5
Flagellar Motion

Rotation

Whether a cell runs or tumbles depends on the direction of rotation of its flagella, but the story turns out to be rather complicated. A tumble involves not only a change in the direction of rotation of one or more of the flagellar filaments, but also a sequence of changes in their handedness and pitch.

During a run, after a cell has gotten up to speed, all of the filaments rotate in the same direction, usually counterclockwise (as seen by an observer behind the cell). Each filament turns individually, but they go around side by side. If you think that the filaments should tangle up or tie in knots, take two thin rods—aluminum welding rods work fine—and wrap them into identical shallow helices by bending them around a pipe. They need not be precisely helical, they just need to be the same shape. Then hold the helices at one end, side by side, and roll them between your fingers. If the helices are left-handed (spiral to the left as they extend away from you) and you turn them clockwise (counterclockwise as seen by someone looking at them from the other end), they will turn smoothly, in parallel. They will do so even if they cross over one another, because the points of crossover travel away from you and are shed at the distal end (Macnab, 1977). If you turn the helices the other way and the wires happen to cross over, then the bundle will jam when the point of crossover reaches your hand. It takes considerable force to break such jams, and the wires rattle as they snap over one another. So the motion in one direction is smooth and quiet and in the other direction rough and noisy.

One of the initial arguments for flagellar rotation (Berg and Anderson, 1973) was the fact that a small amount of bivalent antifilament antibody would jam flagellar bundles, while a large amount of monovalent antifilament antibody (a bivalent antibody

cut in two) had no effect. Evidently, the bivalent antibody worked by cross-linking one filament to another, preventing the rotation, while the monovalent antibody simply made each filament thicker. Another argument was that two cells linked together by their flagella (actually their hooks) counterrotated. A filament is joined to the drive shaft of the motor at its base by a short flexible coupling called the proximal hook. Mutants had been found in which these hooks were abnormally long and filaments were largely absent. Such cells were nonmotile. However, when antihook antibody was added, these cells formed pairs that counterrotated.

This assay was perfected by Silverman and Simon (1974), who cemented filaments (or hooks) to glass using antifilament (or antihook) antibody. If only one filament (or hook) was tethered in this way, the cell body spun at speeds of about 10 Hz (revolutions per second), alternately clockwise or counterclockwise (CW or CCW), changing directions about once per second. Such a tethered cell is shown in Fig. 5.1, spinning CCW.

The correspondence between CCW rotation and runs, on the one hand, and CW rotation and tumbles, on the other, was then established by tethering cells and adding attractants or repellents (Larsen et al., 1974). When a large amount of chemical attractant was added, the cells spun exclusively CCW for several minutes, just as swimming cells ran exclusively in the mixing experiments of Macnab and Koshland (1972). If, instead, a large amount of repellent was added, the cells spun exclusively CW, but only

FIGURE 5.1. Three cells of *E. coli* wild-type strain AW405 tethered to a glass coverslip by a single flagellar filament (top) or simply stuck to the glass (bottom) shown at intervals of 0.1 second beginning at the left. The tethered cell (a long cell about to divide) completes one revolution counterclockwise (CCW). Its axis of rotation is near the right end of the image on the left. Note that the concave side of the cell leads and the convex side lags: the cell is rotating (like a pinwheel) not gyrating (like your arm when you wave it in a circle). (From Berg, 1976, Fig. 1.)

for several seconds, again just as cells tumbled in the mixing experiments.

Filament Shape

Flagellar filaments are relatively stiff, but they can switch between distinct polymorphic forms. Four of these forms are shown in Fig. 5.2. The normal filament is left-handed, and the semicoiled and curly filaments are right-handed. The filament is a polymer of a single protein called flagellin, whose molecules can bond in two different ways. They appear as 11 rows of protofilaments along the surface of a cylinder, as shown in Fig. 5.3. When the flagellin molecules are bonded in one way, the row is short; when they are bonded in the other way, the row is long. If all of the protofilaments in a flagellar filament are identical, the filament is straight. There are two kinds of straight filaments, short or long, but the difference in their lengths is relatively small. However, if some of the protofilaments are short and others are long, the filament is

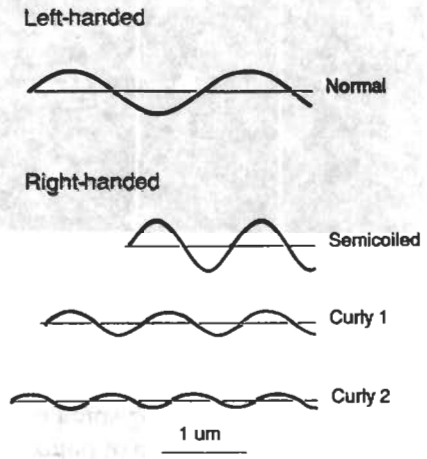

FIGURE 5.2. Drawing of four different flagellar waveforms, each with a contour length of 4 μm. A filament of this length contains about 8000 molecules of flagellin (Hasegawa et al., 1998). The normal filament is left-handed, and the semicoiled, curly 1, and curly 2 filaments are right-handed. The normal and curly 1 filaments have the same overall length. Bar, 1 μm. (Adapted from Calladine, 1978.)

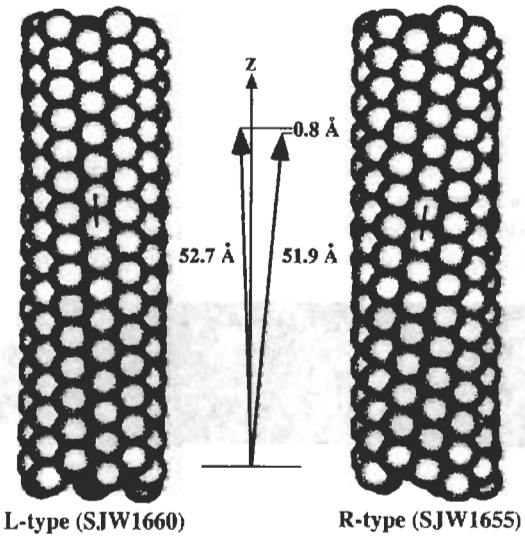

FIGURE 5.3. The surface lattice of L- and R-type straight flagellar filaments. The spacing between flagellin subunits along an 11-start helix (a protofilament) of the R-type is 0.08 nm smaller than between corresponding subunits of the L-type. L and R refer to the handedness of the filament twist. The SJW numbers are numbers of bacterial strains. The distances are measured at a radius of 4.5 nm and are shown magnified in the middle of the drawing. (Namba and Vonderviszt, 1997, Fig. 19, reprinted with permission.)

helical: the short protofilaments run along the inside of the helix. The different shapes shown in Fig. 5.2 arise from different numbers of adjacent protofilaments of a given type. Transformations between these polymorphic forms can be driven by changes in protein structure (i.e., by mutations in the flagellin gene), by changes in the composition of the surrounding medium [e.g., in pH (acidity) or ionic strength (salt content)], or by mechanical twist (i.e., by torsion).

Tumbling

Until recently, it was thought that tumbles occur when all of the flagellar motors switch from CCW to CW, even though experiments in which motors were studied in isolation (in the absence

of large stimuli and without interacting filaments) suggested that each motor switches independently. The resolution to this puzzle was found on labeling flagella with a bleach-resistant fluorescent dye and recording their motion in a fluorescence microscope using strobed laser illumination (Turner et al., 2000).

The simplest case is a cell with a single flagellar filament (Fig. 5.4). A transformation from normal to semicoiled is seen in fields 4 to 10, from semicoiled to curly 1 in fields 12 to 18, and from curly 1 back to normal in fields 24 to 30. The cell swam into the field of view moving toward the 7 o'clock position and left the field of view moving toward the 5 o'clock position. Most of this change in direction occurred while the filament was partially in the semi-

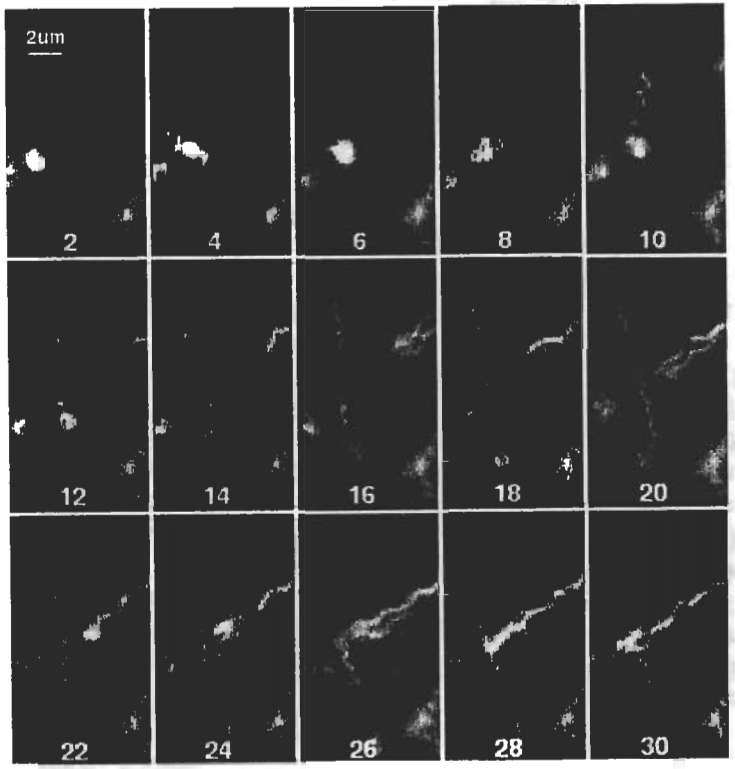

FIGURE 5.4. A cell with one flagellar filament undergoing a polymorphic transformation. The video recording was made at 60 Hz, but only every other field is shown. The numbers are in units of 1/60 second. Note the scale bar (2 μm). (From Turner et al., 2000, Fig. 6.)

coiled form (fields 4 to 12). Evidently, the flagellar motor switched from CCW to CW after field 2 and back again after field 22.

This pattern also occurs in cells with several filaments, where the tumble is generated by changes in the direction of rotation of as few as one or as many as all of the filaments. Generally, the more filaments that are involved, the larger the change in direction. As the filaments regain their normal conformation, they rejoin the normal bundle. Figure 5.5 shows a cell with two flagellar filaments, only one undergoing polymorphic transformations. Note the curly 1 filament wrapping around the normal filament and rejoining the bundle as it reverts to the normal conformation, fields 17 to 20. This cell swam into the field of view moving toward

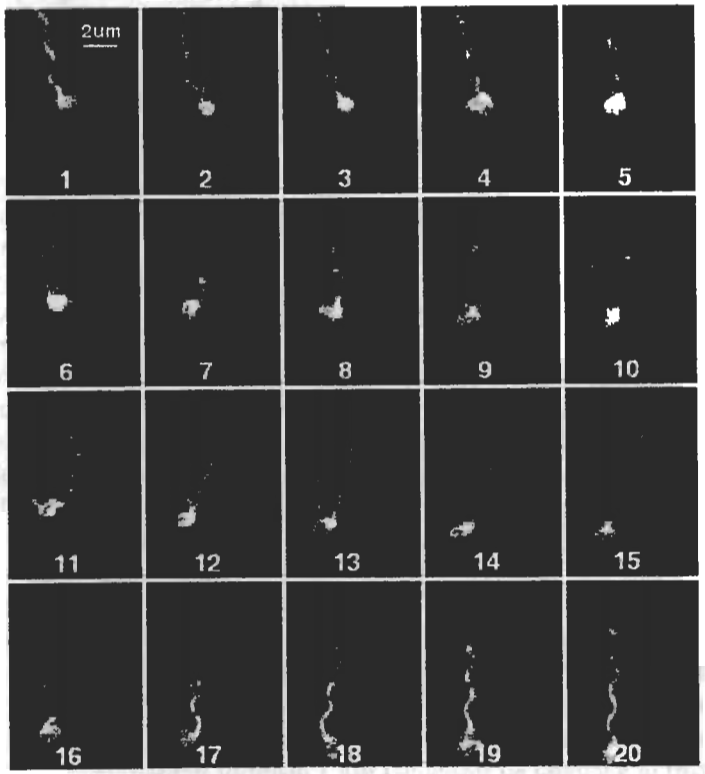

FIGURE 5.5. A cell with two flagellar filaments, only one undergoing polymorphic transformations. (From Turner et al., 2000, Fig. 7.)

the 5 o'clock position and left the field of view moving toward the 6 o'clock position.

The sequence of normal, semicoiled, curly 1, and then back to normal is observed most frequently, as summarized in Fig. 5.6. The change in direction of the cell body generally occurs early on, while the filament is partially in the semicoiled form. This explains why the time required for the cell to change direction, indicated in Fig. 4.3 by the horizontal bars, is substantially shorter than the time required for the cell to get back up to speed, indicated by the corresponding speed trace. The cell in Fig. 5.6 starts out along its new path being pushed by a curly 1 filament spinning CW and a

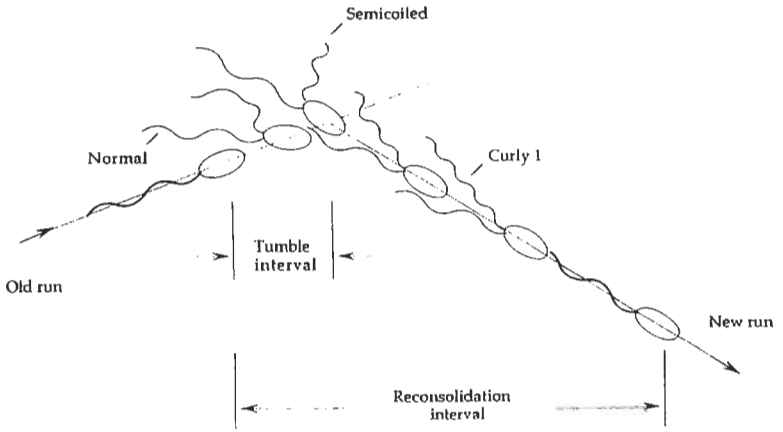

FIGURE 5.6. A schematic drawing of the events that usually occur during a tumble. A cell with a bundle of two flagellar filaments is shown swimming from left to right. The cell alters course as the motor driving one filament changes its direction of rotation and the filament undergoes a normal to semicoiled transformation. This change in course defines the tumble interval, which, according to both the tracking and video data, takes 0.14 second, on average. As the cell begins to move along its new track, the filament undergoes a semicoiled to curly 1 transformation. Both the normal and curly 1 filaments generate forward thrust, but the curly one at a smaller magnitude. Finally, after the direction of flagellar rotation changes again, the filament reverts to normal. As it does so, it rejoins the bundle, and the cell resumes its initial speed. The time from the initial disruption of the bundle to its reconsolidation is defined as the reconsolidation interval. According to the video data, this takes 0.43 second, on average.

normal filament spinning CCW. This propulsion is not as efficient as when both filaments are normal and spinning CCW.

High-speed video recording reveals that transformations from normal to semicoiled or curly 1 are triggered by changes in direction of flagellar rotation from CCW to CW, as expected, while transformations back to normal are triggered by changes in direction from CW back to CCW. But it also is possible for filaments of different kinds to spin backward without changing their overall shape.

In earlier work with swimming cells studied by dark-field microscopy, Macnab and Ornston (1977) observed the curly 1 transformation. Hotani (1982), working with isolated filaments fixed to glass at one end, was able to generate both semicoiled and curly 1 transformations, by flow of a viscous medium. In dark field, an enormous amount of light is scattered by the cell body, so Hotani had an easier task than Macnab and Ornston. The problem of scattering from the cell body is eliminated by the fluorescence technique.

Complications notwithstanding, we are left with the remarkable conclusion that the behavior of the cell depends on the direction of rotation of rotary motors that drive propellers that change their handedness and pitch.

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