its importance in the maintenance of genome integrity has begun to be appreciated[76]. For example, in somatic cells, the frequency of recombinational repair of targeted DSBs varies depending on the location of the homologous sequences that will be used in the recombination reaction: allelic sequences recombine more frequently than non-allelic ones[76]. Similarly, in mouse germ cells, gene conversion frequency between transgenic *LacZ* heteroalleles varies over a sevenfold range for *LacZ* transgenes integrated at different positions, indicating that chromosomal location may be a contributing factor to NAHR[77]. These findings suggest that the nuclear organization of repeats may affect the frequency of NAHR in mammals.

Yeast studies have also shown that many naturally occurring repeats undergo low levels of meiotic NAHR and that crossover outcomes in this case are particularly suppressed. There are as yet no genetic data that directly address this question in mammals. Alu elements are one of the most abundant repetitive elements in the mammalian genome[26]. Genetic disorders mediated by recombination between non-allelic Alu elements have been identified (reviewed in REF. 78), although it remains unknown whether such recombination occurred during meiosis. In both human and mouse somatic cells, a targeted DSB in a LINE inserted into the genome could be repaired by gene conversion using endogenous copies of the repeat[79,80]. Given the prevalence of these multicopy elements in the human genome, understanding their meiotic behaviours is an important challenge.

Restraining NAHR

Because of the substantial impact of NAHR on genome stability, it is not surprising that cells have evolved multiple strategies to suppress or control NAHR. There are several general mechanisms through which NAHR can be discouraged: suppressing Spo11-dependent DSB formation in or near DNA repeats; inhibiting the use of non-allelic homologous templates for recombination and/or favouring the use of allelic templates; and channelling recombination intermediates into pathways that minimize the likelihood of deleterious outcomes. Examples of each type of mechanism have been elucidated and are described below.

Preventing breaks in repeats. The most straightforward way to prevent NAHR is to not form DSBs in susceptible DNA sequences in the first place, and this strategy does indeed seem to operate in many contexts. In *S. cerevisiae*, the ribosomal DNA (rDNA) contains ~ 140 tandem repeats of a 9-kb region that encodes ribosomal RNA genes and constitutes almost 10% of the genome[81]. Despite the large physical distance occupied by this region, interallelic recombination — at least, crossover events — occurs 100-fold less frequently than in other regions[81], and this low recombination level is largely due to suppression of meiotic DSB formation[82]. Suppression of DSBs and of recombination in the rDNA depends strongly on silent information regulator 2 (*SIR2*)[82,83], which encodes a histone deacetylase that promotes the formation of a closed, compact chromatin structure in the rDNA and other regions[84,85]. DSB formation in *S. cerevisiae* is favoured in regions with an open chromatin structure (BOX 1). Therefore, Sir2 may suppress DSBs in the rDNA in part through the formation of a nucleosomal conformation that is not permissive for Spo11 activity. Other features probably contribute as well. For example, Sir2 mediates the exclusion of Hop1, a chromosome structural protein that promotes DSB formation, from the rDNA[86,87].

S. cerevisiae chromosome ends contain ~ 300 base pairs of telomeric repeats and 10–30-kb subtelomeric regions that are enriched in several different repetitive elements[88,89]. Subtelomeric regions are evolutionarily dynamic with respect to the composition of these repetitive elements and the sequence divergence between them[90]. For example, the Y′ element, which is adjacent to telomeric repeats on some chromosomes, exhibits only 1% sequence divergence between copies (other subtelomeric repeats show 10–20% divergence on average)[88,91]. Mitotic NAHR between Y′ elements has been observed[91], suggesting that it contributes to sequence homogenization of Y′ elements[88,90]. It remains unknown to what extent Y′ elements undergo meiotic NAHR and whether meiotic telomere clustering (as discussed above) influences the frequency. However, the distal 20 kb of most chromosomes exhibit much lower meiotic DSB levels[20,21] and undergo few crossovers[23,89]. Similar to what is observed in the rDNA, DSBs in subtelomeric regions are increased in the absence of Sir2, suggesting that there are active mechanisms to suppress DSBs in this part of the genome[82,92].

As noted above, meiotic NAHR is suppressed between Ty elements. It is possible that, by chance, the regions near the analysed Ty elements do not undergo frequent DSB formation. However, a more likely explanation is that an active mechanism suppresses DSB formation near Ty elements, because insertion of a Ty element close to a recombination hotspot reduced the frequency of both DSB formation and recombination in this otherwise recombination-active region[93]. Moreover, insertion of the Ty element converted the chromatin at the hotspot from an open configuration (nuclease hypersensitive) to a closed one (nuclease resistant)[93]. Although the molecular mechanisms behind these effects are not fully understood, these findings are consistent with the view that

Ty element
A retrotransposon in yeast that is ~ 5–6 kb in length and comprises a central domain that is flanked by ~ 330 bp of long terminal repeats (LTRs). The yeast genome contains full Ty elements as well as solo LTRs and LTR fragments.

recombination of DNA repeats is controlled, at least in part, by the establishment of chromatin structures that are not permissive for Spo11-dependent DSB formation.

DSB suppression is likely to be a conserved strategy for minimizing meiotic NAHR for some natural repetitive elements because DSBs are also rare in the large blocks of repetitive DNA in pericentromeric regions in *S. pombe*[24]. Moreover, cytological analyses have shown that recombination-associated protein complexes are rare or absent from the highly repetitive pericentromeric heterochromatin in mice[94] and humans[64]. Although there are no genetic data in mammals to verify that DSB formation is actively inhibited in these regions, it seems likely that DSB suppression in repetitive sequences is a general phenomenon.

Constraining recombination partner choice. Once a DSB is formed in a repetitive sequence, the choice of homologous template for repair becomes an important factor in determining the likelihood that NAHR will occur. In such circumstances, it seems that the presence of additional recombination events at other locations on the same pair of homologues may help to enforce 'good behaviour' of a DSB formed in or near repetitive DNA by promoting the use of an allelic template and/or restricting the use of non-allelic sequences. During early meiotic prophase, homologues find their partners and pair along their entire lengths (FIG. 4a). In many organisms, including fungi (such as *S. cerevisiae* and *Sordaria macrospora*), the plant *Arabidopsis thaliana* and mice, stable homologue pairing depends on DSB formation and early processes of recombination (reviewed in REFS 1,72). As an early step in pairing, homologues become aligned at multiple sites. In organisms in which the chromosomes can be easily visualized and studied, alignment is accompanied by the appearance of roughly 400-nm-long bridges between chromosome axes (known as axial interactions). These bridges are thought to be sites at which recombination reactions are in progress (reviewed in REF. 95). As prophase continues, these interactions become increasingly stable, and homologues pair along their entire lengths. In many organisms, homologue pairing culminates in the formation of the synaptonemal complex, a proteinaceous structure that holds homologues together in close juxtaposition (see REFS 96,97 for details of synaptonemal complex formation and functions of its components). In many fungi, in plants and in mice, neither the initial alignment of homologues nor the subsequent stable pairing interactions are seen in mutants that are defective in DSB formation and strand invasion of DSB ends (for example, *spo11*Δ and *dmc1*Δ mutants in yeast)[72,98], emphasizing the importance of early recombination activities in homologue pairing. Note that *C. elegans* and *D. melanogaster* use DSB-independent mechanisms for homologue pairing (see REFS 1,72).

In principle, the formation of progressively more stable pairing interactions between homologues could inhibit NAHR through two complementary effects: pairing could favour allelic recombination by placing the allelic copy in close proximity, and pairing could place constraints on the ability of broken DNA segments to find and engage non-allelic sequences that are themselves constrained elsewhere in the nucleus. Elegant experiments in yeast tested this scenario using artificial repeats located on two different chromosomes[71] (FIG. 4b). When the homologue of a chromosome carrying a recombination reporter was present (but did not itself carry the recombination reporter), NAHR occurred at a low level. However, when the ability of the homologues to engage in normal pairing was disrupted by substituting their pairing partners with chromosomes from a different yeast strain (≥ 15% sequence divergence, known as homeologues), the frequency of NAHR increased approximately sevenfold (FIG. 4b). These findings indicate that homologous pairing in meiosis can contribute substantially to the suppression of NAHR.

There is also evidence that similar effects occur in mammals. An interesting example is provided by mice that carry two different reciprocal translocations between chromosomes 1 and 13 that have slightly different translocation breakpoints[99]. During meiosis in mice heterozygous for these semi-identical translocations, the two short translocation chromosomes and the two long translocation chromosomes each undergo a separate pairing reaction that culminates in the formation of synaptonemal complexes along most of their lengths. However, at the positions of the translocation breakpoints, the synapsing chromosomes differ from one another and the non-homologous portions form loops that bulge out of the synaptonemal complexes (FIG. 4c). The loop on the larger chromosome pair is homologous to the loop on the smaller chromosome pair. Nevertheless, markers for the presence of unrepaired DSBs often persist[99]. A plausible interpretation of this finding is that the pairing and synapsis of homologous portions impose a physical constraint that impedes the ability of DSB ends in non-homologous portions to search for homology elsewhere in the genome[99].

Controlling the outcome of recombination between divergent sequences. If a DSB forms in a repetitive sequence and a non-allelic homologous partner is then used as the template for recombination repair, opportunities still remain to avoid deleterious consequences from NAHR. Specifically, this can be achieved by channelling the recombination events into pathways that give primarily non-crossover outcomes, which do not result in gross chromosomal rearrangements (FIG. 5).

One way this can be facilitated is through the action of mismatch repair proteins (for example, postmeiotic segregation protein 1 (Pms1) and Msh2 in *S. cerevisiae*). Mismatch repair proteins are conserved among bacteria and eukaryotes and have important roles in mismatch correction during DNA replication and recombination (reviewed in REFS 100,101). Mismatch repair proteins also impede the strand exchange reaction during homologous recombination if a high level of mismatches is present on the heteroduplex DNA (known as heteroduplex rejection)[102], which probably promotes non-crossover recombination pathways such as SDSA[103–106]. Natural repeats (such as retrotransposons) are often more divergent than allelic sequences, thus recombination between non-allelic sequences is expected to generate heteroduplex DNA with more mismatches on average. Although direct evidence

Synaptonemal complex
A proteinaceous structure that is formed between homologues during meiosis. Synaptonemal complexes are composed of lateral elements that are assembled between the entire length of sister chromatids and transverse filaments that connect lateral elements to the central element. In the context of synaptonemal complexes, homologues are intimately connected.

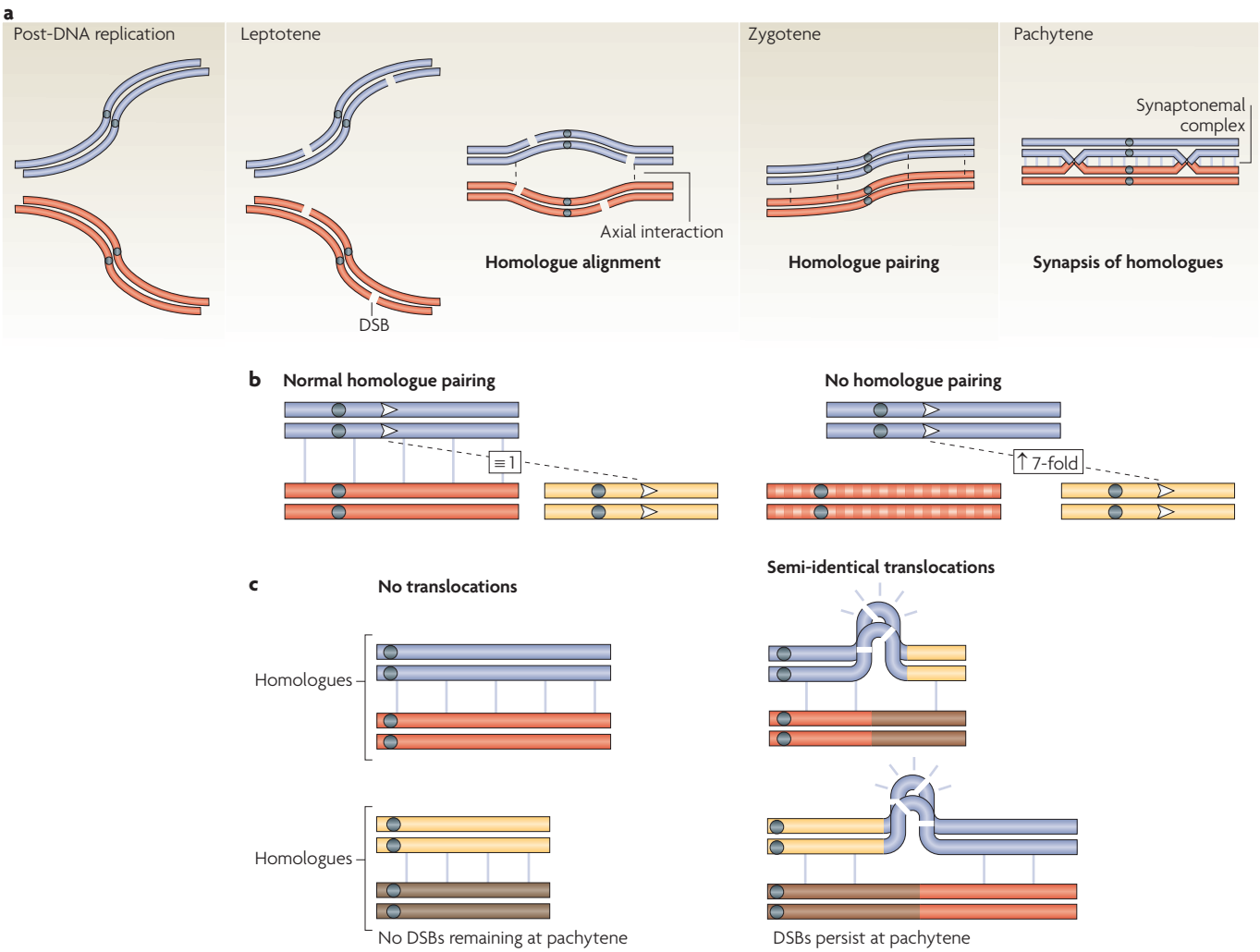

Figure 4 | **The influence of homologue pairing on recombination. a** | Double-strand break (DSB)-dependent homologue pairing in the budding yeast *Saccharomyces cerevisiae* at different stages of meiotic prophase (leptotene, zygotene and pachytene depicted). After pre-meiotic DNA replication, homologous chromosomes are dispersed in the nucleus. DSBs are formed early in meiotic prophase, providing substrates for recombination proteins to carry out homology search and strand invasion, and resulting in the alignment of homologous chromosome axes at multiple sites (axial interactions)[1]. As recombination continues, homologue interactions are progressively stabilized, culminating in the formation of the synaptonemal complex (represented by the vertical lines connecting the homologues)[96]. The development of progressively more stable pairing interactions may inhibit non-allelic homologous recombination (NAHR) by juxtaposing allelic sequences in close proximity. **b** | The influence of homologue pairing on restraining NAHR. Under normal homologue pairing conditions, NAHR between artificial repeats (arrowheads) located on one of the paired homologues and on a heterologue occurs at a low level (set to 1 (≡1)). When pairing between the homologues is disrupted by substituting their pairing partners with chromosomes from a different yeast strain (homeologues, which have sequence divergence; depicted in red stripes), the frequency of NAHR between the artificial repeats on heterologues increases approximately sevenfold. **c** | Persistence of recombinational DSBs in unpaired regions of the genome. Mouse chromosomes with semi-identical reciprocal translocations (shown schematically in the right panel) undergo homologous pairing and synapsis along most of their lengths, and DSBs are repaired in synapsed regions. However, DSBs in sequences that are not shared between homologues persist into pachytene despite the presence of identical sequences on heterologues. The persistance of unrepaired DSBs is inferred from the detection of the phosphorylated form of histone H2AX, which is a cytological marker of DSBs.

is so far lacking to implicate mismatch repair proteins in crossover suppression during NAHR between natural repeats in yeasts and mammals, this is a plausible strategy for stabilizing the genome.

Another means to antagonize NAHR is through the action of any of several helicases that exert anti-recombination activity by disrupting Rad51 or Dmc1 protein–DNA complexes, by dissociating strand exchange intermediates and/or by taking apart Holliday junction recombination intermediates in a manner that disfavours crossover formation. Examples of helicases that can have one or more of these functions include the RecQ family helicases Sgs1 in yeast and Bloom syndrome protein (BLM) in humans, and the unrelated Srs2 helicase in

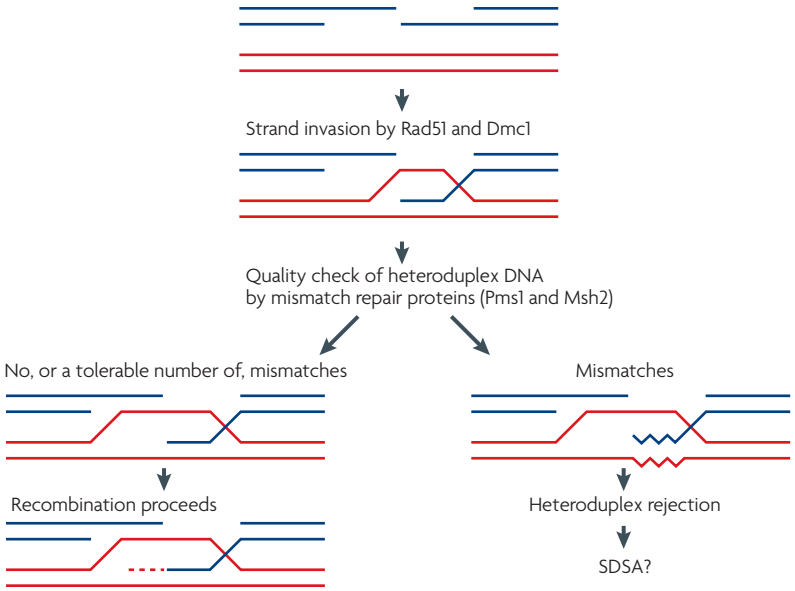

Figure 5 | **Suppression of recombination between divergent sequences.** When a double-strand break (DSB) undergoes strand invasion, heteroduplex DNA is formed. When there are few or no mismatched bases, subsequent recombination processes continue. However, when there are many mismatches, mismatch repair proteins (such as postmeiotic segregation protein 1 (Pms1) and Msh2) impede strand invasion steps that could lead to crossover formation. Subsequently, heteroduplex DNA might be destabilized (known as heteroduplex rejection) and possibly channelled into the synthesis-dependent strand annealing (SDSA) pathway, which leads to a non-crossover outcome.

yeast (reviewed in REFS 101,107). Each of these anti-recombinational activities could potentially be involved in preventing deleterious outcomes of meiotic NAHR, although this has yet to be tested.

Perspectives

Recent work has clearly established that homologous recombination between non-allelic repeated sequences in the human genome influences sequence diversity at both the nucleotide and structural levels. It can result in deletions, duplications, inversions and other rearrangements that can lead to human disorders or contribute to architectural polymorphism in the human genome. Paradoxically, NAHR seems to promote both stability and instability of large repeats on the Y chromosome.

Many questions remain to be examined in our understanding of the molecular mechanisms of NAHR. To what extent are the mechanisms of allelic homologous recombination and NAHR similar? Are the DSBs that initiate NAHR part of the cohort of developmentally programmed DSBs intended for resolution by allelic homologous recombination or do they consist of additional, non-programmed DSBs that must nonetheless be repaired? To begin to answer these questions, a high-resolution map of the human meiotic DSB landscape is required. A caveat to the catalogue of 25,000–50,000 human crossover hotspots identified by population genetic analysis is that it fails to capture new hotspots or to mask hotspots that have recently been extinguished. Indeed, sperm typing has uncovered emerging crossover hotspots concealed in regions of low historical recombination[108,109], and comparative analysis of the human and chimpanzee genomes has shown that hotspots evolve rapidly[110,111]. Therefore, historical crossover analysis reveals an incomplete picture of the contemporary recombination map. Maps of meiotic DSB distributions in the human genome will inform us of how DSBs are distributed in repeats such as LCRs and will provide further insight into the similarities and differences in the mechanisms of allelic homologous recombination and NAHR.

In addition, studies in experimentally tractable organisms, particularly in *S. cerevisiae*, have begun to flesh out the broad principles behind cellular strategies that have evolved to prevent NAHR, but there is a need to translate experimental findings from yeasts to humans. Studies in mice will probably help to bridge this gap, but the present inability to study mammalian meiosis in cell culture is a crucial impediment to progress in this area. It will also be interesting to examine in humans whether sequence variants in different factors involved in recombination pathways or in the prevention of NAHR prove to be NAHR susceptibility loci. Continuing to examine these mechanisms will provide insights into the events that lead to the genomic disorders and conditions that arise by NAHR.

1. Gerton, J. L. & Hawley, R. S. Homologous chromosome interactions in meiosis: diversity amidst conservation. *Nature Rev. Genet.* **6**, 477–487 (2005). **A review of interactions between homologous chromosomes during meiosis.**
2. Hassold, T., Hall, H. & Hunt, P. The origin of human aneuploidy: where we have been, where we are going. *Hum. Mol. Genet.* **16** R203–R208 (2007).
3. Goffeau, A. *et al.* Life with 6000 genes. *Science* **274**, 546–567 (1996).
4. Bailey, J. A. *et al.* Recent segmental duplications in the human genome. *Science* **297**, 1003–1007 (2002). **One of the first papers to report on the extent of LCRs in the human genome.**
5. Keeney, S. in *Recombination and Meiosis: Crossing-Over and Disjunction* (ed. Lankenau, D. H.) 81–123 (Springer-Verlag, Heidelberg, 2007).
6. Hunter, N. in *Topics in Current Genetics, Molecular Genetics of Recombination* (eds. Aguilera, A. & Rothstein, R.) 381–442 (Springer-Verlag, Heidelberg, 2006).
7. Bishop, D. K. & Zickler, D. Early decision; meiotic crossover interference prior to stable strand exchange and synapsis. *Cell* **117**, 9–15 (2004).
8. Dernburg, A. F. *et al.* Meiotic recombination in *C. elegans* initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis. *Cell* **94**, 387–398 (1998).
9. McKim, K. S. *et al.* Meiotic synapsis in the absence of recombination. *Science* **279**, 876–878 (1998).
10. Baudat, F., Manova, K., Yuen, J. P., Jasin, M. & Keeney, S. Chromosome synapsis defects and sexually dimorphic meiotic progression in mice lacking *Spo11*. *Mol. Cell* **6**, 989–998 (2000).
11. Romanienko, P. J. & Camerini-Otero, R. D. The mouse *Spo11* gene is required for meiotic chromosome synapsis. *Mol. Cell* **6**, 975–987 (2000).
12. Yoshida, K. *et al.* The mouse RecA-like gene *Dmc1* is required for homologous chromosome synapsis during meiosis. *Mol. Cell* **1**, 707–718 (1998).
13. Pittman, D. L. *et al.* Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for *Dmc1*, a germline-specific RecA homolog. *Mol. Cell* **1**, 697–705 (1998).
14. Kauppi, L., Jeffreys, A. J. & Keeney, S. Where the crossovers are: recombination distributions in mammals. *Nature Rev. Genet.* **5**, 413–424 (2004). **This review examines the distribution of recombination in humans and mice in comparison with results in *S. cerevisiae*, and discusses the implications of recombination hotspots in genome evolution.**
15. Kong, A. *et al.* A high-resolution recombination map of the human genome. *Nature Genet.* **31**, 241–247 (2002).
16. Lawrie, N. M., Tease, C. & Hulten, M. A. Chiasma frequency, distribution and interference maps of mouse autosomes. *Chromosoma* **104**, 308–314 (1995).
17. Myers, S., Bottolo, L., Freeman, C., McVean, G. & Donnelly, P. A fine-scale map of recombination rates and hotspots across the human genome. *Science* **310**, 321–324 (2005).
18. Baudat, F. & Nicolas, A. Clustering of meiotic double-strand breaks on yeast chromosome III. *Proc. Natl Acad. Sci. USA* **94**, 5213–5218 (1997).
19. Gerton, J. L. *et al.* Inaugural article: global mapping of meiotic recombination hotspots and coldspots in the yeast *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA* **97**, 11383–11390 (2000).
20. Blitzblau, H. G., Bell, G. W., Rodriguez, J., Bell, S. P. & Hochwagen, A. Mapping of meiotic single-stranded DNA reveals double-stranded-break hotspots near centromeres and telomeres. *Curr. Biol.* **17**, 2003–2012 (2007).

21. Buhler, C., Borde, V. & Lichten, M. Mapping meiotic single-strand DNA reveals a new landscape of DNA double-strand breaks in *Saccharomyces cerevisiae*. *PLoS Biol.* **5**, e324 (2007).
References 20 and 21 present high-resolution, genome-wide maps of meiotic DSB hotspots in *S. cerevisiae*.
22. Mancera, E., Bourgon, R., Brozzi, A., Huber, W. & Steinmetz, L. M. High-resolution mapping of meiotic crossovers and non-crossovers in yeast. *Nature* **454**, 479–485 (2008).
23. Chen, S. Y. *et al.* Global analysis of the meiotic crossover landscape. *Dev. Cell* **15**, 401–415 (2008).
24. Cromie, G. A. *et al.* A discrete class of intergenic DNA dictates meiotic DNA break hotspots in fission yeast. *PLoS Genet.* **3**, e141 (2007).
25. Myers, S., Freeman, C., Auton, A., Donnelly, P. & McVean, G. A common sequence motif associated with recombination hot spots and genome instability in humans. *Nature Genet.* **40**, 1124–1129 (2008).
This paper reports the identification of a 13-nucleotide DNA sequence motif that is present in >40% of all human allelic recombination hotspots as well as in several NAHR hotspots.
26. Lander, E. S. *et al.* Initial sequencing and analysis of the human genome. *Nature* **409**, 860–921 (2001).
27. Bailey, J. A. & Eichler, E. E. Primate segmental duplications: crucibles of evolution, diversity and disease. *Nature Rev. Genet.* **7**, 552–564 (2006).
28. Zhang, F., Gu, W., Hurles, M. E. & Lupski, J. R. Copy number variation in human health, disease, and evolution. *Annu. Rev. Genomics Hum. Genet.* **10**, 451–481 (2009).
A comprehensive review of copy number variation in the human genome, including mechanisms of formation and the role of variants in disease.
29. Pentao, L., Wise, C. A., Chinault, A. C., Patel, P. I. & Lupski, J. R. Charcot-Marie-Tooth type 1A duplication appears to arise from recombination at repeat sequences flanking the 1.5 Mb monomer unit. *Nature Genet.* **2**, 292–300 (1992).
30. Chance, P. F. *et al.* Two autosomal dominant neuropathies result from reciprocal DNA duplication/deletion of a region on chromosome 17. *Hum. Mol. Genet.* **3**, 223–228 (1994).
31. Tuzun, E. *et al.* Fine-scale structural variation of the human genome. *Nature Genet.* **37**, 727–732 (2005).
32. Redon, R. *et al.* Global variation in copy number in the human genome. *Nature* **444**, 444–454 (2006).
33. Repping, S. *et al.* High mutation rates have driven extensive structural polymorphism among human Y chromosomes. *Nature Genet.* **38**, 463–467 (2006).
34. Reiter, L. T. *et al.* A recombination hotspot responsible for two inherited peripheral neuropathies is located near a mariner transposon-like element. *Nature Genet.* **12**, 288–297 (1996).
35. Reiter, L. T. *et al.* Human meiotic recombination products revealed by sequencing a hotspot for homologous strand exchange in multiple HNPP deletion patients. *Am. J. Hum. Genet.* **62**, 1023–1033 (1998).
36. Lopez-Correa, C. *et al.* Recombination hotspot in NF1 microdeletion patients. *Hum. Mol. Genet.* **10**, 1387–1392 (2001).
37. Jenne, D. E. *et al.* Molecular characterization and gene content of breakpoint boundaries in patients with neurofibromatosis type 1 with 17q11.2 microdeletions. *Am. J. Hum. Genet.* **69**, 516–527 (2001).
38. Bayes, M., Magano, L. F., Rivera, N., Flores, R. & Perez Jurado, L. A. Mutational mechanisms of Williams-Beuren syndrome deletions. *Am. J. Hum. Genet.* **73**, 131–151 (2003).
39. Bi, W. *et al.* Reciprocal crossovers and a positional preference for strand exchange in recombination events resulting in deletion or duplication of chromosome 17p11.2. *Am. J. Hum. Genet.* **73**, 1302–1315 (2003).
40. Kurotaki, N., Stankiewicz, P., Wakui, K., Niikawa, N. & Lupski, J. R. Sotos syndrome common deletion is mediated by directly oriented subunits within inverted Sos-REP low-copy repeats. *Hum. Mol. Genet.* **14**, 535–542 (2005).
41. Lindsay, S. J., Khajavi, M., Lupski, J. R. & Hurles, M. E. A chromosomal rearrangement hotspot can be identified from population genetic variation and is coincident with a hotspot for allelic recombination. *Am. J. Hum. Genet.* **79**, 890–902 (2006).
42. De Raedt, T. *et al.* Conservation of hotspots for recombination in low-copy repeats associated with the NF1 microdeletion. *Nature Genet.* **38**, 1419–1423 (2006).
References 41 and 42 show the coincidence of allelic recombination hotspots and NAHR hotspots.
43. Frazer, K. A. *et al.* A second generation human haplotype map of over 3.1 million SNPs. *Nature* **449**, 851–861 (2007).
44. Turner, D. J. *et al.* Germline rates of *de novo* meiotic deletions and duplications causing several genomic disorders. *Nature Genet.* **40**, 90–95 (2008).
This paper compares the rates of NAHR events in the germ line and soma, and shows that the events examined are specific to the germ line.
45. Lam, K. W. & Jeffreys, A. J. Processes of copy-number change in human DNA: the dynamics of α-globin gene deletion. *Proc. Natl Acad. Sci. USA* **103**, 8921–8927 (2006).
46. Lam, K. W. & Jeffreys, A. J. Processes of *de novo* duplication of human α-globin genes. *Proc. Natl Acad. Sci. USA* **104**, 10950–10955 (2007).
47. Potocki, L. *et al.* Molecular mechanism for duplication 17p11.2 — the homologous recombination reciprocal of the Smith-Magenis microdeletion. *Nature Genet.* **24**, 84–87 (2000).
48. Hurles, M. E. Gene conversion homogenizes the CMT1A paralogous repeats. *BMC Genomics* **2**, 11 (2001).
49. Skaletsky, H. *et al.* The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* **423**, 825–837 (2003).
50. Aitken, R. J. & Marshall Graves, J. A. The future of sex. *Nature* **415**, 963 (2002).
51. Graves, J. A., Koina, E. & Sankovic, N. How the gene content of human sex chromosomes evolved. *Curr. Opin. Genet. Dev.* **16**, 219–224 (2006).
52. Rozen, S. *et al.* Abundant gene conversion between arms of palindromes in human and ape Y chromosomes. *Nature* **423**, 873–876 (2003).
53. Hughes, J. F. *et al.* Conservation of Y-linked genes during human evolution revealed by comparative sequencing in chimpanzee. *Nature* **437**, 100–103 (2005).
54. Agulnik, A. I. *et al.* Evolution of the DAZ gene family suggests that Y-linked DAZ plays little, or a limited, role in spermatogenesis but underlines a recent African origin for human populations. *Hum. Mol. Genet.* **7**, 1371–1377 (1998).
55. Lange, J. *et al.* Isodicentric Y chromosomes and sex disorders as byproducts of homologous recombination that maintains palindromes. *Cell* **138**, 855–869 (2009).
56. Sun, C. *et al.* Deletion of azoospermia factor a (AZFa) region of human Y chromosome caused by recombination between HERV15 proviruses. *Hum. Mol. Genet.* **9**, 2291–2296 (2000).
57. Kamp, C., Hirschmann, P., Voss, H., Huellen, K. & Vogt, P. H. Two long homologous retroviral sequence blocks in proximal Yq11 cause AZFa microdeletions as a result of intrachromosomal recombination events. *Hum. Mol. Genet.* **9**, 2563–2572 (2000).
58. Blanco, P. *et al.* Divergent outcomes of intrachromosomal recombination on the human Y chromosome: male infertility and recurrent polymorphism. *J. Med. Genet.* **37**, 752–758 (2000).
59. Repping, S. *et al.* Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. *Am. J. Hum. Genet.* **71**, 906–922 (2002).
60. Kuroda-Kawaguchi, T. *et al.* The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. *Nature Genet.* **29**, 279–286 (2001).
61. Repping, S. *et al.* Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. *Nature Genet.* **35**, 247–251 (2003).
62. Bosch, E. & Jobling, M. A. Duplications of the AZFa region of the human Y chromosome are mediated by homologous recombination between HERVs and are compatible with male fertility. *Hum. Mol. Genet.* **12**, 341–347 (2003).
63. Bosch, E., Hurles, M. E., Navarro, A. & Jobling, M. A. Dynamics of a human interparalog gene conversion hotspot. *Genome Res.* **14**, 835–844 (2004).
64. Oliver-Bonet, M., Turek, P. J., Sun, F., Ko, E. & Martin, R. H. Temporal progression of recombination in human males. *Mol. Hum. Reprod.* **11**, 517–522 (2005).
65. Goldman, A. S. & Lichten, M. The efficiency of meiotic recombination between dispersed sequences in *Saccharomyces cerevisiae* depends upon their chromosomal location. *Genetics* **144**, 43–55 (1996).
66. Petes, T. D. & Hill, C. W. Recombination between repeated genes in microorganisms. *Annu. Rev. Genet.* **22**, 147–168 (1988).
67. Schlecht, H. B., Lichten, M. & Goldman, A. S. Compartmentalization of the yeast meiotic nucleus revealed by analysis of ectopic recombination. *Genetics* **168**, 1189–1203 (2004).
Together with reference 65, this study shows that the spatial organization of repeated sequences in a nucleus influences the frequency of meiotic NAHR in *S. cerevisiae*.
68. Steele, D. F., Morris, M. E. & Jinks-Robertson, S. Allelic and ectopic interactions in recombination-defective yeast strains. *Genetics* **127**, 53–60 (1991).
69. Nag, D. K. & Petes, T. D. Meiotic recombination between dispersed repeated genes is associated with heteroduplex formation. *Mol. Cell. Biol.* **10**, 4420–4423 (1990).
70. Allers, T. & Lichten, M. Differential timing and control of noncrossover and crossover recombination during meiosis. *Cell* **106**, 47–57 (2001).
71. Goldman, A. S. & Lichten, M. Restriction of ectopic recombination by interhomolog interactions during *Saccharomyces cerevisiae* meiosis. *Proc. Natl Acad. Sci. USA* **97**, 9537–9542 (2000).
This study provides strong evidence that homologue pairing restricts meiotic NAHR between artificial repeats in *S. cerevisiae*.
72. Bhalla, N. & Dernburg, A. F. Prelude to a division. *Annu. Rev. Cell Dev. Biol.* **24**, 397–424 (2008).
73. Mieczkowski, P. A., Lemoine, F. J. & Petes, T. D. Recombination between retrotransposons as a source of chromosome rearrangements in the yeast *Saccharomyces cerevisiae*. *DNA Repair (Amst)* **5**, 1010–1020 (2006).
74. Kupiec, M. & Petes, T. D. Meiotic recombination between repeated transposable elements in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **8**, 2942–2954 (1988).
75. Kupiec, M. & Petes, T. D. Allelic and ectopic recombination between Ty elements in yeast. *Genetics* **119**, 549–559 (1988).
76. Meaburn, K. J., Misteli, T. & Soutoglou, E. Spatial genome organization in the formation of chromosomal translocations. *Semin. Cancer Biol.* **17**, 80–90 (2007).
77. Murti, J. R., Bumbulis, M. & Schimenti, J. C. Gene conversion between unlinked sequences in the germline of mice. *Genetics* **137**, 837–843 (1994).
78. Batzer, M. A. & Deininger, P. L. Alu repeats and human genomic diversity. *Nature Rev. Genet.* **3**, 370–379 (2002).
79. Tremblay, A., Jasin, M. & Chartrand, P. A double-strand break in a chromosomal LINE element can be repaired by gene conversion with various endogenous LINE elements in mouse cells. *Mol. Cell. Biol.* **20**, 54–60 (2000).
80. D'Anjou, H., Chabot, C. & Chartrand, P. Preferential accessibility to specific genomic loci for the repair of double-strand breaks in human cells. *Nucleic Acids Res.* **32**, 6136–6143 (2004).
81. Eickbush, T. H. & Eickbush, D. G. Finely orchestrated movements: evolution of the ribosomal RNA genes. *Genetics* **175**, 477–485 (2007).
82. Mieczkowski, P. A., Dominska, M., Buck, M. J., Lieb, J. D. & Petes, T. D. Loss of a histone deacetylase dramatically alters the genomic distribution of Spo11p-catalyzed DNA breaks in *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA* **104**, 3955–3960 (2007).
Using a microarray-based approach to map genome-wide DSBs in *S. cerevisiae*, this study shows that meiotic DSBs are suppressed in subtelomeric regions and in the rDNA in a Sir2-dependent manner.
83. Gottlieb, S. & Esposito, R. E. A new role for a yeast transcriptional silencer gene, *SIR2*, in regulation of recombination in ribosomal DNA. *Cell* **56**, 771–776 (1989).
84. Blander, G. & Guarente, L. The Sir2 family of protein deacetylases. *Annu. Rev. Biochem.* **73**, 417–435 (2004).
85. Fritze, C. E., Verschueren, K., Strich, R. & Easton Esposito, R. Direct evidence for SIR2 modulation of chromatin structure in yeast rDNA. *EMBO J.* **16**, 6495–6509 (1997).
86. San-Segundo, P. A. & Roeder, G. S. Pch2 links chromatin silencing to meiotic checkpoint control. *Cell* **97**, 313–324 (1999).
87. Smith, A. V. & Roeder, G. S. The yeast Red1 protein localizes to the cores of meiotic chromosomes. *J. Cell Biol.* **136**, 957–967 (1997).

88. Louis, E. J. The chromosome ends of *Saccharomyces cerevisiae*. *Yeast* **11**, 1553–1573 (1995).
89. Barton, A. B., Pekosz, M. R., Kurvathi, R. S. & Kaback, D. B. Meiotic recombination at the ends of chromosomes in *Saccharomyces cerevisiae*. *Genetics* **179**, 1221–1235 (2008).
90. Louis, E. J., Naumova, E. S., Lee, A., Naumov, G. & Haber, J. E. The chromosome end in yeast: its mosaic nature and influence on recombinational dynamics. *Genetics* **136**, 789–802 (1994).
91. Louis, E. J. & Haber, J. E. Mitotic recombination among subtelomeric Y′ repeats in *Saccharomyces cerevisiae*. *Genetics* **124**, 547–559 (1990).
92. Lichten, M. in *Recombination and Meiosis: Models, Means, and Evolution* (ed. Lankenau, D. H.) 165–193 (Springer-Verlag, Heidelberg, 2008).
93. Ben-Aroya, S., Mieczkowski, P. A., Petes, T. D. & Kupiec, M. The compact chromatin structure of a Ty repeated sequence suppresses recombination hotspot activity in *Saccharomyces cerevisiae*. *Mol. Cell* **15**, 221–231 (2004).
94. Anderson, L. K., Reeves, A., Webb, L. M. & Ashley, T. Distribution of crossing over on mouse synaptonemal complexes using immunofluorescent localization of MLH1 protein. *Genetics* **151**, 1569–1579 (1999).
95. Kleckner, N. Chiasma formation: chromatin/axis interplay and the role(s) of the synaptonemal complex. *Chromosoma* **115**, 175–194 (2006).
96. Page, S. L. & Hawley, R. S. The genetics and molecular biology of the synaptonemal complex. *Annu. Rev. Cell Dev. Biol.* **20**, 525–558 (2004).
97. Anuradha, S. & Muniyappa, K. Molecular aspects of meiotic chromosome synapsis and recombination. *Prog. Nucleic Acid Res. Mol. Biol.* **79**, 49–132 (2005).
98. Burgess, S. M. Homologous chromosome associations and nuclear order in meiotic and mitotically dividing cells of budding yeast. *Adv. Genet.* **46**, 49–90 (2002).
99. Mahadevaiah, S. K. *et al.* Recombinational DNA double-strand breaks in mice precede synapsis. *Nature Genet.* **27**, 271–276 (2001).
100. Schofield, M. J. & Hsieh, P. DNA mismatch repair: molecular mechanisms and biological function. *Annu. Rev. Microbiol.* **57**, 579–608 (2003).
101. Surtees, J. A., Argueso, J. L. & Alani, E. Mismatch repair proteins: key regulators of genetic recombination. *Cytogenet. Genome Res.* **107**, 146–159 (2004).
This review discusses the roles of mismatch repair proteins in repairing mismatches during meiotic recombination and in suppressing recombination between divergent sequences.
102. Worth, L. Jr, Clark, S., Radman, M. & Modrich, P. Mismatch repair proteins MutS and MutL inhibit RecA-catalyzed strand transfer between diverged DNAs. *Proc. Natl Acad. Sci. USA* **91**, 3238–3241 (1994).
103. Borts, R. H. & Haber, J. E. Meiotic recombination in yeast: alteration by multiple heterozygosities. *Science* **237**, 1459–1465 (1987).
104. Borts, R. H. *et al.* Mismatch repair-induced meiotic recombination requires the *pms1* gene product. *Genetics* **124**, 573–584 (1990).
105. Hunter, N., Chambers, S. R., Louis, E. J. & Borts, R. H. The mismatch repair system contributes to meiotic sterility in an interspecific yeast hybrid. *EMBO J.* **15**, 1726–1733 (1996).
106. Elliott, B. & Jasin, M. Repair of double-strand breaks by homologous recombination in mismatch repair-defective mammalian cells. *Mol. Cell. Biol.* **21**, 2671–2682 (2001).
107. Hunter, N. The RecQ DNA helicases: Jacks-of-all-trades or master-tradesmen? *Cell Res.* **18**, 328–330 (2008).
108. Jeffreys, A. J., Neumann, R., Panayi, M., Myers, S. & Donnelly, P. Human recombination hot spots hidden in regions of strong marker association. *Nature Genet.* **37**, 601–606 (2005).
109. Kauppi, L., Stumpf, M. P. & Jeffreys, A. J. Localized breakdown in linkage disequilibrium does not always predict sperm crossover hot spots in the human MHC class II region. *Genomics* **86**, 13–24 (2005).
110. Winckler, W. *et al.* Comparison of fine-scale recombination rates in humans and chimpanzees. *Science* **308**, 107–111 (2005).
111. Ptak, S. E. *et al.* Fine-scale recombination patterns differ between chimpanzees and humans. *Nature Genet.* **37**, 429–434 (2005).
112. Petes, T. D. Meiotic recombination hot spots and cold spots. *Nature Rev. Genet.* **2**, 360–369 (2001).
113. Borde, V. *et al.* Histone H3 lysine 4 trimethylation marks meiotic recombination initiation sites. *EMBO J.* **28**, 99–111 (2009).
114. Jeffreys, A. J. & Neumann, R. Reciprocal crossover asymmetry and meiotic drive in a human recombination hot spot. *Nature Genet.* **31**, 267–271 (2002).
115. Jensen-Seaman, M. I. *et al.* Comparative recombination rates in the rat, mouse, and human genomes. *Genome Res.* **14**, 528–538 (2004).
116. Hillier, L. W. *et al.* Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature* **432**, 695–716 (2004).
117. Paigen, K. *et al.* The recombinational anatomy of a mouse chromosome. *PLoS Genet.* **4**, e1000119 (2008).
118. Buard, J., Barthes, P., Grey, C. & de Massy, B. Distinct histone modifications define initiation and repair of meiotic recombination in the mouse. *EMBO J.* **28**, 2616–2624 (2009).
119. Baudat, F. *et al.* PRDM9 is a major determinant of meiotic recombination hotspots in humans and mice. *Science* 31 Dec 2009 (doi:10.1126/science.1183439).
120. Myers, S. *et al.* Drive against hotspot motifs in primates implicates the *PRDM9* gene in meiotic recombination. *Science* 31 Dec 2009 (doi:10.1126/science.1182363).
121. Hayashi, K., Yoshida, K. & Matsui, Y. A histone H3 methyltransferase controls epigenetic events required for meiotic prophase. *Nature* **438**, 374–378 (2005).
122. Mefford, H. C. *et al.* Recurrent rearrangements of chromosome 1q21.1 and variable pediatric phenotypes. *N. Engl. J. Med.* **359**, 1685–1699 (2008).
123. White, P. C., Vitek, A., Dupont, B. & New, M. I. Characterization of frequent deletions causing steroid 21-hydroxylase deficiency. *Proc. Natl Acad. Sci. USA* **85**, 4436–4440 (1988).
124. Tusie-Luna, M. T. & White, P. C. Gene conversions and unequal crossovers between *CYP21* (steroid 21-hydroxylase gene) and *CYP21P* involve different mechanisms. *Proc. Natl Acad. Sci. USA* **92**, 10796–10800 (1995).
125. Urban, Z. *et al.* 7q11.23 deletions in Williams syndrome arise as a consequence of unequal meiotic crossover. *Am. J. Hum. Genet.* **59**, 958–962 (1996).
126. Somerville, M. J. *et al.* Severe expressive-language delay related to duplication of the Williams-Beuren locus. *N. Engl. J. Med.* **353**, 1694–1701 (2005).
127. Carrozzo, R. *et al.* Inter- and intrachromosomal rearrangements are both involved in the origin of 15q11-q13 deletions in Prader-Willi syndrome. *Am. J. Hum. Genet.* **61**, 228–231 (1997).
128. Sharp, A. J. *et al.* A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures. *Nature Genet.* **40**, 322–328 (2008).
129. Shinawi, M. *et al.* A small recurrent deletion within 15q13.3 is associated with a range of neurodevelopmental phenotypes. *Nature Genet.* **41**, 1269–1271 (2009).
130. Sharp, A. J. *et al.* Characterization of a recurrent 15q24 microdeletion syndrome. *Hum. Mol. Genet.* **16**, 567–572 (2007).
131. Chen, K. S. *et al.* Homologous recombination of a flanking repeat gene cluster is a mechanism for a common contiguous gene deletion syndrome. *Nature Genet.* **17**, 154–163 (1997).
132. Koolen, D. A. *et al.* A new chromosome 17q21.31 microdeletion syndrome associated with a common inversion polymorphism. *Nature Genet.* **38**, 999–1001 (2006).
133. Shaw-Smith, C. *et al.* Microdeletion encompassing MAPT at chromosome 17q21.3 is associated with developmental delay and learning disability. *Nature Genet.* **38**, 1032–1037 (2006).
134. Sharp, A. J. *et al.* Discovery of previously unidentified genomic disorders from the duplication architecture of the human genome. *Nature Genet.* **38**, 1038–1042 (2006).
135. Vnencak-Jones, C. L., Phillips, J. A. 3rd, Chen, E. Y. & Seeburg, P. H. Molecular basis of human growth hormone gene deletions. *Proc. Natl Acad. Sci. USA* **85**, 5615–5619 (1988).
136. Edelmann, L., Pandita, R. K. & Morrow, B. E. Low-copy repeats mediate the common 3-Mb deletion in patients with velo-cardio-facial syndrome. *Am. J. Hum. Genet.* **64**, 1076–1086 (1999).
137. Giorda, R. *et al.* Complex segmental duplications mediate a recurrent dup(X)(p11.22-p11.23) associated with mental retardation, speech delay, and EEG anomalies in males and females. *Am. J. Hum. Genet.* **85**, 394–400 (2009).
138. Bondeson, M. L. *et al.* Inversion of the *IDS* gene resulting from recombination with *IDS*-related sequences is a common cause of the Hunter syndrome. *Hum. Mol. Genet.* **4**, 615–621 (1995).
139. Karsten, S. L. *et al.* Two distinct deletions in the *IDS* gene and the gene *W*: a novel type of mutation associated with the Hunter syndrome. *Genomics* **43**, 123–129 (1997).
140. Simon, D. B. *et al.* Mutations in the chloride channel gene, *CLCNKB*, cause Bartter's syndrome type III. *Nature Genet.* **17**, 171–178 (1997).
141. Zimran, A. *et al.* A glucocerebrosidase fusion gene in Gaucher disease. Implications for the molecular anatomy, pathogenesis, and diagnosis of this disorder. *J. Clin. Invest.* **85**, 219–222 (1990).
142. Saunier, S. *et al.* Characterization of the *NPHP1* locus: mutational mechanism involved in deletions in familial juvenile nephronophthisis. *Am. J. Hum. Genet.* **66**, 778–789 (2000).
143. Wijmenga, C. *et al.* Chromosome 4q DNA rearrangements associated with facioscapulohumeral muscular dystrophy. *Nature Genet.* **2**, 26–30 (1992).
144. Lifton, R. P. *et al.* A chimaeric 11 β-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. *Nature* **355**, 262–265 (1992).
145. Watnick, T. J., Gandolph, M. A., Weber, H., Neumann, H. P. & Germino, G. G. Gene conversion is a likely cause of mutation in *PKD1*. *Hum. Mol. Genet.* **7**, 1239–1243 (1998).
146. Dorschner, M. O., Sybert, V. P., Weaver, M., Pletcher, B. A. & Stephens, K. NF1 microdeletion breakpoints are clustered at flanking repetitive sequences. *Hum. Mol. Genet.* **9**, 35–46 (2000).
147. Mefford, H. C. *et al.* Recurrent reciprocal genomic rearrangements of 17q12 are associated with renal disease, diabetes, and epilepsy. *Am. J. Hum. Genet.* **81**, 1057–1069 (2007).
148. Van Esch, H. *et al.* Deletion of VCX-A due to NAHR plays a major role in the occurrence of mental retardation in patients with X-linked ichthyosis. *Hum. Mol. Genet.* **14**, 1795–1803 (2005).
149. Smahi, A. *et al.* Genomic rearrangement in *NEMO* impairs NF-κB activation and is a cause of incontinentia pigmenti. *Nature* **405**, 466–472 (2000).
150. Aradhya, S. *et al.* A recurrent deletion in the ubiquitously expressed *NEMO* (IKK-γ) gene accounts for the vast majority of incontinentia pigmenti mutations. *Hum. Mol. Genet.* **10**, 2171–2179 (2001).
151. Nathans, J., Piantanida, T. P., Eddy, R. L., Shows, T. B. & Hogness, D. S. Molecular genetics of inherited variation in human color vision. *Science* **232**, 203–210 (1986).
152. Metzenberg, A. B., Wurzer, G., Huisman, T. H. & Smithies, O. Homology requirements for unequal crossing over in humans. *Genetics* **128**, 143–161 (1991).
153. Higgs, D. R., Hill, A. V., Bowden, D. K., Weatherall, D. J. & Clegg, J. B. Independent recombination events between the duplicated human α globin genes; implications for their concerted evolution. *Nucleic Acids Res.* **12**, 6965–6977 (1984).
154. Lakich, D., Kazazian, H. H., Jr., Antonarakis, S. E. & Gitschier, J. Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. *Nature Genet.* **5**, 236–241 (1993).

Acknowledgements
We thank members of the Keeney laboratory for comments on the manuscript. Work from the authors' laboratory is supported in part by the Howard Hughes Medical Institute and in part by grants from the US National Institutes of Health.

Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/gene
SIR2
OMIM: http://www.ncbi.nlm.nih.gov/omim
CMT1A | HNPP | neurofibromatosis 1
UniProtKB: http://www.uniprot.org
BLM | Dmc1 | Hop1 | Msh2 | MSH4 | Pms1 | Rad51 | Sgs1 | Spo11 | Srs2

FURTHER INFORMATION
Scott Keeny's homepages: http://www.ski.edu/keeney; http://www.hhmi.org/research/investigators/keeney.html

ALL LINKS ARE ACTIVE IN THE ONLINE PDF