The interaction of four-way DNA junctions with resolving enzymes

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Abstract
Four-way DNA (Holliday) junctions are resolved into duplex species by the action of the junction-resolving enzymes, nucleases selective for the structure of helical branchpoints. These have been isolated from bacteria and their phages, archaea, yeasts and mammals, including humans. They are all dimeric proteins that bind with high selectivity to DNA junctions and generate bilateral cleavage within the lifetime of the DNA-protein complex. Recent success in obtaining X-ray crystal structures of resolving enzymes bound to DNA junctions has revealed how the structural selectivity of these enzymes is achieved.

Junction-resolving enzymes
Four-way DNA (Holliday) junctions are branchpoints generated by the interconnection of four helices by strand exchange. In principle, they can be created by strand invasion in recombination, double-strand break repair and fork reversal during replication. Four-way junctions can be resolved by the action of junction-resolving enzymes, to create duplex products. These are widespread nucleases that are highly selective for the structure of DNA junctions. The dimeric junction-resolving enzymes introduce paired cleavages at or very close to the branchpoint of the junction, resulting in separation into duplex species.

Junction-resolving enzymes have been isolated from many organisms, from bacteria and their phages, yeasts and archaea, to mammalian cells and their viruses [2,3]. The search for a mammalian junction-resolving enzyme has been long, with several wrong turns along the way, perhaps partly due to a redundancy of pathways that process Holliday junctions in eukaryotes [4–8]. One pathway appears to involve the MUS81–EME1 (essential meiotic endonuclease 1 homologue) complex, although the in vitro properties of this complex are significantly different from those of canonical resolving enzymes, and appear more like flap endonucleases [9]. However, Taylor and McGowan [10] showed this can lead to productive junction resolution. In a second possible pathway, junctions become ‘dissolved’ by a combination of topoisomerase III and Bloom’s helicase [11,12]. A number of additional candidate resolving enzymes have recently emerged. West and colleagues have identified the human GEN1 protein, and the corresponding yeast enzyme Yen1, that generate the symmetrical cleavage of four-way junctions near the branchpoint [13]. We have recently identified the corresponding enzyme in Caenorhabditis elegans (A. Bailly, A.-C. Déclais, D.M.J. Lilley and A. Gartner, unpublished work). However, this gene does not appear to possess a meiotic phenotype. A second candidate enzyme has emerged from studies in our [14] and a number of other [15–17] laboratories. Genetic studies have shown that SLX4 has roles in the survival of cells lacking SGS1 (of the RecQ family) [18], the cleavage of non-homologous DNA sections in homologous recombination [19,20], and in the repair of replication forks blocked by interstrand cross-links [21,22]. We immunoprecipitated SLX4 and found that it forms a 2 MDa complex in which it interacts with a series of proteins involved in DNA repair, SLX1, XPF (xeroderma pigmentosum complementation group F)–ERCC1 (excision repair cross-complementing 1) and MUS81–EME1. Analysis of immunoprecipitated protein from RNAi (RNA interference)-treated cells indicates that the SLX1–SLX4 complex has the appropriate activity for the resolution of four-way junctions [14] (Figure 1).

Recognition and manipulation of junction structure
Studies of the higher eukaryotic junction-resolving enzymes are at a very early stage, and most of our understanding of these enzymes comes from analysis of enzymes found in bacteria and their phages, yeast mitochondria and archaea [23]. The enzymes bind four-way DNA junctions relatively tightly, with dissociation constants close to 1 nM. Binding is highly selective for the structure of junctions; the complex of a dimer of any junction-resolving enzyme and a four-way DNA junction is generally not displaced by a 1000-fold excess of duplex DNA of the same sequence. However, perhaps surprisingly, all of the junction-resolving enzymes distort the very structure that they recognize, and some open the structure of the junction in a major way. Cce1 of budding yeast provides the extreme case, where binding causes the junction to adopt an open-square structure [24] that involves disruption of base pairing adjacent to the point of strand exchange [25]. Although all the resolving enzymes

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Abbreviations used: EME1, essential meiotic endonuclease 1 homologue.
The structural distortion of the DNA junctions induced on binding of the enzymes may ensure a productive resolution event. Experiments using cruciform structures extruded in negatively supercoiled DNA molecules [27] showed that T4 endonuclease VII, T7 endonuclease I and Cce1 introduce paired cleavages within the lifetime of the complexes [28]. Kinetic analysis revealed that there is an acceleration of the second strand cleavage (by a factor of 100 in the case of RuvC), so that it quickly follows cleavage of the first strand [29,30]. The distortion of the junction may play a key role here. It was proposed that the intact junction is strained in the complex initially formed, but that the strain is relieved by the first strand cleavage that results in an acceleration of the second strand cleavage. Thus it seems probable that recognition of the structure of the junction, distortion of that structure and cleavage of the DNA are all interconnected.

**Endonuclease I of bacteriophage T7**

Over recent years, the most effort has been put into the study of the phage T7 junction-resolving enzyme endonuclease I [31–33]. Phages carrying mutations in the gene encoding this enzyme are deficient in recombination [34,35] and accumulate branched DNA intermediates [36]. The enzyme is a stable dimer of 149-amino-acid subunits [37]. It is very basic (pIcalc 9.5), and binds tightly (KD = 2 nM) to junctions in dimeric form. The enzyme falls into the nuclease superfamily [38], and is clearly related to the restriction enzymes. In common with these enzymes, endonuclease I catalyses the breakage of the P–O3′ bond [39] (i.e. the water nucleophile attacks in-line opposite the O3′ atom) and is strongly inhibited by Ca2+ ions. The X-ray crystal structure of the protein was solved a number of years ago [40]. Endonuclease I forms an intimately associated symmetrical homodimer comprising two domains, each formed by residues 17–44 from one subunit and 50–145 from the other. The domains are connected by a bridge that forms part of an extended β-sheet. The active site I conforms to the restriction enzyme consensus [41], i.e. (Asp/Glu20)…Pro42–Asp55…(Asp/Glu65)–Xaa66–Lys67 (amino acid numbering from endonuclease I), and the α and β carbon atoms of the four critical side chains (Glu20, Asp55, Glu65 and Lys67) plus the conserved proline residue (Pro54) can be superimposed with the structure of the corresponding residues of the restriction enzyme BglII [42]. Two Ca2+ ions were observed by X-ray crystallography to be co-ordinated in the active site of BglII in a complex with DNA fragment [42], and we have found two Mn2+ ions in equivalent positions in the crystal structure of wild-type sequence endonuclease I in the absence of DNA [43]. Binding of two metal ions to the protein was also indicated by isothermal titration calorimetry [43], and Fe(II)-mediated cleavage of DNA indicated a very similar location of metal ions in the complex [44]. We built a detailed model of the active-site complex with the phosphodiester backbone [39], consistent with all of the available data, where the pro54 oxygen atom of the scissile phosphate group is co-ordinated directly to both metal ions (Figure 2). This was consistent with the results of methyl phosphate substitution on cleavage rates [39].

The global shape of the complex was found to be significantly altered from that of the free four-way DNA junction. Comparative gel electrophoretic analysis of the endonuclease I complex indicated that there is significant coaxial pairwise alignment of helices, but with a rotation of the axes and a local distortion of the centre [45,46]. This was complicated by the existence of two forms of the complex akin to alternative stacking conformers [44,46]. However, we found that, by selectively shortening two arms of the junction, we could bias the complex to a single form [47]. From this we could determine that the continuous strands of the junction were cleaved selectively. It also turned out to be a key observation, because it enabled us to construct a complex of endonuclease I with a four-way DNA junction that crystallized in a form that diffracted X-rays well [48].
Figure 2 | A model of DNA bound in the active site of phage T7 endonuclease I
Parallel-eye stereoscopic view of the active site with two metal ions and their associated water molecules. The water nucleophile is coloured blue. Reproduced with permission from Biochemistry 2006, 45, 3934–3942 [39]. Copyright 2006 American Chemical Society.

The recognition of junction structure
The structure of the complex (Figure 3) finally revealed how the enzyme achieves its considerable selectivity for four-way DNA junctions [48]. The protein dimer creates two DNA-binding channels that are 30 Å (1 Å = 0.1 nm) in length, formed by the front of one domain and the back of the other. These positively charged channels form extensive contacts with the arms containing the 5’ ends of the continuous strands, resulting in the burial of 4180 Å² of solvent-accessible protein surface. The channels hold the DNA arms in a specific near-perpendicular orientation. This is probably only possible for a four-way junction and thus provides the basis for the selectivity of the enzyme for DNA junctions.

However, this is clearly not the only way in which a resolving enzyme can recognize the structure of a junction. Yang and co-workers solved the structure of a complex of T4 endonuclease VII with a four-way junction [49]. We knew previously that this enzyme opens the structure into a relatively flat X-shape [50], and this was borne out by the crystal structure. The protein dimer adopts an extended S-shape, presenting a large electropositive area to which the junction binds. Two strands of the junction are antiparallel and can be regarded as the continuous strands, whereas the other two strands make a marked change of direction, and can be classed as exchanging strands. The open X-shape of the junction binds with its minor groove face contacting the protein, forming hydrogen bonds and non-bonding interactions in several discrete regions. The active

Figure 3 | The crystal structure of complex between a DNA four-way junction and phage T7 endonuclease I
Two parallel-eye stereoscopic images of the structure are presented, in similar orientations. The two polypeptides are coloured blue and green, and the DNA junction is red. The complex is oriented to view approximately down the axis on the left, whereas the arms are side-on on the right side. The N-terminal 17 amino acids of the protein cannot be located in the crystal structure, but we believe that these play an important role in the function of the enzyme. (A) Both DNA and protein are shown in cartoon form. The metal ions are depicted as yellow spheres, and show the positions of the two active sites juxtaposed with the continuous strands of the junction. (B) In this image, the DNA and protein are shown in space-filling form. Note that the paired arms are deeply embedded in channels formed by the combination of the two polypeptides. These channels are oriented at approximately right angles to each other. Data taken from [48].
sites are located such that they cleave the exchanging strands 2 nt from the central phosphates that define the point of strand exchange. Thus the two active sites of endonuclease VII are much closer to each other (∼25 Å) than those of endonuclease I (∼40 Å).

It is clear that the recognition of the branched DNA structure, its distortion and ultimately the chemical process of resolution are intimately connected. But the detailed processes differ significantly from one enzyme to another.

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