Cells of *Escherichia coli* swim either end forward
(receptor localization/bacterial chemotaxis)

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**ABSTRACT** Chemotactic cells of the bacterium *Escherichia coli* were marked asymmetrically by growth on a rich medium containing tetrazolium red. When this dye is reduced, it tends to form a refractile granule near one end of the cell, readily visualized by dark-field microscopy. In smooth-swimming cells, the marker was found with equal probability in front or behind. In wild-type cells, tumbles changed the cell orientation nearly as often as not. Some cells formed flagellar bundles at one end more frequently than at the other, but the run-interval distributions were the same either way. We conclude that the sensory system does not favor one end of the cell over the other. Thus, chemoreceptors that appear in patches at only one pole do not serve as a nose.

Cells of the bacterium *Escherichia coli* are about 1 μm in diameter by 2 μm long. If motile and chemotactic, they swim steadily forward (run) about 30 μm/s along paths roughly parallel to their longitudinal axes, move erratically with little net displacement (tumble), and then swim off in new directions chosen approximately at random (1). Cells run when pushed forward by a bundle of about six flagellar filaments, each rotating counterclockwise; they tumble when the direction of flagellar rotation changes, the bundle comes apart and the filaments move independently (2, 3). Cells are equipped with chemoreceptors that monitor the concentrations of a variety of chemicals in the external environment, e.g., sugars and amino acids (4). These measurements are made as a function of time (5, 6). Cells compare concentrations measured over the past second with those measured over the previous 3 s and respond to the difference (7): runs that carry cells in favorable directions are extended (1).

It was found recently by immunoelectron microscopy with antibodies against receptors for aspartate and serine and transducers for ribose/galactose (Tar, Tsr, and Trg) that these molecules are nonuniformly distributed (8). Immunogold particles were found in thin sections (~0.1 μm thick) near the cytoplasmic membrane anywhere along the cell body; however, polar clusters were the most frequent (in nonseptating cells, 80% of membrane label at the poles and 81% in clusters predominantly at one pole). This led to the suggestion that *E. coli* might have a nose; i.e., localization of chemoreceptors in a patch at the leading end of a moving cell might be an effective chemotactic strategy (9).

The advantage gained by putting the receptors in front, where they can interact with a few more molecules of ligand, would appear to be offset by the degradation in counting statistics resulting from reduction in detector size. *E. coli* is so small, and the efficiency of diffusion of small molecules over short distances is so great, that swimming has only a marginal effect on diffusive intake. For a spherical cell of radius *a* = 1 μm propelled at velocity *v* = 30 μm/s through a fluid containing molecules of diffusion coefficient *D* = 10⁻⁵ cm²/s, for which the cell is a perfect sink, the fractional increase in intake at the front half of the cell is approximately 5% (10). This gain is largely offset by a decrease in intake at the back half of the cell. The rms fractional error with which a cell of radius *a* can count molecules (whether or not the cell is a perfect sink) decreases as 1/(aDτ)¹/², where *c* is the bulk concentration of ligand and *τ* is the counting time (10). Thus, if one reduces the radius of the detector *a* by a factor of 10, the precision decreases by a factor of (10)⁵/² ≈ 3. Therefore, from the point of view of counting statistics, one does not want to put all of one's receptors in an isolated patch. If *E. coli* chooses to do this, there must be another reason. The receptors of * Caulobacter crescentus*, a polarly flagellated organism, are clustered not at the front of the cell but at the back, near the flagellum (11). This might ensure tight receptor–flagellar coupling. However, the flagella of *E. coli* are distributed widely over the surface of the cell.

Such reservations notwithstanding, the idea of a nose is intriguing and amenable to test. One simply marks cells so that one end can be distinguished from the other and asks whether front and back are randomized by tumbles and whether runs with the marker in front or back are of comparable length. If clustering of receptors is to provide an advantage at the level of sensory reception, then cell orientation must be tightly regulated. This question was not addressed in the original tracking experiments (1), where cells were treated as point particles. The marker should be cytoplasmic, so that it does not mechanically interfere with the flagella, and it should not inhibit chemotaxis. We chose tetrazolium red (12). When cells are grown in a well-buffered medium containing this dye, the dye is reduced, forming a red precipitate, usually near one end of the cell (see below). This does not inhibit motility or chemotaxis, since inclusion of this dye in media used when cells generate complex chemotactic patterns simply enhances their visibility (13).

**MATERIALS AND METHODS**

**Bacteria and Media.** Smooth-swimming cells were strain HCB437, deleted for *tar, tsr, trg*, and genes encoding cytoplasmic chemotaxis proteins (14). Wild-type cells were strain AW405 (15). A 0.1-ml aliquot of a stationary-phase culture [frozen in 10% (vol/vol) dimethyl sulfoxide] was added to 10 ml of tryptone broth containing 0.005% 2,3,5-triphenyltetrazolium chloride (Sigma) in a 10-ml Erlenmeyer flask and grown for about 15 h at room temperature (22°C) without aeration. The cells were washed and resuspended in a motility medium containing 0.01 M potassium phosphate (pH 7.0), 0.1 mM EDTA, and 0.18% methylcellulose (Fisher 4000 cP, used earlier in tracking *E. coli* to enhance motility and reduce Brownian rotation).

**Data Acquisition and Analysis.** The cells were observed in dark field with a Nikon Optiphot microscope equipped with a 100-W tungsten-halogen lamp and a ×40 objective. Unless otherwise noted, the depth of the preparation varied from about 100 to 140 μm. Smooth-swimming cells were observed near the slide; wild-type cells were observed half-way between the coverslip and the slide. The data were recorded on
videotape (Hamamatsu C2400 charge-coupled device camera, Panasonic AG6730 VHS recorder with time-date generator) and analyzed off-line by eye, on a 19-inch monitor. Time spans were computed from the recordings of the time-date generator.

RESULTS AND DISCUSSION

Distribution of Dye Relative to Flagellar Bundle. First, the distribution of the dye relative to the flagellar bundle was determined in cells that do not tumble, strain HCB437. Of 342 cells examined, 139 had a dye granule in front, 142 had it in the back, 42 had it near the middle of the cell, 8 had it at both ends, and 11 had it in a stripe. If an unbiased coin were flipped 139 + 142 = 281 times, one would expect 140 or 141 heads with a standard deviation of (281)^1/2 = 8.4. Thus, we conclude that the positions of the dye and the flagellar bundle are not correlated.

Changes in Orientation on Tumbling. Next, the probability that a cell changes its orientation on tumbling was determined with wild-type strain AW405 using a similar preparation. Cells tended to swim into focus, tumble once or sometimes twice (as shown by abrupt changes in direction), and then swim out of focus (Fig. 1). Of 390 asymmetrically labeled cells examined, 214 first appeared with the dye in front and 176 appeared with the dye in back. Of the former, 116 maintained the same orientation and 98 changed orientation; of the latter, 103 maintained the same orientation and 73 changed orientation. So a total of 219 cells maintained the same orientation, and 171 changed orientation. If an unbiased coin were flipped 390 times, one would expect 195 heads with a standard deviation of 9.9. The result is 2.4 standard deviations from the mean; the probability of this happening by chance with unbiased cells is 2%. So the cell might prefer to maintain its initial orientation with a bias of order 219/390 = 0.56. It is known that the distribution of changes in direction from the end of one run to the beginning of the next peaks in the forward direction (at 68° rather than at 90°, expected from random changes) and that the shortest tumbles, which are the most probable, tend to generate smaller changes (1). So it would not be surprising if there were some preference for the initial orientation. But a bias of 0.56 is not large enough for that orientation to persist for more than a few seconds, given a mean interval between tumbles of order 1 s.

Symmetry of Bundle Formation and Duration of Runs. Finally, the likelihood that a cell spends a large fraction of its time oriented in the same way was determined by following individual wild-type cells for many minutes. Attempts to do this with the tracking microscope (16) failed, because the dye proved very hard to see with the ×20 phase-contrast objective used in that device. So cells were followed by hand in a relatively thin preparation (38 μm between coverslip and slide) in dark field at a similar magnification. Methylcellulose was omitted, because it tended to stabilize the interaction of cells with the glass. A given cell spent only part of its time near the coverslip or the slide, often moving out of focus, so that its image was lost and later regained. We believe that most of these events were associated with tumbles, because they rarely occurred with smooth-swimming cells, which stayed near the coverslip or slide for long periods of time (data not shown). Therefore, a new run was flagged either when a tumble was seen or when the cell moved out of focus. Sometimes tumbles could be inferred, because when the cell was located again, it had changed orientation. We called these events transitions. They were never seen with smooth-swimming cells.

Table 1. Cells tracked by hand for long periods of time

<table>
<thead>
<tr>
<th>Cell</th>
<th>Total time, s</th>
<th>Time, s</th>
<th>Runs, no.</th>
<th>Run interval, s</th>
<th>Tumbles seen, no.</th>
<th>Transitions seen, no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>705</td>
<td>283</td>
<td>284</td>
<td>43</td>
<td>47</td>
<td>6.6 ± 10.4</td>
</tr>
<tr>
<td>2</td>
<td>675</td>
<td>84</td>
<td>154</td>
<td>109</td>
<td>191</td>
<td>0.8 ± 0.6</td>
</tr>
<tr>
<td>3</td>
<td>173</td>
<td>51</td>
<td>52</td>
<td>54</td>
<td>43</td>
<td>0.9 ± 0.8</td>
</tr>
<tr>
<td>4</td>
<td>465</td>
<td>81</td>
<td>268</td>
<td>49</td>
<td>69</td>
<td>1.7 ± 2.2</td>
</tr>
<tr>
<td>5</td>
<td>838</td>
<td>289</td>
<td>481</td>
<td>19</td>
<td>25</td>
<td>15.2 ± 27.3</td>
</tr>
<tr>
<td>6</td>
<td>150</td>
<td>54</td>
<td>61</td>
<td>14</td>
<td>23</td>
<td>3.9 ± 3.2</td>
</tr>
<tr>
<td>7</td>
<td>652</td>
<td>139</td>
<td>273</td>
<td>77</td>
<td>135</td>
<td>1.8 ± 1.8</td>
</tr>
<tr>
<td>8</td>
<td>489</td>
<td>147</td>
<td>222</td>
<td>41</td>
<td>56</td>
<td>3.6 ± 4.5</td>
</tr>
<tr>
<td>9</td>
<td>720</td>
<td>230</td>
<td>359</td>
<td>50</td>
<td>85</td>
<td>4.6 ± 10.2</td>
</tr>
<tr>
<td>10</td>
<td>231</td>
<td>129</td>
<td>41</td>
<td>26</td>
<td>14</td>
<td>5.0 ± 10.0</td>
</tr>
<tr>
<td>11</td>
<td>383</td>
<td>295</td>
<td>32</td>
<td>54</td>
<td>10</td>
<td>2.1 ± 8.8</td>
</tr>
<tr>
<td>12</td>
<td>645</td>
<td>147</td>
<td>521</td>
<td>68</td>
<td>140</td>
<td>2.2 ± 2.7</td>
</tr>
</tbody>
</table>

During a given run, the marker was seen either at the head (H) or at the tail (T). The run-interval columns give the mean interval ± its standard deviation. Transitions refer to events in which a cell moved out of focus in one orientation and was located again in the other.
The data for wild-type cells are summarized in Table 1. The total tracking time is larger than the time the marker was seen at the head (H) plus the time it was seen at the tail (T), because it includes the time the cell was out of focus. Whereas some cells spent the same amount of time H or T (e.g., cells number 1 and 3), most did not. But this was not true because intervals of one type were much longer than those of the other, but rather because they differed in number. The mean intervals were roughly the same in either case, as were the shapes of the distributions; only their areas varied. The distributions for cell number 2 are shown in Fig. 2. All distributions were approximately exponential except for the paucity of short events, as expected for runs under conditions in which one cannot detect such events (as judged by the criteria applied earlier in tracking experiments). We conclude that a tumble occurs with equal probability regardless of the orientation of the cell body. Therefore, the machinery that controls the direction of flagellar rotation is not sensitive to cell orientation.

Why then do some cells run more often with the marker in one orientation? One possibility is that when a tumble ends and a bundle forms, it is more likely to form at one end of the cell than at the other. This seems likely if the distribution of flagella over the cell surface is asymmetric. If flagella arise at random points, asymmetry will be common. A rough estimate can be made by dividing the cell in two (H or T) and flipping coins, i.e., by applying the binomial distribution. For example, if there are six flagella, the chance is 5/16 that they are distributed 3 and 3, 15/32 that they are 2 and 4, 3/16 that they are 1 and 5, and 1/32 that they are all at one end. In real life, the problem is more complicated, because the total number of flagella varies from cell to cell. A second possibility is that our experimental set-up favors observation of runs with the marker in the tail, since the cells in Table 1 spent 59% of their time (58% of their runs) in that configuration. However, the bias in the earlier tumble experiments was the other way around, so this difference is probably not significant. If asymmetric distribution of flagella over the cell surface does favor bundle formation at one end of a cell, then tumbles should leave cells in the same orientation more often than not, as observed. So these data are internally consistent.

Summary. Tumbles tend to randomize cell orientation, and runs of either orientation are of similar duration. Therefore, the cell sensory system does not determine asymmetric cell orientation. If there is an advantage to localizing chemoreceptors in patches at only one pole, that advantage must accrue at some step after signal detection.

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