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Adaptation by target remodelling

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Abstract

Bacteria direct their movement in response to certain chemicals by controlling the rotation of whip-like appendages called flagella. The sensitivity of the response can be adjusted at the signal's target, the flagellar motor.

When we switch on a lamp in a dark room, our eyes adapt quickly to the lighting. Similarly, in a process known as chemotaxis, specific sensory systems in bacteria detect and adapt to changes in the concentration of molecules such as nutrients, so that the microbes can control the motion of their flagella and swim to more favourable environments. Many adaptive mechanisms act on cell-surface receptor proteins that detect stimuli and generate signals across the cell membrane and within the cytoplasm. Indeed, the sensitivity of the bacterial chemo-taxis machinery is regulated by addition or removal of methyl groups on the chemical-sensing receptors (chemoreceptors)¹. But, as Yuan *et al.*² report on page 233 of this issue, the machinery can also adapt by remodelling the target of signalling — the flagellar motor. Motor adaptation differs from receptor adaptation in its time frame, mechanism and function.

In the bacterial chemotaxis system, different chemoreceptors can bind to either attractant or repellent ligand molecules, and by doing so they alter the activity of a receptor-associated kinase enzyme² (Fig. 1). The kinase adds a phosphate group to the protein CheY, which can bind only in its phosphorylated form (CheY-P) to FliM, a protein component of the flagellar rotary motor. Binding leads to a switch in flagellar rotation from anticlockwise — the default state — to clockwise. As anticlockwise rotation powers forward swimming ('runs') and clockwise rotation causes abrupt directional changes ('tumbles'), their alternation generates a three-dimensional random walk. When the bacterium swims towards increasing concentrations of attractant, the interaction of attractant with its receptors reduces kinase activity, and thus CheY-P levels and the probability of tumbles, biasing the random walk in favourable directions.

But when ligand concentration remains constant, the system adapts by reverting to its null state, so that it can then respond to any additional concentration changes. Adaptation occurs through the addition or removal of methyl groups to the receptors in response to occupancy by attractants or repellents. Methylation and demethylation shift receptor conformation and thus kinase activity, CheY-P levels, rotational bias and swimming behaviour in the opposite direction to attractant and repellent occupancy, respectively. These receptor adaptive changes occur more slowly than those generated by ligand binding, and this difference

provides the bacterium with a means of comparing current and recent ligand concentrations through the opposing influences of receptor occupancy and the extent of methylation. The overall result is that the microbe can sense temporal changes in the concentration of relevant molecules and therefore can swim in the 'right' direction.

The flagellar motor is extremely sensitive to small variations in CheY-P concentration³, and this poses a challenge to the system because the steady-state CheY-P concentration varies among individual cells⁴. Given this variation, some cells would be expected to run only or to tumble only, and therefore to be unable to perform proper chemotaxis. As this does not seem to occur, a mechanism must exist to keep a correspondence between steady-state CheY-P levels and the narrow CheY-P concentration range over which the rotational switch is sensitive. However, previous research⁵ did not find one possible mechanism — feedback from the flagellar rotary motor to the kinase.

Yuan *et al.*² investigated an alternative possibility: could the motor itself adapt to changes in CheY-P concentration? The authors used a strain of *Escherichia coli* that lacked the enzymes that carry out receptor methylation and demethylation, as otherwise the process under study would have been masked by the rapid and complete adaptation mediated by these enzymes. When the bacteria were stimulated by an attractant, the authors observed that the CheY-P concentration and the motor's clockwise bias both decreased rapidly, as expected. However, over the next few minutes, even though CheY-P levels remained unchanged, the clockwise bias gradually increased to an intermediate steady-state value that was lower than in the absence of attractant. A similar slow, partial adaptation of the flagellar motor was reported^{6,7} 25 years ago in bacteria lacking the receptor methylation and demethylation enzymes.

The researchers² also measured the clockwise bias of flagellar motors in individual bacterial cells before stimulation, at maximum response and after partial adaptation. In the presence of persistently decreased CheY-P concentration, they documented a shift in the motor's sensitivity to CheY-P to a lower, but still narrow, concentration range. When they analysed these data using a mathematical model for cooperative motor switching, the results indicated that the adaptation probably reflected an increased number of binding sites for CheY-P in the flagellar motor — in other words, more copies of FliM.

To test this hypothesis, the authors expressed a modified version of FliM (fused to a yellow fluorescent protein) in the *E. coli* cells, and then observed these cells using total internal reflection fluorescence (TIRF) microscopy. The researchers did indeed detect increases of up to about 25% in the number of FliM copies (from 34 to 42 copies) in flagellar motors upon partial adaptation. This result is consistent with a previous finding⁸ of CheY-P-dependent turnover of FliM units, and suggests a function for such a process: the addition of FliM copies to the flagellar motor would increase the probability of CheY-P binding to the motor and therefore the sensitivity of the rotational switch.

Yuan and colleagues' results² generate questions for future research. For instance, how does the flagellar motor sense changes in the level of CheY-P? And how does sensing lead to an increased number of FliM copies? How few and how many copies can be accommodated,

and what structural interactions between FlIM units allow such variability? Answering these questions will be challenging, but insight could be gained by identifying and characterizing mutant bacteria that are defective in motor adaptation, and by extending high-resolution fluorescence analyses such as the TIRF microscopy experiments done by the authors. Full answers are likely to require both biochemical and structural studies.

It also needs emphasizing that motor adaptation is a slow process, occurring over minutes. So, in the absence of methylation-dependent adaptation of the receptors, which occurs within seconds, bacteria cannot perform chemotaxis^{9,10}. Nonetheless, motor adaptation is likely to be crucial for accommodating stochastic variations in the dosages of protein components of the chemotaxis sensory machinery, such as CheY. The adjustment of the sensitivity of a signalling system by adaptive remodelling of its output end is a tantalizing observation, because experience tells us that if a new strategy is discovered in one biological system, then there are undiscovered examples in other systems. Therefore, as the authors suggest, other biological molecular machines may adapt to signals by resetting the sensitivity of their response.

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Figure 1. Microbial sense and sensibility

Protein receptors on a bacterial cell's surface bind to specific attractant or repellent molecules, which act as inputs for the bacterium's sensory system. In the absence of attractant, a receptor-associated kinase enzyme donates a phosphoryl group (P) to the protein CheY. Phosphorylated CheY (CheY-P) is an intracellular signal that interacts with the protein FliM at the flagellar motor. This interaction generates a change in rotational direction of the motor — the system's output. Upon attractant binding, the receptor undergoes a conformational change that reduces kinase activity and consequently the levels of CheY-P and the probability of a change in the direction of rotation. Binding of repellent has the opposite effects. If attractant or repellent concentrations do not change for a while, the sensory system 'resets' rapidly and completely, within seconds, to a null state through receptor methylation (for attractant) or demethylation (for repellent) at the input end. Yuan *et al.*² find that the system also slowly and partially adapts at the output end to different, persistent levels of CheY-P. It does so by changing the number of FliM copies in the flagellar motor, thus shifting the narrow sensitivity range of the motor to correspond to different steady-state levels of CheY-P.