The crystal structures of DNA Holliday junctions

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Nearly 40 years ago, Holliday proposed a four-stranded complex or junction as the central intermediate in the general mechanism of genetic recombination. During the past two years, six single-crystal structures of such DNA junctions have been determined by three different research groups. These structures all essentially adopt the antiparallel stacked-X conformation, but can be classified into three distinct categories: RNA–DNA junctions; ACC trinucleotide junctions; and drug-induced junctions. Together, these structures provide insight into how local and distant interactions help to define the detailed and general physical features of Holliday junctions at the atomic level.

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Abbreviations

bp	base pairs
HMT	4′-hydroxymethyl-4,5′,8-trimethylpsoralen
Lh-X	left-handed stacked-X
NER	nucleotide excision repair
Rh-X	right-handed stacked-X

Introduction

DNA recombination was first recognized as a means to introduce genetic diversity in cells. More recently, the mechanism of recombination has become implicated as an important cellular mechanism to repair (reviewed in [1]) or replicate through (reviewed in [2•]) DNA lesions. All of these processes are thought to undergo a mechanism analogous to that proposed by Holliday [3] in 1964 for homologous recombination, which involves a four-stranded intermediate [3–8] in which DNA strands cross-over between two homologous duplexes to effect an exchange of genetic material (Figure 1a). The central role of the four-stranded Holliday junction in recombination has lead to numerous studies to characterize its physical properties and innumerable attempts to crystallize the structure. In the past two years, four-way junctions have been seen in the crystal structures of DNA complexes with two different proteins and of six nucleic acid-only constructs. In this review, we will focus on the intrinsic non-protein-dependent structures of the junction. Although none of these constructs was originally designed to study junctions, our collective good fortune now provides us with the most detailed models of Holliday junctions to date and insights into how they are stabilized.

Molecular models of the four-way junction

It is now generally accepted that there are two structural forms of the Holliday junction (reviewed in [9•]). Low salt conditions favor an open-X form, in which the four arms are extended in a square planar geometry (Figure 1b), thereby minimizing the repulsion between the negatively charged phosphates concentrated at the junction. This is also the form observed in complexes with the enzymes RuvA [10,11,12•] and Cre [13].

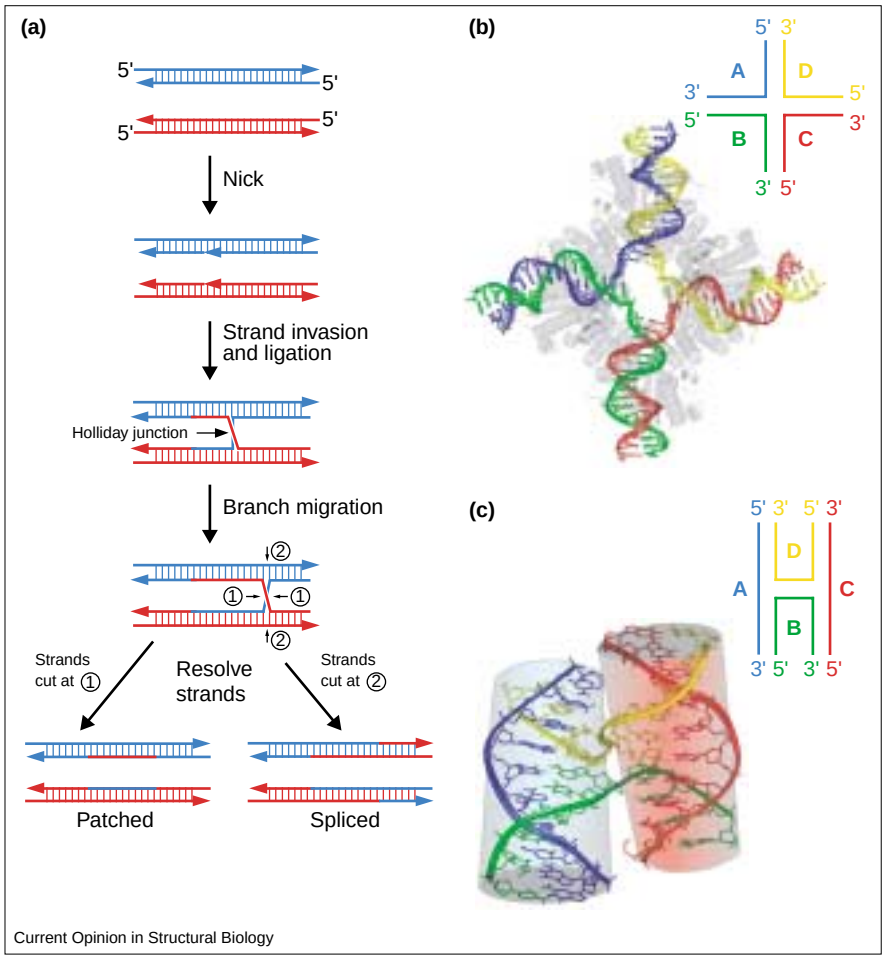
In contrast, the presence of multivalent cations effectively shields these negative charges, allowing the arms to coaxially pair and stack into the more compact stacked-X junction (Figure 1c). Before the recent crystal studies, the most detailed models of four-way junctions were derived from studies using gel electrophoresis, fluorescence resonance energy transfer, NMR spectroscopy and atomic force microscopy [9•]. These models showed that the stacked duplex arms across the junction of the antiparallel stacked-X conformation are related in a right-handed sense by an angle of approximately 60° [14,15,16•]. Furthermore, there are two distinct stacked-X conformational isomers (or conformers), depending on which DNA strands are exchanged between duplexes across the junction [17–19].

Molecular models of the stacked-X junction have been constructed by simply redirecting the phosphodiester linkages of two adjacent duplexes from theoretical models [20,21] or from crystal structures of resolved DNA double helices [22–25] to generate four-stranded assemblies. The lattices of certain B-DNA crystals place the grooves of adjacent duplexes in close proximity and properly oriented to model a junction that maintains the base pairing between nucleotides. NMR studies have supported the general features of these models, showing that the base pairs stacked across the junction are similar to those of B-DNA [18,26,27]. Although useful in describing general structural features, these molecular models cannot elucidate the details of the Holliday junction at the atomic level in the manner that single-crystal structures can.

Three classes of junction in crystal structures

Since the mid-1980s, there have been sporadic reports of various laboratories having crystallized a DNA Holliday junction. Unfortunately, none of these resulted in a published structure. It is ironic that the structures of the first nucleic acid junction [28••], the first DNA junction [29••] and the first junction in a homologous DNA sequence [30••] were all published within 12 months of each other and none was designed to study four-way junctions. Currently, there are six available crystal structures of DNA containing junctions, which fall into three classes: RNA–DNA junctions (Figure 2a); ACC trinucleotide

Figure 1



Recombination and the Holliday junction. (a) The model for homologous recombination proposed by Holliday [3]. (b) The open-X form of the Holliday junction, as seen in the complex with the junction-resolving enzyme Cre [13]. (c) The antiparallel Rh-X junction, as seen in the ACC-type crystal structures.

DNA junctions (Figure 2b); and psoralen (drug) cross-linked junctions (Figure 2c).

The first RNA–DNA complex was designed originally to study a DNA construct with catalytic RNAse activity (a DNAzyme), but it crystallized as a right-handed stacked-X junction [28••]. The most recent variation of this class of junction is an RNA–DNA junction with the stacked duplexes related in a left-handed sense; this has been termed the crossed conformation [31••]. However, as the two structures differ only in the handedness of the interduplex angle, in this review we will refer to them as the right-handed (Rh-X) and the left-handed stacked-X (Lh-X) RNA–DNA junctions.

The two related DNA sequences d(CCGGGACCGG) [29••] and d(CCGGTACCGG) [30••] were designed to elucidate the effects on B-DNA of tandem G•A mismatched base pairs and of cross-linking by the photochemotherapeutic drug psoralen, respectively; however, both crystallized as near identical antiparallel Rh-X junctions. Together, these two structures show how a fully DNA type junction is stabilized by a core ACC trinucleotide sequence [30••].

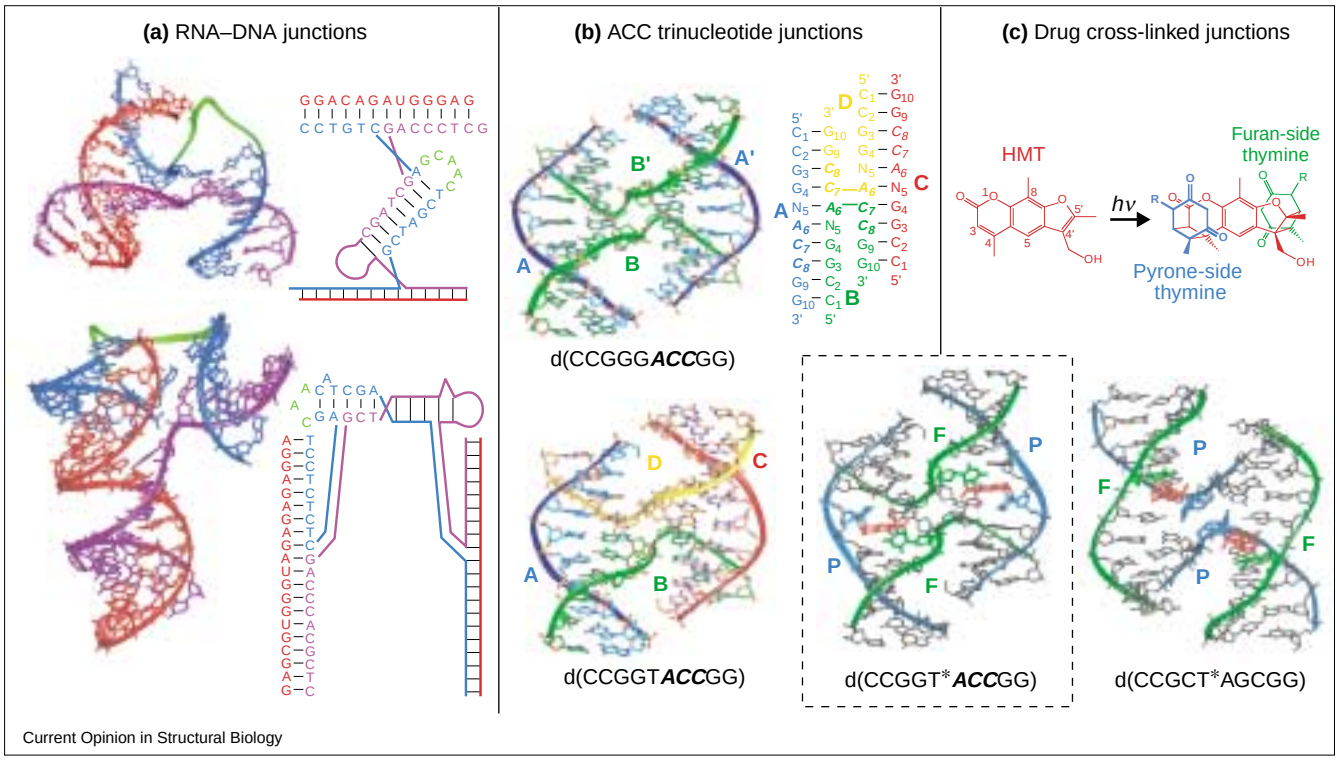
The two most recently determined structures reveal DNAs that are cross-linked by 4′-hydroxymethyl-4,5′,8-trimethyl-psoralen (HMT) [32••]. The junction in HMT–d(CCGGTACCGG) is a consequence of the stabilizing ACC trinucleotide in the DNA sequence, whereas the junction in HMT–d(CCGCTAGCGG) is induced by the drug — the unmodified DNA crystallizes as resolved duplexes [30••]. The psoralen-bound structures are examples of the competing stacked-X conformers. The HMT–d(CCGGTACCGG) junction places the four-base-pair arms stacked over the six-base-pair arms, with the furan-linked strands exchanging across the junction. Alternatively, the HMT–d(CCGCTAGCGG) junction has a six-over-four stacking arrangement and the pyrone strands exchanging across the junction.

From these six crystal structures, five main principles have emerged concerning the structure and stability of four-way junctions.

Junctions do not distort the conformation of the stacked arms

Except for the gaps in the phosphoribose backbones across the junction, the stacked duplex arms are effectively

Figure 2



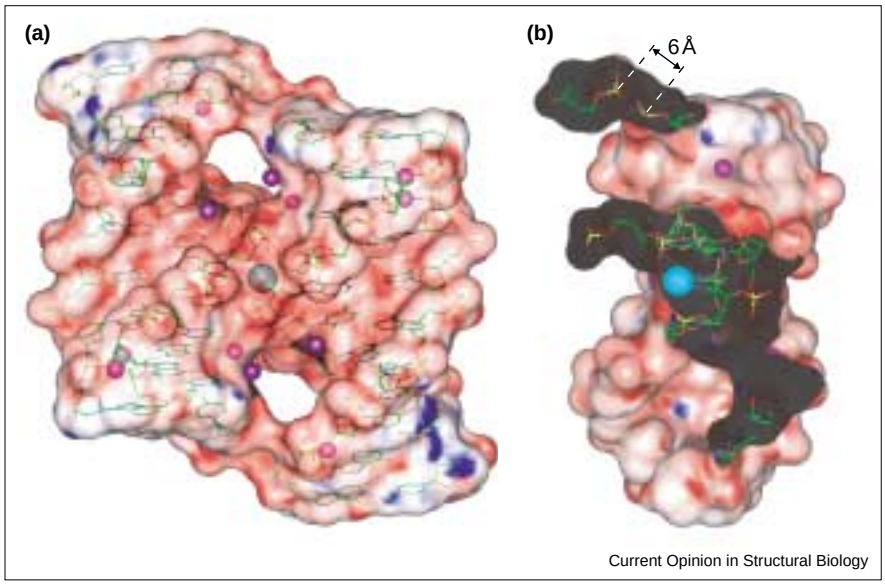
Single-crystal structures of nucleic acid junctions. (**a**) The RNA–DNA junctions [28••,31••]. The RNA chains (red) assemble with DNA to form junctions with Watson–Crick base-paired duplex arms (blue). One arm in each structure has only a single Watson–Crick base pair in a short loop (green). The structure and sequence of one unique junction are shown for each RNA–DNA junction. The symmetry related strands are shown in purple. (**b**) The ACC trinucleotide junctions [29••, 30••]. The common trinucleotide sequence (italics) forms the stable core of these junctions. In the d(CCGGGACCGG) structure, the four-stranded junction is formed by two unique DNA strands (blue and green) and their symmetry mates. The d(CCGGTACCGG) junction is formed by four strands with unique conformations in the crystal. (**c**) The psoralen (drug) cross-linked junctions [32••]. Psoralens intercalated between the base pairs of a d(TA) dinucleotide will, upon UV radiation, covalently cross-link the two thymine residues, one at the six-membered pyrone (P) ring and the other at the five-membered furan (F) ring of the drug (top). The HMT–d(CCGCTAGCGG) junction is induced by the drug, whereas the HMT–d(CCGGTACCGG) structure can also be classified as an ACC-type junction.

continuous double helices. As with the structures of double-helical RNAs and DNAs, the conformations of the duplex arms of the junctions are defined by the nucleotide sequence. For example, the d(GpT/A*C) dinucleotide step in the d(CCGGTACCGG) junction (A* is at the point of strand exchange) [30••] has a nearly identical helical conformation to the same step in the B-DNA structure of d(CCAGTACTGG) [33•]. Similarly, the mismatched d(G•A) base pairs impose the same distortions on the duplex arms of the d(CCGGGACCGG) junction [29••] as on the B-DNA structure of d(CCAAGATTGG) [34]. In the RNA–DNA complexes [28••,31••], the 3′-*endo* sugars of the RNA strands induce the hybrid arms to adopt the A-form conformation. This is generally true for all duplex RNA–DNA crystal structures [35–37]. Finally, the positions of the ordered solvent molecules in the DNA junctions (Figure 3a) are nearly identical to those of the analogous B-DNA crystal structures. Thus, the conformations of the stacked duplexes across the junction show the same sequence dependence as the structures of resolved duplexes.

Base stacking and hydrogen bonds define the structure and stability of Holliday junctions

We have seen that base stacking and hydrogen-bonding interactions within a conserved ACC trinucleotide are essential to the crystallization of junctions with the sequence motif d(CCGGN***ACC***GG). Within this motif, the d(C8•G3) base pair forms direct and solvent-mediated hydrogen bonds with the exchanging phosphates at the cross-over [29••,30••]. Replacing cytosine C8 with a thymine, as in d(CCAGTACTGG), results in crystals of standard B-DNA [33•], suggesting that these hydrogen-bonding interactions are critical in stabilizing the junctions. The d(A6•N5) and d(C7•G4) base pairs, however, show no direct contacts either within or across the junction, other than base stacking. Still, replacing either the A6 or C7 nucleotides, as in the sequences d(CCGGCGCCGG) [38] and d(CCGCTAGCGG) [30••], also results in crystals of standard B-DNA duplexes. This suggests that base stacking also plays a critical role in the stability of junctions in crystals. The relevant stacking interaction that spans the gap in the duplex is between the

Figure 3



The solvent structure and solvent-accessible surface of d(CCGGTACCGG). (a) The solvent molecules (purple spheres) and the single Na^+ ion (blue sphere) are shown overlaid on the solvent-accessible surface [30••] of this DNA junction. Surface with negative electrostatic potential is red, neutral potential is white and positive potential is blue. (b) Cut-away side view showing the buried Na^+ ion and the close P–P contact (6 Å) between the adjacent arms of the junction.

A6 nucleotide and the d(C7•G4) base pair. The nucleotide that complements A6 is inconsequential, as it can be a thymine [30••], a mismatched guanine [29••] or a psoralen-adducted thymine [32••] base. In this last case, the two complementary bases are not hydrogen bonded and the highly inclined thymine base shows no stacking interactions with the neighboring base pairs. Thus, both base stacking and hydrogen bonding contribute to the ability of these sequences to crystallize as a junction.

Distant interactions along the arms affect the overall geometry of junctions

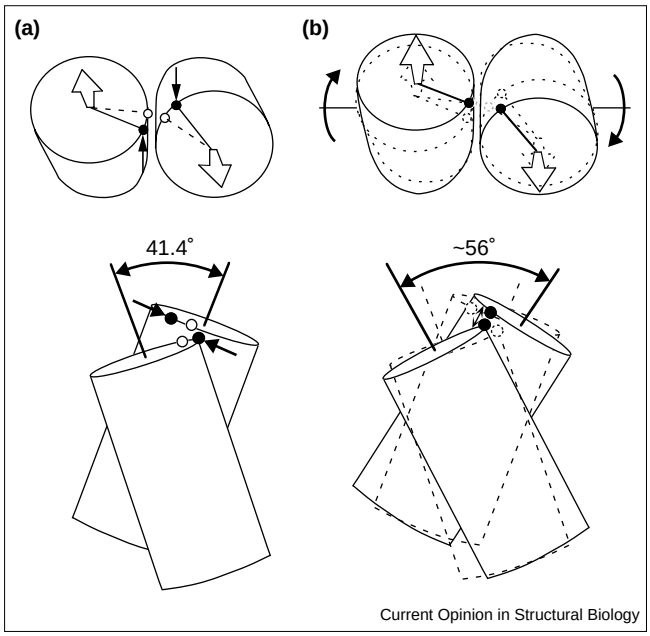
The interduplex angles relating the helices of the stacked-X junctions range from –80° to 55° in the crystal structures. This angle in the DNA-only structures (ranging from 36° to 42°), however, is much shallower than the approximately 60° angle estimated by other methods [14,15,16•]. This suggests that the crystal lattice may perturb the geometry of the detailed interactions within the junction. We believe, however, that the structural features of the junction are defined predominantly by conserved close contacts across the junction between phosphate oxygens at the ends of the arms (with phosphorus–phosphorus or P–P distances ≤ 6 Å) (Figure 3b). Although no ordered solvent molecules were observed bridging the negatively charged phosphates, these close contacts must be mediated by cations. We propose that the interhelical angle varies to accommodate perturbations to the helical structure of the stacked arms while maintaining this close contact; thus, it is the overwinding of the B-DNA arms that accounts for the shallow angles in the crystal structures.

In support of this, the interduplex angles in the d(CCGGTACCGG) and d(CCGGGACCGG) junctions are both approximately 41° and the duplex arms are overwound; the average helical repeat is 9.7 base pairs/turn (bp/turn) relative to the 10.5 bp/turn for B-DNA in solution [39,40] (see also Update). It is known that high concentrations of Na^+, as present in the crystallization solutions, induce overwinding of the DNA double helix [41]. By simply considering the geometry of the junction as two adjacent cylinders of DNA, we can see that the close P–P contacts are lost when the arms in the junctions adopt the helical repeat of B-DNA in solution (Figure 4). In order to reinstate this contact, the arms must be sheared to an interhelical angle of approximately 56°. Thus, the approximately 60° angle observed in solution probably stems from a requirement to maintain the close P–P contact in junctions whose stacked duplexes adopt the solution conformation of B-DNA.

However, the psoralen-linked junctions also have an approximately 10.5 bp/turn helical twist in the arms, but their interduplex angles are more shallow (by ~5°) than the unmodified structures. The interactions at the ACC trinucleotides of the drug-bound and the unmodified sequences are nearly identical. In addition, both of the psoralen junctions have interhelical angles of approximately 36°, but the two structures are conformers of each other. Thus, the interduplex angle across the junction is not defined by the interactions within the junction. It appears that the more than 5 Å extension of the DNA duplexes by the drug requires a shallower interduplex angle in order to establish the close P–P contact in the psoralenated structures.

Finally, we propose that the interduplex angles in the Rh-X [28••] and Lh-X [31••] RNA–DNA junctions are more variable (55° and –80°) because this P–P contact is lost. In both of these structures, one of the four helical arms has only a single Watson–Crick base pair, with the remainder of the nucleotides in that arm forming a short loop. Thus, without the constraint of the close P–P contact,

Figure 4



Effect of helical twist on the interduplex angle across the junction. (a) The stacked duplexes of the ACC-type junctions are viewed down the helices (top) and along the junction (bottom), with the two phosphates in close contact (open circle) across the adjacent arms. These same phosphates (filled circles) are more separated in B-DNA arms, which have a 10.5 bp/turn repeat. (b) Shearing the ACC-type junction (dashed structure) re-establishes the close P–P contact in duplex arms with a 10.5 bp/turn repeat (solid structure) and results in an interhelical angle of approximately 56°.

the overall structure of the junction can be highly variable, even if it adopts a stacked-X geometry.

The junctions show very few specific ion interactions

A detailed comparison of the solvent environments of all these junctions has led us to ask where are the ions? There are specific ion interactions in both of the RNA–DNA junctions [28••,31••]. A hydrated Mg^{2+} ion was identified at the disrupted stacked base pairs of the A-helix arm in the Lh-X RNA–DNA structure [31••]; however, it is unlikely that this will be relevant in the DNA-only structures, where the base-stacking interactions are unperturbed. In the Rh-X RNA–DNA junction [28••], a Co^{3+} ion was observed bridging a phosphate to the bases in the major groove across the junction. Although the narrower major groove of the DNA junctions cannot accommodate the large ion, this may be a more generally significant interaction.

Given the highly negative electrostatic potential at the junctions (Figure 3a) and the presence of divalent cations in the crystallization solutions, it is surprising that no divalent cations were observed in any of the DNA structures. A putative Na^+ ion was located in the major groove of the d(CCGGTACCGG) junction [30••]. This ion is completely buried beneath the solvent-accessible surface, suggesting that it was encapsulated during assembly of the junction (Figure 3b). In the mismatched d(CCGGGACCGG) junction [29••] and the HMT–d(CCGGTACCGG) [32••] junction, this Na^+ ion is replaced by one or two water molecules (see also Update). Thus, the specific binding of cations is seen to contribute to the formation of junctions in the crystals, but a majority of the cation interactions are apparently nonspecific.

Psoralen cross-links induce the formation of Holliday junctions

Four-way junctions are important intermediates in the repair of DNA lesions and adducts. The drug-induced junction in HMT–d(CCGCTAGCGG) shows how psoralen is capable of accommodating and even influencing its own damage repair. This is consistent with psoralen cross-links being highly recombinagenic [42–44].

Mammalian and bacterial cells respond to psoralen adducts by activating the nucleotide excision repair (NER) system to splice out the cross-linked DNA [45]. The junction formed in HMT–d(CCGCTAGCGG) shows how destabilization of the DNA duplex by psoralen can facilitate repair by the NER system, even though this is not generally considered a recombination-dependent mechanism. In mammalian cells, the DNA is excised by the repair enzymes on either strand of the psoralen cross-link with equal probability [45]; however, the enzymes are 10 times more likely to fill in the resulting gap on the furan-side strand [46•]. This is consistent with the opposite pyrone strand being the one that is displaced from the duplex to form the junction in the crystal.

The drug-induced Holliday junction may also be relevant as a model for the cellular response to psoralen in the mechanism for reinitiating replication at DNA lesions. Recombination is believed to be necessary in this mechanism, which uses newly synthesized DNA to bypass single- and double-stranded breaks and lesions that cause the collapse of the replication complex [2•]. Thus, it is interesting to speculate how the drug-induced junction may play a role in the repair of cross-links or in the cell's ability to bypass these adducts during replication.

Conclusions

With the currently available crystal structures, we see that the four-way Holliday junction involved in recombination can be fixed at a stable position through hydrogen bonding and base stacking. The more general features, including the angular relationship between duplexes of the stacked-X structure, may be influenced more by contacts that are distant from the point of exchange. Future studies should focus on how these local and distant interactions contribute to the properties of the junctions in the crystals and in solution, and how they affect the ability of the stacked-X junctions to migrate — junctions locked into this conformation can spontaneously migrate along the sequence [47]. There remains a paucity of information on

specific ion interactions at the junctions, even though cations have been mapped in solution to sites at and near the exchanged DNA strands [48]. These interactions may reveal themselves in higher resolution crystal structures and in crystals that incorporate cations that have higher specificity. Finally, the role that various drugs and covalent adducts play in influencing the formation of junctions and their roles in recombination repair and in reinitiating stalled replication forks need to be explored in greater detail in solution and in the cellular environment.

Update

We have recently solved the structure of a methylated variant of d(CCGGTACCGG) as a Holliday junction to better than 1.6 Å resolution (J Vargason, PS Ho, unpublished data). The effect of cytosine methylation at cytosine C* in the ACC core was seen to shift one set of the stacked duplexes by the equivalent of one base pair (~3.4 Å) along the helix axis relative to the opposing stacked arms across the junction. The interduplex angle, however, remains approximately 41°, while maintaining the close P–P contact seen in other ACC core junctions. In addition, the junction itself becomes more compact and therefore cannot accommodate any of the solvent interactions seen in the nonmethylated junctions [29••,30••,32••].

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