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⊕ Online links

TIMELINE

Happy Hollidays: 40th anniversary of the Holliday junction

Yilun Liu and Stephen C. West

Abstract | In 1964, the geneticist Robin Holliday proposed a mechanism of DNA-strand exchange that attempted to explain gene-conversion events that occur during meiosis in fungi. His proposal marked the birthday of the now famous cross-stranded DNA structure, or Holliday junction. To understand the importance of the Holliday model we must look back in the history of science beyond the last 40 years, to a time when theories of heredity were being proposed by Gregor Johann Mendel.
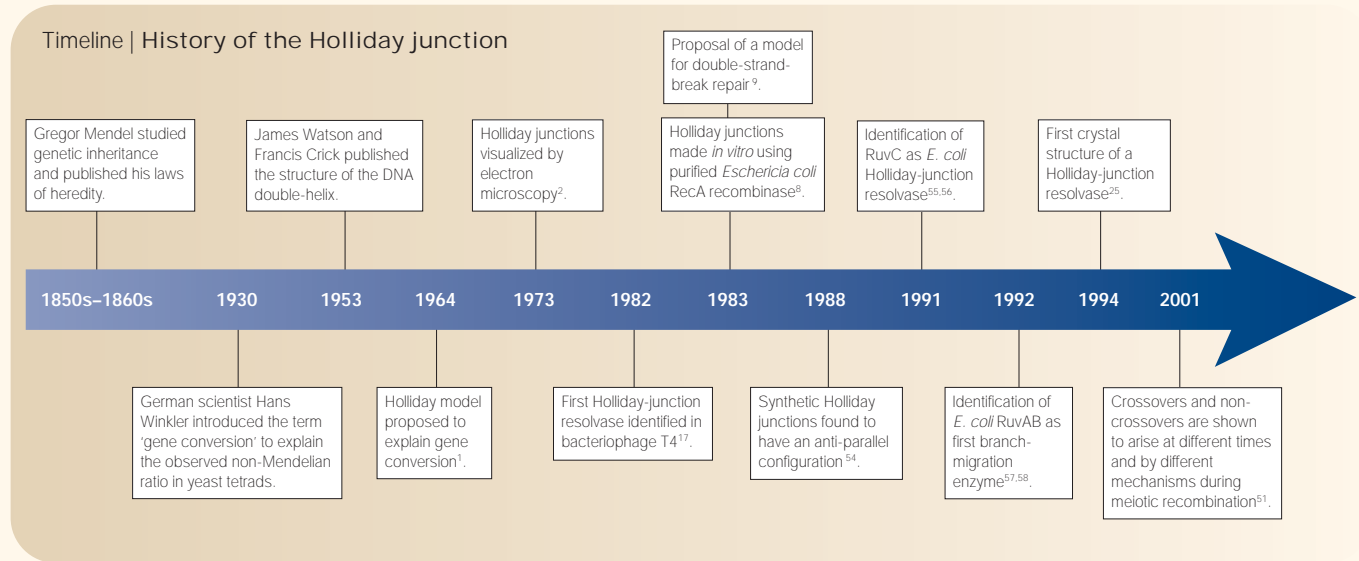
Gregor Mendel, an Augustinian monk who taught natural science, was a man who paid attention to detail. In 1866 (TIMELINE), on the basis of his studies with pea plants, Mendel published a series of observations describing how characters or traits (now known as genes) are passed from parents to their offspring. One important conclusion from his study was that hereditary factors do not combine, but are passed intact to the offspring, and that each member of the parental generation transmits only half of its hereditary factors to each offspring (with some factors being dominant over others). His work became the foundation for modern genetics; we now interpret it as showing that a parental cell with a pair of heterozygous (that is, different) alleles will produce gametes with a 2:2 ratio, such that each allele is represented equally in the haploid gametes (FIG. 1). However, although Mendel's law of segregation was mostly shown to hold true, subsequent studies indicated that this was not always the case. Deviations from the expected 2:2 ratio were first reported by the German scientist Hans Winkler who, in 1930, introduced the term gene conversion to define the aberrant 3:1 ratio that had been observed in yeast tetrads. That is, during the process of segregation of the gametes, a gene-conversion event takes place that converts one allele to the other, so that the ratio of the alleles in the haploid gametes changes from 2:2 to 3:1.

How does gene conversion work? In 1964, Robin Holliday (FIG. 2) from the John Innes

> "The structure at the point of strand exchange later became known as a Holliday junction, and is embedded in history as a central intermediate in the process of homologous recombination."

Timeline | **History of the Holliday junction**

Proposal of a model for double-strand-break repair [9].

Gregor Mendel studied genetic inheritance and published his laws of heredity.

James Watson and Francis Crick published the structure of the DNA double-helix.

Holliday junctions visualized by electron microscopy [2].

Holliday junctions made *in vitro* using purified *Eschericia coli* RecA recombinase [8].

Identification of RuvC as *E. coli* Holliday-junction resolvase [55,56].

First crystal structure of a Holliday-junction resolvase [25].

| 1850s–1860s | 1930 | 1953 | 1964 | 1973 | 1982 | 1983 | 1988 | 1991 | 1992 | 1994 | 2001 |

German scientist Hans Winkler introduced the term 'gene conversion' to explain the observed non-Mendelian ratio in yeast tetrads.

Holliday model proposed to explain gene conversion [1].

First Holliday-junction resolvase identified in bacteriophage T4 [17].

Synthetic Holliday junctions found to have an anti-parallel configuration [54].

Identification of *E. coli* RuvAB as first branch-migration enzyme [57,58].

Crossovers and non-crossovers are shown to arise at different times and by different mechanisms during meiotic recombination [51].
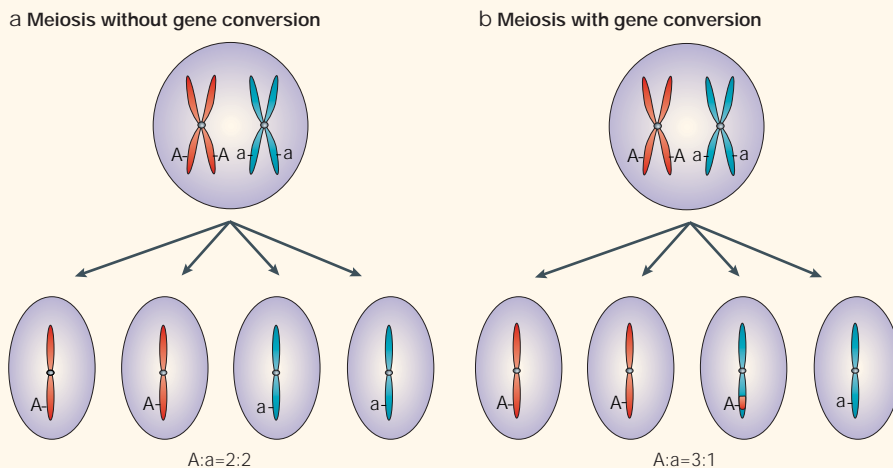
Institute in the United Kingdom — who was studying DNA damage and genetic recombination in the smut fungus *Ustilago maydis* and the budding yeast *Saccharomyces cerevisiae* at the time — proposed a model for recombination that provided a molecular basis for both gene conversion and crossing over (that is, how genes linked on the same chromosome could segregate from each other)[1]. The Holliday model suggested that after DNA replication, which generates two copies of each of the two heterozygous alleles, recombination is initiated by the introduction of nicks at the same position in two DNA molecules that have different alleles (FIG. 3a). These breaks in the DNA strands allow single strands to anneal to the complementary sequences in the other duplex (FIG. 3b). The

switching of strands between DNA molecules results in the formation of a cross-stranded structure that physically links the two interacting DNA strands. The structure at the point of strand exchange later became known as a Holliday junction, and is embedded in history as a central intermediate in the process of homologous recombination (FIG. 3c). A second, critical aspect of the Holliday model invokes cutting (or 'resolution') of the crossover so that the two DNA helices can separate. Owing to the symmetry of the junction, it was assumed that there might be two possible orientations of resolution, each with a different outcome. If the breaks are introduced in the strands that are complementary to the initiating nicks (FIG. 3c, green arrows), the arms that flank the junc-

tion are crossed over. By contrast, cutting the other pair of strands results in recombination without crossing over (FIG. 3c, purple arrows). In both cases, however, the strand exchange produces heteroduplex DNA that contains mismatched nucleotides. Holliday postulated that mechanisms must exist to repair these mismatches, a suggestion that was later proven to be true when the enzymes of mismatch repair were discovered. Holliday proposed that heteroduplex DNA serves as the substrate for the gene-conversion event and, depending on which strand is used as the template for mismatch repair, the allelic ratio can be maintained as 2:2 (FIG. 3d), or changed to the observed aberrant ratio of 3:1 (FIG. 3e). This model provided a mechanistic basis for 'gene conversion'.

Forty years after the Holliday model was proposed, we must now look back to see what biophysical analyses have told us about the structure of the Holliday junction, and how biochemical studies have defined the properties of enzymes that can specifically recognize four-way junctions and promote reactions that, originally, could only be imagined by Holliday. We will also reinvestigate the dogma that the Holliday junction is the central intermediate of recombination, and discuss how the Holliday model has evolved and been tailored to fit into the present picture of DNA recombination and DNA-strand-break repair.

Existence of the Holliday junction
The first physical evidence to support Holliday's proposal of a cross-stranded DNA intermediate was provided by electron-microscope studies that were carried out in the early 1970s. Work with S13 and ØX174 —

a Meiosis without gene conversion

A—A a—a

A A a a

A:a=2:2

b Meiosis with gene conversion

A—A a—a

A A A a

A:a=3:1

Figure 1 | **The Mendelian ratio.** Based on the laws of segregation, published as part of the theory of heredity by Gregor Mendel in the mid-nineteenth century, a parental cell with a pair of heterozygous alleles (designated *A* and *a*), will produce gametes with an *A:a* = 2:2 ratio (left). However, this is not always the case as the 2:2 rule is violated on rare occasions when the ratio is 3:1 (right). This problem led Robin Holliday to propose his model for recombination to explain gene conversion.

Figure 2 | **Robin Holliday.** In May 2004, Robin Holliday attended the EMBO workshop 'Recombination Mechanisms' and presented a lecture to celebrate the 40th anniversary of his model. This photograph was taken at the Chateau de Chenonceau, France, by David Roth.

two *Escherichia coli* phages that use their host's enzyme systems for recombination — revealed the presence of intermediates in which DNA molecules were linked by a 'Holliday junction'[2–4]. A spectacular image of two plasmid DNA molecules linked by a Holliday junction is shown in FIG. 4a (REF. 5). Soon afterwards, Holliday junctions that were made by the recombination of 2μ plasmid DNA were observed in eukaryotic cells[6].

Our understanding of the detailed mechanism of recombination and the formation of Holliday junctions was advanced significantly in the 1980s when several of the key recombination proteins from *E. coli* were purified and characterized. Using appropriately constructed DNA substrates, it was shown that purified RecA protein could initiate strand-exchange reactions to form Holliday junctions *in vitro*[7,8]. The concept of the Holliday junction was, by this time, well accepted by the scientific community, and was embraced as a *de facto* intermediate in recombination for at least the next decade.

However, further studies of recombination in eukaryotic cells, which were boosted by the emergence of sophisticated molecular-genetic approaches in yeast, indicated that the Holliday model was too symmetrical, and failed to account for data that had been obtained in *S. cerevisiae* where little evidence of reciprocal heteroduplex DNA could be found. It also became clear that there were alternative pathways that could lead to the formation of recombinant products. In the 1980s, two models were proposed that, at present, continue to

form the basis of our understanding of recombination. In 1983, Jack Szostak, Terry Orr-Weaver, Rodney Rothstein and Frank Stahl proposed a model that was novel in two respects: first, recombination was initiated by a DNA double-strand break (DSB) rather than by nicks; and second, the recombining DNA helices became linked by two Holliday junctions[9]. Soon afterwards, work from Sternberg's laboratory indicated that recombination could occur between repetitive sequences by a mechanism that became known as single-strand annealing[10]. More recently, other models have gained in popularity, most notably the concept that recombination can occur by synthesis-dependent strand annealing (SDSA) without

Holliday-junction formation[11]. Whether or not the Holliday junction could survive these 'revolutions' will be discussed below.

The structure of a Holliday junction

The physical existence of Holliday junctions, as indicated by electron microscopy, stimulated biophysicists to try to determine its structure. But their goal was not an easy one, as it was extremely difficult to study the structure of a crossover that was just a small part of a larger DNA molecule. The description, by Ned Seeman and colleagues in 1983, of a four-way junction that could be made simply by annealing short synthetic oligonucleotides was therefore a defining moment that led to
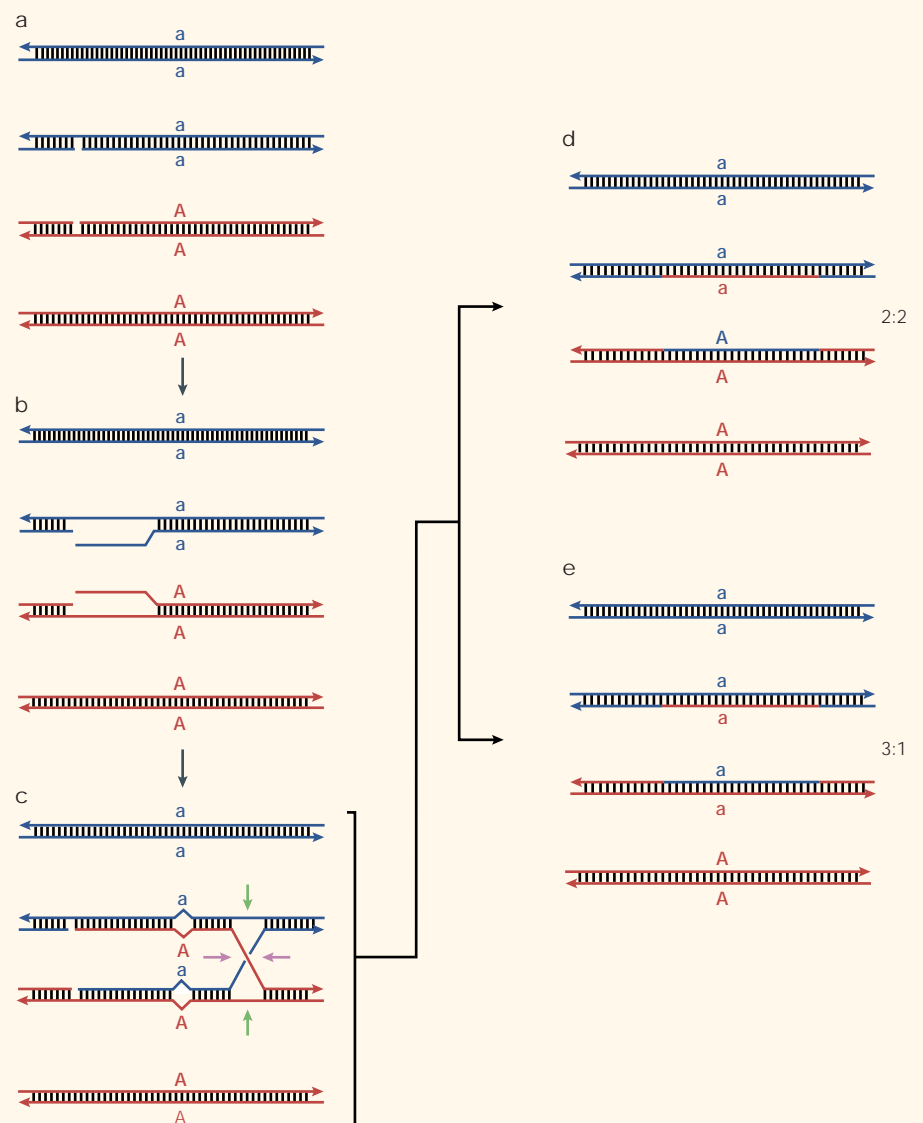

Figure 3 | **The Holliday model.** After DNA replication and before meiotic cell division, nicks are introduced at a defined point on two homologous chromosomes (**a**). Strand exchanges take place to generate a crossover, or Holliday junction (**b** and **c**). Symmetric resolution in the two possible orientations (indicated by purple and green arrows) allows separation of the recombining chromosomes (**c**). Crossover or non-crossover products are formed, dependent on the orientation of resolution. DNA mismatches present in the heteroduplex DNA might be corrected, leading to gene conversion (**d** and **e**).
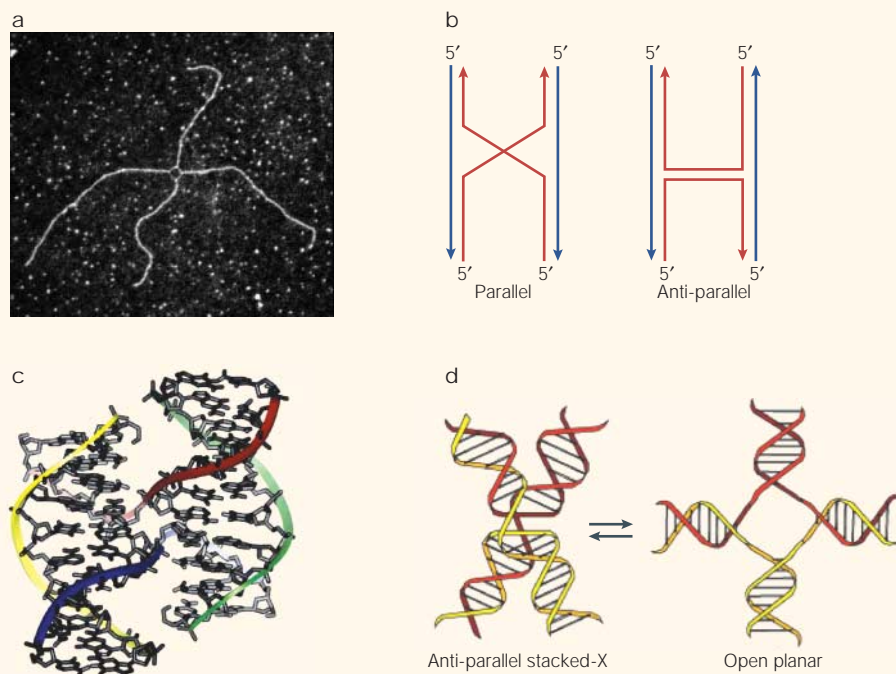
Figure 4 | **Structure of the Holliday junction. a** | Electron-microscope image of a recombination intermediate. In this image, the Holliday junction was partially denatured to assist its visualization. **b** | Two possible configurations for the Holliday junction, with the DNA shown in the parallel (left) or anti-parallel configuration (right). **c** | Three-dimensional view of a Holliday junction, as determined by X-ray crystallography. **d** | The Holliday junction shown in the anti-parallel stacked-X (left) and open planar (right) configuration. Part **a** is reproduced with permission from REF. 5 © (1979) Cold Spring Harbor Laboratory Press. Part **c** is modified with permission from REF. 16 © (2000) the National Academy of Sciences.

significant progress in understanding the three-dimensional structure of the junction[12]. But it was still many years before scientists were able to determine the structure of Holliday's junction at the atomic level.

Initial studies of the Holliday-junction structure were both surprising and controversial. Gel electrophoresis and analyses using fluorescent energy transfer (FRET; a technique that measures the distance between the excited states of two fluorescent dyes) revealed that the four-way junction could exist in a variety of structures, none of which resembled the expected structure in which the two linked DNA helices would lie parallel to each other. Robin Holliday was bemused by the physical studies and found it difficult to accept that these structures were truly representative of the Holliday junction as it exists within the cell. What was so controversial was that, in the presence of a divalent metal ion such as Mg^{2+}, the junction adopts a twofold symmetric X-shape in which the DNA helices lie anti-parallel to each other[13] (FIG. 4b). But this was no artefact, as the anti-parallel structure of the Holliday junction was later confirmed by three independent X-ray crystallographic structures[14–16] (FIG. 4c).

In the anti-parallel orientation, the exchanging strands do not cross with each other. The torsional angle of the sugar-phosphate backbone of the DNA at the point of exchange adapts a *gauche* conformation, instead of the *trans* conformation that is found in normal duplex DNA. This allows it to bend into a U shape and pair with the complementary strand that is running in the opposite direction. This structure, which was observed *in vitro*, raises interesting questions about its existence *in vivo*. Within the cell, it is expected that homologous chromosomes at meiosis (or sister chromatids undergoing mitotic recombination) will be aligned parallel to each other, at least in a global sense. So if the duplexes at the Holliday junction lie anti-parallel to each other, then, at the local level, one of the two DNA molecules will have to rotate 180° into the anti-parallel orientation. Whether this can occur *in vivo*, where physical constraints will be imposed by the flanking arms and by proteins that bind to the DNA, remains a puzzle.

In addition to the anti-parallel X-structure that is observed in the presence of divalent cations, Holliday junctions are also seen to form an unstacked fourfold symmetric planar structure (FIG. 4d). The square-planar structure

is seen in the absence of metal ions, which indicates that the metal ions neutralize the electrostatic repulsions that are caused by the phosphates in the DNA backbone at the point of the crossover[13]. Interestingly, enzymes that bind Holliday junctions and catalyse the key reactions that are involved in junction processing (branch migration and nucleolytic resolution) have been shown to bind and stabilize the open planar structure. Therefore, it can be argued that the fourfold, symmetric, unstacked structure might be the more physiologically relevant of these two structures.

Holliday-junction-processing enzymes
In the Holliday model, recombination occurs by a two-step process: symmetric nicking initiates crossover formation, and symmetric nicking resolves the crossover. But in 1964, Robin was not really thinking about the enzymes that were involved and how they might promote such reactions. Indeed, it took until 1982, when biochemical evidence provided the first example of an enzyme that could recognize Holliday junctions and promote a specific cleavage reaction, for us to realize that Holliday-junction-specific proteins existed. The relevant protein from bacteriophage T4 is the product of gene 49. The enzyme, T4 endonuclease VII, is a structure-specific endonuclease that is required for the separation of highly branched DNA before its packaging into phage heads[17]. Subsequently, Holliday-junction resolvases were identified in various species, including bacteriophage-T7 endonuclease I, pox virus A22, *E. coli* RuvC and RusA, archaeal Hjc and Hje, and *Saccharomyces cerevisiae* mitochondrial Cce1 (or Ydc2 in *Schizosaccharomyces pombe*)[18]. All of these enzymes recognize Holliday junctions and resolve them by the introduction of symmetrically positioned nicks in strands with the same polarity, thereby forming nicked duplex products that can be repaired in a simple nick-ligation reaction.

Mammalian resolvases. It has been more difficult to identify Holliday-junction resolvases in eukaryotes, although mammalian activities that fit the resolvase paradigm were first observed in 1990 (REF. 19). The issue has also been complicated by the presence of Mus81, a flap/fork endonuclease from yeast and humans that has a weak Holliday-junction cleavage activity *in vitro*[20,21]. Because Mus81 is required for the formation of crossover products that result from homologous recombination during meiosis in *S. pombe*, it was suggested that Mus81 could be the eukaryotic

Holliday-junction resolvase. However, further studies showed that the mechanism by which Mus81 cuts Holliday junctions differs from that of all other Holliday-junction resolvases, and that the nuclease had a very potent structure-specific activity on replication-fork or flap structures. It is therefore not a Holliday-junction resolvase in the classic sense[22]. Moreover, in mammalian cell extracts, Holliday-junction-resolution activity can be separated from MUS81 (REF. 23), and the resolvase complex in these cell extracts contains the recombination proteins RAD51C and XRCC3 (REF. 24). Further work to identify the nuclease component of the latter complex is underway.

Surprisingly, even though Holliday-junction resolvases from different species are functionally conserved, they bear little similarity to each other at the level of amino-acid sequence. This makes it particularly difficult to use sequence homology and database analyses to identify related nucleases from higher organisms. The lack of primary sequence conservation indicates that they are unlikely to have evolved from the same ancestral gene, which presents us with a mystery: why are the Holliday-junction resolvases, which function in highly conserved recombination/repair processes, so evolutionarily diverse? And, which features give them the ability to recognize and cleave four-way junctions?

Atomic structure of the prokaryotic resolvases. When the crystal structures of various Holliday-junction resolvases were solved, it became clear that most belong to two families: the integrase superfamily (RuvC and Ydc2) or the nuclease superfamily (T7 endonuclease I and Hjc)[25–30] (FIG. 5). So, even though the resolvases are not conserved at the amino-acid level, they do resemble each other on the structural level.

"…since the Holliday model was first proposed, we have seen its re-evaluation and continued modification … Perhaps the most significant change lies in the complexity of the process, in which alternative events can occur."

By contrast, T4 endonuclease VII and *E. coli* RusA (which itself is phage-derived) seem to have evolved independently. Exactly what the Holliday-junction resolvases have in common remains a difficult question to answer, as the only obvious feature is a catalytic domain that contains clusters of basic aspartate and glutamate residues that are required for metal-ion binding.

E. coli RuvC is the most well-characterized resolvase and functions as a paradigm for other Holliday-junction resolvases. The mechanism of Holliday-junction resolution can be broken down into a number of experimentally separable steps including DNA binding, modification of DNA structure and cleavage[31]. RuvC binds specifically to a Holliday junction as a dimer and unfolds the junction into an open planar configuration (FIG. 6a). In the presence of divalent cations, RuvC introduces symmetrical nicks in strands of the same polarity. Although the protein binds junctions in a structure-specific manner, the cleavage reaction has extra selectivity at the sequence level such that the degenerate sequence 5′-(A/T)TT↓(G/C)-3′ is the preferred cleavage site.

Junction migration. The concept that junctions might 'slide' along DNA to extend the length of the heteroduplex was also proposed in Holliday's 1974 paper[32]. We now refer to this reaction as branch migration, and from studies of bacterial proteins we have a good understanding of how it takes place[31]. In *E. coli*, Holliday junctions are branch migrated by the products of the DNA-damage-inducible *ruvA* and *ruvB* genes. RuvA protein, a tetramer, binds the junction in the unfolded open-square configuration, with the four DNA arms lying in positively charged grooves on its surface (FIG. 6b). The unfolded configuration favours branch migration, as do four acidic amino acids on the surface of the RuvA tetramer, which function as guides to facilitate the transient opening of base pairs as strands pass from one helical axis to another[33,34]. The motor of branch migration is RuvB, a hexameric ring protein, which associates with RuvA to form the tripartite structure that is shown in FIG. 6b (REF. 35,36). The rings are positioned in the opposite orientation relative to the Holliday junction, and therefore exert equal and opposite forces after ATP hydrolysis in a reaction that results in the passage of DNA helices through the protein complex.

In vivo, it is thought that the RuvA–RuvB complex functions together with RuvC resolvase as part of a 'resolvasome' complex.
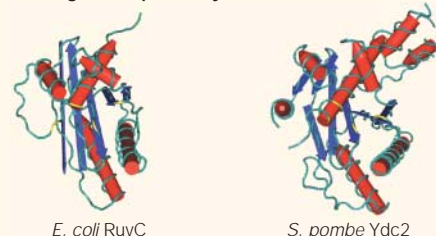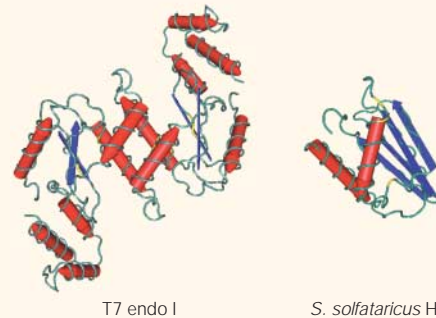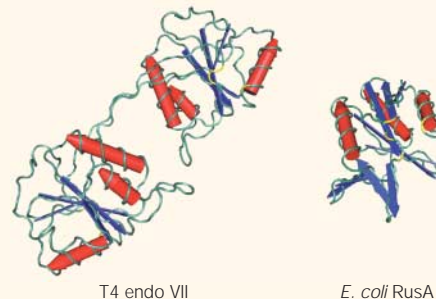

Figure 5 | **Structural comparison of the Holliday-junction resolvases.** Comparison of the crystallographic structures of various Holliday-junction resolvases indicate that *Escherichia coli* RuvC and *Schizosaccharomyces pombe* Ydc2 belong to the integrase superfamily, whereas T7 endonuclease I (T7 endo I) and *Sulfolobus solfataricus* Hjc are members of the nuclease superfamily. The origins of T4 endonuclease VII and RusA are unknown. The structures were obtained from the National Center for Biotechnology Information (NCBI) structure database (see the online links box) and illustrated using CN3D software.

Analogous branch-migration and resolution activities have been observed in fractionated mammalian extracts[24,37], but the proteins that are responsible for branch migration have yet to be identified. Members of the RecQ family of DNA helicases, such as Bloom's syndrome protein (BLM), and Werner's syndrome protein (WRN) and RecQ5β, have been shown, at least *in vitro*, to be capable of catalysing branch migration in addition to unwinding duplex DNA[38–40]. Defects in BLM or WRN lead to inheritable diseases known as Bloom's syndrome (which

a

b
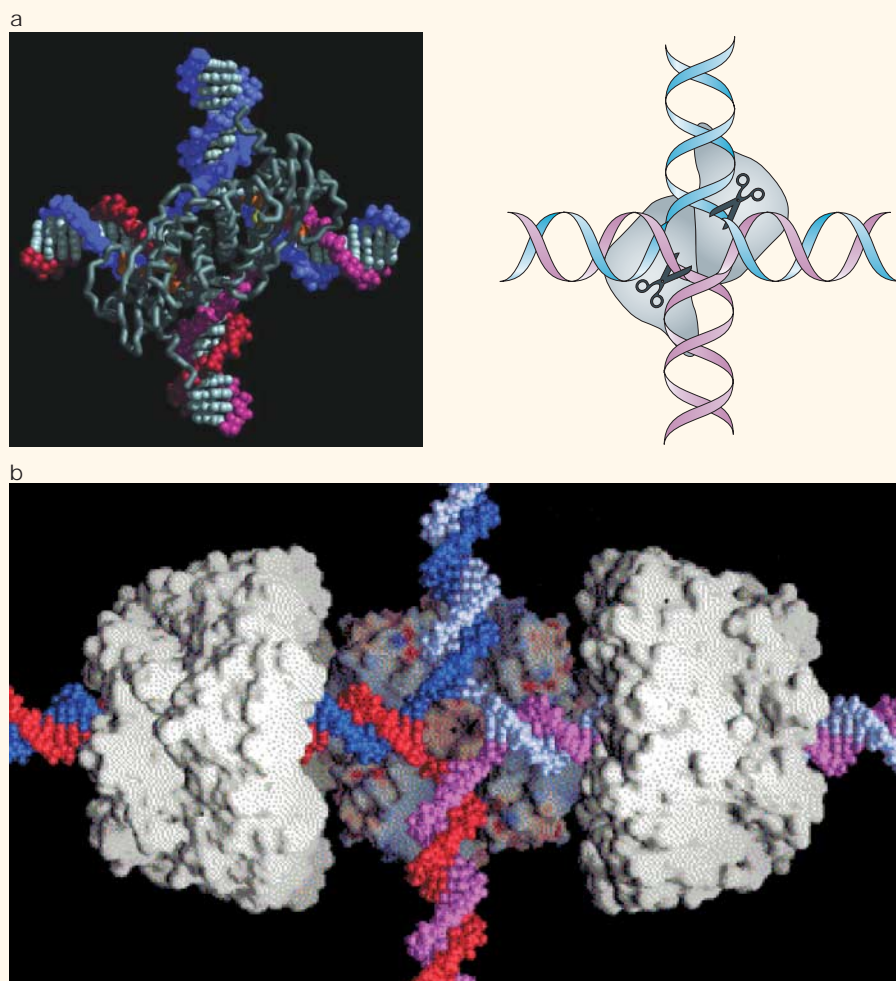
Figure 6 | **Three-dimensional structure of RuvC– and RuvAB–Holliday-junction complexes.**
a | Atomic model of RuvC binding to a Holliday junction. The dimeric RuvC protein introduces nicks at symmetric positions in strands with the same polarity. **b** | Model of the RuvAB–Holliday-junction complex[53]. Part **a** is modified from REF. 53 © (1996) American Association for the Advancement of Science. The image in part **b** is reproduced with permission from the University of Sheffield web site (see online links box).

afflicts more than 1 in a 100 Ashkenazi Jews) and Werner's syndrome (which is characterized by premature ageing). On a cellular level, mutations in RecQ-family proteins give rise to genomic instability and a sensitivity to DNA-damaging agents[41]. However, RecQ-family proteins, BLM in particular, are unlikely to be branch-migration motors equivalent to RuvB, because mutations in the *BLM* gene lead to a phenotypic increase in the frequency of sister-chromatid exchanges (that is, crossovers) as a result of homologous recombination at stalled replication forks. In this respect, BLM could be regarded as an anti-recombinase. New insight into this phenomenon was recently gained when it was shown that BLM and topoisomerase IIIα function together to dissociate structures that contain double

Holliday junctions[42]. As Holliday-junction 'dissolution' by BLM and topoisomerase IIIα gives rise to non-crossover products, the disruption of this pathway in Bloom's syndrome cells provides a satisfying explanation for the elevated level of crossovers that are observed in the mutant. The *S. cerevisiae* homologues of BLM and topoisomerase IIIα — Sgs1 and Top3, respectively — promote similar reactions in yeast[43], so in some situations (that is, recombination at blocked replication forks), this system might provide an alternative pathway to process double Holliday junctions (FIG. 7).

Crossover versus non-crossover
In the 40 years since the Holliday model was first proposed, we have seen its re-evaluation and continued modification, which has led

to the dynamic picture that represents our present understanding of how recombinants form in eukaryotic cells (FIG. 7). Perhaps the most significant change lies in the complexity of the process, in which alternative events can occur. Most recombination events are thought to result from the formation of a DSB: in meiotic recombination DNA-strand breaks are a consequence of DSBs that are introduced by a topoisomerase-like protein known as SPO11 (REF. 44), whereas in a mitotic cell they might be radiation-induced breaks or DSBs that arise from stalled and broken replication forks[45,46]. The ends of the DNA are resected to produce single-stranded DNA that recruits recombination proteins such as replication protein A (RPA), RAD52 and RAD51. The assembly of a RAD51 nucleoprotein filament leads to interactions with homologous duplex DNA and strand invasion. This process is known as single-end invasion (SEI), and RAD54 is thought to stabilize this recombination intermediate to allow the subsequent events to take place.

In some recombination pathways, SEI is followed by the annealing of the second DNA end in a reaction that might involve the single-strand-annealing activity of RAD52 (FIG. 7). This intermediate can proceed to form double Holliday junctions, and any remaining gaps might be filled by new DNA synthesis. The resulting Holliday junctions might then serve as the substrate for a classic Holliday-junction-resolution reaction — which involves RAD51C, XRCC3 and other as-yet-unidentified factors[24] — or be dissociated by the combined actions of BLM and topoisomerase IIIα (REF. 42).

Recombinants can also form by pathways that do not involve Holliday junctions (FIG. 7). For example, the formation of double Holliday junctions can be prevented by the MUS81 complex, which cleaves strand-invasion intermediates (FIG. 7, green arrows) before they can mature into Holliday junctions[47–49]. Similarly, DSBs can be repaired by SDSA, a pathway that is dependent on the SRS2 helicase[43,50]. In SDSA, nucleoprotein filaments of the RAD51 recombinase

"So, 40 years on, the importance of the Holliday model is evidenced by the fact that it has evolved rather than having been replaced…"

Figure 7 | **Homologous recombination, the big picture.** Summary of our current understanding of recombination pathways that are initiated by a DNA double-strand break (DSB) and which lead to gene conversion with or without crossover. First, the ends of the DSB are resected to produce single-stranded DNA that recruits the recombination protein RAD51. The assembly of a RAD51 nucleoprotein filament leads to interactions with homologous duplex DNA and strand invasion. This process is known as single-end invasion (SEI) and the intermediate structures might be stabilized by the RAD54 protein. In some pathways for recombination (centre), SEI is followed by capture of the second DNA end in reactions that are likely to involve RAD52. This intermediate can proceed to form double Holliday junctions, and any remaining gaps might be filled by new DNA synthesis. The resulting Holliday junctions might then serve as the substrate for a classic Holliday-junction-resolution reaction, involving RAD51C, XRCC3 and other as-yet-unidentified factors, or be dissociated by the combined actions of BLM (Bloom's syndrome protein) and topoisomerase IIIα (Topo III). The BLM–Topo-III reaction primarily leads to the formation of non-crossover products, as mutations in BLM cause an increase in crossover formation. Recombinants can also form by a MUS81-dependent pathway that does not involve Holliday-junction formation (right). Similarly, DSBs can be repaired by synthesis-dependent strand annealing (SDSA), a pathway that is dependent on the SRS2 helicase (left).
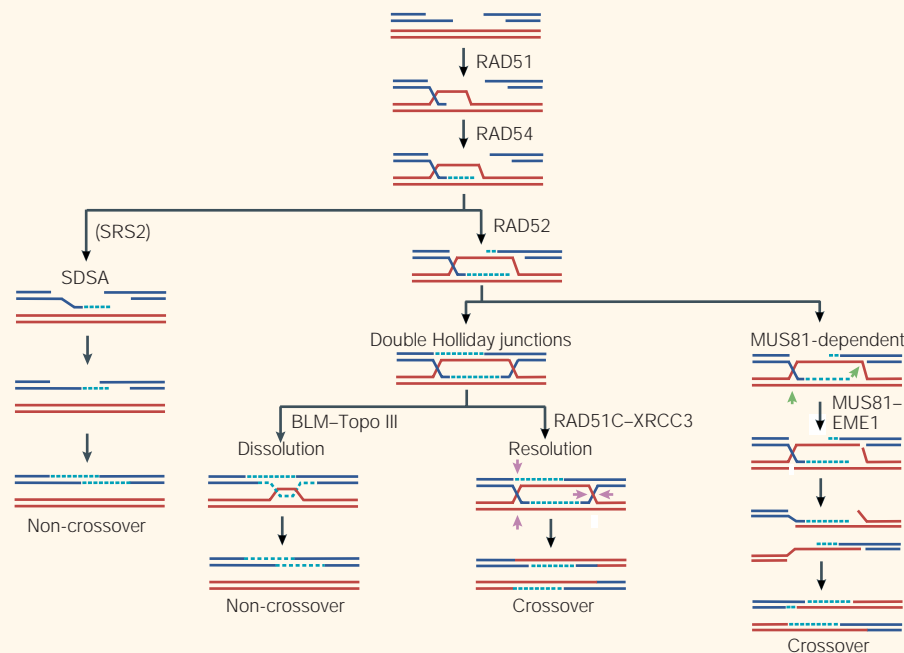
Holliday junctions, the intermediate is already destined to be resolved in a specific orientation that leads to crossover[51,52]. Whether or not this is also true for organisms other than yeast remains to be answered.

So, 40 years on, the importance of the Holliday model is evidenced by the fact that it has evolved rather than having been replaced, even in the face of the genetic revolution that has taken place during this time. The Holliday junction still exists and takes its rightful place in many recombination pathways. We know its atomic structure, and we have discovered protein complexes that move it and cut it. But we are still left with so many mysteries, in particular, what factors control how, and into what, it is resolved. These puzzles cannot be explained by our present knowledge of the structure of the Holliday junction and the enzymes that resolve it. These challenges are for the future, for us to investigate now that the model has been celebrated four decades after it was born.

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promote SEI, and the reaction is followed by DNA synthesis to fill the gap. At this stage, SRS2 helicase is thought to dissociate heteroduplex intermediates that are formed within the RAD51 filament, so that the invading strand that has a newly synthesized 3′ end is available to reanneal with the second end of the same DNA molecule at the break site. DNA synthesis can then take place to restore the integrity of the DNA. Although all these recombination pathways can result in gene conversion, there is a very significant difference between them in that some pathways commit to the formation of either a crossover or a non-crossover product. For example, BLM/topoisomerase-IIIα-dependent Holliday-junction dissolution and SDSA events generally produce non-crossover products, whereas MUS81-dependent reactions primarily yield the crossovers.

In the Holliday model it was suggested that a junction could be resolved in one of two possible orientations to yield either the crossover or non-crossover product. However, this concept is not necessarily true as studies of yeast meiosis indicate that non-crossovers appear earlier than crossovers. Moreover, mutations in Ndt80 — a yeast meiosis-specific transcription factor that is important for the completion of prophase in meiosis I — cause meiotic arrest with unresolved Holliday-junction intermediates. Surprisingly, the mutant cells show a significant reduced frequency of crossover products, whereas the non-crossover products remain at a normal level. This finding indicates that, at least in yeast meiosis, crossover and non-crossover products are formed by different mechanisms, and, more importantly, that once the DSB-repair reaction has committed to a resolution pathway that involves double

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FURTHER INFORMATION
Bill Engels's laboratory (movies of Holliday junctions and mechanisms of recombination):
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