Inventory of Supplemental Information

Supplemental Information

Phage Mu transposition target immunity: protein distribution pattern formation along DNA by a diffusion-ratchet mechanism

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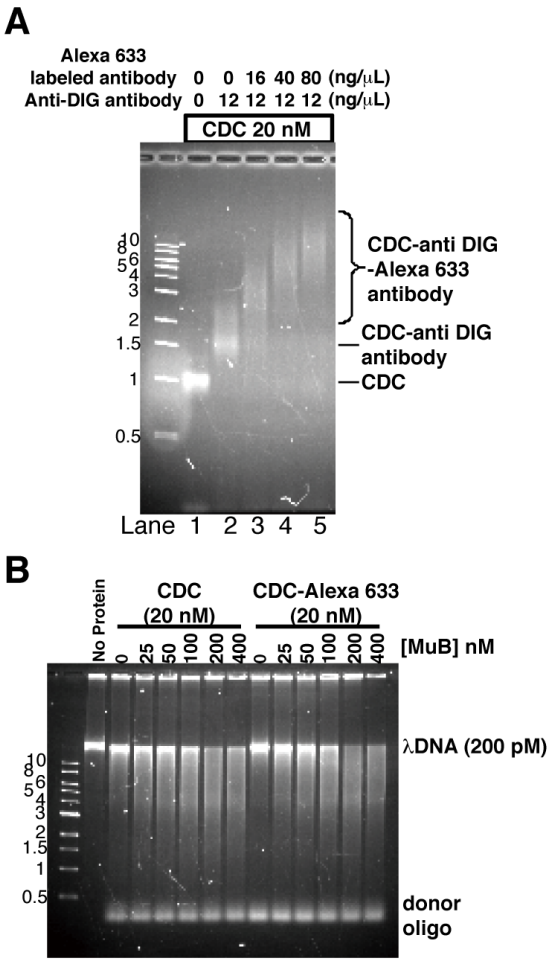

Figure S1, related Figure 1. Construction and strand transfer activity of fluorescence-labeled transpososomes. (A) Construction of fluorescence labeled transpososome. CDCs (lane 1) were incubated in a buffer containing anti-DIG antibody at the indicated concentration (lane 2). The indicated amount of Alexa-633 labeled antibody was mixed with CDC-anti-DIG antibody complex and the mixtures were incubated 30 ºC for 20 min (lane 3-5). The samples were analyzed by 1.5 % agarose gel electrophoresis containing heparin and BSA (Mizuuchi and Mizuuchi, 2001). (B) Strand transfer activity was examined in a buffer (25 mM Hepes pH 7.6, 2 mM ATP, 1 mM DTT, 0.1 mg/mL BSA, 7.5 % glycerol, 150 mM NaCl, 10 mM $MgCl_2$, 15 % DMSO and 0.04 % Triton) containing 200 pM λ DNA, the indicated amount of MuB and 20 nM unlabeled or fluorescent labeled transpososome. The mixtures (20 μL) were incubated at 30 ºC for 30 min. The reactions were terminated by addition of 5 μL stop buffer (100 mM Tris-HCl (pH 7.5), 1 % SDS and 60 mM EDTA) and incubation at 70 ºC for 10 min.

The samples were analyzed by 0.8 % agarose gel electrophoresis. Both strands of the λ DNA are cut by the insertion of the transpososome, but held together within the stable transpososome complex. After protein removal, the λ DNA is fragmented at the sites of transpososome insertion. Decrease in the full-size λ DNA and increase of the shorter fragments reflected the strand transfer activity of the transpososome.

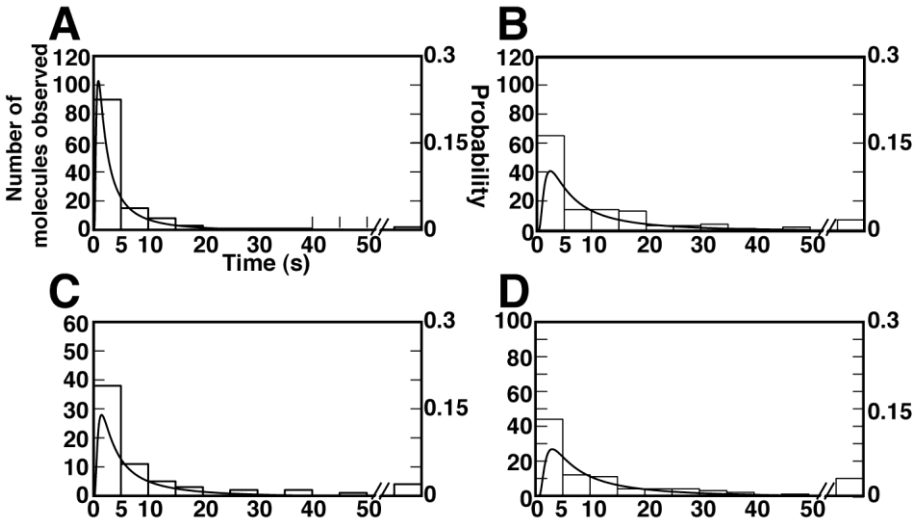

Figure S2, related Figure 3. DNA loop lifetime measurements. A, B, C and D indicate the results with the buffer flow interruption times of 15, 30, 60 and 120 second, respectively. Data were fitted to an inverse Gaussian distribution, which is a two-parameter probability (0-∞) distribution function that describes the distribution of time that takes for a Brownian motion with positive drift to reach a fixed level, and the shape parameter λ allows increased contribution of the tail portion of the distribution relative to the Gaussian distribution. The fitting parameters are shown in Table S3.

We took advantage of the stable tetrameric MuA cluster in the form of the STC to observe DNA loops that persist many seconds. We interpreted that the multiple MuB interacting domains of the MuA tetramer could processively activate the ATPase activity of multiple MuB molecules during disassembly of a MuB cluster and the multiple rounds of the iterative process would take many seconds (Greene and Mizuuchi, 2002c). This local processivity model suggests that the lifetime of the DNA loops may correlate with the size of the MuB clusters. With this hypothesis in mind, we measured the lifetime of the individual DNA loops. The lifetime distribution observed after different durations of free Brownian motion qualitatively looked similar to each other, but the observed average lifetime was shorter with shorter durations of free Brownian motion and the distribution tail grew longer with longer durations of free Brownian motion (Table S3).

The above result suggests that with longer durations of free Brownian motion, the MuA clusters have a tendency to interact with larger clusters of MuB compared to

earlier times, for example because very small MuB clusters might be depleted earlier during buffer flow interruption. At the beginning of the free Brownian motion of DNA, the MuB cluster size distribution would not be independent of the distance from a MuA cluster because during the initial 30 min of buffer flow, MuB cluster distribution would have reached a steady state without the impact of the looping interaction with the MuA cluster. As expected, we did not find any correlation between the observed loop size and the loop lifetime after a 15 sec buffer flow interruption (data not shown). After an extended period of free Brownian motion, larger MuB clusters located near a MuA cluster might have become scarce. However, MuB removal was relatively slow (Fig. 4A) and therefore we did not detect a correlation between the observed loop size and loop lifetime after 2 min of free Brownian motion either.

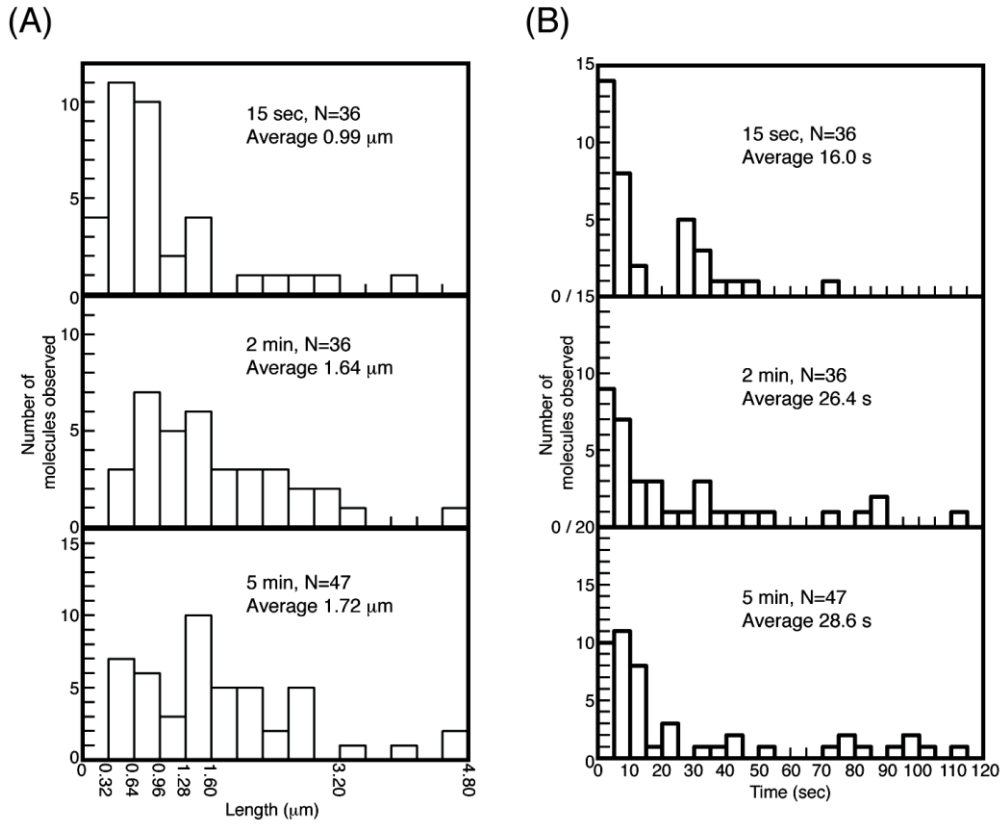

Figure S3, related to Figure 3. (A) **DNA loop length measurement using a mixture of wild type MuB and EGFP-MuB.** DNA loop length was measured after different flow interruption durations, 15 sec, 2 min and 5 min from the top. The experiments were carried out as for Figure 3. The concentrations of wild type and EGFP-MuB were 22.5 and 2.5 nM, respectively. (B) **DNA loop lifetime measurement using a mixture of wild type MuB and EGFP-MuB.** DNA loop lifetime was measured after different flow interruption durations, 15 sec, 2 min and 5 min from the top. The experiments were carried out as for Figure 3. The concentrations of wild type and EGFP-MuB were 22.5 and 2.5 nM, respectively.

Table S1, related to Figure 2. Number of fluorescence labeled MuA clusters on a λ DNA.

Number of fluorescent labeled CDC on a single λ DNA.	**Number of examples observed. Input λ/CDC ratio 1:20[a].**	**Number of examples observed. Input λ/CDC ratio 1:50[b].**
0	28	2
1	45	5
2	20	5
3	18	5
4	6	10
5	3	5
6	0	2
7	0	1
8	0	1
Total[c]	120	36
Average[d]	1.5	3.4

[a]Number of examples of the λ DNA molecules observed with the number of fluorescence labeled MuA clusters indicated in the left column when the λ DNA and the CDC were mixed at a ratio of 1 to 20.

[b]Number of examples of the λ DNA molecules observed with the number of fluorescence labeled MuA clusters indicated in the left column when the λ DNA and the CDC were mixed at a ratio of 1 to 50.

[c]Total number of λ DNA samples scored.

[d]Average number of fluorescence labeled MuA clusters on a single λ DNA.

Table S2, related to Figure 3. Fraction of the MuA clusters that had detectable loop formation.

Time of Buffer Flow Interruption (sec)	Number of Loops Detected	Number of STCs	Ratio
15	119	490	0.24
30	126	404	0.31
60	66	340	0.19
120	95	337	0.28

Table S3, related to Figure 3. DNA loop statistics.

(A). Fitting parameters for the detected DNA loop size distribution.

Time of Buffer Flow Interruption (sec)	**Mean (μm)**	**Variance (μm^2)**	**λ[a]**
15	0.99	1.03	0.94
30	1.43	1.85	1.59
60	2.01	2.66	3.09
120	2.02	2.72	3.05

(B). Fitting parameters for the detected DNA loop lifetime distribution.

Time of Buffer Flow Interruption (sec)	**Mean (sec)**	**Variance (sec^2)**	**λ[a]**
15	6.1	87.2	2.67
30	12.3	233	8.02
60	11.4	307	4.83
120	17.3	576	8.99

[a] λ is the shape parameter calculated as follows. $\lambda = \frac{M^3}{V}$, where M and V are Mean and Variance, respectively. This parameter is used for inverse Gaussian distribution function fitting as follows. $f(x) = \left(\frac{\lambda}{2\pi x^3}\right)^{\frac{1}{2}} \exp\frac{-\lambda(x-M)^2}{2M^2 x}$

Movie S1, related to Figure 2. Simultaneous observation of Alexa-633 marked MuA clusters and EGFP-MuB on a single λ DNA. This movie shows EGFP-MuB binding to λ DNA containing Alexa-633 marked MuA clusters. Top panel shows fluorescent signal from EGFP-MuB and bottom panel shows fluorescent signal from Alexa-633 marked MuA clusters. Buffer containing 25 nM EGFP-MuB was infused. The video rate is 150 times as fast as real time. The area displayed is 80 μm x 80 μm.

Movie S2, related to Figure 2. One-step extension of the DNA loop bridged by an Alexa-633 marked MuA cluster and an EGFP-MuB target complex (wide field view). Buffer flow interruption and restart. Top panel shows fluorescent signal from EGFP-MuB and the bottom panel shows the fluorescent signal from Alexa-633 marked MuA clusters. Buffer containing 25 nM EGFP-MuB was infused. Buffer flow was interrupted for 15 sec and restarted. The video rate is 10 times as fast as real time. The area displayed is 80 μm x 80 μm.

Movie S3, related to Figure 2. One-step extension of the DNA loop bridged by an Alexa-633 marked MuA cluster and an EGFP-MuB target complex (an individual molecule). This movie shows the fluorescent signal from EGFP-MuB (left panel) and that of the Alexa-633 marked MuA clusters (right panel), which corresponds to Figure 2C. From 5 sec after buffer flow stop to 10 sec before buffer flow restart, the data was not recorded to avoid excessive beam exposure. The video rate is 10 times as fast as real time. The area displayed is 16 μm x 3.2 μm.

Supplemental Experimental Procedures

Oligo-nucleotides. Oligos used to make fluorescent labeled Cleaved Donor Complex (CDC) were Y005, 5'-CAG AGT GAA GCG GCG CAC GAA AAA CGC GAA AGC GTT TCA CGA TAA ATG CGA, and Y006, 5'-DTC CAT CGG AAC CGT AGA CCG TGT TTT CGC ATT TAT CGT GAA ACG CTT TCG CGT TTT TCG TGC GCC GCT TCA. "D" at the 5' end of Y006 stands for digoxigenin (DIG) to be complexed with anti-DIG antibody. Oligos were annealed to make donor dsDNA. Oligos used for DNA binding assay were Y014, 5'-T*GA GTG TGT AAA TTT TAA TTT A and Y015, 5'-T*TA CAC ACT CAT AAA TTA AAA T. Both were phosphorylated at the 5' ends and "T*" at the 5' ends stands for amino-modified thymine.

DNA binding assay. Amino-modified oligos were fluorescence labeled using Alexa-514 carboxylic acid, succinimidyl ester (Invitrogen) and purified according to the protocol of the manufacturer. Two 22 nt oligos, complementary to each other by a 11 nt stagger, were mixed in a buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 1 mM ATP and 10 mM DTT), annealed overnight and ligated to generate poly-dispersed fragments

(average size of several kbp) with sequence repeats containing a dye at every 11 bp. The reaction mixture was incubated at 65 ºC for 20 min to inactivate Ligase, and the buffer was changed to TE using a spin column.

All kinetic measurements were carried out using a stopped-flow spectrafluorometer (Model SX20; Applied Photophysics) equipped with dual photomultiplier tubes. Filters used for the PMTs were Semrock FF01-494/41-25 (for ECFP) and Semrock FF01-542/41-25 (for Alexa-514). Excitation was at 425 nm for ECFP-MuB. The donor signal quenching was used for ECFP-MuB DNA binding analysis. Fluorescence energy transfer was calculated from the signals from the two channels with corrections for spillover. Time course curves based on the donor signal quenching and the FRET efficiency were superimposable. For the wild type MuB binding to Alexa-514 labeled DNA, one PMT with Semrock filter FF01-542/41-25 was used. Excitation was at 495 nm. All kinetic data curves represent the average of at least 4 experiments.

Supplemental References

Mizuuchi, M., and Mizuuchi, K. (2001). Conformational isomerization in phage Mu transpososome assembly: effects of the transpositional enhancer and of MuB. EMBO J

20, 6927-6935.