Homologous recombination

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Primer

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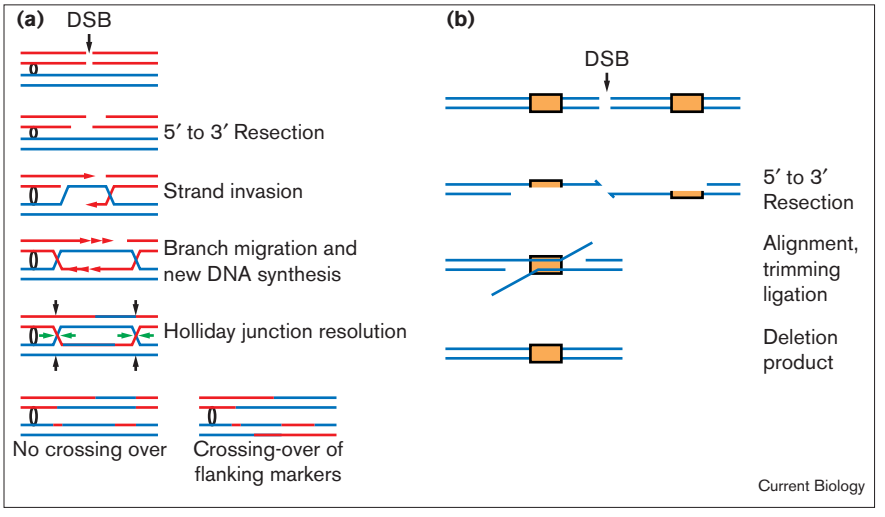
DNA damage, in the form of DNA double-strand breaks, poses a considerable threat to genomic integrity and cell survival. If left unrepaired, a single double-strand break is sufficient to cause cell death and, if repaired inappropriately, a double-strand break may give rise to a potentially oncogenic translocation.

Double-strand breaks in genomic DNA may arise accidentally in a number of ways, including through exposure to harmful exogenous agents such as ionizing radiation or cytotoxic chemicals, as a consequence of mechanical stress, or through the breakdown of a replication fork. Alternatively, they may be introduced by endonucleases, as intermediates in normal cellular processes such as meiosis, or the mating type switch in yeast, or during V(D)J gene rearrangements in vertebrate T-cell development.

The repair of double-strand breaks is so critical that eukaryotic organisms have developed two distinct mechanisms to deal with it. One mechanism involves the simple rejoining of the broken DNA ends, regardless of sequence, a process known as nonhomologous end joining. This mechanism has the major drawback that it is somewhat inaccurate, as small deletions may be introduced at the site of the DNA break. In the G1 phase of the cell cycle, however, when there is only one copy of each chromosome, the repair of double-strand breaks by nonhomologous end joining is crucial for cell survival. The second mechanism of double-strand break repair takes advantage of the fact that, in S-phase, DNA replication generates an identical copy of each DNA molecule. In this situation double-strand breaks may be repaired by using the undamaged copy of the DNA as a template, to restore the break in the damaged DNA without the loss of genetic information. This high-fidelity DNA repair mechanism is homologous recombination. Repair of double-strand breaks by homologous recombination not only helps protect cells against the potentially lethal effects of DNA damage but, in the process, helps generate the genetic diversity that drives evolution.

Figure 1



(a) Double-strand break repair model for homologous recombination. The double-strand break (DSB) is 5′ to 3′ resected, producing DNA ends with 3′ ssDNA tails. The 3′ ends invade a homologous DNA duplex, forming a DNA crossover or Holliday junction that provides a primer to initiate new DNA synthesis. Branch migration of the Holliday junction extends the region of heteroduplex away from the initial site of crossover. Holliday junctions are resolved by endonucleolytic cleavage of either the crossed strands (green arrows) or non-crossed strands (black arrows) of the junction. Resolution of the two Holliday junctions in different orientations (that is, crossed with non-crossed) will result in the exchange of flanking markers, whereas resolution in the same orientation (crossed with crossed or non-crossed with non-crossed) does not result in exchange of flanking markers. **(b)** Single-strand annealing model. Single-strand annealing occurs between directly repeated homologous sequences. Resection of a DSB continues until complimentary sequences are revealed. The complimentary sequences are aligned, the non-homologous ends are trimmed and the gaps repaired by DNA synthesis. Recombination of direct repeats by single-strand annealing results in deletion of the intervening DNA and the restoration of a single copy of the repeated sequence.

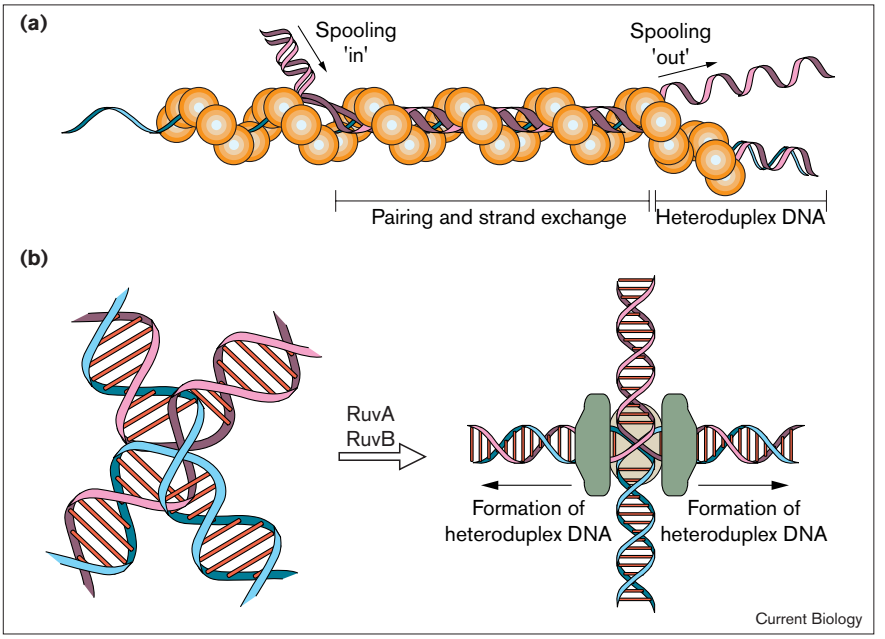
Mechanisms

Clearly, the use of one DNA molecule as a template for repairing another requires a close physical association between the two DNAs. In 1964 Robin Holliday proposed that recombination was achieved through the physical exchange of DNA strands from separate DNA duplexes, via a crossover intermediate that became known as a Holliday junction. The Holliday model has since been refined to accommodate increasing knowledge of homologous recombination and additional models have been developed to account for recombination in particular circumstances, such as that between repeated homologous sequences. Two models are described in Figure 1.

DNA breaks are initially ‘resected’ (or chewed back) by cellular nucleases to generate DNA ends with 3′ single-stranded tails. These regions of single-stranded DNA (ssDNA) are critical for both the recruitment of the cellular recombination machinery and for initiating the homologous pairing that precedes strand exchange. For

the majority of DNA breaks, repair by homologous recombination probably occurs via the formation, branch migration and resolution of Holliday junction intermediates as described by the double-strand break repair model (Figure 1a). For double-strand breaks that occur within directly repeated homologous sequences, however, repair may also occur through a single strand annealing mechanism without the formation of a Holliday junction (Figure 1b). Instead, the resected homologous ends are aligned and the intervening sequences deleted, to produce a single copy of the repaired sequence.

Figure 2



(a) RecA (orange) forms a helical nucleoprotein filament on ssDNA. The ssDNA is stretched to 1.5 times its normal length to facilitate the search for homology. The nucleoprotein filament interacts with naked duplex DNA until homology is found, then strand exchange is initiated, producing a region of heteroduplex DNA. (Adapted with permission from West SC, *Ann Rev Biochem* 1992, **61**:603-640.) **(b)** The RuvA (light green) and RuvB (dark green) proteins bind to a Holliday junction, opening it out into a planar, cruciform structure. A tetramer of RuvA sits at the crossover point of the junction, while two hexameric RuvB rings sit on opposite arms of the junction with the DNA passing through the centre of each ring. Branch migration is driven by RuvB 'pumping' DNA through each ring, in opposite directions, moving the Holliday junction along the DNA.

Recombination in prokaryotes

Most of our knowledge concerning the biochemistry of recombination has been gained through studies of bacteria such as *Escherichia coli*. In fact, most of the 20 or so proteins known to be involved in homologous recombination in *E. coli* have been purified, and their biochemical functions have been determined. Of these, a few key proteins carry out the fundamental processes of strand exchange, branch migration and resolution.

In *E. coli*, resection of broken DNA ends to produce 3′ ssDNA tails is carried out primarily by the RecBCD complex, which comprises the products of the *recB*, *recC* and *recD* genes. This complex has both exonuclease and endonuclease activities, as well as an ability to unwind double-stranded DNA. Although double-strand breaks may also be resected by other nucleases, recombination is much less efficient in the absence of RecBCD.

The key processes of homologous pairing and strand exchange are carried out by the RecA protein. This 38 kDa protein binds to and polymerises along ssDNA, forming a right handed nucleoprotein filament (Figure 2a). Importantly, filament formation by RecA occurs with a defined 5′ to 3′ polarity, ensuring that 3′ ssDNA ends are completely covered. Homology is established through the interaction of the filament with naked duplex DNA. Next, RecA catalyses strand exchange, with a defined 5′–3′ polarity relative to the ssDNA in the filament, to form a region of heteroduplex DNA (duplex DNA comprising DNA strands from different DNA molecules). The region of heteroduplex DNA is extended, by branch migration of the Holliday junction, away from the site of the DNA break. Branch migration does not take place within the RecA–nucleoprotein filament but is carried out by two additional proteins, RuvA and RuvB, which bind to the Holliday junction, altering its conformation and displacing RecA from the DNA (Figure 2b).

Finally, Holliday junctions are resolved to separate duplex DNAs by a junction-specific endonuclease, RuvC. The RuvC piggybacks on the RuvAB complex, where it monitors sequences until a consensus sequence (A/T)–T–T–(C/G) is identified, whereafter it makes symmetrical nicks in opposite strands of the junction. It is the resolution of these Holliday junctions that determines whether or not flanking sequences are exchanged after recombination (see Figure 1).

Recombination in higher organisms

Much of our knowledge concerning recombination in eukaryotic organisms has been gained from yeast genetics, but most of the factors identified in yeast have direct homologues in humans. Furthermore, one of the key factors, the Rad51 protein, has considerable structural and functional similarity to the bacterial RecA protein described

above. Like RecA, Rad51 binds DNA to form a nucleoprotein filament that catalyses homologous pairing and strand exchange, albeit with lower efficiency than its bacterial counterpart. But strand exchange by Rad51 is stimulated by additional factors such as the Rad52 protein or a complex containing the Rad55 and Rad57 proteins. Beyond these basic steps, little is known about the biochemical mechanism of recombination in higher organisms and many issues remain unclear.

One problem of current interest involves the polarity of strand exchange in eukaryotic organisms. In biochemical assays, human RAD51 protein promotes strand exchange with 3′ to 5′ polarity, which is opposite to that for bacterial RecA (5′ to 3′). Interestingly, the yeast Rad51 protein catalyses strand exchange *in vitro* with either polarity, depending on the assay conditions.

Another interesting problem surrounds the role of the RAD52 protein. Whereas deletion of the *rad52* gene in yeast produces a severe defect in homologous recombination, mice that lack RAD52 show no apparent phenotype. One possible explanation is that higher organisms encode another homologue of RAD52 with overlapping function, but no such gene has so far been identified.

Questions concerning the initial resection of double-strand breaks also remain unresolved. Genetic studies in yeast have identified a role for a complex comprising the Mre11, Rad50 and Xrs2 proteins in the resection of DNA ends. But biochemical studies have shown that this complex has a 3′ to 5′ exonuclease activity, which produces a DNA with a 5′ single-stranded end. Perhaps resection of the double-strand break requires additional factors. This paradox has not yet been resolved.

Two genetically tractable systems have recently been developed to study recombination in mammalian cells. First, in Japan, Takeda and colleagues have used the chicken DT40 cell line, in which targeted recombination occurs at a particularly high level, to make a series of cell lines in which genes encoding specific recombination factors have been knocked out. Second, Maria Jasin's group in the USA has developed a genetic reporter system to study the repair of DNA breaks introduced by the expression of a rare-cutting endonuclease, I-Sce1. This system has been used to demonstrate roles in recombination for the *RAD51* paralogues *XRCC2* and *XRCC3*, as well as for the breast cancer-associated gene *BRCA1*.

Genetic diversity in meiosis

Whereas in mitosis homologous recombination occurs mainly between identical sister chromatids, in meiosis recombination occurs between homologous pairs of chromosomes (that is, non sister chromatids). Cytological studies have shown that during meiosis each pair of homologous chromosomes exhibits one or more crossovers, which may play a part in keeping the pairs of chromosomes together before segregation into daughter cells. Recombination seems to be initiated by the introduction of double-strand breaks in particular 'hot spot' regions of the chromosome, by the Spo11 protein. Genetic studies have shown that although some of the proteins involved in mitotic recombination are also involved in meiotic recombination, some meiosis-specific proteins, such as DMC1 and RDH54, are also involved.

As homologous pairs of chromosomes encode different alleles they are not genetically identical. Hence, the repair of meiotically-induced double-strand breaks by homologous recombination may result in the transfer of genetic information from one non-sister chromatid to another, a process known as gene conversion. As each of the 23 homologous pairs of chromosomes may undergo several gene conversion events during a single meiotic division the capacity for creating new genetic associations is huge. Upon segregation of the homologues each new combination can be acted upon by natural selection providing the molecular basis for evolutionary change.

Homologous recombination in cancer

In recent years much excitement has been generated by reports linking the recombination protein RAD51 with two genes associated with heritable breast cancer, *BRCA1* and *BRCA2*. Indeed, there is a direct physical interaction between the RAD51 and BRCA2 proteins. In support of this association, studies in mice have shown that homozygous deletion of either *RAD51* or *BRCA2* is lethal at around days 6–8 of embryogenesis. Furthermore, mice encoding a truncated form of the *BRCA2* gene, although viable, show a marked sensitivity to DNA-damaging agents such as the crosslinker mitimycin C, suggesting a deficiency in recombinational DNA repair. The evidence that BRCA2 is associated with RAD51 and is possibly involved DNA repair is both intriguing and exciting. Above all, the clinical significance of this finding serves to underscore the importance of future studies on homologous recombination.

Key references

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