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The chemotactic response and motor function of a bacterial flagellar motor in a single cell.

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An *Escherichia coli* cell transduces extracellular stimuli sensed by chemoreceptors to the state of an intracellular signal molecule, which regulates the switching of the rotational direction of the flagellar motors from counterclockwise (CCW) to clockwise (CW) and from CW back to CCW. We performed high-speed imaging of flagellar motor rotation and show that the switching of two different motors on a cell is controlled coordinately by an intracellular signal protein, phosphorylated CheY (CheY-P). The switching is highly coordinated with a sub-second delay between motors in clear correlation with the distance of each motor from the chemoreceptor patch localized at a cell pole, which would be explained by the diffusive motion of CheY-P molecules in the cell. Our results suggest that a transient increase and decrease in the concentration of CheY-P caused by a spontaneous burst of its production by the chemoreceptor patch at steady state.
In response to an attractant or repellant, an *E. coli* cell controls the rotational direction of its flagellar motor by a chemotaxis system. In response to an attractant, a change in the intracellular concentration of a chemotaxis protein, CheY-P, switches the direction of the flagellar motor. To elucidate the signaling process occurring inside an *E. coli* cell after attractant recognition, we examined the cellular response to serine instantaneously photoreleased from a caged compound. The results showed that the response time, the initial CW duration, depended on the distance between the receptor and motor, indicating that the intracellular signal induced by serine propagates through the cytoplasm from the receptor-kinase cluster toward the motor. The response time included the time required for diffusion of the signaling molecule and the 240 ms for enzymatic reactions. These quantitative findings increase our understanding of the signal transduction process occurring inside cells during bacterial chemotaxis.
It was believed that the binding of CheY-P to the flagellar motor induced a switch in rotation from CCW to CW direction. However, it has not been directly demonstrated in a functioning motor. Here we demonstrated that the rotational switch of a functioning motor is directly regulated by the binding and dissociation of CheY-P. By simultaneously visualizing the rotational switching of a motor and the localization of green fluorescent protein (GFP)-labeled CheY, we found that CheY-P molecules bind to and dissociate from a motor rapidly (~100 ms) during rotational switching and that the binding of 13 CheY-P molecules is sufficient to induce the CW rotation. This is the first direct measurement of the regulation of output from a signal transduction pathway by the binding of intracellular signaling proteins in living cells.

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