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Rotation and Switching of the Flagellar Motor Assembly in *Halobacterium halobium*

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Halobacterium halobium **swims with a polarly inserted motor-driven flagellar bundle. The swimming direction of the cell can be reversed by switching the rotational sense of the bundle. The switch is under the control of photoreceptor and chemoreceptor proteins that act through a branched signal chain. The swimming behavior of the cells and the switching process of the flagellar bundle were investigated with a computer-assisted motion analysis system. The cells were shown to swim faster by clockwise than by counterclockwise rotation of the flagellar bundle. From the small magnitude of speed fluctuations, it is concluded that the majority, if not all, of the individual flagellar motors of a cell rotate in the same direction at any given time. After stimulation with light (blue light pulse or orange light step-down), the cells continued swimming with almost constant speed but then slowed before they reversed direction. The cells passed through a pausing state during the change of the rotational sense of the flagellar bundle and then exhibited a transient acceleration. Both the average length of the pausing period and the transient acceleration were independent of the stimulus size and thus represent intrinsic properties of the flagellar motor assembly. The average length of the pausing period of individual cells, however, was not constant. The time course of the probability for spontaneous motor switching was calculated from frequency distributions and shown to be independent of the rotational sense. The time course further characterizes spontaneous switching as a stochastic rather than an oscillator-triggered event.**

Halobacteria thrive facultative-phototrophically in brines and salt ponds with saturating concentrations of sodium chloride and other salts under strong sunlight. The photobiochemistry of these organisms is based on two light-driven ion pumps, bacteriorhodopsin and halorhodopsin, which absorb light with their retinal chromophors in the visible range (500 to 600 nm) and translocate protons and chloride ions to the outside and the inside of the cell, respectively (29). In addition to its use for light-energy conversion, retinal is the chromophore of sensory rhodopsins I and II (P-480, phoborhodopsin), two photoreceptor pigments that control the swimming behavior of the cell (for reviews, see references 27, 28, 34, and 36). During heterotrophic growth when oxygen is abundant, *Halobacterium* spp., like many heterotrophic organisms, avoid blue light which is received by sensory rhodopsin II. When oxygen is scarce, the light-driven ion pumps and, in parallel, sensory rhodopsin I are induced (21, 30). Sensory rhodopsin I acts as a green and near-UV light receptor and aids the cell in finding photosynthetically efficient green to orange light.

Halobacterium halobium cells are propelled by a monopolarly inserted flagellar bundle. In the stationary growth phase bipolarly flagellated cells also occur, but generally one of the flagellar bundles is much shorter than the other and thus contributes less to the overall movement of the cell (2). Each flagellar bundle consists of 5 to 10 flagellar filaments (11) that form a right-handed semirigid helix. In the electron microscope, it is seen that each of the isolated flagellar filaments is equipped with a basal body and therefore most likely driven by an individual flagellar motor (12a).

Bacterial flagella are too thin to be resolved by the light microscope. Fortunately, they can be visualized by high-intensity dark-field microscopy (17, 31) so that their action can be directly observed in vivo. Alam and Oesterhelt (2) used this method to study the flagellation and swimming mechanism of *H. halobium*. Their experiments revealed that clockwise (CW) rotation of the right-handed helical flagellar bundle exerts a pushing force on the cell body and propels it forward. In the counterclockwise (CCW) rotational sense, the bundle pulls the cell, which then swims with the flagellated pole in front. Thus, in each rotational sense a translational movement results. This is quite different from the chemotactic bacterium *Escherichia coli*, which swims only when the left-handed flagella are rotating CCW, whereas CW rotation or pauses of rotation produce tumbling (1, 3, 7, 14, 18, 35).

In the absence of stimuli, the halobacterial cells occasionally spontaneously switch the rotational sense of their bundle and continue swimming but in the reverse direction (2). After each switching event, the cell does not necessarily take the original path. Because they spend on the average the same time in both rotational modes, the cells carry out a random walk that keeps them evenly distributed in their suspending medium. The random walk may become biased because the switch of the flagellar motor is under the control of a signal chain. That part of the flagellar motor apparatus which interacts with the ultimate member of the signal chain we call the switch. The signal chain integrates the information coming from both photo- and chemoreceptors (38). An increase in intensity of blue or near-UV light or a decrease in intensity of orange light induces motor switching and thus a photophobic response of the cell (9). Inverted stimulation with these light qualities suppresses spontaneous motor switching for a certain time so that the cell continues swimming in the preferred direction. The sensory input is transmitted from the receptors to the motor via a chemical signaling path (26) that depends on fumarate (23).

Although photoreceptors and signal chains are intensively studied, very little is known about the final target of the

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sensory input, the flagellar motor apparatus of *H. halobium*. In this study, we analyzed the switching behavior of the flagellar bundle and demonstrated that the rotation and switching of the individual filaments are synchronous.

MATERIALS AND METHODS

Bacterial strains, culture conditions, and preparation of specimens. *H. halobium* M407 (BR^- SRI^+ $SRII^+$; 21), M416 (BR^- SRI^+ $SRII^-$; derived from M407; 15), and Fl×37 (BR^- SRI^+ $SRII^+$; 37) were grown under standard conditions in the dark (25). If not indicated otherwise, the cultures were grown for 2 days to obtain only monopolarly flagellated cells and diluted to a density of 3.5×10^8 cells per ml with fresh medium that contained 0.1% arginine prior to measurement. All experiments were carried out at 21°C.

Dark-field microscopy. Observation of the flagellar bundles was carried out by high-intensity dark-field microscopy essentially as described by Alam and Oesterhelt (2). Observation light was produced by a 900-W xenon lamp, filtered through 2 cm of a 1% $CuSO_4$ solution, and immersed in a 20-cm water bath and KG 3 and KG 4 heat protection filters (Schott, Mainz, Federal Republic of Germany), both 3 mm in thickness, and an OG 570 filter to remove any blue light. Observation was carried out with a 50:1 oil immersion objective.

For experiments in which the flagellar bundle was to be observed, a 33-h-old culture was used since the flagellar bundles are most visible in this phase (26). Before measurement, the cell suspension was diluted 10 times with normal growth medium that contained 0.1% arginine (pH adjusted to 7.0). A 5-μl sample of this cell suspension was put on a slide and covered with a slip (20 by 20 mm; Menzel Gläser, Braunschweig, Federal Republic of Germany), both of which were cleaned with acetone and double-distilled water. After 30 min of incubation at room temperature, the visibility of the flagellar bundles was optimal and the experiment was started. When a photophobic response of the cell was to be induced, the OG 570 filter was removed for a fraction of a second so that the cells were exposed to the blue light.

Motion analysis. The motion of the cells was recorded on line by a VP-110 videoprocessor (Motion Analysis Corp., Santa Rosa, Calif.) and evaluated essentially as described previously (22). Frames were digitized at a rate of 10 or 15 Hz for 5- or 6-s time intervals. The program, written in Turbo-Pascal, allowed for the simultaneous display of each individual track and the parameters of motion.

RESULTS

Cells are propelled by CW and CCW rotation of the flagellar bundle with different efficiencies. When the swimming behavior of monopolarly flagellated cells was observed, often faster and smoother swimming during CW than during CCW rotation could be seen. A typical example of this behavior is shown in Fig. 1. To quantitate the efficiency of flagellar propulsion in the two modes, we recorded the swimming pattern and the velocity of single cells with a computer-assisted motion analysis system under simultaneous observation of the rotational sense of the flagellar bundle by high-intensity dark-field microscopy.

Figure 2 shows the average speed of a single cell over 5 s, as propelled by CW or CCW rotation of the flagellar bundle. It can clearly be seen that the average velocity of the cell during CW rotation was nearly twice (1.85 times) that during CCW rotation. The swimming velocity in each rotational mode was relatively constant in time; i.e., the standard deviation was ±12% of the mean for CW and ±24% for CCW rotation. This result indicated that most if not all of the 5 to 10 flagellar filaments rotated synchronously in the CW or CCW direction, since a larger fluctuation in speed would be expected otherwise.

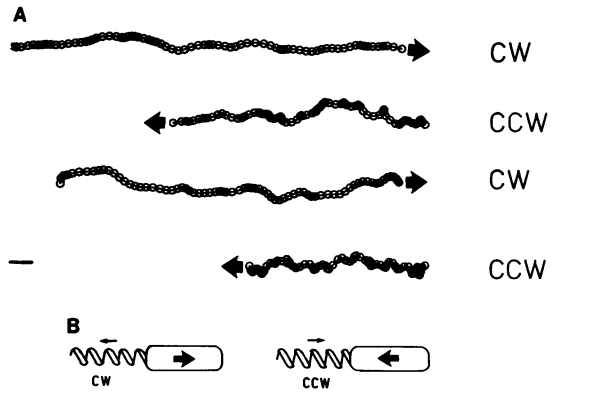

FIG. 1. (A) Motion analysis of a halobacerial cell in two modes of swimming. The pattern of a single cell during successive intervals of CW and CCW rotation of its flagellar bundle was recorded, and the centroids of the cell were displayed with a 67-ms time resolution. The bar represents 1 μm. (B) Swimming direction of halobacterial cells with respect to the rotational sense of the flagellar bundle.

The visibility of the halobacterial flagellar bundle in dark-field microscopy was often much better during CW than during CCW rotation. In many cells, the flagellar bundle was visible only in the CW mode and seemingly disappeared upon switching to the CCW mode. This finding might be due either to partial dissociation of the flagellar bundle, since CCW rotation destabilizes the right-handed helical arrangement, or to an asynchronous rotation of individual flagellar filaments in the CCW mode that dissociates the flagellar bundles as well.

Cells can pause for several hundred milliseconds during flagellar motor switching. The swimming pattern of individ-

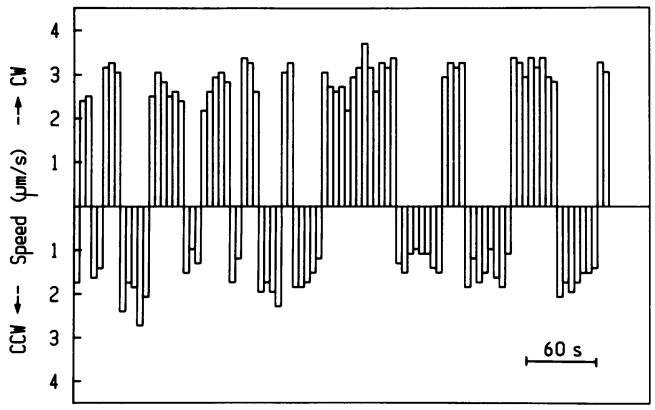

FIG. 2. Histogram of speed distribution of a swimming halobacterial cell. The speed of a single cell during successive intervals of CW and CCW rotation of its flagellar bundle was recorded as in Fig. 1 for periods of 5 s. Each bar represents the average velocity of the cell during this period. From time to time, the cell was stimulated with light to induce motor switching. The cell had a speed of 2.94 ± 0.34 μm/s (mean ± standard deviation; $n = 48$) during CW rotation and a speed of 1.59 ± 0.39 μm/s ($n = 45$) during CCW rotation.

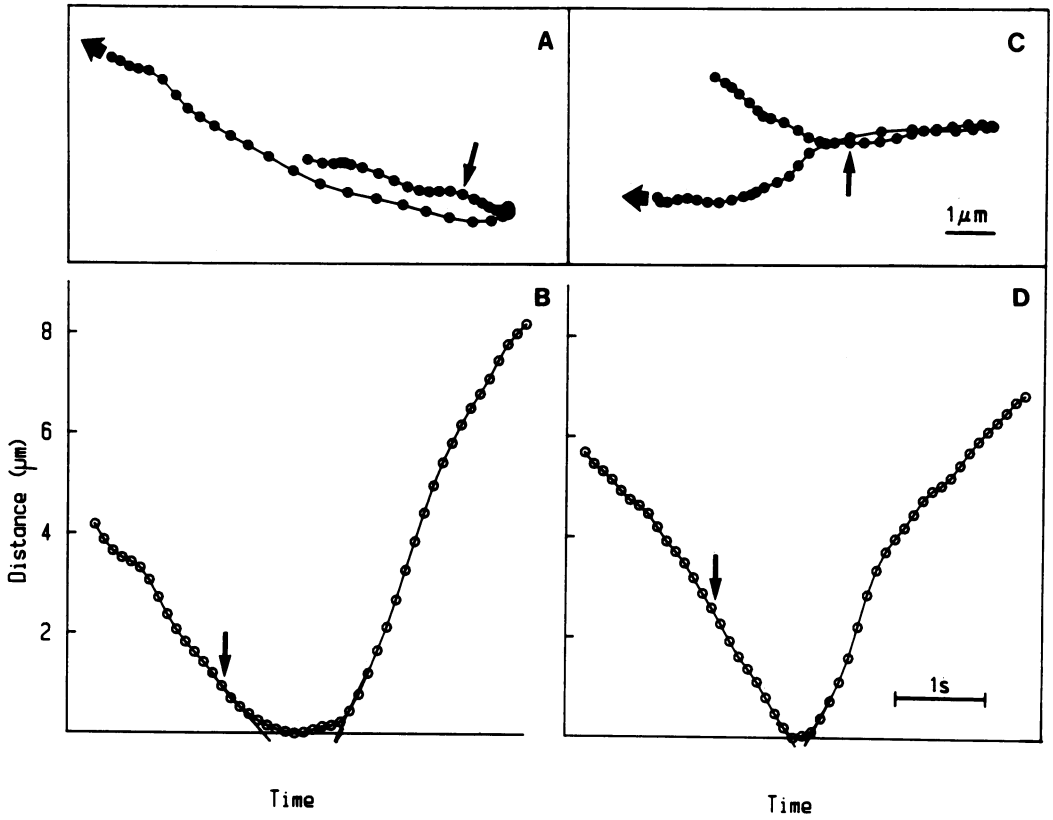

FIG. 3. Motion patterns of *Halobacterium* cells during motor switching. The behavior of single cells was recorded and evaluated for the pausing period. Two typical examples are shown. (A and C) Swimming path of a cell; the successive centroids are displayed in 100-ms time intervals. The thin arrow marks the onset of light; the thick arrow indicates the swimming direction of the cell. (B and D) Distance of the position of a cell at time t from the point of reversal (distance = 0) as calculated from the tracks in panels A and C, plotted against time with a 100-ms time resolution. The thin arrow marks the onset of light. Both cells stopped during switching of the flagellar motor and subsequently showed a transient acceleration of swimming speed. Two cells from strain M416 were observed.

ual cells during motor switching that was induced by blue light stimulation was recorded. A transient acceleration of the swimming speed immediately after reversal of the rotational sense was often observed (Fig. 3A and C). Most of the cells apparently paused in the course of a switching event for several hundred milliseconds before they resumed swimming (Fig. 3A). By plotting the distance of the cell's center from the point of reversal against time, a period of drastically reduced speed during motor switching becomes obvious; in the text that follows, this period is called pausing of the cell. The length of this pausing period was estimated by extrapolation of both branches of the curve to the abscissa, i.e., to the zone of speed zero (Fig. 3B and D). The duration of individual pausing periods determined in this way was found to be quite variable. Figure 4 shows a frequency distribution of pausing intervals measured in a cell population. The vast majority (82%) of the cells examined paused for longer than 114 ms. This average length of the pausing interval was 330 ms for M416 cells at 21°C. This value coincides with that for the most abundant class of the histogram in Fig. 4.

Data obtained by repeated stimulation of a single cell are shown in Fig. 5. It is clearly seen that the cell paused for various times. Thus, the broadness of the pausing interval distribution in Fig. 4 is not only due to different behaviors of individual cells.

Pausing of a cell could be the result of either a real stop in the rotation of all individual motors of the flagellar bundle or the antagonistic rotation of individual motors within the bundle at the same time (see Discussion).

Cells swim with nearly constant speed prior to motor switching. To measure the kinetics of motor switching, cells

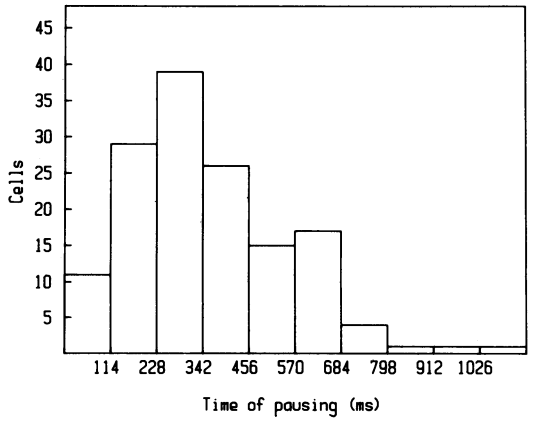

FIG. 4. Distribution of pausing times in a cell population. The pausing times of single cells from strain M416 upon near-UV light stimulation as measured at 21°C was evaluated from plots as in Fig. 3. Of 179 cells examined, 146 paused. In 33 cells (i.e., 18%), no pausing was detected during motor reversal.

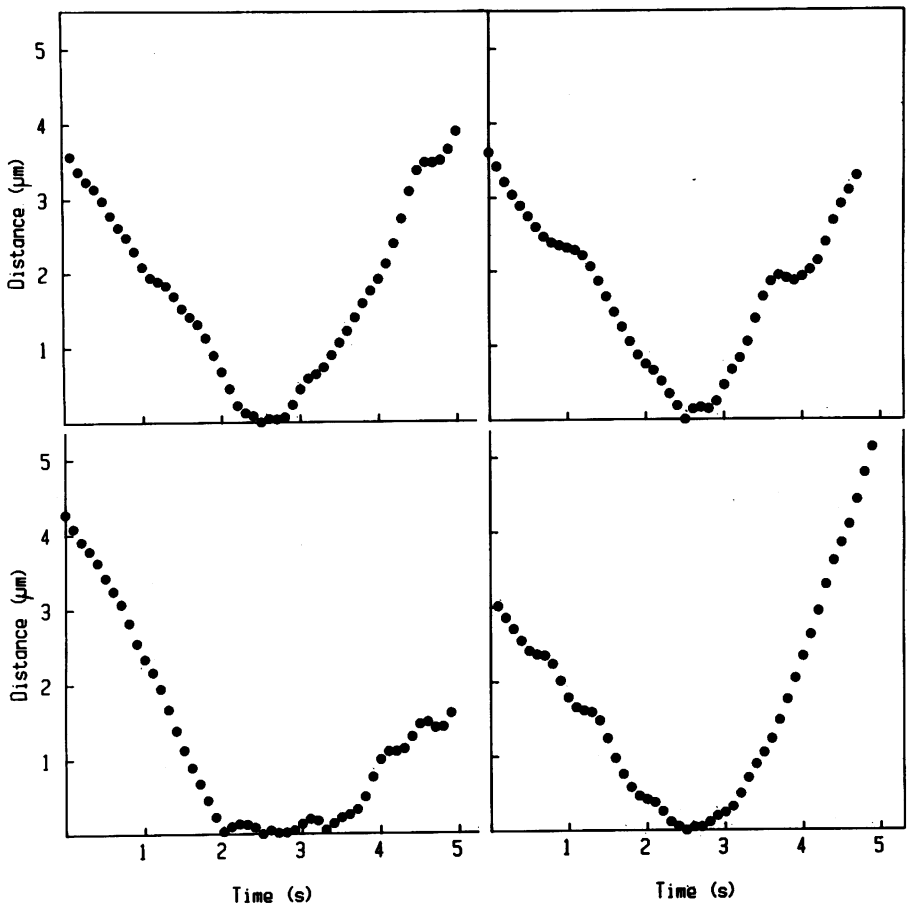

FIG. 5. Different pausing intervals measured in a single cell.

were stimulated with a step-down in orange light intensity and their swimming path was recorded. Since the response time, i.e., the time elapsing after stimulus application until reversal, is not identical in every cell even upon repeated stimulation at a given intensity, the distance-versus-time plots had to be superimposed with respect to the time of reversal of the flagellar motor. Figure 6 shows a plot for which the data from several hundred cells were averaged. After application of the stimulus (arrow), the cells continued swimming with almost constant speed. About 500 ms prior to the switching event, the speed was reduced to about 80% of its initial value. Thus, the signal formed did not slow down the speed of the motor assembly for at least 1.3 s after stimulus application. The plot shows that switching occurs as a sudden event. The discontinuity of the curve presumably is due to a short tumbling movement of the cells which can be observed in the moment at which the flagellar bundle is switched.

Stopping period and transient acceleration are independent of the stimulus size. If halobacterial cells are stimulated with a blue light flash, the response time (the time elapsed between the onset of light and the photophobic response) is inversely proportional to the flash intensity (21). The same set of experiments was carried out with two different light intensities to analyze the duration of the stopping period. As expected, the different intensities of the blue flash caused a difference in the average response time (data not shown). The average pausing period evaluated from extrapolation to the abscissa as in Fig. 3 did not change significantly upon

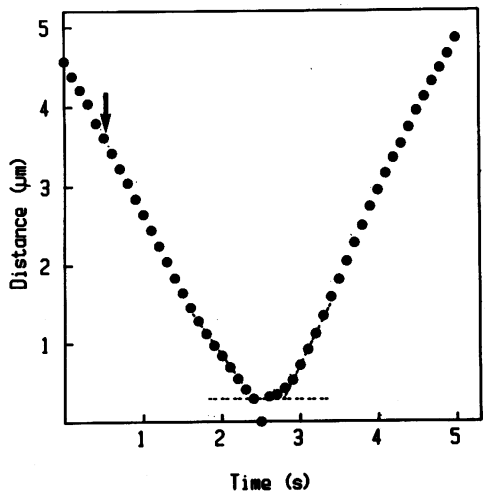

FIG. 6. Average distance of the position of an individual cell of a population from the point of reversal of the flagellar motor. Cells were stimulated by a step-down in intensity of orange light (579 nm) from 64 W m^{-2} to zero, and their motion was recorded with a 100-ms time resolution. The time at which the flagellar motor switched was determined as described previously (22), and the time-dependent difference in position of the cell was plotted versus time. The data for 495 cells of strain Fl×37 were superimposed and calibrated to their point of reversal. On average, the cells responded 2.04 s after stimulus application (arrow). A 60-h-old culture was used. The average pausing period of the cells was evaluated, as indicated by the dashed lines.

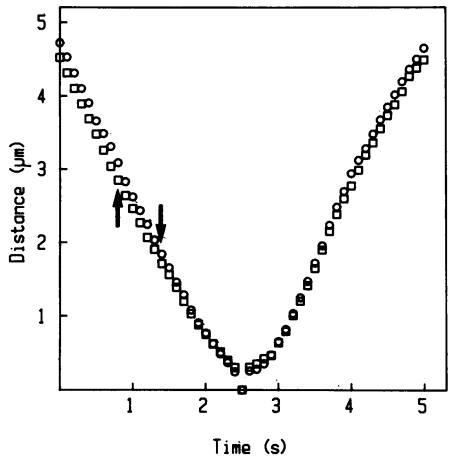

FIG. 7. Pausing of halobacterial cells upon stimulation with different intensities of blue light. Cells of a 24-h culture of strain Fl×37 were stimulated with a 100-ms pulse of 481-nm light. The data for individual cells were superimposed as described in the legend to Fig. 6. At 9.8×10^{-4} mol m^{-2} (○), the cells responded on average 1.16 s (n = 226) after stimulus application (↓). When stimulated with 1.23×10^{-5} mol m^{-2} (□), the mean response time that elapsed after stimulus application (↑) was 1.67 s (n = 226).

increase of the stimulus intensity 80-fold (Fig. 7). This was true also for the transient acceleration (by a factor of 1.5 in the experiment of Fig. 7) of the swimming speed after the switching event. This finding demonstrates that both stopping and transient acceleration are due to intrinsic properties of the flagellar motor switch rather than to the strength of the signal.

Switching from CW to CCW and vice versa occurs with the same time-dependent probability. Halobacteria on the average spend equal times in the CW and CCW rotational modes (10, 20), but the swimming speeds of the cells are different in the two modes (Fig. 1 and 2). Therefore, further experiments were done to determine whether this asymmetry includes the flagellar motor switch. Time spans between spontaneous reversals were measured under simultaneous observation of the flagellar bundle in a dark field, and the swimming intervals in the CW and CCW modes were recorded and grouped into classes.

Figure 8A shows the data obtained from experiments with six cells. The logarithm of the number of switching events that have not occurred at time t after the preceding event is plotted versus time. The straight line with a negative slope indicated an exponential decrease of the number of reversals still to be expected. During the exponential decay, switching occurs with a constant probability per unit of time, with equal time constants for transitions from CW to CCW and back to CW. The early phase of the curve, i.e., the behavior up to 7 s, is not fitted by the exponential. In contrast, the number of cells switching immediately after a spontaneous event is very low (21). To quantify this early phase, we calculated the time-dependent switching probability p_t from the data in Fig. 8A, again separately for transitions to the CW and CCW modes.

The fraction of switching events d[CCW] (i.e., transitions from state CW to state CCW) occurring per time interval dt is a function of the time-dependent switching probability (p_t) and the number of cells that are still in the CW mode: $d[\mathrm{CCW}]/dt = p_t\,[\mathrm{CW}]$. The time-dependent switching probability in the interval Δt can hence be approximated by $p_t = (1/[\mathrm{CW}])\,(\Delta[\mathrm{CCW}]/\Delta t)$ and therefore evaluated from the data presented in the curves of Fig. 8A. The accuracy of the calculated values depends on the total number of cells in the CW mode observed in the experiment. The probability p_t obtained from these data is independent of any specific model for the flagellar motor switch. Figure 8B shows the

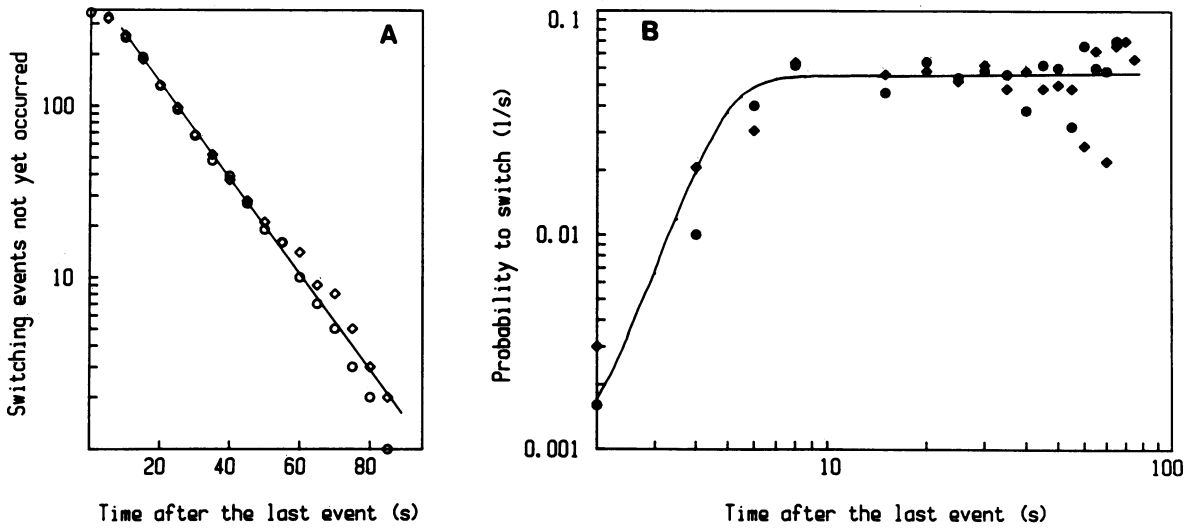

FIG. 8. Time course of spontaneous motor switching after a preceding switching event. Cells of strain Fl×37 were observed under simultaneous observation of the flagellar bundles, and the lengths of swimming intervals were recorded for the CW (diamonds) and CCW (circles) directions separately. In each mode, 346 events were observed. (A) The number of switching events that had not occurred at time t was plotted versus time after the preceding event. The slope defines the rate constant k_2 in the four-state model. The data for six cells were combined. The time constants of the exponential decay of each cell did not differ significantly. (B) The switching probability was calculated as described from the data shown in panel A and plotted against time after the preceding spontaneous switching event. To make the early phase of the curve more clearly visible, a logarithmic time scale was chosen. The line was calculated from the four-state model of the motor (21) with time constants $k_1 = 0.00405\ s^{-1}$ and $k_2 = 0.065\ s^{-1}$.

time dependence of p_t for both transition CW → CCW and transition CCW → CW (the latter calculated in the same way). After a spontaneous event, the switching probability is very low but rises in time until it approaches a constant value. When this plateau, i.e., constant switching probability, is reached, the frequency distribution declines exponentially (Fig. 8A). Again, no difference in the analysis of the CW and CCW modes is apparent. The straight line represents the prediction of the switching probability p_t as calculated from the four-state model of the flagellar motor switch (21; see Discussion).

DISCUSSION

Does the assembly act as a functional unit? We have analyzed the rotation and switching behavior of the flagellar motor assembly of the lophotrichously flagellated bacterium *H. halobium*. The flagellar bundle of the cells consists of 5 to 10 individual filaments that are inserted in close proximity into the cell membrane. Each of the filaments most likely is driven by an individual flagellar motor, as concluded from the fact that intact flagellar filaments each equipped with a basal body can be isolated (12a). Freeze-etching micrographs of the lophotrichously flagellated *Aquaspirillum serpens* demonstrated that the filaments of the polarly inserted flagellar bundle emerge from distinct motor units (6). Similarly, Remsen et al. (32) found individual motors in *Ectothiorhodospira mobilis* but mounted in addition to a special organelle. The *E. coli* cell, which has several flagellar motors inserted at random points on the cell surface (16) (peritrichous type of flagellation), does not carry out synchronous motor switching (19). Therefore, we were interested in the question of whether synchronization exists in *H. halobium*.

The statement that rotation and switching of the flagellar motors are synchronous in *H. halobium* is based on two lines of evidence: (i) the swimming speed of the cells does not fluctuate to an extent that could be explained by independent motor switching events of 5 to 10 filaments, and (ii) motor switching was shown to be an all-or-none process; i.e., the cells did not gradually slow down to zero speed after light stimulation prior to motor switching.

It is more difficult to answer the question as to the time scale on which switching is synchronous. The cells were shown to undergo a pausing period of several hundred milliseconds during motor switching. Since for bacterial motility inertial forces can be neglected, the pausing of the cell directly correlates to a stoppage of net propulsion by the flagellar bundle (4). During the stopping period of the bundle, which also can be directly observed in the dark-field microscope, part of the motors might be in the CW mode and the other part might be in the CCW mode. This could lead to a resting bundle because adhesional forces emerging from interaction of flagellar filaments might block rotation in either sense. Alternatively, bundle stops could be due to a real pausing of the motor rotation. Pausing has been described for individual motors in *E. coli* (13) and is the third observable state of the motor, in addition to CW and CCW rotation. In the case of *E. coli*, the probability for pausing to occur is under the control of the signal chain, as is switching (8). However, there are no data available as to whether the duration of pausing is regulated as well.

For *H. halobium*, we showed that stimulus strength, and thus the amount of signal applied to the motor assembly, does not influence the pausing period (Fig. 7), i.e., does not promote resumption of the swimming state. On the other hand, the response time elapsing after stimulus application until motor switching occurs strongly depends on the stimulus strength (21). This clearly characterizes the switching as a cooperative process of the flagellar motor assembly.

Two different mechanisms of how synchronization might be brought about are obvious. The more likely one is in analogy to *E. coli*. Synchronization is mediated by the adhesional forces the flagellar filaments are exposed to in the bundle. These forces prevent rotation opposite to that of the majority of the filaments. At the present state of knowledge, however, the alternative possibility that the switch complex of all of the flagellar filaments is activated synchronously cannot be excluded.

The origin of spontaneous motor switching events. Two different views of how spontaneous motor switching events occur exist in the literature. Schimz and Hildebrand proposed a regulator substance that oscillates in a sawtooth-shaped manner within a certain concentration range and triggers the flagellar motor switch whenever its concentration reaches a critical level (33, 34). This was concluded from the finding that the responsiveness of bacteria to attractant stimuli (370 nm step-down) depends on the delay time between spontaneous reversal and stimulus application. All evidence that spontaneous reversals are evoked by an oscillator is based on light stimulation experiments.

Given the exponential decay of the frequency distribution, we suggested spontaneous motor switching to be a stochastic event and introduced a four-state model of the flagellar motor switch sufficient to fit the experimental data quantitatively (21). Both the exponential decay and the interpretation of motor switching as a stochastic event were confirmed by McCain et al., who in addition showed that the length of a given swimming interval is independent of the length of the preceding one (24). In this context, it is of interest that a four-state model has also been proposed for the motor switch in *E. coli* (12) and that threshold crossings of a regulatory substance subjected to statistical fluctuations have been excluded from causing spontaneous motor switching (5).

The time-dependent probability for motor switching that is plotted in Fig. 8B contributes to the resolution of the discrepancy between the oscillator and stochastic event hypotheses, since it is directly calculated from the experimental data without any assumptions. According to this curve, immediately after a spontaneous motor switching event, the probability for a consecutive event is low but grows with time to approach a constant level (resulting in the exponential decay shown in Fig. 8A). The final switching probability is reached as early as 7 s after the preceding event and remains constant for at least 80 s. This finding seems to be in obvious contradiction to the assumption of a regulatory substance oscillating in a sawtooth-shaped manner. Therefore, it is not clear how the frequency distribution of spontaneous intervals in *H. halobium* could be explained by the oscillating concentration of a regulatory substance.

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REFERENCES

1. **Adler, J.** 1965. Chemotaxis in *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. **30**:289–292.
2. **Alam, M., and D. Oesterhelt.** 1984. Morphology, function and isolation of halobacterial flagella. J. Mol. Biol. **176**:459–475.
3. **Asakura, S., G. Eguchi, and T. Iino.** 1966. *Salmonella* flagella: in vitro reconstitution and over-all shapes of flagellar filaments. J. Mol. Biol. **16**:302–316.

4. **Berg, H. C.** 1983. Random walks in biology. Princeton University Press, Princeton, N.J.
5. **Block, S. M., J. E. Segall, and H. C. Berg.** 1983. Adaptation kinetics in bacterial chemotaxis. J. Bacteriol. **154**:312–323.
6. **Coulton, J. W., and R. G. E. Murray.** 1978. Cell envelope associations of *Aquaspirillum serpens* flagella. J. Bacteriol. **136**:1037–1049.
7. **Eisenbach, M.** 1990. Functions of the flagellar modes of rotation in bacterial motility and chemotaxis. Mol. Microbiol. **4**:161–167.
8. **Eisenbach, M., A. Wolf, M. Welch, R. S. Caplan, I. R. Lapidus, R. M. Macnab, H. Aloni, and O. Asher.** 1990. Pausing, switching and speed fluctuation of the bacterial flagellar motor and their relation to motility and chemotaxis. J. Mol. Biol. **211**:551–563.
9. **Hildebrand, E., and N. Dencher.** 1975. Two photosystems controlling behavioural responses of *Halobacterium halobium*. Nature (London) **257**:46–48.
10. **Hildebrand, E., and A. Schimz.** 1985. Behavioral pattern and its sensory control in *Halobacterium halobium*, p. 129–142. *In* M. Eisenbach and M. Balaban (ed.), Sensing and response in microorganisms. Elsevier, Amsterdam. 129–142.
11. **Houwink, A. L.** 1956. Flagella, gas vacuoles and cell-wall structure in *Halobacterium halobium*; an electron microscope study. J. Gen. Microbiol. **15**:146–150.
12. **Kuo, S. C., and D. E. Koshland.** 1989. Multiple kinetic states of the flagellar motor switch. J. Bacteriol. **171**:6279–6287.

12a. **Kupper, J., W. Marwan, and D. Oesterhelt.** Unpublished data.

13. **Lapidus, I. R., M. Welch, and M. Eisenbach.** 1988. Pausing of flagellar rotation is a component of bacterial motility and chemotaxis. J. Bacteriol. **170**:3627–3632.
14. **Larsen, S. H., R. W. Reader, E. N. Kort, W.-W. Tso, and J. Adler.** 1974. Change in direction of flagellar rotation is the basis of the chemotactic response in *Escherichia coli*. Nature (London) **249**:74–77.
15. **Lebert, M.** 1987. Isolierung und Charakterisierung von Verhaltensmutanten von Halobacterium halobium. Diplomarbeit, Universität Marburg, Marburg, Federal Republic of Germany.
16. **Leifson, E.** 1960. Atlas of bacterial flagellation. Academic Press, New York.
17. **Macnab, R. M.** 1976. Examination of bacterial flagellation by dark-field microscopy. J. Clin. Microbiol. **4**:258–265.
18. **Macnab, R. M.** 1977. Bacterial flagella rotating in bundles: a study in helical geometry. Proc. Natl. Acad. Sci. USA **74**:221–225.
19. **Macnab, R. M., and D. P. Han.** 1983. Asynchronous switching of flagellar motors on a single bacterial cell. Cell **32**:109–117.
20. **Marwan, W., M. Alam, and D. Oesterhelt.** 1987. Die Geißelbewegung halophiler Bakterien. Naturwissenschaften **74**: 585–591.
21. **Marwan, W., and D. Oesterhelt.** 1987. Signal formation in the halobacterial photophobic response mediated by a fourth retinal protein (P 480). J. Mol. Biol. **195**:333–342.
22. **Marwan, W., and D. Oesterhelt.** 1990. Quantitation of photochromism of sensory rhodopsin-I by computerized tracking of *Halobacterium halobium* cells. J. Mol. Biol. **215**:277–285.
23. **Marwan, W., W. Schäfer, and D. Oesterhelt.** 1990. Signal transduction in *Halobacterium* depends on fumarate. EMBO J. **9**:355–362.
24. **McCain, D. A., L. A. Amici, and J. L. Spudich.** 1987. Kinetically resolved states of the *Halobacterium halobium* flagellar motor switch and modulation of the switch by sensory rhodopsin I. J. Bacteriol. **169**:4750–4758.
25. **Oesterhelt, D., and G. Krippahl.** 1983. Phototrophic growth of Halobacteria and its use for isolation of photosynthetically deficient mutants. Ann. Microbiol. (Inst. Pasteur) **134B**:137–150.
26. **Oesterhelt, D., and W. Marwan.** 1987. Change of membrane potential is not a component of the photophobic transduction chain. J. Bacteriol. **169**:3515–3520.
27. **Oesterhelt, D., and W. Marwan.** 1989. Signal transduction in halobacteria, p. 282–301. *In* A. E. Evangelopoulos et al. (ed.), Receptors, membrane transport and signal transduction. NATO ASI series, H29. Springer-Verlag, Berlin.
28. **Oesterhelt, D., and W. Marwan.** 1990. Signal transduction in *Halobacterium halobium*. Symp. Soc. Gen. Microbiol. **46**:219–239.
29. **Oesterhelt, D., and J. Tittor.** 1989. Two pumps, one principle: light-driven ion transport in halobacteria. Trends Biochem. Sci. **14**:57–61.
30. **Otomo, J., W. Marwan, D. Oesterhelt, H. Desel, and R. Uhl.** 1989. Biosynthesis of the two halobacterial light sensors P480 and SR and variation in gain of their signal transduction chains. J. Bacteriol. **171**:2155–2159.
31. **Reichert, K.** 1909. Über die Sichtbarmachung der Geißeln und die Geißelbewegung der Bakterien. Centralbl. Bakteriol. Abt. I **51**:14–93.
32. **Remsen, C. C., S. W. Watson, J. B. Waterbury, and H. G. Trüper.** 1968. Fine structure of *Ectothiorhodospira mobilis* Pelsh. J. Bacteriol. **94**:2374–2392.
33. **Schimz, A., and E. Hildebrand.** 1985. Response regulation and sensory control in *Halobacterium halobium* based on an oscillator. Nature (London) **317**:641–643.
34. **Schimz, A., and E. Hildebrand.** 1988. Photosensing and processing of sensory signals in *Halobacterium halobium*. Bot. Acta **101**:111–117.
35. **Silverman, M., and M. Simon.** 1974. Flagellar rotation and the mechanism of bacterial motility. Nature (London) **249**:73–74.
36. **Spudich, J. L., and R. A. Bogomolni.** 1988. Sensory rhodopsins of halobacteria. Annu. Rev. Biophys. Biophys. Chem. **17**:193–215.
37. **Spudich, E. N., and J. L. Spudich.** 1982. Control of transmembrane ion fluxes to select halorhodopsin-deficient and other energy-transduction mutants of *Halobacterium halobium*. Proc. Natl. Acad. Sci. USA **79**:4308–4312.
38. **Spudich, J. L., and W. Stoeckenius.** 1979. Photosensory and chemosensory behavior of *Halobacterium halobium*. Photobiochem. Photobiophys. **1**:43–53.