Supplemental Data

RecBCD Enzyme Switches Lead Motor Subunits

in Response to χ Recognition

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Table S1. Analysis of Wild-Type RecBCD Enzyme-Mediated Unwinding of χ-Containing DNA

molecule	initial rate (bp/s) ± standard error*	pause duration (s) +/- standard error†	paused at (bp from the free end)	rate after chi (bp/s) ± standard error*	unwound (bp)
BCD-01	324 ± 11	4.7 ± 0.9	8480	305 ± 21	12516
BCD-02	610 ± 47	6.5 ± 1.3	6230	395 ± 21	11555
BCD-03	576 ± 41	4.1 ± 0.5	6341	513 ± 22	13686
BCD-04	1287 ± 105	8.7 ± 4.8	6208	70 ± 13	7365
BCD-05* *	**987 ± 84**	**4.6 ± 1.3**	**7635**	**336 ± 6**	**21096**
BCD-06	480 ± 52	9.1 ± 2.1	5780	436 ± 5	28892
BCD-07	967 ± 127	0.9 ± 0.5	6391	372 ± 4	28142
BCD-08	594 ± 67	11.1 ± 2.2	5894	567 ± 6	**30870‡**
BCD-09	933 ± 68	12.2 ± 1.7	8550	649 ± 9	**30870‡**
BCD-10	91 ± 6	*dissociated before chi*			2586
BCD-11	970 ± 11	*didn't stop at chi*			26951
BCD-12	1014 ± 69	3.2 ± 0.6	6756	417 ± 13	14425
BCD-13	1039 ± 81	5.6 ± 1	8421	768 ± 14	**30870‡**
BCD-14	324 ± 26	*dissociated before chi*			4258
BCD-15	1050 ± 83	0.3 ± 0.1	6124	314 ± 16	11091
BCD-16	303 ± 36	*dissociated before chi*			5798
BCD-17	151 ± 23	3.4 ± 1.5	4089	70 ± 2	7693
BCD-18	165 ± 4	*didn't stop at chi*			6516
BCD-19	126 ± 6	*dissociated before chi*			2968
BCD-20	313 ± 13	5.92 ± 0.7	4239	193 ± 1	16675
BCD-21	703 ± 48	2.81 ± 0.6	7628	603 ± 7	**30870‡**
BCD-22	786 ± 44	1.66 ± 0.3	6272	345 ± 4	18345
Average ± standard deviation	*629 ± 368*	*5.3 ± 3.5*	*6,500 ± 1,300*	*397 ± 194*	*16,000 ± 10,000*

26 molecules were trapped. DNA unwinding was detected in 22 cases. Only molecules that unwound DNA are presented in the table. Representative traces are shown on the right.

* Standard error resulted from fitting experimental data;
† The duration of the pause was calculated by subtracting the time when the pause began from the time when the pause ended. Standard error for each pause was calculated using following formula:

$$\text{St. error} = (t2-t1) * \sqrt{(\text{st. error on } t1)^2/t1 + (\text{st. error on } t2)^2/t2}$$, where t1 and t2 are the beginning and the end of the pause.
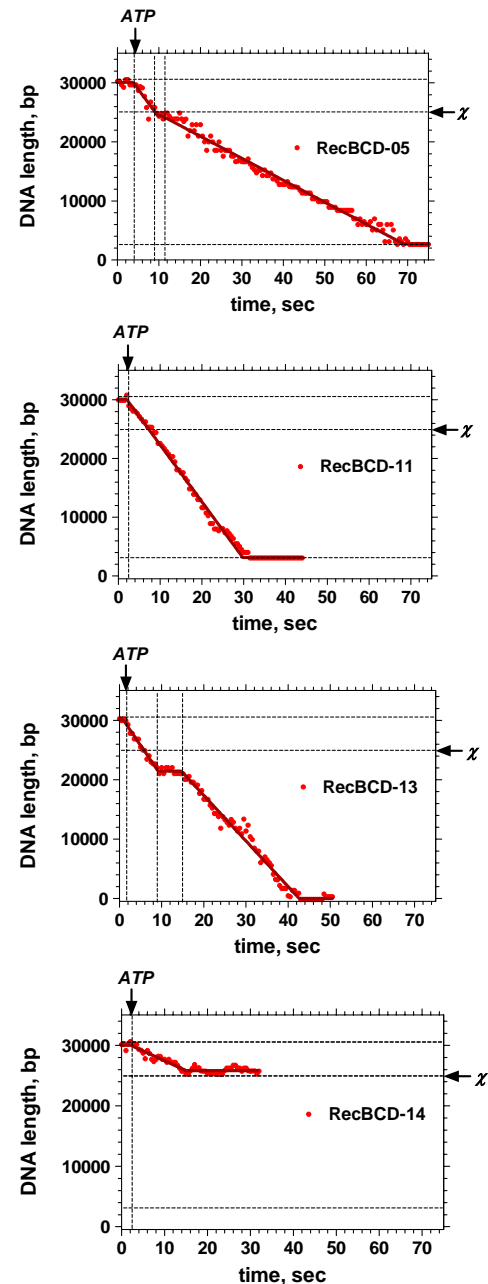‡ DNA was unwound completely;
** Molecule depicted in Figure 2.

Table S2. Analysis of RecBCD^K177Q Enzyme-Mediated Unwinding of χ-Containing DNA

molecule	*initial rate (bp/s) +/- standard error**	*pause duration (s) +/- standard error†*	*paused at (bp from the free end)*	*rate after chi (bp/s) +/- standard error**	*unwound (bp)*
BCDkq-01	282 ± 6	4.53 ± 0.8	6469	311 ± 134	6646
BCDkq-02	399 ± 31	*dissociated before chi*			4369
BCDkq-03	239 ± 18	*dissociated before chi*			4881
BCDkq-04	157 ± 20	6.5 ± 2.6	5979	142 ± 25	6866
BCDkq-05	217 ± 11	5.4 ± 1.7	5980	216 ± 82	6409
BCDkq-06	400 ± 33	4.4 ± 1.4	5611	383 ± 121	6455
BCDkq-07	718 ± 55	2.2 ± 0.5	6688	767 ± 114	9847
BCDkq-08	307 ± 22	8.4 ± 1.8	5560	304 ± 37	7294
BCDkq-09	295 ± 20	*didn't stop at chi*			7329
BCDkq-10	260 ± 19	15.2 ± 3.8	5419	264 ± 25	8996
BCDkq-11	585 ± 35	*didn't stop at chi*			6882
BCDkq-12	383 ± 16	*didn't stop at chi*			7299
BCDkq-13	376 ± 30	*dissociated before chi*			4739
BCDkq-14	455 ± 34	6.2 ± 1.9	4600	340 ± 167	5756
BCDkq-15	281 ± 19	*dissociated before chi*			4108
BCDkq-16	190 ± 12	*dissociated before chi*			4114
BCDkq-17	227 ± 12	*dissociated before chi*			4491
BCDkq-18	214 ± 10	*dissociated before chi*			4758
BCDkq-19	290 ± 19	15.5 ± 3.3	6735	197 ± 15	9153
BCDkq-20*	**361 ± 13**	**4.5 ± 0.9**	**5276**	**325 ± 49**	**8664**
BCDkq-21	349 ± 23	10.7 ± 2.7	6440	337 ± 122	7078
Average ± standard deviation	*333 ± 133*	*7.6 ± 4.4*	*5,890 ± 670*	*326.0 ± 163*	*6,500 ± 1,700*

26 molecules were trapped. DNA unwinding was detected in 21 cases. Only molecules that unwound DNA are presented in the table. Representative traces are shown on the right.

* Standard error resulted from fitting experimental data;
† The duration of the pause was calculated by subtracting the time when the pause began from the time when the pause ended. Standard error for each pause was calculated using following formula

St. error $=(t2-t1) * \sqrt{(\text{st. error on } t1)^2/t1+(\text{st. error on } t2)^2/t2}$,where t1 and t2 are the beginning and the end of the pause
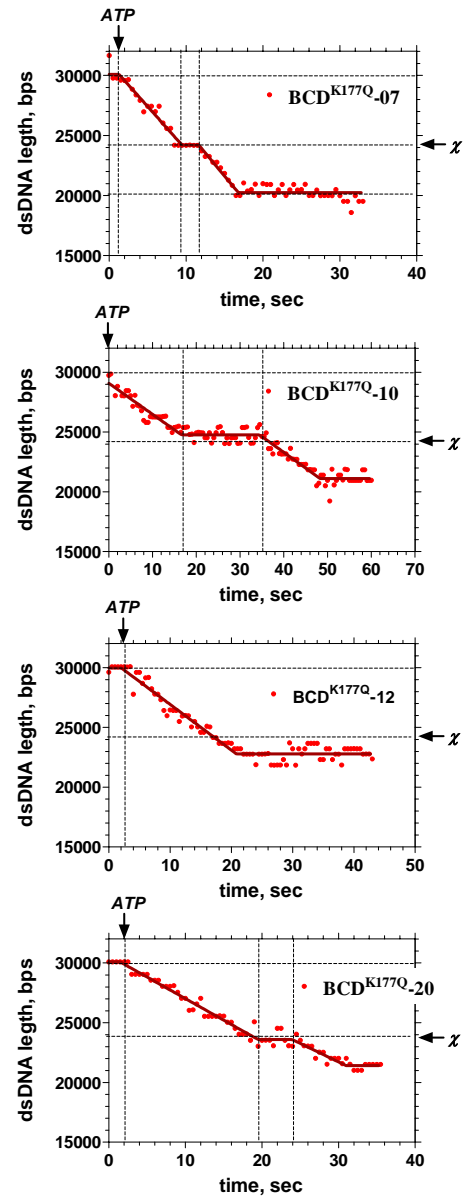** Molecule depicted in Figure 2.

Table S3. Analysis of Wild-Type RecBCD Enzyme-Mediated Unwinding of χ-Containing DNA in the Presence of P1 Nuclease

molecule	initial rate (bp/s)+/- (standard error)*	comment	unwound (bp)
BCD-P1_01	836 ± 10	*didn't stop at chi*	**30870**‡
BCD-P1_03	90 ± 5	*dissociated before chi*	4415
BCD-P1_04	449 ± 4	*didn't stop at chi*	21685
BCD-P1_05	215 ± 5	*didn't stop at chi*	7425
BCD-P1_06	337 ± 19	*dissociated before chi*	3713
BCD-P1_07	623 ± 12	*didn't stop at chi*	17816
BCD-P1_08	187 ± 5	*didn't stop at chi*	6240
BCD-P1_09	367 ± 6	*didn't stop at chi*	11267
BCD-P1_10	844 ± 40	*didn't stop at chi*	6389
BCD-P1_11	440 ± 18	*dissociated before chi*	5050
BCD-P1_12	354 ± 19	*dissociated before chi*	4940
BCD-P1_13	389 ± 6	*didn't stop at chi*	17604
BCD-P1_14	658 ± 15	*didn't stop at chi*	7845
BCD-P1_15	823 ± 7	*didn't stop at chi*	18821
BCD-P1_16	785 ± 6	*didn't stop at chi*	**30870**‡
BCD-P1_17	720 ± 19	*didn't stop at chi*	6644
BCD-P1_18**	**911 ± 6**	*didn't stop at chi*	**30870**‡
BCD-P1_19	416 ± 33	*dissociated before chi*	3502
BCD-P1_21	960 ± 7	*didn't stop at chi*	25536
BCD-P1_22	921 ± 18	*didn't stop at chi*	11726
BCD-P1_23	812 ± 43	*didn't stop at chi*	6065
BCD-P1_25	636 ± 19	*didn't stop at chi*	7028
BCD-P1_26	1043 ± 7	*didn't stop at chi*	**30870**‡
BCD-P1_28	805 ± 13	*didn't stop at chi*	12112
Average ± standard deviation	*609 ± 274*		*13,700 ± 9,800*

28 molecules were trapped. DNA unwinding was detected in 23 cases. Only molecules that unwound DNA are presented in the table. Representative traces are shown on the right.

* Standard error resulted from fitting experimental data.

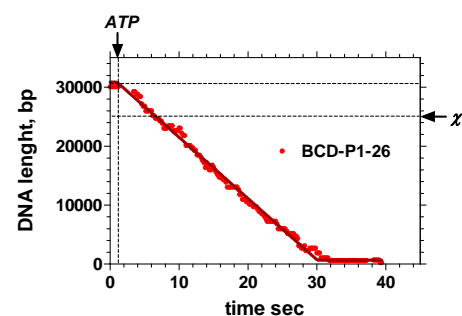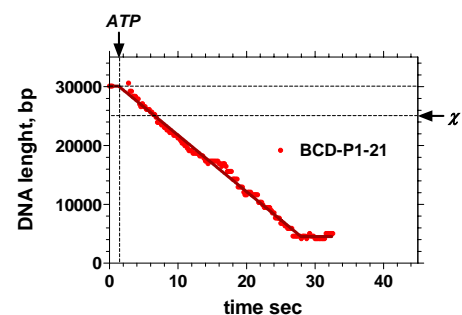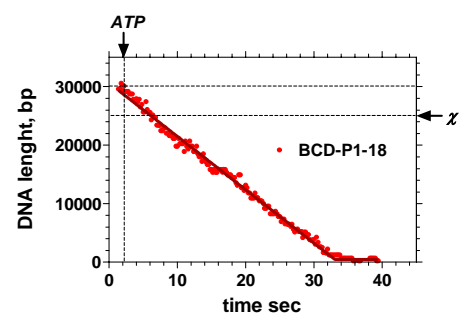‡ DNA was unwound completely.
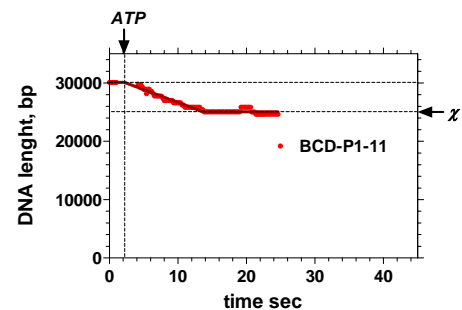
** Molecule depicted in Figure 3.

Table S4. Analysis of RecBCD^{K177Q} Enzyme-Mediated Unwinding of χ-Containing DNA in the Presence of P1 Nuclease

Table S4. Analysis of RecBCD^{K177Q} Enzyme-Mediated Unwinding of χ-Containing DNA in the Presence of P1 Nuclease

molecule	initial rate (bp/s) +/- standard error*	pause duration (s) +/- standard error†	paused at (bp from the free end)	rate after chi (bp/s) +/- standard error*	unwound (bp)
BCDkq-P1-01	476 ± 18	didn't stop at chi			6449
BCDkq-P1-02	392 ± 32	stopped near chi			5533
BCDkq-P1-03	369 ± 21	11.4 ± 3.1	6739	207 ± 57	7426
BCDkq-P1-04	398 ± 11	5.3 ± 0.8	7480	414 ± 95	8459
BCDkq-P1-05	466 ± 26	6.3 ± 1.2	5433	379 ± 102	6053
BCDkq-P1-06	511 ± 55	4.4 ± 0.9	4761	517 ± 157	5826
BCDkq-P1-07	397 ± 22	stopped before chi			3541
BCDkq-P1-08	412 ± 23	1.5 ± 0.4	5061	461 ± 200	5509
BCDkq-P1-09	335 ± 11	didn't stop at chi			6019
BCDkq-P1-10	364 ± 14	didn't stop at chi			5732
BCDkq-P1-11	437 ± 22	3 ± 0.7	6894	378 ± 58	8514
BCDkq-P1-12**	454 ± 44	8.3 ± 2.1	5114	458 ± 18	13141
Average ± standard deviation	*417.4 ± 52*	*5.7 ± 3.3*	*5,900 ± 1,000*	*402 ± 99*	*6850.2*

14 molecules were trapped. DNA unwinding was detected in 12 cases. Only molecules that unwound DNA are presented in the table.

* Standard error resulted from fitting experimental data.
† The duration of the pause was calculated by subtracting the time when the pause began from the time when the pause ended. Standard error for each pause was calculated using following formula:

$$\text{St. error} = (t2-t1) * \sqrt{(\text{st. error on } t1)^2/t1 + (\text{st. error on } t2)^2/t2}\ ,$$

where t1 and t2 are the beginning and the end of the pause.
** Molecule depicted in Figure 3.