Supporting Text

Supporting Materials and Methods

Materials. pUC18 was used for the construction of the 1-kbp arm of the Holliday junction. pYWH1050, which is a derivative of pUC18, was constructed by inserting a 2027-bp HindIII fragment of λ phage DNA (23130-25157) into the HindIII site of pUC18 and used for construction of the 4.7-kbp arm of the Holliday junction (see Fig. 6).

Construction of Holliday Junction. The sequences of 12 nucleotides (imHJ-1D, imHJ-1DH, imHJ-1B, imHJ-2, imHJ-4, imHJ-4H, B1, D1, HJY-1, HJY-2, HJY-3, and HJY-4) that were used for preparation of the long arm of the Holliday junction are shown in Table 4. Construction of Holliday junctions for single-molecule and biochemical analyses are depicted in Fig. 6 and 7, respectively, and were performed as described (1). Holliday junction DNA for the single-molecule analysis was constructed as follows: the 4,700-bp fragment I (FI) was prepared by PCR using a pair of primers, imHJ-1D and imHJ-2, and pYWH1050 as a template. The 4,700-bp fragment II (FII) was prepared by PCR using a pair of primers, imHJ-1B and imHJ-2, and pYWH1050 as a template. The digoxigenin labeled fragment (FIII) was prepared by PCR using a pair of primers, D1 and imHJ-2, and FI as a template. The biotin-labeled fragment (FIV) was prepared by PCR using a pair of primers, B1 and imHJ-2 and FII as a template. Synthetic partial duplex DNA, HJY-12 was prepared by annealing HJY-1 and HJY-2. HJY-34 was prepared by annealing HJY-3 and HJY-4. HJY-12 was ligated to the EcoRI-digested FIII, resulting in SI, Y-form digoxigenin labeled DNA. HJY-34 was ligated to the EcoRI-digested FIV, resulting in SII, Y-form biotin labeled DNA. An equal quantity (final concentration, 2 nM) of SI and SII was mixed in TNM buffer (10 mM Tris·HCl pH 8.0/0.1 mM EDTA/50 mM NaCl/10 mM $MgCl_2$) and incubated at 50°C for 2 min to anneal and construct the long arm of the Holliday junction.

Construction of the Holliday junction for biochemical analysis was performed as follows: the 1-kbp fragment 1 (F1) was prepared by PCR using a pair of primers, imHJ-1DH and imHJ-4 and pUC18 as a template. The 1-kbp fragment 2 (F2) was prepared

by PCR using a pair of primers, imHJ-1D and imHJ-4H, and pUC18 as a template. HJY-1 was 5'-end labeled with [γ-^{32}P]ATP. HJY-12 was prepared by annealing 5' end-labeled HJY-1 with HJY-2, and the product was ligated to the EcoRI-digested F1, resulting in S1. HJY-34 was ligated to EcoRI digested F2, resulting in S2. The ^{32}P-labeled S1 (final concentration. 0.4 nM) was mixed with a 10-fold molar excess of unlabeled S2 (final concentration. 4 nM) in TNM buffer and incubated for 2 min at 50°C.

Biochemical Analysis of Branch Migration Kinetics. Biochemical time courses of Holliday junction branch migration by RuvA-RuvB were analyzed by using a set of difference equations followed by the scheme shown in Fig. 4*A*. In this scheme, the initial state was defined as a semi-mobile Holliday junction randomly positioned in the 10-bp region located between the heterologous sequence formed by the single-stranded DNA tails and the mutation in the vicinity of the EcoRI site. After assembly of the RuvA-RuvB complex on a Holliday junction (HJ2* or HJ3*), translocation can proceed in two opposite directions with equal probability. Branch migration to the "left" brings the RuvA-RuvB complex to the heterology sequence formed by the single stranded DNA tails (HJ2*). This causes the translocation of the RuvA-RuvB complex to stop, and the RuvA-RuvB complex can return to the initial state or go through the heterology sequence, resulting in unpaired branch migration substrates (S1*+S2). Unpaired branch migration substrates are capable of reannealing and reforming Holliday junctions (return to the initial state). If a length of Holliday junction DNA is longer than the maximal processivity, the RuvA-RuvB complex may dissociate from the junction on the way of branch migration to the "right." If a length of Holliday junction is shorter than the processivity, branch migration progresses continuously to the distal end, resulting in the irreversible formation of two hetero-duplex products (P1*+P2). Previously, Dennis *et al.* reported that the processivity is more than 4,411 bp (1). Therefore, we used the Holliday junction DNA containing the 1-kbp arm so that we did not need to take account of the processivity and consequently were able to simplify the equations. We formulated the equations as described below with characteristic times and velocity: the time of assembly of RuvA-RuvB on the junctions (τ_{AB}), the time of reannealing of the

unpaired substrates (τ_{anneal}), the time of bypass through the sequence heterology (τ_{het}), the lifetime of the complex stalled at a sequence heterology (τ_{life}) and the mean velocity of branch migration (V). The proportions of HJ*, HJ2*, HJ3*, S1* and P1* are depicted as $f_{HJ*}(n)$, $f_{HJ2*}(n)$, $f_{HJ3*}(n)$, $f_{S1*}(n)$ and $f_{P1*}(n)$, respectively. The probabilities for Δt time steps of each event, the assembly of the complex, the dissociation of the complex at the heterologous sequence, the reannealing of the unpaired substrates and the branch migration through the heterologous sequence are expressed as $\Delta t/\tau_{AB}$, $\Delta t/\tau_{life}$, $\Delta t/\tau_{anneal}$ and $\Delta t/\tau_{het}$, respectively. In this study, we formulate $f_{HJ*}(n)$, $f_{HJ2*}(n)$, $f_{HJ3*}(n)$, $f_{S1*}(n)$ and $f_{P1*}(n)$ as difference equations at Δt = 0.1 sec and set n in 0.1-s time intervals. The equations, $f_{HJ*}(n)$, $f_{HJ2*}(n)$ and $f_{S1*}(n)$ are given in Eqs. **1-3**, respectively.

$$f_{HJ*}(n)=\left(1-\frac{0.2}{\tau_{AB}}\right)f_{HJ*}(n-1)+\frac{0.1}{\tau_{life}}f_{HJ2*}(n-1)+\frac{0.1}{\tau_{anneal}}f_{S1*}(n-1) \quad [\mathbf{1}]$$

$$f_{HJ2*}(n)=\left(1-\frac{0.1}{\tau_{life}}-\frac{0.1}{\tau_{het}}\right)f_{HJ2*}(n-1)+\frac{0.1}{\tau_{AB}}f_{HJ*}(n-1) \quad [\mathbf{2}]$$

$$f_{S1*}(n)=\left(1-\frac{0.1}{\tau_{anneal}}\right)f_{S1*}(n-1)+\frac{0.1}{\tau_{het}}f_{HJ2*}(n-1) \quad [\mathbf{3}]$$

Without consideration of the conversion HJ3* into the irreversible products, P1*+P2, we formulate the equation of the proportion of HJ3* as $g_{HJ3*}(n)$.

$$g_{HJ3*}(n)=g_{HJ3*}(n-1)+\frac{0.1}{\tau_{AB}}f_{HJ*}(n-1) \quad [\mathbf{4}]$$

It takes 1,000/V s to convert HJ3* into P1*+P2. Assuming that all HJ3* is converted into P1* after 1,000/V s of branch migration, when n is below 10,000/V, P1* does not appear, and when n is 10,000/V or more, $f_{P1*}(n)$ can be formulated by moving $g_{HJ3*}(n)$ in parallel by 10,000/V. Therefore, $f_{P1*}(n)$ is given as follows:

$$f_{P1*}(n)=\begin{cases}0 & \left(n<\dfrac{10000}{\mathrm{V}}\right)\\ g_{HJ3*}\left(n-\dfrac{10000}{\mathrm{V}}\right) & \left(n\geq\dfrac{10000}{\mathrm{V}}\right)\end{cases} \quad [\mathbf{5}]$$

$f_{HJ3*}(n)$ is given by subtraction of $f_{P1*}(n)$ from $g_{HJ3*}(n)$.

$$f_{HJ3*}(n)=g_{HJ3*}(n)-f_{P1*}(n) \quad [\mathbf{6}]$$

The difference equations were written and the value of the parameter was formulated to fit the obtained data using the Solver in Microsoft Excel.

Supporting Results

Kinetic Parameters. In this study, we could estimate the branch migration rate by the biochemical analysis (Fig. 4 and 5). Furthermore, we also obtained the other kinetic parameters (see Table 3). The two kinetic parameters τ_{AB} and τ_{het} depend on ATP concentration. Branch migration rates through the heterologous region could be calculated based on τ_{het} (Table 3). These results provide a *Km* value of 130 μM and a Vmax of 0.70 bp·s^{-1}. The Km value is a 4-fold increase relative to that through the homologous region. The Vmax value is a 30-fold decrease relative to the rate of branch migration through the homologous region. The decrease of branch migration rates is consistent with the results reported in ref. 1. The time constants for the reannealing of S1* and S2 and the lifetime of the RuvA-RuvB complex stalled at a sequence heterology were more than several thousand seconds (data not shown), indicating that the reannealing of S1* and S2 and the dissociation of the RuvA-RuvB complex at a sequence heterology did not occur particularly frequently.

Biochemical Analysis Under Various Conditions. In this study, the *V*max value of the rotation rate was one-third of that determined by biochemical analysis. One of the possibilities to explain the reduction of the maximal rate is the differences in buffer conditions between the single-molecule and the biochemical analysis. Therefore, we also determined the kinetic parameters on various buffer conditions shown in Fig. 8 to ascertain whether the reduction of the maximal rate was caused by the difference of buffer conditions between two analyses. As shown in Fig. 8 *A*, *C*, and *E*, under the buffer conditions at pH 7.5 or including 1 mg/ml casein, the branch migration rates are consistent with the maximal rate of the biochemical analysis (Fig. 4), indicating that pH or addition of casein is not the cause of the reduction of the maximal rate. On the other hand, under the buffer condition containing 300 mM KCl or 300 mM KCl and 1 mg/ml casein, the branch migration rate is ~70 % of that without KCl, indicating that high concentrations of KCl is the cause of reduction of the maximal rate (Fig. 8 *B*, *D*, and *E*). However, the *Vmax* value of the rotation rate was one-third of that determined by biochemical analysis; therefore, only high concentrations of KCl is not the cause of the reduction of the branch migration rate.

1. Dennis, C., Fedorov, A., Kas, E., Salome, L. & Grigoriev, M. (2004) *EMBO J.* **23,** 2413-22.