The Holliday junction in an inverted repeat DNA sequence: sequence effects on the structure of four-way junctions

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SUMMARY
Holliday junctions are important structural intermediates in recombination, viral integration and DNA repair. We have solved the 2.1 Å resolution single-crystal structure of the inverted repeat sequence d(CCGGTACCGG) as a Holliday junction. This junction has B-DNA arms with all standard Watson-Crick base pairs; it therefore represents the intermediate proposed by Holliday as being involved in homologous recombination. The junction is in the stacked-X conformation, with two interconnected duplexes formed by coaxially stacked arms, and criss-crossed at an angle of 41.4˚ as a right-handed X. Comparing this sequence with those crystallized as B-DNA and previously as a junction, we define an ACC trinucleotide as the core of a stable junction in this system. The 3'-C·G base pair of this ACC core forms direct and water mediated hydrogen bonds to the phosphates at the cross-over strands. Interactions within this core define the conformation of the Holliday junction, including the angle relating the stacked duplexes and how the base pairs are stacked in the stable form of the junction.

INTRODUCTION
When genetic information is exchanged, *e.g.*, during recombination across sister chromatids or integration of viral DNA into host genomes, the DNA double-helix is disrupted to form an intermediate structure. Holliday proposed that this intermediate during homologous recombination is a four-way junction (Figure 1).[1] Studies on synthetic four-stranded complexes and on extruded DNA cruciforms show that four-way junctions can adopt either an open extended-X or the more compact stacked-X conformation depending on the counter-cation (for a recent review, see reference 2). During recombination, four-way junctions are resolved by enzymes to complete the process of strand exchange between duplexes.

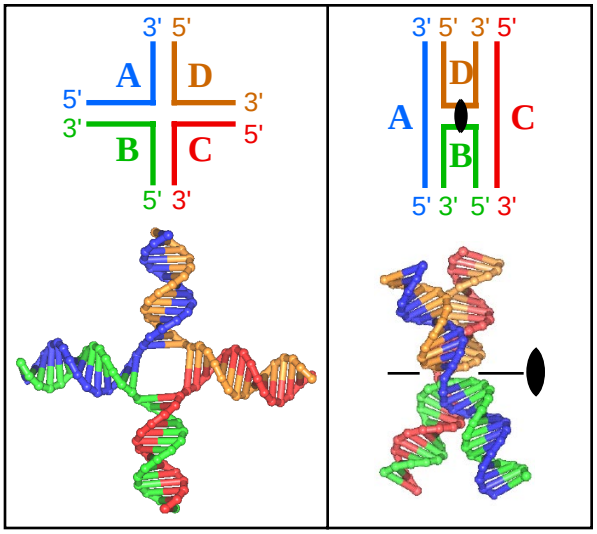

Figure 1. Conformations of four-way junctions. The left-hand panel shows the association of DNA strands A (blue), B (green), C (red) and D (yellow) to form a junction (top) with four duplex arms extended in a square planar geometry (extended-X form, bottom). The right-hand panel shows these same strands (top) associated to form the stacked-X structure of the junction, with pairs of arms coaxially stacked as double-helices related by two-fold symmetry (bottom).

Recently, the crystal structures of junctions in a DNA-RNA complex[3] and in the sequence d(CCGGGACCGG)[4] have been reported. In the first structure, the DNA/RNA arms are in the A-conformation, while in the latter structure two G·A mismatched base pairs sit adjacent to the cross-over between the duplexes. These structures left us with the question: what is the structure of a Holliday junction with B-DNA arms and standard base pairs?

DATA COLLECTION

X-ray diffraction data was collected on very thin diamond shaped (300 x 100 x 20 μm^3) crystals of the sequence d(CCGGTACCGG) using beamline 5.0.2 (λ = 1.1 Å) at the Advanced Light Source (ALS) in Berkeley, CA. The crystals were kept at 100 K using a liquid nitrogen stream to reduce radiative damage during data collection. The crystals are in the monoclinic *C2* space group, with unit cell dimensions a = 66.5 Å, b = 23.5 Å, c = 76.9 Å, and β = 114.8˚. The data was limited to 2.1 Å resolution according to ‹I/σ›, completeness, and R_{merge} statistics.[5]

RESULTS AND DISCUSSION

The structure of d(CCGGTACCGG) was solved as a four-stranded Holliday junction (Figure 2). Despite attempts to solve the structure as B-DNA double-helices, the electron density maps indicated that the duplexes were connected by the crossed-over strands of a junction. The electron density was consistently observed at all resolution limits to be discontinuous between nucleotides A6 and C7 in one strand of each duplex, but to connect these nucleotides across adjacent duplexes. The F_o-F_c maps drawn with the backbone atoms of nucleotides A6 and C7 omitted showed these same connections regardless of whether the structure was refined as two resolved double-helices or as a four-way junction. The structure refined as a Holliday junction (R=23.0%, R_{free}=31.8%) clearly shows the backbone trace between the adjoining duplex.[5]

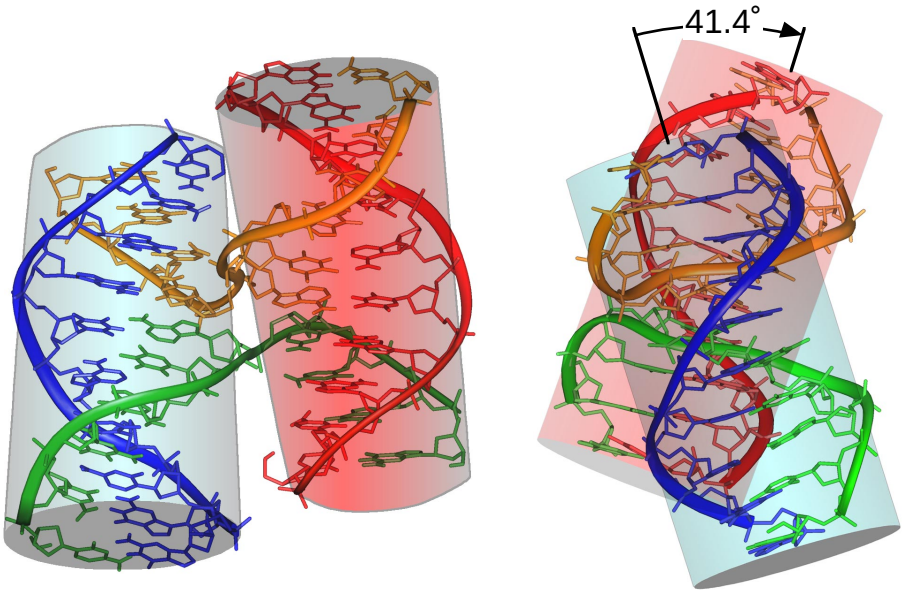

Figure 2. The Holliday junction structure of d(CCGGTACCGG). The asymmetric unit contains four strands of the sequence, which assemble into the stacked-X conformation of a four-way junction. Left: view down the two-fold axis of the junction. Right: view along the Holliday junction. Two duplexes, formed by stacking of the four arms of the junction, are related by a right-handed twist of 41.4˚.

The junction resulting from the assembly of the four identical DNA strands has approximate two-fold symmetry, but with the dyad sitting between A6 and C7 rather than bisecting the sequence. Pairing the complementary nucleotides of the DNA strands A, B, C, and D results in six-base pair A-B and C-D arms, and four-base pair C-D and D-A arms. The A-B arm is coaxially stacked on the D-A arm and B-C stacked on C-D to form the two continuous antiparallel duplexes in the stacked-X form of the junction. The conformation resembles an H with the two arms twisted 41.4˚ in a right-handed sense. The stacked arms form continuous B-DNA duplexes with very little disruption in base pairing, base stacking and solvent interactions by the junction.

The junction itself is a compact and relatively rigid structure. We observe that the temperature factors (B-factors) of the trinucleotides A6-C7-C8 in strands B and D that span the junction and

the complementary G3 and G4 nucleotides are generally lower (< 30 Å^2) than the remainder of the DNA. If we accept that B-factors reflect the thermal disorder of atoms in a crystal, the low B-factors of these nucleotides reflect a relatively inflexible junction. This local rigidity can be attributed to specific hydrogen bonds between the C8·G3 base pairs of the B-C and D-A arms and the phosphates at the strands that cross-over.[5]

The compactness of the junction results in a close approach of four phosphates (within 6.5 Å of each other) at the cross-over and thus a highly negative electrostatic potential that must be compensated by counterions. We observe a single well-ordered solvent molecule (B-factor = 18.6 Å^2 *versus* an average of 44.6 Å^2 for other added solvent) which sits less than 2.5 Å to the phosphates of the two A6 nucleotides spanning the junction. Because this is shorter than standard hydrogen bonding distances for water, we have assigned this as sodium ion directly coordinated to the phosphate oxygens at the junction. Thus, a sodium ion apparently sits in the center of the junction to help compensate for the high density of negative charges, and to cross-link through metal coordination the strands of the junction.

Comparing the sequences of similar decanucleotide crystals, we see that an A6-C7-C8 trinucleotide is common to the junction forming sequences.[5] Changing any one of these nucleotides results in a B-DNA duplex. Immediately preceding A6 is either a guanine or thymine; however, since guanine G5 of d(CCGGGACCGG) is mispaired, this position can be any nucleotide. The CC/GG dinucleotide at the 5' and 3'-termini are common to both the junction and B-DNA sequences, while G3 and G4 complement the cytosines of the ACC trinucleotide. This ACC trinucleotide forms the cross-over of the four-way junction. The hydrogen bonds from cytosine C8 to an adjacent phosphate at the cross-over and the water mediated hydrogen bonds from G3 to the phosphates of adenine A6 across the junction play important roles in defining the geometry and stability of the junction in both sequences. For example, these well defined interactions fix the orientation between the two duplexes across the junction, and thus account for the 41.4˚ twist of these duplexes. We can thus define this ACC trinucleotide as the core of the Holliday junction in these crystal systems.

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