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Motor Adaptive Remodeling Speeds Up Bacterial Chemotactic Adaptation

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ABSTRACT Bacterial chemotaxis is a canonical system for the study of signal transduction. One of the hallmarks of this system is its robust adaptive behavior. However, how fast the system adapts remains controversial. The adaptation time measured at the level of the kinase activity was tens of seconds, whereas that measured at the level of the flagellar motor was <10 s. The flagellar motor was recently shown to exhibit adaptive remodeling, its main physiological function being to provide a robust match between the chemoreceptor output and the motor input, whereas its adaptation timescale was thought to be too slow to contribute much to the overall adaptation timescale of the chemotaxis system. Here, through theoretical modeling of the motor adaptive remodeling and experimental tests, we show that this motor adaptation contributes significantly to speeding up the overall chemotactic adaptation, thereby resolving the previous inconsistency.

INTRODUCTION

The chemotaxis system allows bacteria to sense and respond to changes in concentrations of chemical attractants or repellents in the environment (1,2). Receptor clusters process input (1–3), with signal relaying to the flagellar motor to generate output (4). Binding of the chemicals to receptors modulates the activity of an associated histidine kinase, CheA, thereby changing the level of phosphorylation of a small diffusible protein, CheY. Phosphorylated CheY (called CheY-P) binds to FliM, a component of the switch complex at the base of the flagellar motor, and modulates the direction of motor rotation (5–7). A phosphatase, CheZ, dephosphorylates CheY-P. The chemotaxis system exhibits robust, perfect adaptation (8–10). After a stepwise stimulus, the system output, measured by the CheY-P level or the directional bias of the motor rotation, changes abruptly before slowly readapting to its pre-stimulus level. Adaptation at the receptor level is mediated by receptor methylation and demethylation by the corresponding enzymes (CheR and CheB).

The adaptation time, measured using the CheY-P level or the motor rotation directional bias as the system output, has been controversial. The measurements performed by monitoring the motor-rotation directional bias led to an adaptation time ranging from 4 to 9 s in a wild-type *Escherichia coli* cell at room temperature (11–13), whereas measurements performed by monitoring the CheY-P level resulted in an adaptation timescale of ~20 s (14). Recently, the flagellar motor was shown to exhibit adaptive remodeling (15). Thus, the chemotaxis system exhibits a tandem architecture of adaptation at the receptor and motor levels (16). An intuitive guess would be that the motor adaptation contributes to the difference between those two types of measurement: whereas adaptation time measured at the level of CheY-P concentration results from receptor-level adaptation, that measured at the level of motor-rotation directional bias results from receptor-level adaptation and an additional contribution of motor adaptive remodeling. However, the motor-adaptation timescale was thought to be in the range of 1 min, too long to play a critical role in the overall adaptation timescale (15). Here, by investigating the dynamics of motor adaptive remodeling, we will show that it surprisingly contributes significantly to speeding up the overall chemotactic adaptation.

MATERIALS AND METHODS

Experiments to measure overshoot phenomenon

HCB316 [Δ(5201(*tar-tap*) and Δ7021(*tsr*))], were derived from *E. coli* K12 strain RP437. The filament gene *fliC* was further deleted from HCB316, yielding CZ1. The plasmid pFD313 constitutively expresses sticky filament $FliC^{st}$ (17). The plasmid pLC113 expresses Tar under a salicylate-inducible promoter. CZ1 transformed with pFD313 and pLC113 was used in the

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experiment. Cells were grown, and the bead assay was carried out in a procedure described previously (13). During cell growth, 1 μM salicylate was used to induce Tar expression to a level approximating that in wild-type cells. In the experiments, 2.5 μM MeAsp was used as the attractant. Twenty experiments were performed, and the mean and standard deviation were plotted in Fig. 1 *A*. The clockwise (CW) biases were normalized by dividing by the average pre-stimulus value.

Förster resonance energy transfer measurements

The experimental setup is similar to that described previously (18). *cheZ* and *cheY* were further deleted from HCB316. CheZ-eCFP and CheY-eYFP were expressed from pVS88, a plasmid that encodes both fluorescent fusion proteins under control of an isopropyl β-D-thiogalactopyranoside-inducible promoter (19). The setup was based on a Nikon Ti-E microscope with a 40× 0.60 NA objective. The illumination light was provided by a 75 W xenon lamp through an excitation bandpass filter (FF02-438/24-25, Semrock, Rochester, NY) and a dichroic mirror (FF458-Di02-25x36, Semrock). The epifluorescent emission was split into cyan and yellow channels by a second dichroic mirror (FF509-FDi01-25x36, Semrock), and collected through emission bandpass filters (FF01-483/32-25 and FF01-542/32-25, Semrock) by two photon-counting photomultipliers (H7421-40, Hamamatsu, Hamamatsu City, Japan). Signals from the two photomultipliers were recorded at a sampling rate of 1 Hz using a data-acquisition card installed in a computer (USB-1901(G)-1020, ADlink, New Taipei, Taiwan). The ratio of the signals from the yellow and cyan channels was used to represent the fluorescence resonance energy transfer (FRET) value. The FRET value is normalized by subtracting the lowest FRET value reached after attractant addition and then dividing by the average pre-stimulus value (Fig. 1 *B*).

Stochastic simulations of motor adaptive remodeling

The simulations started with a steady-state CheY-P level, Y, of 2.90 μM and a corresponding steady-state CW bias, B. At $t = 0$ s, the motor is in the counterclockwise (CCW) state, switching randomly between the two states. The rate of switching from CCW to CW is $B/0.11$ s^{-1}, and the rate of switching from CW to CCW is $(1 - B)/0.11$ s^{-1}, as measured previously (20). The rate of change with time in the number of FliM molecules followed Eq. 1, and was different for the CCW and CW intervals. The instantaneous CW bias was calculated using Eq. 3. The time step in the simulations was 0.01 s. For simulation of the overshoot response in Tar-only cells with intact receptor-level adaptation, at $t = 100$ s, the CheY-P level abruptly reduces to 2.60 μM, and then recovers back to 2.90 μM with a timescale, τ_m, of 20 s: $Y = 2.60 + 0.30 \times (1 - \exp(-(t - 100)/\tau_m)$ for $t > 100$ s. For the simulation to reproduce the motor partial adaptation, at $t = 100$ s, the CheY-P level rapidly reduces to 2.6 μM according to $Y = 2.60 + 0.30 \times \exp(-(t - 100)/\tau_A)$, with a time constant, τ_A, of 8 s, mimicking the time it takes for medium exchange in a typical motor adaptation experiment with a stepwise addition of attractant. For the simulation of response to a small stepwise stimulus, at $t = 100$ s, the CheY-P level dropped abruptly from the initial value of 2.90 to 2.82 μM, and then recovered to the initial value with a timescale, τ_m, of 20 s: $Y = 2.82 + 0.08 \times (1 - \exp(-(t - 100)/\tau_m)$ for $t > 100$ s. For the simulation of the spontaneous fluctuation of motor rotation direction in a steady state, the fluctuation of CheY-P level was described using the Langevin equation (21): $dY/dt = -(Y - Y_0)/\tau_m + \eta(t)$, where τ_m is the relaxation time, the same as the timescale of receptor methylation/demethylation, and $\eta(t)$ is a Gaussian white noise with zero mean and $\langle \eta(t)\eta(t')\rangle = 2\sigma_Y^2/\tau_Y \delta(t - t')$. The deviation, δY, of the CheY-P level from its steady-state value was updated using the Ornstein-Uhlenbeck formula (20,22):

$$\delta Y(t + \Delta t) = \delta Y(t) \times e^{-\Delta t/\tau_Y} + \sigma_Y \times \sqrt{1 - e^{-2\Delta t/\tau_Y}} \times n(0, 1)$$

where $n(0,1)$ is the standard normal distribution with zero mean and unit variance, σ_Y is the standard deviation of the fluctuation of CheY-P level, and τ_Y is equivalent to τ_m.

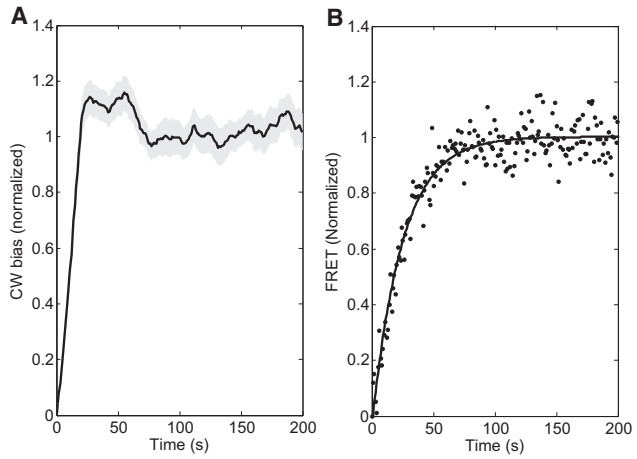

FIGURE 1 Responses of cells with a single type of receptor (Tar) subjected to a stepwise stimulus of 2.5 μM MeAsp at time 0. (*A*) The overshoot phenomenon observed at the level of motor CW bias with a bead assay. The solid line is the average of 20 measurements and the shaded area represents the standard deviation. (*B*) There is no overshoot at the level of CheY-P concentration measured by FRET between CheZ-eCFP and CheY-eYFP. The solid line is a fit with $1 - \exp(-t/\tau)$, where τ is the fitting parameter.

RESULTS

Experimental observation of overshoot response in cells with a single type of chemoreceptor

In studying the dynamics of motor adaptive remodeling, we observed an overshoot phenomenon in the step response of *E. coli* cells with only one type of chemoreceptor and intact receptor-level adaptation. We used a Tar-only strain and studied its response to a stepwise stimulus of 2.5 μM α-methyl-DL-aspartate (MeAsp). The motor CW bias (the probability of the motor rotating clockwise) was used as the indicator of chemotactic output, and its kinetics was monitored with a bead assay. After the stepwise stimulus of attractant at time 0, the motor CW bias dropped abruptly, after which it slowly recovered but overshot to a higher level and then returned back to its pre-stimulus level (Fig. 1 *A*). Four examples of the raw data (un-normalized) are shown in Fig. S1.

The overshoot phenomenon has been observed before, but only in the step response of wild-type *E. coli* cells with multiple types of chemoreceptors (23,24). This was due to methylation cross talk in cells with multiple types of receptors, as suggested by a recent model for the dynamics of receptor clusters (25). Therefore, it was surprising to observe the overshoot response in the Tar-only strain, as there is no methylation cross talk. As the model dealt with the chemotactic output at the level of CheY-P concentration,