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[1]Centre for Behavioural Biology, School of Veterinary Science, University of Bristol, Langford, BS40 5DU, UK. [2]Queen Mary University of London, Biological and Experimental Psychology Group, School of Biological & Chemical Sciences, London, E1 4NS, UK.
E-mail: mike.mendl@bris.ac.uk

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Thermal Robustness: Lessons from Bacterial Chemotaxis

Temperature changes affect reaction kinetics. How do signaling pathways cope with such global perturbation? A recent study dissects the solution found by bacterial chemotaxis.

Yann S. Dufour, Michael W. Sneddon, and Thierry Emonet*

Biological systems living in complex environments must perform well across a wide range of environmental conditions. Therefore, signaling pathways and regulatory networks controlling cellular functions are expected to operate in the presence of environmental perturbations [1]. Temperature fluctuations in the cellular environment affect the rate of all chemical reactions in a cell. How cells remain functional in the face of such global perturbations remains relatively unexplored. A recent study published in *Cell* by Oleksiuk *et al.* [2] takes us a step closer to understanding the molecular mechanisms used by signaling pathways to cope with temperature change. Using the *Escherichia coli* chemotaxis pathway as model system [3], the authors present a comprehensive picture of the various strategies evolved by this system to compensate for the effect of temperature change on two key functional parameters: the steady state of the system output and the rate of adaptation to a constant stimulus.

Like many flagellated bacterial species, *E. coli* biases its random walk (runs and tumbles) toward favorable conditions by making temporal comparisons of environmental signals and by suppressing changes in swimming direction accordingly [3]. The basic functional ingredients necessary to swim up a signal gradient are high sensitivity to stimuli and the capability to adapt to constant stimulus. In both regards, bacterial populations of *E. coli* perform extremely well, operating close to the theoretical limit of sensitivity [4] while exhibiting nearly perfect adaption over five orders of magnitude in signal intensity [5,6]. High sensitivity is achieved through the cooperative activity of receptors that form clusters in the cell membrane [7,8], and adaptation results from the slow methylation or demethylation of these receptors by the antagonistic enzymes CheR and CheB, respectively (Figure 1A). The activity of the receptors is transmitted to the flagellar motors through a phosphorylation cascade initiated by the receptor-bound histidine kinase CheA and relayed by the cytoplasmic response-regulator CheY. The switching rate of the flagellar motors, which determines the mean run length, is ultra-sensitive to the level of the phosphorylated form of CheY (CheY-P) [9]. CheY-P steady-state levels are maintained in the sensitive range of the motor by the basal level of CheA kinase activity and constitutive dephosphorylation by the phosphatase CheZ. Despite its simplicity, the bacterial chemotaxis pathway exhibits rich functions and continues to reveal remarkable properties of biological pathways to researchers.

Probing how *E. coli* chemotaxis might be affected by temperature variation represents a significant technical challenge. Thanks to current theoretical understanding of the behavior of the pathway and a clever use of genetic backgrounds, Oleksiuk *et al.* [2] were able to monitor the activities of different components of the system *in vivo.* Taking advantage of the rapid kinetics of the phosphorylation cascade, they monitored the Förster resonance energy transfer (FRET) between CheY-P and CheZ, both labeled with fluorescent proteins, as a readout of the kinase activity [8]. Using ordinary

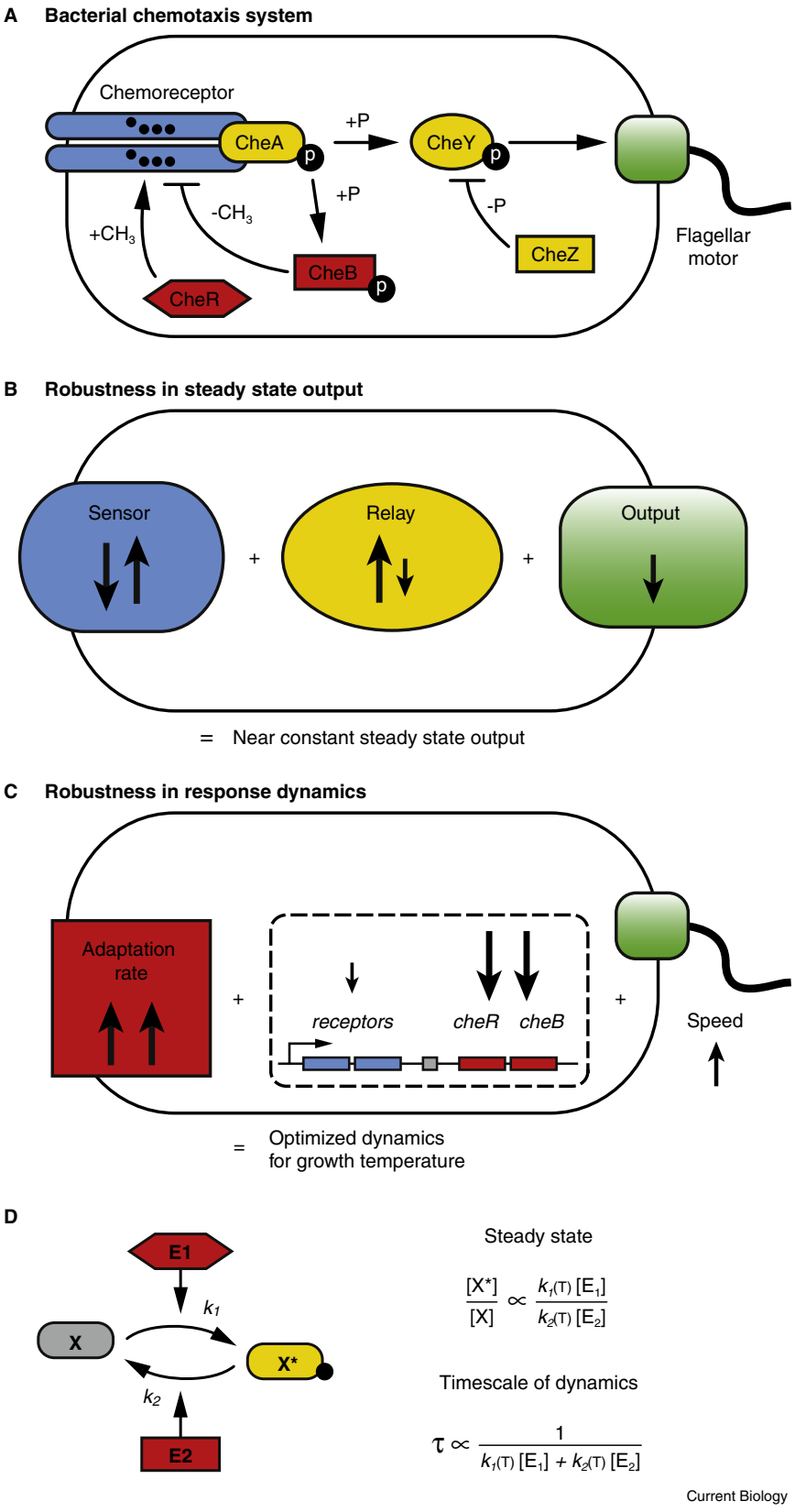

Figure 1. Thermal compensation mechanisms of the *E. coli* chemotaxis system.

(A) The chemotaxis network consists of sensory chemoreceptors (blue) wired to a signaling cascade (yellow) that modulates the rotational state of the flagellar motors (green). Adaptation to persistent stimuli is mediated by methylation and demethylation of the receptors by CheR and CheB (red). (B) The system achieves robustness of the adapted behavior to temperature variation by the combined effects of the intrinsic compensation of receptor activity (sensor), the adjustment of phosphorylation and dephosphorylation rates (relay), and the adjustment of motor sensitivity (output). (C) The system achieves partial compensation of increased swimming speed at higher temperatures by an increased rate of adaptation. An added genetic regulatory control mechanism adjusts the ratio of receptors to the adaptation enzymes to further optimize the average adaptation rate to different growth temperatures. (D) Thermal dependency of a futile cycle working in the first-order regime. Robustness of the steady state to thermal fluctuations can be achieved by ensuring that the first-order rates, $k_1(T)$ and $k_2(T)$, have proportional temperature dependencies when the ratio of enzyme concentrations $[E_1]$ to $[E_2]$ remains unchanged (in the chemotaxis system, *cheR* and *cheB* are co-transcribed [15]). In contrast, thermal robustness of the characteristic timescale to reach equilibrium, τ, requires adjustment of $[E_1]$ and $[E_2]$ to compensate for the shorter timescale that would result from an increase in $k_1(T)$ and $k_2(T)$ as temperature increases.

differential equations to express the relationship between the kinetic parameters of the system, Oleksiuk *et al.* [2] determined the relevant combinations of genetic backgrounds and environmental conditions that would fix key parameters of the system. These experimental conditions enabled the determination of the temperature dependence of several specific enzymatic activities by monitoring the interaction between CheY-P and CheZ over an ensemble of 300–500 isogenic cells. Their results therefore provide information about how dynamical properties of the pathway are affected by temperature when averaged over the population.

Oleksiuk *et al.* [2] asked first how the activity of the receptor–kinase complex depends on temperature. For this experiment, they used aspartate and serine receptors genetically modified to carry glutamines at different methylation sites, which make them functionally equivalent to native receptors with different methylation states [8]. The modified receptors were expressed in a mutant background lacking the adaptation proteins CheR and CheB and the native receptors. Monitoring the FRET response of these cells to

temperature variations provided information about the temperature dependency of the activity of the receptors as a function of their methylation states. Interestingly, receptor–kinase complexes with two or more glutamines showed an increased basal activity with increased temperature, whereas fewer than two glutamines resulted in an inverted response. The authors could fit their data with a Monod–Wyman–Changeux model of the activity of receptor complexes [10,11] by assuming that the methylation-dependent free energy of the receptors in units of k_BT changed linearly with respect to temperature. Next, Oleksiuk *et al.* [2] hypothesized that the effect of temperature variation on the receptor–kinase complex could be compensated by modulating the relative proportions of receptors with different methylation states. Indeed, cells with receptor–kinase complexes containing the aspartate receptor at a ratio of 2.5 receptors with 3 glutamines for 1 receptor with 1 glutamine were temperature insensitive.

Given the ultra-sensitive response of the motors, the steady-state level of CheY-P needs to be tightly controlled to maintain an optimal flagellar motor switching rate. Therefore, the activities of the kinase, CheA, and phosphatase, CheZ, must be matched even when temperature varies. Again using the CheY-P/CheZ FRET signal, Oleksiuk *et al.* [2] determined the temperature dependency of CheA and CheZ kinetics. The rates of CheZ-dependent CheY-P dephosphorylation were measured by monitoring the decrease in FRET signal after the suppression of CheA activity by the addition of saturating concentrations of L-aspartate. The rate of kinase activity of CheA was calculated by measuring the increased steady-state level of the FRET signal in cells expressing highly modified receptors to mimic the full activation of the receptor–kinase complex. The experiments revealed that CheA and CheZ enzymatic activities are imperfectly compensated as temperature varies. Although this would seem to pose a problem, previous experimental results showed that the flagellar motor may be able to compensate for this discrepancy by becoming less sensitive to CheY-P levels [12,13].

Thus, for each stage within the signaling pathway — at the receptor complex, the phosphorylation cascade, and possibly at the motor level — it seems that *E. coli* has evolved balanced sensitivities to temperature to achieve thermal robustness of the adapted state (Figure 1B). However, even though antagonistic reactions can maintain a steady-state level across a range of temperatures, the rate at which this equilibrium is reached should increase with temperature, thereby altering the duration of time that cells will swim in response to an increase in attractant. In theory, this effect would imply that the drift velocity of a bacterial population in gradients is temperature sensitive.

To investigate the dynamics of adaptation, Oleksiuk *et al.* [2] used the dynamics of the CheY-P/CheZ FRET signal one last time to characterize the rates of methylation by CheR and demethylation by CheB after addition or removal of methyl-aspartate. They found that the adaptation rate is not robust to temperature variations but instead follows the expected temperature dependency. However, the relevant functional parameter in this case is not the adaptation rate, but rather the mean run length, which needs to be calibrated to the particular signal gradient to maximize drift velocity along the gradient [14]. Therefore, a change in adaptation rate could potentially be compensated by a change in cell speed, as demonstrated theoretically by the authors.

Oleksiuk *et al.* [2] determined that cell speed does increase moderately with temperature, but not sufficiently to compensate for the large increase in adaptation rate. Therefore, in the absence of direct feedback, the authors hypothesized that *E. coli* has a pre-programmed compensatory mechanism to adjust the adaptation rate close to its optimal range. Cells grown at a specific temperature exhibited an adaptation rate closer to the theoretical optimal range for that temperature than cells that were first grown at a different temperature and were later transferred to the target temperature. Ultimately, Oleksiuk *et al.* [2] demonstrated, using quantitative immunoblotting and genetic constructs, that a genetic element upstream of the *cheR* gene, possibly a temperature-sensitive secondary RNA structure, is responsible for modulating the protein expression ratio between the receptor proteins and the adaptation proteins, CheR and CheB. Therefore, the increased enzymatic activity of the adaptation proteins at high temperature is compensated by a decrease in protein expression to keep the chemotaxis adaptation rate within a functional range (Figure 1C).

What did we learn about strategies for thermal robustness? Overall, *E. coli* achieves robust performance by relying primarily on direct feedback integrated into the network, but with the addition of pre-programmed compensatory regulation [2,15]. The latter strategy may be an indication of the limits on how much temperature compensation can be achieved by a network itself. Maintaining steady-state activity in an antagonistic reaction system can be achieved with direct temperature feedbacks built into the reaction rates but maintaining the characteristic timescale of such a system is more difficult (Figure 1D). In the case of the adaptation mechanism mediated by CheR and CheB, *E. coli* apparently relies on an additional pre-programmed regulatory mechanism to achieve robustness.

In the context of homogeneous populations, pre-programmed regulation may appear to be an inadequate solution because the population will not be able to adapt quickly to rapid fluctuations in the environment. Examples from other organisms, however, have demonstrated that an additional layer of robustness to perturbations may emerge in large clonal populations [16] by taking advantage of phenotypic and behavioral variability. These sources of diversity naturally arise from stochastic fluctuations in gene expression and signal transduction and have been demonstrated experimentally and theoretically in bacterial chemotaxis [17–20]. By tuning each individual cell differently, such variations may enable cell populations to cover a larger region of the functional parameter space, thus increasing their odds of success. For example, we mentioned earlier that the adaptation rate of the chemotaxis system should be calibrated to the cell swimming speed and the shape of the signal gradient. Since the specific shape of a gradient at a given time will be difficult for a cell to predict, survival of the population may be enhanced by allowing cells in a population to explore a range of adaptation rates. Thus, phenotypic and behavioral variability may provide an additional layer of

robustness to the system. While the precise role that variability plays on thermal robustness remains unknown, the work of Oleksiuk *et al.* [2] does elucidate various strategies that cells use to cope with temperature perturbations. We expect that the examples provided by *E. coli* chemotaxis will be valuable to further our understanding of the structure and dynamics of many other signaling networks.

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Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520, USA.
*E-mail: thierry.emonet@yale.edu

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Conservation Biology: The Many Ways to Protect Biodiversity

Protecting hotspots of marine species richness may not be an effective strategy to conserve biodiversity because these sites do not coincide with hotspots of functional and phylogenetic diversity.

Isabelle M. Côté

In his inaugural address at the Fisheries Exhibition of London in 1883, T.H. Huxley affirmed with confidence that "in relation to our present modes of fishing, a number of the most important sea fisheries… are inexhaustible". Modes of fishing have changed drastically since Huxley's lecture. Technology has improved to the point where virtually every part of the world's oceans is now accessible to fishing and many fish stocks have been exhausted [1,2]. The effects of fishing are felt not only on species that are sought, but also on habitats and non-target species that become inadvertently caught in fishing gear. These indirect effects have prompted a revolution in the management of marine resources: a focus on ecosystems rather the traditional consideration of single species or issues [3]. One essential tool of ecosystem-based management is the use of marine protected areas (MPAs) — areas of the sea within which some or all extractive activities, especially fishing, are prohibited. Full protection can successfully increase the density, size and biomass of organisms within MPA boundaries [4], and the vast majority of MPAs do work if given enough time [5]. As a result, there has been a global resolve to place at least 10% of coastal and marine areas within MPAs by 2020 [6]. But where should we establish these MPAs? It would make sense, at least initially, to target areas that are particularly species-rich, as these might be of "particular importance for biodiversity and ecosystem services" [6]. A study by David Mouillot and colleagues in this issue of *Current Biology* [7] shows that the current system of Mediterranean MPAs does capture hotspots of fish species diversity very well, but that this approach fails to capture other important aspects of biodiversity, such as diversity of function and of evolutionary history.

The Mediterranean as a Case Study

Mouillot *et al.* [7] focus on fish diversity in the Mediterranean. This large, nearly enclosed sea is remarkable in many ways. It is a global hotspot of marine species richness, reflecting the unique combination of its sub-tropical location, Atlantic heritage, periodic isolation, and varied climate and hydrology. An unusually high proportion of Mediterranean marine species — about one fifth — is endemic. As a result of millennia of human occupation, it has both the best inventoried and some of the most threatened marine fauna in the world [8]. There are currently some 100 MPAs in the Mediterranean, covering only less than 0.5% of the total area of the region. In the course of their study, Mouillot *et al.* [7] discovered that the location of these MPAs coincides well with areas that have particularly high numbers of fish species (total, endemic, as well as threatened). Not a bad performance for a collection of MPAs that were established largely at