Supporting Materials

Coordinated reversal of flagellar motors on a single *Escherichia coli* cell

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Supplementary Materials and Methods

Calculation of diffusion of CheY-P molecules from chemoreceptor patch

It is difficult to estimate the absolute value of delay time of switching for the activation of chemoreceptors because we could not know the time of the activation of them. Therefore, the $\Delta\tau$ value (see Eq. 2) was plotted against $M2^2 - M1^2$, where $M1$ and $M2$ were the distances from the chemoreceptor patch to the closest motor and to the furthest of the two motors, respectively. If switching of the motors is caused by CheY-P spread from chemoreceptors by a simple one-dimensional diffusion, the relationship between $\Delta\tau$ values and $M2^2 - M1^2$ should be linear (Eq. 2).

$$\langle M2^2 \rangle = 2 \cdot D \cdot t_2$$

$$\langle M1^2 \rangle = 2 \cdot D \cdot t_1$$

$$\langle M2^2 \rangle - \langle M1^2 \rangle = 2 \cdot D \cdot (t_2 - t_1) = 2 \cdot D \cdot \Delta\tau \qquad \text{Eq. 2}$$

where D and t are the diffusion coefficient and delay time of switching for the activation of chemoreceptors, respectively. $t_2 - t_1$ is defined as $\Delta\tau$. Assuming the CheY-P molecules spread by simple one-dimensional diffusion, the concentration of CheY-P (C) at a certain position (x) against a time (t) is expressed by Eq. 3.

$$C \propto \frac{1}{\sqrt{4 \cdot \pi \cdot D \cdot t}} \cdot e^{-\frac{x^2}{4 \cdot D \cdot t}} \qquad \text{Eq. 3}$$

Table S1
Bacterial strains and plasmids

	Description	Reference
Strains		
RP437	Wild-type for motility and chemtaxis	(1)
EFS031	RP437 Δ*motAmotB fliC-sticky*	This work
EFS032	RP437 Δ*motAmotB* Δ*cheZ fliC-sticky*	This work
Plasmids		
pMMB206	Cm^r $P_{tac\text{-}lac}$	(2)
pBAD24	Ap^r P_{BAD}	(3)
pFLAG-CTC	Ap^r P_{tac}	Sigma-Aldrich
pTH2300	*motAmotB* in pMMB206	This work
pBAD24-GFP-CheW	*gfp-cheW* in pBAD24	Prof. I. Kawagishi
pAH115	*cheY*-D13K in pFLAG-CTC	Prof. I. Kawagishi
pFSZ1	*cheZ* in pBAD24	This work

Ap^r, ampicillin-resistant; Cm^r, chloramphenicol-resistant; P_{lac}, *lac* promoter; P_{tac}, *tac* promotor; P_{BAD}, *araBAD* promoter.

Supplementary Figure Legends

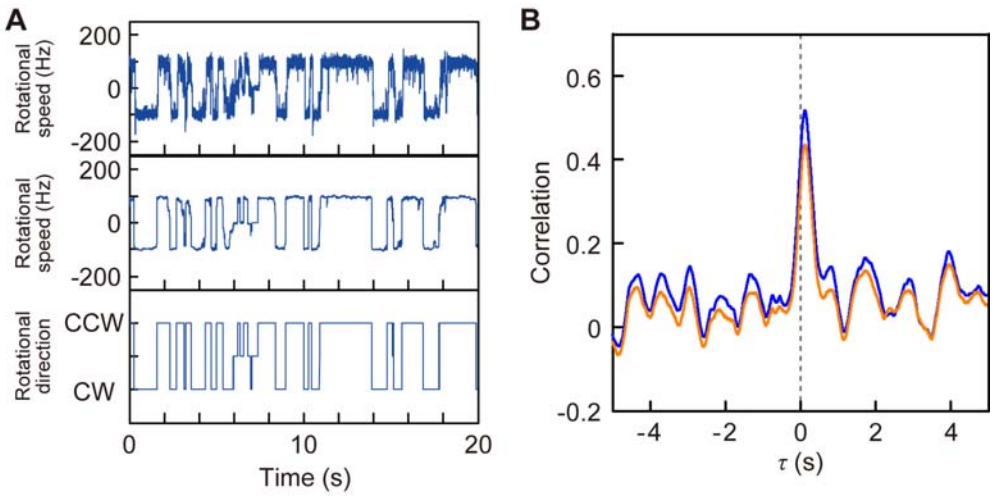
FIGURE S1

Correlation analysis for raw traces and for the traces assigned to 3 states. (A) To extract the time course of switching from raw trace of rotational speed, we performed following procedure. The top panel shows the typical raw trace of rotational speed. The raw trace of rotational speed was filtered by the Chug-Kennedy filtering algorithm (C-K filter) (middle trace). An analytical window of 100 data-points and a weight of 10 were used in the filtering procedure. From the trace of rotational speed run through the C-K filter, rotational speed more than 20 Hz, between ±20 Hz, and less than -20 Hz were assigned CCW, pause, and CW, respectively (bottom trace). Resultant traces of rotational direction against time were subjected to a correlation analysis. (B) Orange and blue lines indicate the correlation analyses for the raw traces and for the traces assigned to 3 states, respectively. The data that were shown Fig. 2A were used for the analyses. Both correlation analyses showed similar profile, and the apparent near 0-s peaks were shown. Therefore, procedure for the classification does not affect to the correlation analysis.

FIGURE S2

Sequential images of 2 beads attached to flagellar filaments on the same cell. The time shown on the images corresponds to Fig. 5B. Images are shown every 0.8 ms. These images include ones shown in Fig. 5A.

FIGURE S1


A
Rotational speed (Hz)
200
0
-200
Rotational speed (Hz)
200
0
-200
Rotational direction
CCW
CW
0
10
20
Time (s)
B
Correlation
0.6
0.4
0.2
0
-0.2
-4
-2
0
2
4
τ (s)

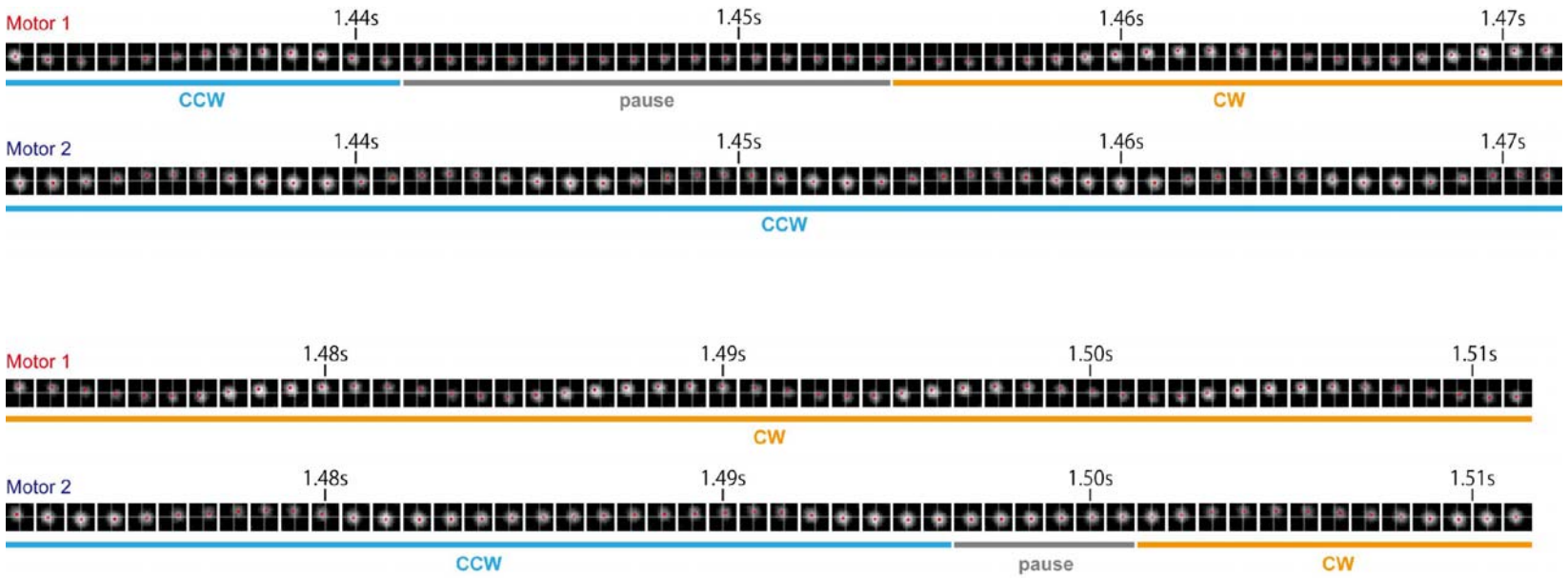

Motor 1
1.44s
1.45s
1.46s
1.47s
CCW
pause
CW
Motor 2
1.44s
1.45s
1.46s
1.47s
CCW
Motor 1
1.48s
1.49s
1.50s
1.51s
CW
Motor 2
1.48s
1.49s
1.50s
1.51s
CCW
pause
CW

FIGURE S2

Supplementary References

1. Parkinson, J. S., and S. E. Houts. 1982. Isolation and behavior of *Escherichia coli* deletion mutants lacking chemotaxis functions. *J. Bacteriol.* 151:106-113.
2. Morales, B. M., A. Backman, and M. Bagdasarian. 1991. A series of wide-host-range low-copy-number vectors that allow direct screening for recombinants. *Gene* 97:39-47.
3. Guzman, L. M., D. Belin, M. J. Carson, and J. Beckwith. 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *J. Bacteriol.* 177:4121-4130.

Supplementary Movie legends

Movie S1 The rotational motions of three motors in cells with expressing GFP-CheW. The fluorescence image (green) is superimposed on the phase-contrast image. Green spot at the pole of cells indicate the localization of GFP-CheW, which represents the position of chemoreceptor patch. The sampling and play rates were 1,250 and 30 frames/s, respectively. Therefore, the play speed of video is 42 times slowed. The movie represents the switching behavior that is shown in Fig. 5B (from 0 to 2.4 s). Note that switching of the motor closer to the chemoreceptor patch consistently precedes switching of the motor farther from the patch.