The thermal impulse response of *Escherichia coli*

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Swimming *Escherichia coli* responds to changes in temperature by modifying its motor behavior. Previous studies using populations of cells have shown that *E. coli* accumulate in spatial thermal gradients, but these experiments did not cleanly separate thermal responses from chemotactic responses. Here we have isolated the thermal response by studying the behavior of single, tethered cells. The motor output of cells grown at 33°C was measured at constant temperature, from 10° to 40°C, and in response to small, impulsive increases in temperature, from 23° to 43°C. The thermal impulse response at temperatures < 31°C is similar to the chemotactic impulse response: Both follow a similar time course, share the same directionality, and show biphasic characteristics. At temperatures > 31°C, some cells show an inverted response, switching from warm- to cold-seeking behavior. The fraction of inverted responses increases nonlinearly with temperature, switching steeply at the preferred temperature of 37°C.

thermotaxis | taxis | strategy

The bacterium *Escherichia coli* will migrate and aggregate in spatial thermal gradients (1, 2). Swimming cells explore their environment by performing what is essentially a random walk; the bacteria make a series of runs and tumbles, where runs are intervals of smooth swimming, and tumbles are events that randomly reorient the cell (3). The thermotactic behavior of bacteria is similar to its chemotactic behavior: Cells measure thermal stimuli over time and bias their run and tumble statistics to promote movement toward their preferred temperature. Typically, an increase in temperature causes bacteria to swim smoothly, and a decrease in temperature causes bacteria to tumble (1), but the response will reverse when cells are adapted to certain chemoattractants (4, 5). This stochastic strategy biases the cell's random walk, so that on average it will migrate along spatial temperature gradients (6).

Studies on the molecular details of the thermosensory system have shown that it uses the same biochemical pathway as chemotaxis (7). In chemotaxis, ligands are detected through a set of membrane-bound chemoreceptors (Tsr, Tar, Trg, and Tap) that transduce this information to a phosphorelay signaling cascade (8). Mutants that are nonchemotactic generally have no thermal response (9). The primary temperature transducers are Tsr and Tar, the high-abundance chemoreceptors for the attractants serine and aspartate, respectively (10). Cells with only Tsr or Tar show a wild-type thermal response, whereas cells with only Trg and Tap, at normal levels, show no thermal response (11). Cells adapt to temperature changes via the chemosensory adaptation pathway, where the activity of the receptors is modulated by changing their methylation state (4). The methylation state of the receptors also affects the direction of the thermal response. Cells adapted to 0.1 mM of serine and aspartate—which fully methylates the Tsr and Tar receptors—increase their tumbling probability in response to an increase in temperature, the opposite of nonadapted cells.

Previous thermotaxis assays have generally involved populations of cells. One method is to directly measure the steady state aggregation of swimming bacteria in a spatial thermal gradient. Maeda *et al.* (1) placed cells in a linear temperature gradient (19–39°C) and found that cells accumulated at 30°C. However, cells have higher metabolic rates at higher temperatures, and so

the cells produced oxygen gradients in their assay. The aggregation of bacteria in the thermal gradients could not, therefore, be attributed to thermal effects alone in their experiments. Salman *et al.* (2) observed swimming cells confined to narrow PDMS channels in linear thermal gradients and found that after 45 min, the cell concentration would peak at 34°C. At later times the peak would drift to lower temperatures, and more importantly, the velocity of the drift was proportional to the total initial cell density, implicating cell–cell interactions either via secreted signals (12) or by consumption of nutrients creating chemical gradients, as observed in soft-agar swarm plates. Another method is to measure the swimming behavior of cells in response to a temporal change in temperature. Maeda and Imae (9) applied large temperature ramps (>Δ10°C, 0.2°C/s) to a population of swimming cells and measured the fraction of tumbling versus swimming cells over time. Although this assay is capable of quantifying the thermal response, the time resolution is slow (minutes), the behavioral variability is large, and there is no universally accepted classification of run and tumbling patterns for swimming cells. The sizeable temperature ramps necessary to elicit robust behavioral responses is another concern, because the temperature changes are large enough to induce gene expression (heat-shock response) (13) and may be saturating the sensory system.

To avoid all of these complications associated with using a population of cells to study thermal responses, we have adopted the tethered-cell assay to measure the thermal responses of single cells. In the tethered-cell assay (14), a cell is attached by a single flagellar motor to a glass coverslip, and its motor direction is monitored by measuring the rotation of the cell's body. The run and tumble pattern of swimming cells corresponds respectively to the counter clockwise (CCW) and clockwise (CW) rotation of the flagellar motor (15), and so sensitive measurements of the taxis response can be made by using this technique. To measure the thermotactic behavior of cells, we monitored their position using real-time, computer-video microscopy over a range of temperatures and also in response to impulses of heat delivered by an infrared laser.

The benefit of studying the impulse response is that its structure reflects the details of how input signals are processed, assuming that the response is measured in the linear regime (16). The chemotactic impulse response of *E. coli* has been successfully measured by stimulating cells with iontophoretic pulses of attractants and repellents (17), and the phototactic impulse response of *Rhodobacter sphaeroides* has been measured by using pulses of visible light (18). Here we perform an analogous experiment on tethered cells of *E. coli*, using pulses of heat. Because we are studying single cells and not populations of cells, we are isolating the thermotactic response from the chemotactic gradients that have complicated previous studies.

For thermal aggregation to occur, cells must invert their thermal response when they are above their preferred temper-

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Fig. 1. CCW bias versus temperature. The motor output of single, tethered, wild-type cells, grown at 33°C, was measured for at least 10 minutes. Each data point is the mean of the average CCW bias per cell. The error bars are the weighted standard deviation. The number of cells assayed at each temperature, from left to right, are: 82, 69, 72, 53, 60, 68, 45, and 59.

ature. To discover the thermal preference of cells, we looked for this inversion and found that cells begin to change their response at temperatures above 31°C. This switch in behavior happens most steeply at 37°C, with half the cells displaying an inverted response. At temperatures above 40°C, almost all of the responsive cells show an inverted response.

Results

Adapted Temperature Behavior. Cells of *E. coli* were tethered to glass coverslips by a single flagellum. The flagellar motor spins the cells in the CW and CCW direction, and the motor output of single cells can be measured by following the motions of the cell body. To determine the tendency of cells to swim (CCW) or tumble (CW), we measured their angular velocity and calculated the fraction of time they spent spinning in the CCW direction (CCW bias). To study how cells adapt to thermal stimuli, we measured the CCW bias at different temperatures using a custom temperature-controlled stage. Fig. 1 shows the CCW bias of the cells from 10°C to 40°C. The adapted bias varies non-monotonically with temperature from 0.8 to 0.5, indicating that the temperature compensation by the thermosensory network is not precise. In comparison, chemosensory adaptation is precise over many orders of magnitude of concentration (19). However, there are exceptions. It has been shown that cells do not fully adapt to serine; the average run interval of swimming cells rises and falls as the steady-state serine concentration increases from 10^{-6} to 10^{-2} M (20). Tsr, the chemoreceptor for serine, is a major thermoreceptor, and so perhaps this failure to adapt precisely to serine might be the reason why cells only partially adapt to temperature.

Impulse Response. We measured the response of cells to temporal changes in temperature by stimulating them with an impulse of heat. An infrared laser (1,440 nm) was focused to a diffraction limited volume around the tethered cell body. The laser, when pulsed (50 ms), produced a positive temperature jump (3°C) with a duration of about 100 ms (cooling time constant, $\tau = 0.08$ s $^{-1}$). The cell's motor direction was monitored while it was repeatedly stimulated (once every 10 s), and the pulse-aligned data were averaged to calculate the CCW bias (17). The resulting impulse

Fig. 2. Impulse responses at 31°C. (a) The points are the CCW biases averaged per wild-type cell in response to an impulse of heat. The thick curve is the CCW bias averaged over all cells. The ratio of the area of the positive to the area of the negative lobe is 0.4. The data were computed from 802 cycles from 21 cells. Each cycle lasted 10 seconds, and the laser pulse started at 0 s and lasted for 50 ms. (b) The double impulse response. The open diamonds show the response when the laser was pulsed twice, once at $t=0$ sec and again at $t=0.66$ sec. The response from a single pulse is shown by dots. The expected double impulse response assuming linearity is shown as a solid line. The single impulse response data were computed from 24 cells with a total of 1,075 cycles. The double impulse response data were computed from 34 cells with a total of 1,378 cycles. The laser power was half of that used in a. (Inset) Step response from 16 cells with a total of 359 cycles. Bar indicates when laser was turned on. Laser power was approximately 1/60th the power used in a.

response at 31°C is shown in Fig. 2a. Smaller and larger temperature changes produced smaller and larger responses, respectively, so our stimulus was above threshold and below saturation (data not shown). The time course of the thermal impulse response is similar to the chemotactic impulse response (21). The initial increase in CCW bias indicates that cells respond by increasing their smooth swimming probability, whereas the overshoot below the baseline indicates that cells are making a differential measurement.

To test the linearity of the system, we measured the response of cells to a small step in temperature and also to two sequential impulses of heat. The step response is shown in Fig. 2b (Inset) and

Fig. 3. Inverted impulse responses. The open diamonds indicate the impulse response from wild-type cells adapted to 0.1 mM α -methyl-DL-aspartate and 0.1 mM L-serine at 31°C (9 cells, 328 cycles). The dots indicate the impulse response from wild-type cells adapted to a temperature of 40°C (23 cells, 683 cycles).

follows the expected time course assuming a biphasic impulse response (17): the CCW bias changes in the direction of the temperature step but adapts to the baseline bias within 10 s. Fig. 2*b* shows the response of cells stimulated by two impulses of heat separated by 0.66 s. This response roughly follows the theoretical response calculated from the sum of two single impulse responses offset by 0.66 s.

It is possible that the flagellar motor itself is responding to the thermal impulses. It has been shown that the motor is sensitive to cold temperatures: “guttled” strains deleted of their chemotaxis proteins will start to switch at temperatures near 0°C (22). To determine whether motors were sensitive to our thermal pulses, we used a strain in which we could induce different levels of CheY^{**}, a mutant version of CheY that mimics the phosphorylated version CheY-P (23). Because this strain has no native CheY, the sensory network is effectively disconnected from the motor. We measured the impulse response from cells that were induced to produce levels of CheY^{**} that correspond to a CCW bias of ≈ 0.5 , but we saw no change in the CCW bias, suggesting that the wild-type thermal response results only from signaling upstream from the motor.

Impulse Response Inversion. It is known that the directionality of the thermal response depends on the methylation state of the Tsr and Tar receptors (24). Increasing the methylation state by stimulating the receptors with attractants (10) or by genetically modifying the receptors (24) will cause an inversion of the response. We verified these results on tethered cells by measuring the impulse response of cells adapted to 100 μ M α -methyl-DL-aspartate and 100 μ M L-serine at 31°C (Fig. 3). The response is similar in shape to the impulse response shown in Fig. 2*a* but is now inverted.

Because the presence of chemical attractants can invert the thermal response, we were curious if an increase in temperature would have the same effect. Using a heating stage, we increased the ambient temperature to 40°C and measured the thermal impulse response (Fig. 3). We found that it too was inverted, but the response lacks the biphasic character of the noninverted or the chemically inverted impulse response. The time course of the first lobe is similar to that of the noninverted and the chemically inverted responses; however, the second lobe has essentially

Fig. 4. Impulse response of a single cell at 30°C and 40°C. The impulse response of a single wild-type cell at 30°C is shown by the dots, and at 40°C by the open diamonds. Note that by looking at the direction of the first lobe, the response at 30°C is positive while the direction of the response at 40°C is negative. A total of 94 cycles were measured. Cycles 1–29 and 60–94 were taken at 40°C, while cycles 30–59 were taken at 30°C. The system was given 15 minutes to equilibrate between temperature changes. Data were smoothed with a 120-ms averaging window.

disappeared, indicating that cells are no longer making a differential measurement. To verify that this inversion happens in the same cell at different temperatures and not just by different cells at different temperature, we measured the thermal response of single cells shifted to 30°C and 40°C, and did indeed find that single cells will invert their response (Fig. 4).

Impulse Response as a Function of Temperature. For cells to aggregate in a spatial thermal gradient, the direction of their thermal response has to switch as they swim past their preferred temperature. We tested the directionality of the thermal response as a function of temperature by looking at the impulse responses of single cells like the example shown in Fig. 4. For each cell, we measured the directionality of the response over 50 cycles and then calculated the fraction of the responses in the positive and negative direction at each temperature (Fig. 5). Cells that showed no response or ambiguous responses were not considered (see *Materials and Methods*). At room temperature, the impulse responses of all cells were positive (warm-seeking), but at 31°C, a small fraction of the cells showed an inverted, negative (cold-seeking) response. This trend continued with a steep switch in behavior occurring at 37°C, with equal subpopulations of warm- and cold-seeking cells. By 43°C, all of the cells displayed negative responses. We verified that this inversion also occurs with swimming cells by monitoring swimming patterns of cells subjected to 5°C ramps of increasing and decreasing temperature (1°C/min) at room temperature and at 40°C (data not shown).

Discussion

We believe we are the first to show that the thermal response of *E. coli* begins to invert when the temperature is $>31^{\circ}\text{C}$, and that their thermal preference is 37°C . Previous studies have shown that adaptation to serine and aspartate (10), genetic modification of the methylation state of Tsr and Tar (4), and high cell concentrations (5), will cause cells to reverse their thermal response. Here we have shown that thermal stimuli alone will do. We mapped this thermally induced inversion from 23°C to 43°C and found that it is nonlinear—the change from a warm to a cold

Fig. 5. Directionality of thermal response versus temperature. Dots indicate the fraction of cells with a positive-directed (warm-seeking) thermal impulse response, a positively directed first lobe. Diamonds indicate the fraction of cells with a negative-directed response, which is simply $1 - (\text{positive fraction})$ but is plotted to help guide the eye. If the directionality of the impulse response was ambiguous, those data were not considered. The number of cells assayed at each temperature, from left to right: 20, 19, 21, 37, 25, 34, 32, 20, 23, 8.

preference switches steeply at 37°C. There is at least one compelling reason why 37°C is the preferred temperature. The natural environment of *E. coli* is the gastrointestinal tract of animals, and so it is not surprising that the thermal preference of the bacteria is approximately the average body temperature of mammals.

Cells adapt to temperature changes but not precisely—the CCW bias varies over the measured range of 10–40°C. This is in contrast with adaptation to many chemical attractants where the CCW bias returns precisely to prestimulus levels over a wide range of concentration ($\approx 10^4$ -fold) (20). In general, this resetting of the zero point is necessary for any sensory system to remain sensitive to small changes in input stimuli. However, it has been shown that cells only partially adapt to serine, and because Tsr is a major thermoreceptor, perhaps this is why cells only partially adapt to temperature.

We found that the thermal impulse response at 31°C (Fig. 2a) is similar to the chemical impulse response. Because thermal information is transduced through the same receptors involved in chemotaxis (10), it is not surprising that they share common features: Both have a similar time course, the same directionality, and biphasic characteristics. The thermal response is not as linear as the chemotactic response (17), but it is linear enough to make qualitative conclusions of how thermal signals are processed. The biphasic nature of the response suggests that cells make thermal measurements differentially, as has been shown for chemotaxis. Cells continuously compare measurements made early in the past with measurements made further in the past and respond to the difference.

With the impulse response measured both below and above the preferred temperature, we can begin to understand how cells might migrate and aggregate in a linear thermal gradient. The impulse response above the preferred temperature is inverted but is not biphasic—consisting of a single negative-directed lobe. It has been shown that a single positive lobe in the response function will transiently move cells up an attractive gradient; however, at steady-state, the accumulation is not optimal and, in fact, is in the reverse direction (25, 26). So the inverted thermal impulse response suggests that above the preferred temperature, accumulation occurs on the warmer side, the wrong direction for

accumulation at the preferred temperature. Working in the opposite direction, however, is orthokinesis—the tendency for organisms to accumulate where their speed is slow (27). The swimming velocity of cells decreases as the temperature decreases (data not shown), and so orthokinesis will bias the distribution toward lower temperatures because of the slower speeds. A further complication is that cells will normally be in the presence of other signaling gradients, either from cell-concentration dependent effects or from environmental chemical gradients. This interaction between temperature and chemical gradients is likely to be important to both thermotaxis and chemotaxis because both sensory modalities share the same biochemical pathway. It remains to be seen how these sensory modalities interact to produce advantageous tactic behavior.

From a broad perspective, exposure to environmental temperature changes is a universal condition of living organisms, and in principle, all steps of biochemical signaling networks can be temperature-dependent. Enzymatic rates are generally exponential functions of temperature and typical Q10 values (increase in enzymatic rate per 10°C) are from 1.2 to 2.5 (28), implying rate increases from 2- to 16-fold over 30°C. This wide variation of reaction rates presents an obvious challenge for robust design, particularly for systems that need to transmit quantitative information, e.g., signaling systems. Understanding how the bacterial thermotactic network remains robust and responsive to changes in temperature will likely lead to a broader understanding of how temperature compensation is achieved in general.

Materials and Methods

Bacterial Strains. Strains KF84 and HCB902 were obtained from H. C. B. at Harvard University.

Growth Conditions. Cells were grown at 33°C with shaking (185 rpm) with appropriate antibiotics: 4-h cultures were grown in TB (10 g/liter BactoTryptone, 5 g/liter NaCl, in H₂O) from overnight cultures grown in LB (10 g/liter BactoTryptone, 5 g/liter yeast extract, 5 g/liter NaCl, in H₂O) by adding 100 μ l of the overnight into 10 ml of TB. Cells were rinsed two times in tether buffer (10 mM KPO₄, 0.1 mM EDTA, 0.067 NaCl, 1 μ M L-methionine, and 50 mM glycerol) via centrifugation ($1,900 \times g$ for 5 min).

Chemicals. Chemicals were obtained from the following sources: Bacto Tryptone, Bacto yeast extract and sodium chloride: Fisher Scientific; L-serine, L-methionine, α -methyl-aspartate, ampicillin, ethylenediamine tetraacetic acid, glycerol, and kanamycin: Sigma; potassium phosphate dibasic anhydrous and potassium phosphate monobasic monohydrate: Becton Dickinson; IPTG: Ambion.

Temperature Control. Custom temperature-controlled stages were built for an inverted and upright microscope. All temperature stages used controllers from Oven Industries (5C7-001). A cooling and heating stage used two water-cooled 77-W peltier elements on either side of a $5 \times 2 \times 1/8$ -inch steel stage. The cooling water was set at 10°C and supplied by a water chiller (Haake DC-10). A heating-only stage was built by using a pair of 10-W 10- Ω power resistors attached to an aluminum stage insert. For both stages, feedback temperature was measured next to the sample position by using a small thermistor. Temperature calibration was performed by using a small thermocouple and Fluke digital thermometer (Fluke 531I). The thermocouple was calibrated with a mercury thermometer certified by the National Institute of Standards and Technology. The microscope was given 20 min to reach equilibrium after each temperature change. The sample temperature was controlled to within 0.5°C.

Impulse Response Instrument. A 1,440-nm diode laser (FOL1404QQM, Fitel) was focused to an ≈ 3 - μ m-diameter spot at the sample plane by using an optical design similar to a laser tweezer (29). Briefly, the beam from the laser was expanded and steered by a set of infrared lenses in the 2F configuration and directed into a TE2000 inverted microscope configured with a laser-port step-up kit (Nikon). A custom dichroic mirror and blocking filter (Chroma) were used to reflect the light into the objective. The laser overfilled the back focal aperture of the 40 $\times$ DIC objective (ELWD Plan Fluor, NA = 0.60, Nikon). The laser was controlled and powered by a commercial driver (Thorlabs).