= Holliday junction =

A Holliday junction is a branched nucleic acid structure that contains four double @-@ stranded arms joined together . These arms may adopt one of several conformations depending on buffer salt concentrations and the sequence of nucleobases closest to the junction . The structure is named after the molecular biologist Robin Holliday , who proposed its existence in 1964 .

In biology , Holliday junctions are a key intermediate in many types of genetic recombination , as well as in double @-@ strand break repair . These junctions usually have a symmetrical sequence and are thus mobile , meaning that the four individual arms may slide though the junction in a specific pattern that largely preserves base pairing . Additionally , four @-@ arm junctions similar to Holliday junctions appear in some functional RNA molecules .

Immobile Holliday junctions , with asymmetrical sequences that lock the strands in a specific position , were artificially created by scientists to study their structure as a model for natural Holliday junctions . These junctions also later found use as basic structural building blocks in DNA nanotechnology , where multiple Holliday junctions can be combined into specific designed geometries that provide molecules with a high degree of structural rigidity .

= = Structure = =

Holliday junctions may exist in a variety of conformational isomers with different patterns of coaxial stacking between the four double @-@ helical arms . Coaxial stacking is the tendency of nucleic acid blunt ends to bind to each other , by interactions between the exposed bases . There are three possible stacking conformers : an unstacked form and two stacked forms . The unstacked form dominates in the absence of divalent cations such as Mg2 + , because of electrostatic repulsion between the negatively charged backbones of the strands . In the presence of at least about 0 @.@ 1 mM Mg2 + , the electrostatic repulsion is counteracted and the stacked structures predominate . As of 2000 , it was not known with certainty whether the electrostatic shielding was the result of site @-@ specific binding of cations to the junction , or the presence of a diffuse collection of the ions in solution .

The unstacked form is a nearly square planar , extended conformation . On the other hand , the stacked conformers have two continuous double @-@ helical domains separated by an angle of about 60 ° in a right @-@ handed direction . Two of the four strands stay roughly helical , remaining within each of the two double @-@ helical domains , while the other two cross between the two domains in an antiparallel fashion .

The two possible stacked forms differ in which pairs of the arms are stacked with each other ; which of the two dominates is highly dependent on the base sequences nearest to the junction . Some sequences result in an equilibrium between the two conformers , while others strongly prefer a single conformer . In particular , junctions containing the sequence A @-@ CC bridging the junction point appear to strongly prefer the conformer that allows a hydrogen bond to form between the second cytosine and one of the phosphates at the junction point . While most studies have focused on the identities of the four bases nearest to the junction on each arm , it is evident that bases farther out can also affect the observed stacking conformations .

In junctions with symmetrical sequences , the branchpoint is mobile and can migrate in a random walk process . The rate of branch migration varies dramatically with ion concentration , with single @-@ step times increasing from 0 @.@ 3 ? 0 @.@ 4 ms with no ions to 270 ? 300 ms with 10 mM Mg2 + . The change in rate is correlated with the formation of the stacked versus the unstacked structures .

Holliday junctions with a nick , or break in one of the strands , at the junction point adopt a perpendicular orientation , and always prefer the stacking conformer that places the nick on a crossover strand rather than a helical strand .

RNA Holliday junctions assume an antiparallel stacked conformation at high magnesium concentrations , a perpendicular stacked conformation at moderate concentrations , and rotate into a parallel stacked conformation at low concentrations , while even small calcium ion concentrations

favor the antiparallel conformer .

= = Biological function = =

The Holliday junction is a key intermediate in homologous recombination , a biological process that increases genetic diversity by shifting genes between two chromosomes , as well as site @-@ specific recombination events involving integrases . They are additionally involved in repair of double @-@ strand breaks . In addition , cruciform structures involving Holliday junctions can arise to relieve helical strain in symmetrical sequences in DNA supercoils . While four @-@ arm junctions also appear in functional RNA molecules , such as U1 spliceosomal RNA and the hairpin ribozyme of the tobacco ringspot virus , these usually contain unpaired nucleotides in between the paired double @-@ helical domains , and thus do not strictly adopt the Holliday structure .

The Holliday junctions in homologous recombination are between identical or nearly identical sequences , leading to a symmetric arrangement of sequences around the central junction . This allows a branch migration process to occur where the strands move through the junction point . Cleavage , or resolution , of the Holliday junction can occur in two ways . Cleavage of the original set of strands leads to two molecules that may show gene conversion but not chromosomal crossover , while cleavage of the other set of two strands causes the resulting recombinant molecules to show crossover . All products , regardless of cleavage , are heteroduplexes in the region of Holliday junction migration .

Many proteins are able to recognize or distort the Holliday junction structure . One such class contains junction @-@ resolving enzymes that cleave the junctions , sometimes in a sequence @-@ specific fashion . Such proteins distort the structure of the junction in various ways , often pulling the junction into an unstacked conformation , breaking the central base pairs , and / or changing the angles between the four arms . Other classes are branch migration proteins that increase the exchange rate by orders of magnitude , and site @-@ specific recombinases . In prokaryotes , Holliday junction resolvases fall into two families , integrases and nucleases , that are each structurally similar although their sequences are not conserved .

In eukaryotes , two primary models for how homologous recombination repairs double @-@ strand breaks in DNA are the double @-@ strand break repair (DSBR) pathway (sometimes called the double Holliday junction model) and the synthesis @-@ dependent strand annealing (SDSA) pathway . In the case of double strand breakage , the 3 ' end is degraded and the longer 5 ' end invades the contiguous sister chromatid , forming a replication bubble . As this bubble nears the broken DNA , the longer 5 ' antisense strand again invades the sense strand of this portion of DNA , transcribing a second copy . When replication ends , both tails are reconnected to form two Holliday Junctions , which are then cleaved in a variety of patterns by proteins . An animation of this process can be seen here .

Double @-@ strand DNA breaks in bacteria are repaired by the RecBCD pathway of homologous recombination . Breaks that occur on only one of the two DNA strands , known as single @-@ strand gaps , are thought to be repaired by the RecF pathway . Both the RecBCD and RecF pathways include a series of reactions known as branch migration , in which single DNA strands are exchanged between two intercrossed molecules of duplex DNA , and resolution , in which those two intercrossed molecules of DNA are cut apart and restored to their normal double @-@ stranded state . Homologous recombination occurs in several groups of viruses . In DNA viruses such as herpesvirus , recombination occurs through a break @-@ and @-@ rejoin mechanism like in bacteria and eukaryotes . In bacteria , branch migration is facilitated by the RuvABC complex or RecG protein , molecular motors that use the energy of ATP hydrolysis to move the junction . The junction must then be resolved into two separate duplexes , restoring either the parental configuration or a crossed @-@ over configuration . Resolution can occur in either a horizontal or vertical fashion during homologous recombination , giving patch products (if in same orientation during double strand break repair) or splice products (if in different orientations during double strand break repair) . RuvA and RuvB are branch migration proteins , while RuvC is a junction @-@ resolving enzyme .

There is evidence for recombination in some RNA viruses , specifically positive @-@ sense ssRNA viruses like retroviruses , picornaviruses , and coronaviruses . There is controversy over whether homologous recombination occurs in negative @-@ sense ssRNA viruses like influenza .

= = = Resolution = = =

In budding yeast Saccharomyces cerevisiae , Holliday junctions can be resolved by four different pathways that account for essentially all Holliday junction resolution in vivo . The pathway that produces the majority of crossovers in S. cerevisiae budding yeast , and possibly in mammals , involves proteins EXO1 , MLH1 @-@ MLH3 heterodimer (called MutL gamma) and SGS1 (ortholog of Bloom syndrome helicase) . The MLH1 @-@ MLH3 heterodimer binds preferentially to Holliday junctions . It is an endonuclease that makes single @-@ strand breaks in supercoiled double @-@ stranded DNA . The MLH1 @-@ MLH3 heterodimer promotes the formation of crossover recombinants . While the other three pathways , involving proteins MUS81 @-@ MMS4 , SLX1 and YEN1 , respectively , can promote Holliday junction resolution in vivo , absence of all three nucleases has only a modest impact on formation of crossover products .

Double mutants deleted for both MLH3 (major pathway) and MMS4 (minor pathway) showed dramatically reduced crossing over compared to wild @-@ type (6- to 17 @-@ fold) ; however spore viability was reasonably high (62 %) and chromosomal disjunction appeared mostly functional .

Although MUS81 is a component of a minor crossover pathway in the meiosis of budding yeast , plants and vertebrates , in the protozoan Tetrahymena thermophila , MUS81 appears to be part of an essential , if not the predominant crossover pathway . The MUS81 pathway also appears to be the predominant crossover pathway in the fission yeast Schizosaccharomyces pombe .

The MSH4 and MSH5 proteins form a hetero @-@ oligomeric structure (heterodimer) in yeast and humans . In the yeast Saccharomyces cerevisiae MSH4 and MSH5 act specifically to facilitate crossovers between homologous chromosomes during meiosis . The MSH4 / MSH5 complex binds and stabilizes double Holliday junctions and promotes their resolution into crossover products . An MSH4 hypomorphic (partially functional) mutant of S. cerevisiae showed a 30 % genome wide reduction in crossover numbers , and a large number of meioses with non exchange chromosomes . Nevertheless , this mutant gave rise to spore viability patterns suggesting that segregation of non @-@ exchange chromosomes occurred efficiently . Thus in S. cerevisiae proper segregation apparently does not entirely depend on crossovers between homologous pairs .

= = Use in DNA nanotechnology = =

DNA nanotechnology is the design and manufacture of artificial nucleic acid structures as engineering materials for nanotechnology rather than as the carriers of genetic information in living cells . The field uses branched DNA structures as fundamental components to create more complex , rationally designed structures . Holliday junctions are thus components of many such DNA structures . As isolated Holliday junction complexes are too flexible to assemble into large ordered arrays , structural motifs with multiple Holliday junctions are used to create rigid " tiles " that can then assemble into larger " arrays " .

The most common such motif is the double crossover (DX) complex , which contains two Holliday junctions in close proximity to each other , resulting in a rigid structure that can self @-@ assemble into larger arrays . The structure of the DX molecule forces the Holliday junctions to adopt a conformation with the double @-@ helical domains directly side @-@ by @-@ side , in contrast to their preferred angle of about 60 ° . The complex can be designed to force the junctions into either a parallel or antiparallel orientation , but in practice the antiparallel variety are more well @-@ behaved , and the parallel version is rarely used .

The DX structural motif is the fundamental building block of the DNA origami method , which is used to make larger two- and three @-@ dimensional structures of arbitrary shape . Instead of using individual DX tiles , a single long scaffold strand is folded into the desired shape by a number of

short staple strands . When assembled , the scaffold strand is continuous through the double @-@ helical domains , while the staple strands participate in the Holliday junctions as crossover strands .

Some tile types that retain the Holliday junction 's native 60 ° angle have been demonstrated . One such array uses tiles containing four Holliday junctions in a parallelogram arrangement . This structure had the benefit of allowing the junction angle to be directly visualized via atomic force microscopy . Tiles of three Holliday junctions in a triangular fashion have been used to make periodic three @-@ dimensional arrays for use in X @-@ ray crystallography of biomolecules . These structures are named for their similarity to structural units based on the principle of tensegrity , which utilizes members both in tension and compression .

= = History = =

Robin Holliday proposed the junction structure that now bears his name as part of his model of homologous recombination in 1964 , based on his research on the organisms Ustilago maydis and Saccharomyces cerevisiae . The model provided a molecular mechanism that explained both gene conversion and chromosomal crossover . Holliday realized that the proposed pathway would create heteroduplex DNA segments with base mismatches between different versions of a single gene . He predicted that the cell would have a mechanism for mismatch repair , which was later discovered . Prior to Holliday 's model , the accepted model involved a copy @-@ choice mechanism where the new strand is synthesized directly from parts of the different parent strands .

In the original Holliday model for homologous recombination , single @-@ strand breaks occur at the same point on one strand of each parental DNA . Free ends of each broken strand then migrate across to the other DNA helix . There , the invading strands are joined to the free ends they encounter , resulting in the Holliday junction . As each crossover strand reanneals to its original partner strand , it displaces the original complementary strand ahead of it . This causes the Holliday junction to migrate , creating the heteroduplex segments . Depending on which strand was used as a template to repair the other , the four cells resulting from meiosis might end up with three copies of one allele and only one of the other , instead of the normal two of each , a property known as gene conversion .

Holliday 's original model assumed that heteroduplex DNA would be present on both chromosomes , but experimental data on yeast refuted this . An updated model by Matt Meselson and Charley Radding in 1975 introduced the idea of branch migration . Further observations in the 1980s led to the proposal of alternate mechanisms for recombination such as the double @-@ strand break model (by Jack Szostak , Frank Stahl , and others) and the single @-@ strand annealing model . A third , the synthesis @-@ dependent strand annealing model , did not involve Holliday junctions .

The first experimental evidence for the structure of the Holliday junction came from electron microscopy studies in the late 1970s , where the four @-@ arm structure was clearly visible in images of plasmid and bacteriophage DNA . Later in the 1980s , enzymes responsible for initiating the formation of , and binding to , Holliday junctions were identified , although as of 2004 the identification of mammalian Holliday junction resolvases remained elusive (however , see section " Resolution of Holliday junctions , " above for more recent information) . In 1983 , artificial Holliday junction molecules were first constructed from synthetic oligonucleotides by Nadrian Seeman , allowing for more direct study of their physical properties . Much of the early analysis of Holliday junction structure was inferred from gel electrophoresis , FRET , and hydroxyl radical and nuclease footprinting studies . In the 1990s , crystallography and nucleic acid NMR methods became available , as well as computational molecular modelling tools .

Initially , geneticists assumed that the junction would adopt a parallel rather than antiparallel conformation , because that would place the homologous duplexes in closer alignment to each other . Chemical analysis in the 1980s showed that the junction actually preferred the antiparallel conformation , a finding that was considered controversial , and Robin Holliday himself initially doubted the findings . The antiparallel structure later became widely accepted due to X @-@ ray crystallography data on in vitro molecules , although as of 2004 the implications for the in vivo structure remained unclear , especially the structure of the junctions is often altered by proteins

bound to it .

The conceptual foundation for DNA nanotechnology was first laid out by Nadrian Seeman in the early 1980s . A number of natural branched DNA structures were known at the time , including the DNA replication fork and the mobile Holliday junction , but Seeman 's insight was that immobile nucleic acid junctions could be created by properly designing the strand sequences to remove symmetry in the assembled molecule , and that these immobile junctions could in principle be combined into rigid crystalline lattices . The first theoretical paper proposing this scheme was published in 1982 , and the first experimental demonstration of an immobile DNA junction was published the following year . Seeman developed the more rigid double @-@ crossover (DX) motif , suitable for forming two @-@ dimensional lattices , demonstrated in 1998 by him and Erik Winfree . In 2006 , Paul Rothemund first demonstrated the DNA origami technique for easily and robustly creating folded DNA structures of arbitrary shape . This method allowed the creation of much larger structures than were previously possible , and which are less technically demanding to design and synthesize . The synthesis of a three @-@ dimensional lattice was finally published by Seeman in 2009 , nearly thirty years after he had set out to achieve it .