Structural dynamics of individual Holliday junctions

Sean A. McKinney[1], Anne-Cécile Déclais[2], David M.J. Lilley[2] and Taekjip Ha[1]

Published online 23 December 2002; doi:10.1038/nsb883

The four-way DNA (Holliday) junction is the central intermediate of genetic recombination, but the dynamic aspects of this important structure are presently unclear. Although transitions between alternative stacking conformers have been predicted, conventional kinetic studies are precluded by the inability to synchronize the junction in a single conformer in bulk solution. Using single-molecule fluorescence methodology we have been able to detect these transitions. The sequence dependence, the influence of counterions and measured energetic barriers indicate that the conformer transition and branch migration processes share the unstacked, open structure as the common intermediate but have different rate-limiting steps. Relative rates indicate that multiple conformer transitions occur at each intermediate step of branch migration, allowing the junction to reach conformational equilibrium. This provides a mechanism whereby the sequence-dependent conformational bias could determine the extent of genetic exchange upon junction resolution.

Genetic recombination is important in the repair of double-strand DNA breaks, the processing of stalled replication forks[1] and the generation of genetic diversity, which is important for evolution. During this process, two homologous DNA molecules undergo strand exchange to form a four-way DNA (Holliday) junction[2–4], and the recognition and resolution of this species by junction-resolving enzymes determines the genetic outcome of the process[5].

The four-way DNA junction is structurally polymorphic (reviewed in ref. 6). In the absence of divalent metal ions, the center is open and the four helical arms are directed towards the corners of a square[7] (Fig. 1*a*). However, upon addition of magnesium ions, the structure folds by coaxial pairwise stacking of helical arms into the stacked X-structure (Fig. 1*b*). The X-structure was deduced to be a right-handed, antiparallel structure[8,9], recently confirmed by X-ray crystallography[10–12]. In this structure, two of the DNA strands run continuously through a given pair of stacked helices, while the other two are exchanged between helical pairs. A given junction might adopt two possible stacked structures, depending on the choice of stacking partners (Fig. 1*d*). In one conformer, arm B is coaxially stacked with arm H (*isoI*), whereas in the other it stacks with arm X (*isoII*). The stacking conformation seems to be important for the outcome of genetic recombination because there is evidence that the stacking conformer determines the orientation of the junction resolution by junction-resolving enzymes[8,13,14]. This orientation determines whether genetic information is exchanged over a long or short range.

Holliday junctions have been crystallized in single stacking conformers, implying strong biases toward one conformer[10–12]. However, evidence for the coexistence of both conformers was found in some junction sequences by time-resolved fluorescence resonance energy transfer (FRET) and NMR experiments[15] and by comparative gel electrophoresis[16], in which there was also indirect evidence for exchange between the two conformers. The relative populations of the two conformers are determined by the junction sequence[8,17,18] but not by the solution conditions (see below). Therefore, studying the kinetics of stacking conformer transitions using ensemble techniques that require synchronization is not possible. Until now, the only estimate for the transition rates comes from NMR linewidth studies[19].

We used the single-molecule FRET method, capable of measuring conformational changes of single biological molecules in real-time without the need for synchronization[20–22], to study the stacking conformer transitions, an example of truly non-synchronizable dynamics. Not only could we make the first real-time observation of these stacking conformer transitions, but we were also able to measure the transition rates as a function of DNA sequence, types and concentrations of counterions, and temperature. The new kinetic information we obtained provides clues to the relation between the stacking conformer transitions and branch migration, as well as a plausible mechanism whereby the sequence-dependent conformational bias could determine the orientation of the junction resolution in the context of a fully branch-migratable junction.

Conformer transitions of single junctions

For single molecule studies, we assembled DNA junctions from 4 component strands, each of 22 nucleotides. The resulting junction had four helical arms (B, H, R and X), each of 11 base pairs (bp). The sequences were chosen so as to prevent branch migration. Three of the four helices were conjugated at the 5′ termini by various species (Fig. 1*d*): helix H by Cy3 (FRET donor), helix B by Cy5 (FRET acceptor) and helix R by biotin (for surface immobilization). In one stacking conformer (*isoI*), helix B stacks on helix H, maximizing the interfluorophore distance (minimizing FRET), whereas in the other conformer (*isoII*), helix B stacks on helix X, minimizing the distance (maximizing FRET). Our experiments were carried out using junctions with two different central sequences (Fig. 1*c*). Junction 3 is heavily biased towards stacking conformer *isoII*[8], whereas junction 7 has near-equal populations in each conformer[16].

[1]Physics department, University of Illinois, Urbana-Champaign, Urbana, Illinois 61801, USA. [2]Cancer Research UK Nucleic Acid Structure Research Group, Department of Biochemistry, The University of Dundee, Dundee DD1 5EH, UK.

Correspondence should be addressed to T.H. *e-mail: tjha@uiuc.edu*

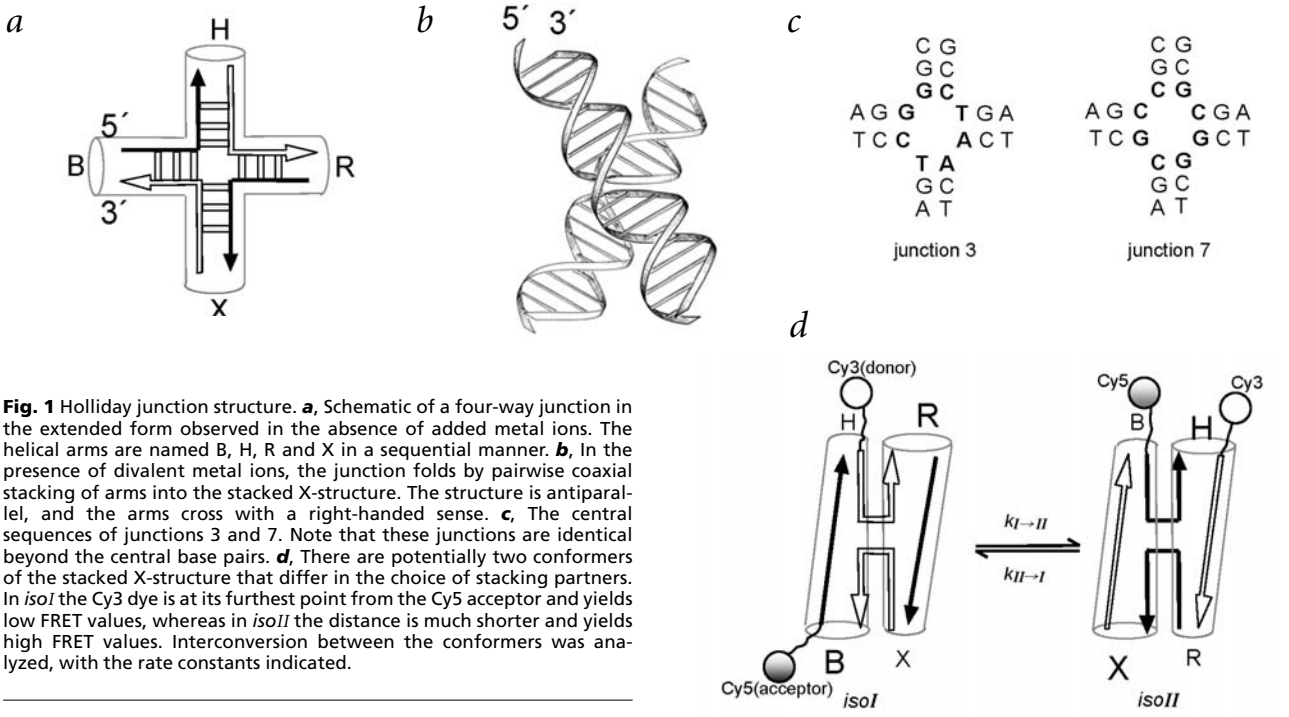

Fig. 1 Holliday junction structure. ***a***, Schematic of a four-way junction in the extended form observed in the absence of added metal ions. The helical arms are named B, H, R and X in a sequential manner. ***b***, In the presence of divalent metal ions, the junction folds by pairwise coaxial stacking of arms into the stacked X-structure. The structure is antiparallel, and the arms cross with a right-handed sense. ***c***, The central sequences of junctions 3 and 7. Note that these junctions are identical beyond the central base pairs. ***d***, There are potentially two conformers of the stacked X-structure that differ in the choice of stacking partners. In *isoI* the Cy3 dye is at its furthest point from the Cy5 acceptor and yields low FRET values, whereas in *isoII* the distance is much shorter and yields high FRET values. Interconversion between the conformers was analyzed, with the rate constants indicated.

Single-molecule donor and acceptor intensity time records of junctions 7 and 3 (Fig. 2*a*) show anti-correlated, two-state fluctuations of donor and acceptor signals, indicating changes in FRET. The transitions between the two states were instantaneous within 8 ms bin time. Junction 7 displays almost no bias between the two states, whereas junction 3 shows a strong bias toward the high FRET state — that is, the *isoII* stacking conformer — consistent with previous ensemble studies[8,16].

The rates of conformer transitions, $k_{I\to II}$ and $k_{II\to I}$, averaged over several hundred transitions from at least 12 molecules each were 12 s^{-1} and 3.5 s^{-1}, respectively, for junction 3, and 5.7 s^{-1} and 6.1 s^{-1}, respectively, for junction 7 in 50 mM Mg^{2+} and no NaCl at 25 °C (Fig. 2*b*). The fluctuation rates did not depend on laser intensity but were strongly dependent on the solution conditions. Junctions labeled with only the donor showed no such fluctuations. Those molecules exhibiting acceptor signal showed single-step photobleaching events for both acceptor and donor. On the basis of these observations, we conclude that the FRET fluctuations faithfully reflect the stacking conformer transitions of individual junction molecules.

The rates showed statistically significant variations among molecules for all conditions used. The histogram of the free energy difference (ΔG) between the two conformers, defined as $-RT \ln(k_{I\to II}/k_{II\to I})$, could be fit by a Gaussian function with a width of $\leq RT$ (data not shown). In the following discussions, we focus on the average values of the rates for junction 7.

Influence of counterions on transitions

The dependence of $k_{I\to II}$ and $k_{II\to I}$ on magnesium ion concentration differs in the presence and absence of 50 mM NaCl (Fig. 3). The transition rates between conformers decrease significantly with increased Mg^{2+} concentration but maintain an ~1:1 ratio. This implies two things: first, that the main effect of changing Mg^{2+} concentration is in altering the activation energy and not in the relative stability of the two conformers and, second, that the open structure, stable in the absence of Mg^{2+} ions, is a necessary transient in conformer transitions. At higher Mg^{2+} concentrations, displacing Mg^{2+} ions decorating the negatively charged phosphate groups at the exchange point becomes increasingly harder, resulting in lower transition rates. Monovalent ions, which do not fold the junction at low concentrations, would act only to screen electrostatic interactions between the Mg^{2+} ions and the phosphates and should accelerate the transitions; indeed, the addition of 50 mM NaCl increases the transition rates consistently for all Mg^{2+} concentrations examined (Fig. 3). Although the Holliday junction fully folds into the stacked X-structure at submillimolar Mg^{2+} concentration, the rate of conformer transitions continues to change for at least another two orders of magnitude increase in Mg^{2+} concentration.

Comparison to branch migration kinetics

Earlier observations showed that spontaneous branch migration becomes accelerated by 1,000-fold if Mg^{2+} is removed[23,24] and that the unstacked, open structure of the junction (O), stabilized in the absence of Mg^{2+}, is probably the common intermediate for both processes. Despite the similar effects of Mg^{2+}, the rate-limiting step or the transition state must differ between the two processes because (i) $k_{I\to II}$ and $k_{II\to I}$ are substantially higher than the estimated base pair stepping rate of spontaneous branch migration (k_{BM}) under the equivalent conditions (~17 s^{-1} in 10 mM Mg^{2+}, 50 mM NaCl, pH 8 at 25 °C (Fig. 3) compared with 0.7 s^{-1} for k_{BM}[24,25]), and (ii) the temperature dependence of the conformer transitions gives an activation enthalpy ($\Delta H^{\ddagger}$) of 100–120 kJ mol^{-1} (Fig. 4*a*), substantially smaller than $\Delta H^{\ddagger}$ for branch migration of ~160 kJ mol^{-1} (refs. 24–26). This difference in $\Delta H^{\ddagger}$ is not entirely unexpected: branch migration requires the breakage of base pairs at the exchange point, an energetically unfavorable process, whereas the conformer transitions do not necessarily involve the disruption of base pairing. An immediate corollary of the rate comparisons here is that the conformer transitions should occur several times before the branch migrates by one step; hence, stacking conformer transitions must be an integral feature of branch migration.

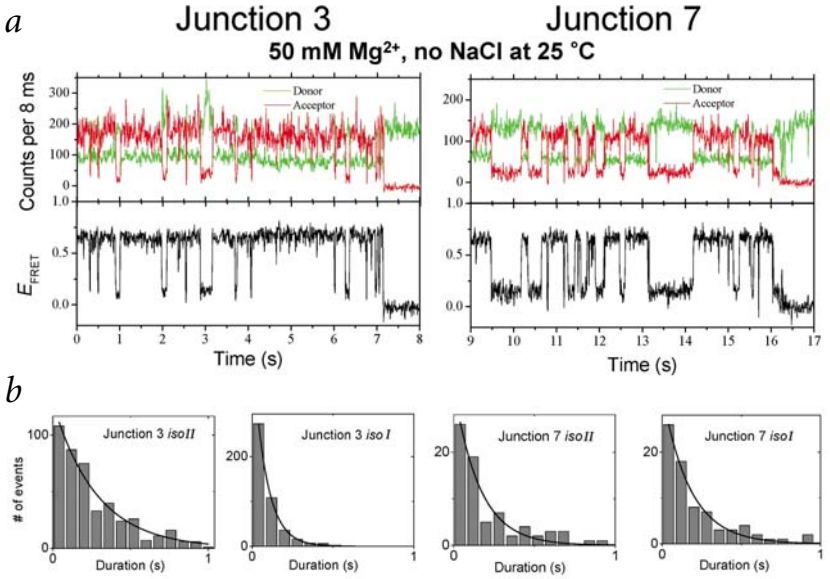

Fig. 2 Single-molecule time records and analysis ***a***, Stacking conformer transitions of individual molecules of junction 3 and 7. The top graphs show time traces of donor (green) and acceptor (red) intensities (I_D and I_A) corrected for crosstalk between the two detectors, direct acceptor excitation and background. The bottom graphs show the corresponding $E_{FRET} \equiv \int I_A / (I_A + I_D)$, a good approximation of FRET efficiency. Acceptor photobleaching towards the ends led to near zero E_{FRET}. ***b***, Histograms for the duration of each state are fit by single exponential decay. The two lifetimes are roughly equal for junction 7, but *isoII* is favored by ~4:1 for junction 3, corresponding to ΔG ~3 kJ mol^{-1}. These ratios are consistent with published comparative gel electrophoresis studies[16].

Implications for junction resolution

Strong correlations have been observed between the dominant stacking conformation and the preferred orientation of junction resolution for several junction-resolving enzymes[8,13,14]. These observations suggest that the local DNA sequences can determine the outcome of genetic recombination *via* the bias in the stacking conformation. However, it has not been clear how this bias can be enforced in a fully branch-migratable junction because if the conformer transition were slower than the stepping rate of branch migration, the conformational dynamics would not reach equilibrium before the next migration step occurs. NMR studies determined the conformer transitions rates of 5.6 s^{-1} and 2.3 s^{-1} under a single solution condition[19] (15 mM Mg^{2+} and 50 mM NaCl, pH 6.5, at 21 °C). However, as we show here, these rates strongly depend on the solution conditions, so the direct comparison to the branch migration was not possible. Our study shows that the conformer transitions are substantially faster than the branch migration steps; hence, the sequence-dependent conformer bias can be fully manifested even in a fully branch-migratable junction and can determine the extent of genetic information exchange upon junction resolution.

Energy landscape of junction dynamics

On the basis of our observations, we propose a unified energy landscape of stacking conformer transitions and branch migration by Holliday junctions (Fig. 4*b*). Along the reaction coordinates of conformer transitions and branch migration (where *n* denotes the branch point location), we proposed the open structure (O_n, O_{n+1},…) as the common intermediate state for both processes. As Mg^{2+} concentration increases, the stacking conformers ($isoI_n$, $isoII_n$, $isoI_{n+1}$, $isoII_{n+1}$,…) become more stable relative to both the open structures and the transition states (T^{XO}_n, T^{XO}_{n+1},…) separating *O* from *X* states and leading to the large decrease in $k_{I\to II}$ and $k_{II\to I}$. At the same time, k_{BM} decreases with increasing Mg^{2+} concentration because *O* states are less populated. Because branch migration is 1,000-fold slower in the presence of Mg^{2+}, the intermediate state must be populated at least 0.1% of the time, setting the upper limit for the free energy difference between the open structure and the stacking conformers (ΔG_{XO}) at ~15 kJ mol^{-1}. The branch migration from the open structure must go through a transition state ($T^{BM}_{n,n+1}$,…), which is the main barrier in branch migration. The nature of this transition state is not clear yet but probably involves disruption of the base pairs at the exchange point, consistent with the large enthalpic cost.

In this model, the increase in Mg^{2+} concentration will decrease the population of the intermediate open structure, thereby slowing down both the branch migration and conformer transition. Our studies also suggest that Mg^{2+} ions have additional effects other than changing the population of the intermediate state. The rate of branch migration reached a maximum at 0.5 mM Mg^{2+} (ref. 26), but the conformer transition rates continued to change steeply at least until 50 mM Mg^{2+} (Fig. 3). This observation indicates that Mg^{2+} ions influence the energy barriers for the conformer transition and branch migration differently. Conclusions drawn here are probably general for other sequences because even the heavily biased junction 3 has conformer transition rates that are far higher than the rate of branch migration.

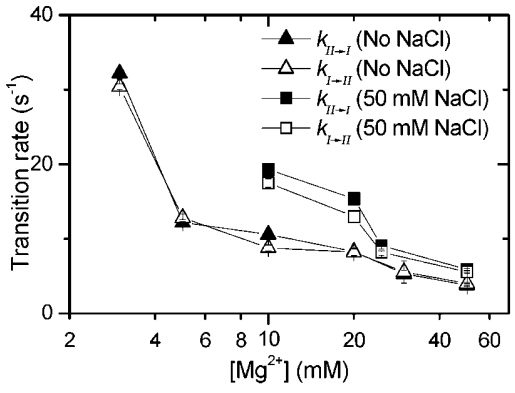

Fig. 3 Na^+ and Mg^{2+} influence the conformer transition rates differently. The conformer transition rates, $k_{I\to II}$ (open symbols) and $k_{II\to I}$ (filled symbols), are averaged over at least 30 molecules of junction 7 for various [Mg^{2+}] with (squares) and without (triangles) 50 mM NaCl.

Conclusions

In summary, we can construct a comprehensive model for the processes of conformer exchange and branch migration, linked by common open states. The faster rate of conformer exchange will ensure that equilibrium between stacked forms is achieved

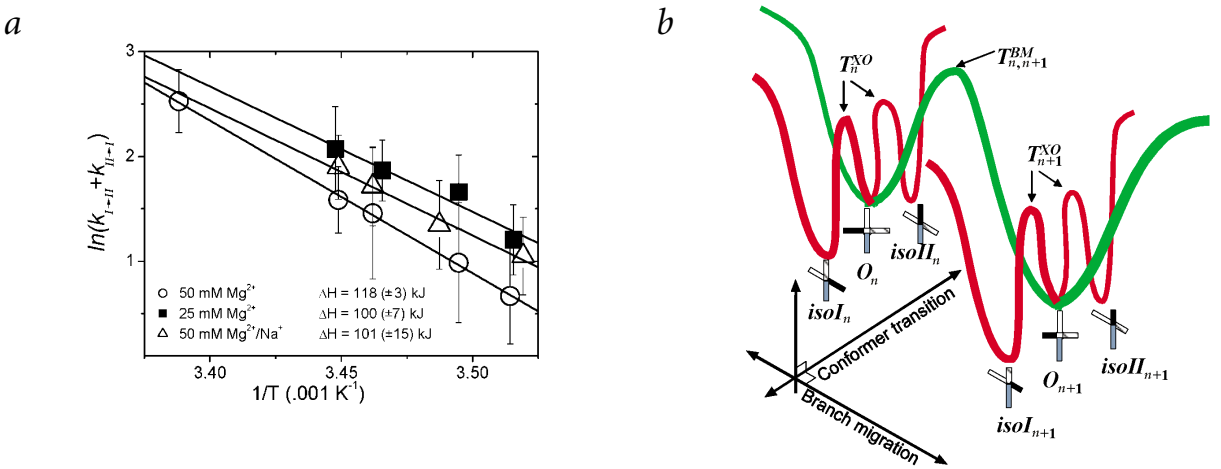

Fig. 4 Energetics of Holliday junction dynamics. ***a***, Temperature dependence in kJ of ($k_{I\to II}$ + $k_{II\to I}$) averaged over 60 molecules of junction 7 under each condition. The large error bars result from the inhomogeneous distribution of the transitions rates among individual molecules. The activation enthalpy ($\Delta H^{\ddagger}$) was obtained by fitting the data using the function $ln(k)$ = Const. – $\Delta H^{\ddagger}$ / RT. ***b***, The proposed energy landscape of conformer transitions and branch migration. Red and green lines are the free energy function along the reaction coordinate of conformer transitions ($isoI_n \leftrightarrow O_n \leftrightarrow isoII_n$) and branch migration ($O_n \leftrightarrow O_{n+1}$), respectively. $T^{BM}_{n,n+1}$ is the transition state between O_n and O_{n+1}. Two lines intersect at the open structure, a common intermediate for both processes. See text for the definition of symbols.

at each stage of branch migration. The decrease in the rate of branch migration in the presence of Mg^{2+} ions probably results from depopulation of the open state because of the stabilization of the stacked X-structure.

The conformer transitions observed here raise interesting questions about the Holliday junction dynamics in a cellular context. The genomic DNA molecules are likely constrained *in vivo* by other cellular components; how such frequent, large swiveling of DNA helices involved in stacking conformer transitions can be accommodated is not known. Combining the single-molecule fluorescence method with mechanical manipulation tools to apply force or torque may address this issue. One possibility is that the Holliday junction conformational dynamics are altered by junction-specific proteins. Indeed, RuvA and RuvB (two branch migration proteins) in *E. coli* maintain the junction in the open conformation to facilitate branch migration[27], and many junction-resolving enzymes induce global changes in junction conformations[5]. Single-molecule approaches illustrated here should also be useful in understanding other dynamic aspects of Holliday junction during recombination, including its formation, branch migration and final resolution into duplexes.

Methods

DNA preparation. Oligonucleotides of the following sequences were synthesized using phosphoramidite chemistry implemented on ABI394 DNA synthesizers. Cy5-J7b is 5′-Cy5-CCCTAGCAAGCCG CTGCTACGG; Cy3-J7h, 5′-Cy3-CCGTAGCAGCGCGAGCGGTGGG; Biot-J7r, 5′-Biot-CCCACCGCTCGGCTCAACTGGG; J7x, 5′-CCCAGTTGAGCGC TTGCTAGGG; Cy5-J3b, 5′-Cy5-CCCTAGCAAGGGGCTGCTACGG; Cy3-J3h, 5′-Cy3-CCGTAGCAGCCTGAGCGGTGGG; Biot-J3r, 5′-Biot-CCCAC-CGCTCAACTCAACTGGG; and J3x, 5′-CCCAGTTGAGTCCTTGCTAGGG. The junction was annealed by first mixing 45, 50 and 55 pmol of the oligonucleotides labeled with biotin, Cy3 and no attachment, respectively, in 25 mM Tris-HCl, pH 7.5, and 25 mM NaCl. This mixture was slowly cooled from 65 to 37 °C, at which point 55 pmol of the Cy5-labeled oligonucleotide was added, followed by 15 min incubation and chilling on ice. Assembled junctions were purified by electrophoresis using polyacrylamide gels and electroelution.

Single-molecule measurements. Strepatvidin-coated glass or quartz surfaces were prepared by successive application of 1 mg ml^{-1} biotinylated BSA (Sigma) and 0.2 mg ml^{-1} steptavidin (Molecular Probes) in buffer A (10 mM Tris-HCl, pH 8.0, and 50 mM NaCl). Biotinylated junction molecules (10–50 pM in buffer A) were then added to the treated surface and immobilized. A 532 nm laser (Crystalaser) was used to excite the donor, Cy3. Single-molecule data were obtained using a confocal scanning microscope[28], except for the temperature-dependence studies that used a wide-field evanescence field microscope[22]. For temperature regulation, a water-circulating bath (NESLAB) was connected to all parts in contact with the sample, including the prism mount, the sample mounting platform and the microscope objective (*via* a brass tubing collar). The sample temperature was monitored directly using a thermocouple. Unless otherwise specified, all measurements were made at 25 °C in a 50 mM Tris-HCl, pH 8.0, oxygen scavenger system (7% (w/v) glucose, 1% (v/v) 2-mercaptoethanol, 0.1 mg ml^{-1} glucose oxidase and 0.02 mg ml^{-1} catalase) with specified amounts of $MgCl_2$ and NaCl. We also performed measurements on PEG surfaces[22] and found similar results both in terms of molecular heterogeneity and Mg^{2+}-dependent changes in the transition rates.

Data analysis. To analyze the traces, we first filtered out blinking events (defined as a reversible transition of the acceptor to an inactive state, giving rise to an unquenched donor emission) and photobleaching of either fluorophore, and then performed a threshold analysis on the E_{FRET} time traces. For all but the very lowest [Mg^{2+}], two distinct E_{FRET} states were observed at ~0.2 and 0.6, in reasonable agreement with the predictions based on the stacked X-structure. The threshold varied ranging from 0.35 to 0.5 depending on [Mg^{2+}]. Transitions were not counted unless the state lasted at least for two data points, with exceptions being made for one data point transitions that showed anti-correlated behavior in I_D and I_A. To calculate the average rate, the duration of all events from a specified number of molecules were plotted as a histogram and fit to an exponential decay. This method yielded similar decay times with those obtained by autocorrelation analysis. We used autocorrelation analysis to determine the temperature dependent rates.

Acknowledgments

We thank I. Rasnik for experimental help. Funding was provided by the NIH, NSF, Searle Scholars Award (T.H.) and by Cancer Research UK (D.M.J.L.). S.A.M. was partially supported by the NIH molecular biophysics training grant.

Competing interests statement

The authors declare that they have no competing financial interests.

Received 10 October, 2002; accepted 19 November, 2002.

1. Cox, M.M. *et al*. The importance of repairing stalled replication forks. *Nature* **404**, 37–41 (2000).
2. Holliday, R. A mechanism for gene conversion in fungi. *Genet. Res.* **5**, 282–304 (1964).
3. Potter, H. & Dressler, D. On the mechanism of genetic recombination: electron microscopic obervation of recombination intermediates. *Proc. Natl. Acad. Sci. USA* **73**, 3000–3004 (1978).
4. Schwacha, A. & Kleckner, N. Identification of double Holliday junctions as intermediates in meiotic recombination. *Cell* **83**, 783–791 (1995).
5. Lilley, D.M.J. & White, M.F. The junction-resolving enzymes. *Nat. Rev. Mol. Cell. Biol.* **2**, 433–443 (2001).
6. Lilley, D.M.J. Structure of helical junctions in nucleic acids. *Q. Rev. Biophys.* **33**, 109–159 (2000).
7. Clegg, R.M., Murchie, A.I.H. & Lilley, D.M.J. The solution structure of the four-way DNA junction at low-salt conditions — a fluorescence resonance energy transfer analysis. *Biophys. J.* **66**, 99–109 (1994).
8. Duckett, D.R. *et al*. The structure of the Holliday junction, and its resolution. *Cell* **55**, 79–89 (1988).
9. Murchie, A.I. *et al*. Fluorescence energy transfer shows that the four-way DNA junction is a right-handed cross of antiparallel molecules. *Nature* **341**, 763–766 (1989).
10. Nowakowski, J., Shim, P.J., Prasad, G.S., Stout, C.D. & Joyce, G.F. Crystal structure of an 82-nucleotide RNA–DNA complex formed by the 10–23 DNA enzyme. *Nat. Struct. Biol*. **6**, 151–156 (1999).
11. Ortiz-Lombardia, M. *et al*. Crystal structure of a DNA Holliday junction. *Nat. Struct. Biol.* **6**, 913–917 (1999).
12. Eichman, B.F., Vargason, J.M., Mooers, B.H.M. & Ho, P.S. The Holliday junction in an inverted repeat DNA sequence: sequence effects on the structure of four-way junctions. *Proc. Natl. Acad. Sci. USA* **97**, 3971–3976 (2000).
13. Mueller, J.E., Kemper, B., Cunningham, R.P., Kallenbach, N.R. & Seeman, N.C. T4 endonuclease VII cleaves the crossover strands of Holliday junction analogs. *Proc. Natl. Acad. Sci. USA* **85**, 9441–9445 (1988).
14. Bennett, R.J. & West, S.C. RuvC protein resolves Holliday junctions *via* cleavage of the continuous (noncrossover) strands. *Proc. Natl. Acad. Sci. USA* **92**, 5635–5639 (1995).
15. Miick, S.M., Fee, R.S., Millar, D.P. & Chazin, W.J. Crossover isomer bias is the primary sequence-dependent property of immobilized Holliday junctions. *Proc. Natl. Acad. Sci. USA* **94**, 9080–9084 (1997).
16. Grainger, R.J., Murchie, A.I.H. & Lilley, D.M.J. Exchange between stacking conformers in a four-way DNA junction. *Biochemistry* **37**, 23–32 (1998).
17. Chen, J.H., Churchill, M.E., Tullius, T.D., Kallenbach, N.R. & Seeman, N.C. Construction and analysis of monomobile DNA junctions. *Biochemistry* **27**, 6032–6038 (1988).
18. Carlstrom, G. & Chazin, W.J. Sequence dependence and direct measurement of crossover isomer distribution in model Holliday junctions using NMR spectroscopy. *Biochemistry* **35**, 3534–3544 (1996).
19. Overmars, F.J.J. & Altona, C. NMR study of the exchange rate between two stacked conformers of a model Holliday junction. *J. Mol. Biol.* **273**, 519–524 (1997).
20. Weiss, S. Measuring conformational dynamics of biomolecules by single molecule fluorescence spectroscopy. *Nat. Struct. Biol.* **7**, 724–729 (2000).
21. Zhuang, X.W. *et al*. Correlating structural dynamics and function in single ribozyme molecules. *Science* **296**, 1473–1476 (2002).
22. Ha, T. *et al*. Initiation and reinitiation of DNA unwinding by the *Escherichia coli* Rep helicase. *Nature* **419**, 638–641 (2002).
23. Johnson, R.D. & Symington, L.S. Crossed-stranded DNA structures for investigating the molecular dynamics of the Holliday junction. *J. Mol. Biol.* **229**, 812–820 (1993).
24. Panyutin, I.G. & Hsieh, P. The kinetics of spontaneous DNA branch migration. *Proc. Natl. Acad. Sci. USA* **91**, 2021–2025 (1994).
25. Mulrooney, S.B., Fishel, R.A., Hejna, J.A. & Warner, R.C. Preparation of figure 8 and cruciform DNAs and their use in studies of the kinetics of branch migration. *J. Biol. Chem.* **271**, 9648–9659 (1996).
26. Panyutin, I.G., Biswas, I. & Hsieh, P. A pivotal role for the structure of the Holliday junction in DNA branch migration. *EMBO J.* **14**, 1819–1826 (1995).
27. Parson, C.A., Stasiak, A., Bennett, R.J. & West, S.C. Structure of a multisubunit complex that promotes DNA branch migration. *Nature* **374**, 375–378 (1995).
28. Ha, T.J. *et al*. Single-molecule fluorescence spectroscopy of enzyme conformational dynamics and cleavage mechanism. *Proc. Natl. Acad. Sci. USA* **96**, 893–898 (1999).