Eur Biophys J (1997) 26: 163–173

ARTICLE

Holger Naber

The response of halobacteria to single light stimuli: a theoretical analysis

Received: 23 September 1996 / Accepted: 20 February 1997

Abstract Halobacteria are propelled by rotating flagella. Under stationary environmental conditions, the direction of rotation of the flagella switches spontaneously at intervals ranging from 5 to 50 s. Sudden changes of light intensity induce (repellent response) or transiently suppress (attractant response) a switching event. For repellent and non-saturating attractant stimuli, the average time between stimulus application and succeeding switching event apparently varies as a function of the time of stimulation relative to the preceding switching event. However, thorough evaluation of the experimental data suggests that the way in which a stimulus changes the internal state of a cell is independent of both the time of stimulation and the spinning direction of the flagellar motors. Flagellar switching is described in terms of a switch complex of the flagellar motor apparatus. It is assumed that a switching event is triggered with constant probability per unit time if the switch complex is in its active state and no stimulus was applied. After a switching event, the switch complex regains activity in a multi-step process. Stimuli modulate the switching step through an intracellular signal which is interpreted as the effective output of the sensory chain (including adaptation processes). This signal is assumed to be independent of the status of the motor apparatus. The model yields a quantitative description of spontaneous switching behavior. Attractant responses can be understood by assuming a transient block of the switching step. The explanation of repellent responses requires the assumption of an oscillating signal and a concomitant acceleration of reactivation of the switch complex.

Key words Archaea · Flagellar switch · Intracellular oscillation · Phototaxis · Signal processing

H. Naber
Institut für Biologische Informationsverabeitung,
Forschungszentrum Jülich, PO Box 1913, D-52425 Jülich, Germany
(Fax: +49-2461 614216; e-mail: h.naber@fz-juelich.de)

Abbreviations CW Clockwise · CCW Counterclockwise · fpt First-passage time

1 Introduction

Halobacteria are able to convert light energy into metabolic energy and to sense changes of light intensity in different wavelength ranges. These processes are based on the activation of rhodopsin-like proteins that either directly feed into the energy-metabolism of the cell (bacterio- and halorhodopsin; for review, see Lanyi 1984) or are coupled to a sensory pathway through which light stimuli are signalled to the flagellar motor apparatus and thus affect behavior (sensory rhodopsin I and II; for review, see Spudich and Bogomolni 1988). In a spatially and temporally uniform environment, the cells reverse their swimming direction at intervals ranging from 5 to 50 s. The reversals are caused by spontaneous switchings of the flagella from clockwise[1] (CW) to counterclockwise (CCW) rotation or vice versa (Alam and Oesterhelt 1984). Light stimuli modulate the spontaneous behavior by inducing or delaying reversals. In analogy to chemotaxis, the stimuli are termed repellent or attractant stimuli according to whether the average duration of a swimming interval is decreased or increased, respectively (Spudich and Stoeckenius 1979). A repellent response is observed if the light intensity decreases in the green to red or increases in the blue or ultraviolet range. An attractant response is observed for opposite intensity changes. The deactivation of photoreceptor complex molecules, most probably by reversible methylation, allows the cells to adapt to persistent illumination and ensures that changes in their environment only transiently influence the behavior of the cells (Marwan et al. 1995). This light sensitivity enables halobacteria to avoid regions of high intensity of harmful ultraviolet light and to

[1] The rotational sense is defined looking along the flagella towards the cell body

accumulate in regions where the conversion of light into metabolic energy is efficient.

Important details of the molecular basis of signal transduction in the archaeon *Halobacterium salinarium* have been revealed recently, suggesting a close relationship to signal transduction in chemotaxis of the eubacterium *Escherichia coli* (Yao and Spudich 1992; Rudolph and Oesterhelt 1995, 1996; Rudolph et al. 1995). However, there is no complete model of the signal chain at the present time. To get insight into its functionality, physiological experiments were performed, in which the input-output relation for various stimulation programs was measured (for review, see Petracchi et al. 1994). Recent experiments have demonstrated that after strong repellent stimulation a few switching events occur at regular intervals (Krohs 1995). This was interpreted as the consequence of stimulus-induced oscillations in the signal chain.

An important question for a model description of the system is whether the sensitivity to a stimulus depends on the rotational state of the flagellar motor and on the time of stimulation during a swimming interval, i.e. on the time interval between stimulus and preceding switching event. Recent results obtained by Krohs (1994b, 1995) suggest that the way in which both attractant and repellent stimuli change the internal state of the cells is independent of the status of the motor apparatus if the cells are fully adapted to the light conditions before stimulation. However, the time interval between application of a stimulus and succeeding reversal does in some cases depend on the interval between the stimulus and the preceding reversal.

The objective of this paper is understand by using theoretical methods the results of experiments in which halobacteria were stimulated by a single step-like increase or decrease of light intensity. Focus is on two questions: (1) Can a change in responsiveness occur during a swimming interval although the action of the stimulus on the flagellar motor is independent of the status of the motor apparatus? (2) Has the occurrence of some switching events at regular intervals after repellent stimulation to be explained by an oscillation in the signal chain, or can it alternatively be explained by the fact that the flagellar motor cannot switch at arbitrarily short intervals? The model being discussed is a straightforward extension of a model for spontaneous flagellar motor switching which has been proposed previously (Naber 1996). Switching is described in terms of a switch complex of the flagellar motor, which runs through a fixed sequence of states between every two switching events. This model yields a quntitative description of the spontaneous switching behavior characterized by the probability distribution of swimming interval lengths and correlation functions for sequences of successive switching events.

2 Summary of experimental methods and results

In this paper, I refer to a systematic study of responses of halobacteria to single stimuli, which has been performed by Krohs (1994a, b, 1995). The experiments were based on the direct observation of single cells out of a population of bacteria. Cells that were adapted to persistent illumination were stimulated by switching light on or off, respectively, at different intervals after an arbitrarily chosen reversal of some cell. The time until the succeeding reversal of the same cell was measured regardless of whether this occurred before or after the stimulus. By plotting the duration τ of the swimming interval versus the interval t_s between reference reversal and stimulation, phase-response plots were obtained, that were analysed without further data processing. Two examples are shown in Fig. 1. These plots of raw data are comparable to contour-plots of the frequency distribution of interval lengths as a function of the time of stimulation t_s. A high density of dots indicates a high probability of a reversal within a certain time interval. Because switching events are plotted regardless of whether they were potentially influenced by the stimulus, such plots represent a superposition of spontaneous and stimulus-dependent events.

2.1 Response to attractant stimuli

Phase-response plots for attractant stimuli show two populations of dots clearly separated by an area of very low dot density, which correspond to spontaneous and delayed switching events (Fig. 1a). This means that attractant stimuli result in a transient suppression of reversals. The period of reversal suppression does not depend on the interval between stimulus and preceding reversal, but increases with increasing light intensity change. On the average, the time to reversal $\tau^* = \tau - t_s$ after an attractant stimulus is constant for saturating stimuli, but decreases with increasing t_s for sub-maximal stimuli (Krohs 1994b).

A finding which is common to both attractant and repellent responses is that the cells apparently do not immediately respond to a stimulus. Switching events that occur up to approximately 2 s after stimulation obviously belong to the population of spontaneous events. For repellent stimuli, this period is slightly shorter than for attractant stimuli. The effect is clearly discernible in the data by an abrupt change of the dot density for $\tau^* \approx 1-2$ s. It is explained by a signal processing time t_p. The value of $t_p \approx 1-2$ s estimated from phase-response plots is in agreement with the results by Sundberg et al. (1986).

2.2 Response to repellent stimuli

A repellent stimulus induces a reversal within 2–3 s if a cell is stimulated two or more seconds after a reversal ($t_s \gtrsim 2$ s). In a phase-response plot, this is visible as a narrow strip of high dot density (Fig. 1b). At $t_s \lesssim 2$ s the time to reversal of about half of the cells increases by several seconds and a second population of dots appears, which is separated from the first one by a gap of low dot density. The period of increased average time to reversal, that is accompanied with the splitting of reversals, is usually called

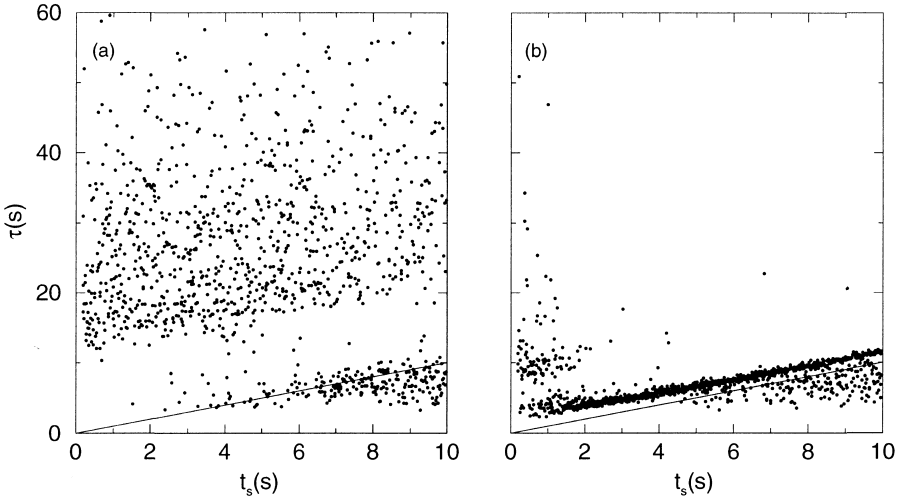

Fig. 1 Experimental phase-response plots for attractant (**a**) and repellent (**b**) stimuli (Krohs 1994a, b). Each *dot* represents a single measurement; each *plot* shows the results from three series of experiments. The UV-sensitive photosystem (intermediate S_{373} of the photocycle of sensory rhodopsin I) was stimulated by a step-like decrease (**a**) or increase (**b**) of light intensity at a wavelength of 370 nm by 6.0×10^{12} $h\nu$ mm^{-2} s^{-1}. White background light was used. The straight lines ($\tau=t_s$) separate events which occurred before and after stimulation. Reproduced with permission of U. Krohs

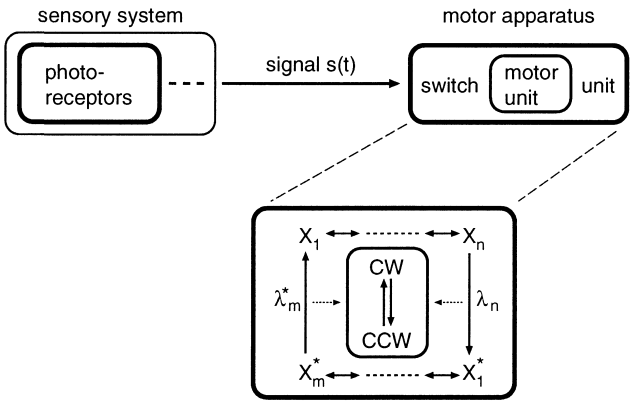

Fig. 2 Outline of the model. A light stimulus modulates the behavior of the flagellar motor through an intracellular signal $s(t)$, which is independent of the status of the flagellar motor. The motor apparatus is considered to consist of a switch and a motor unit as described in the text

"refractory period" (Schimz and Hildebrand 1985; McCain et al. 1987). However, instead of being equivalent to spontaneous reversals, reversals which belong to the second population are equivalent to second reversals after stimulation (Krohs 1995). Counting reversals accordingly, the average time interval between stimulation and both the first and the second reversal is independent of t_s, except for a slight increase of the interval between stimulus and first reversal for small t_s.

In a joint histogram of the time intervals between stimulation and first, second, and third reversal, the respective maxima become less sharp with increasing time, but they are clearly discernible and separated from each other by intervals of approximately 6 s (Krohs 1995). This means that a single repellent stimulus induces a few switching events at regular intervals, regardless of whether the first reversal after stimulation belongs to the first or second population of dots. This phenomenon was ascribed to a damped oscillation of the signal chain evoked by strong repellent stimulation (Krohs 1995).

3 Theory

3.1 General structure of the model

The period of reversal suppression after attractant stimulation as well as the interval between repellent stimulus and induced reversal(s) are independent of both the time interval between stimulus and preceding switching event and the sense of flagellar rotation. This means that the way in which a stimulus influences the internal state of a cell does not change during a swimming interval, which, in turn, motivates the assumption that sensitivity to a stimulus also does not change. On the modelling level, this is considered by interpreting the response to a stimulus as the response of the motor apparatus to an intracellular signal $s(t)$ generated by the sensory system, that is independent of the status of the motor apparatus (Fig. 2). The signal $s(t)$ is regarded as the effective output of the sensory pathway (including amplification and adaptation processes). It could, for example, represent the concentration change of phosphorylated chemotaxis protein CheY (Rudolph and Oesterhelt 1996) or of the switch factor fumarate, which is released upon repellent stimulation (Montrone et al. 1993).

The notion of an effective sensory signal allows one to discriminate between sensitivity, which means a property

of the sensory system, and responsiveness, which refers to the cells' final behavior. In the following, I shall concentrate on the question of whether variable responsiveness may be consistent with the assumption of constant sensitivity (reflected in a signal $s(t)$ which is independent of the time of stimulation t_s). I will not discuss the mechanisms leading to a specific signal. For the purpose of stimulation, $s(t)$ is chosen on the basis of general considerations and experimental results.

3.2 Modelling switching behavior

In *Halobacterium* as well as in *E. coli*, the process of switching the flagella from CW to CCW rotation or vice versa is described in terms of a "motor switch". The switch is thought of as an assembly of subunits of the motor apparatus, which directly interacts with the motor units and determines the direction of rotation of the flagella (Francis et al. 1994). On a molecular level, there is a far-reaching correspondence between *E. coli* and *Halobacterium* regarding the activation mechanism of the switch. The flagella only rotate CCW in *E. coli* and CW in *Halobacterium* if the cells lack (phosphorylated) CheY (Wolfe et al. 1987; Rudolph and Oesterhelt 1996). In both species, a second cytoplasmic component, which has been identified as fumarate (or a fumarate metabolite), is necessary to switch the sense of rotation (Marwan and Oesterhelt 1990; Barak and Eisenbach 1992).

Despite these analogies, there is a basic difference in behavior. Cells of *E. coli* only swim if their flagella rotate CCW. A switching to CW rotation leads to a short period of tumbling. Chemical stimuli alter the ratio of swimming and tumbling periods by decreasing and increasing the phosphorylation rate of CheY and thus suppress or favor a reorientation of the cells (for review, see Eisenbach 1991). In *Halobacterium*, the CW and CCW states of the motor are equivalent with respect to the cells' behavior. Halobacteria swim equally well with flagella rotating in either direction, the distribution of swimming interval lengths between spontaneous reversals is the same for both CW and CCW rotation (Hildebrand and Schimz 1985; Marwan et al. 1991), and there is no indication that stimuli affect the duration of CW and CCW periods in an opposite manner as observed in *E. coli* (Krohs 1994b; 1995).

Modelling spontaneous switching behavior, this equivalence was transferred to the structure of the model by assuming that the switch complex cyclically runs through a sequence of states between every two switching events (Naber 1996). This was in accordance with a proposition by McCain et al. (1987). However, the finding that the flagellar motor exclusively spins CW in the absence of phosphorylated CheY shows that functional equivalence of the CW and the CCW states can probably not be explained by a structural equivalent. In accordance with Marwan and Oesterhelt (1987), it looks more appropriate to distinguish between CW and CCW states of the switch complex. Extending my previous ansatz, I assume that the switch complex can exist in states $X_1, \ldots, X_n$ and $X_1^*, \ldots, X_m^*$, where a transition from X_n to X_1^* (X_m^* to X_1) coincides with a switching from CW to CCW (CCW to CW) rotation of the flagella, and the transitions $X_1 \rightarrow \cdots \rightarrow X_n (X_1^* \rightarrow \cdots \rightarrow X_m^*)$ are interpreted as a reactivation of the switch complex (Fig. 2). The states $X_1, \ldots, X_n$ and $X_1^*, \ldots, X_m^*$ could, for example, represent different conformational states of the switch complex. The transitions coinciding with switching events are assumed to be operationally irreversible. All other transitions may be reversible.

In this model, functional equivalence means that the frequency distribution of times that the system needs to run from X_1 to X_1^* (via $X_2, \ldots, X_n$) is at least very similar to the distribution of run-times from X_1^* to X_1 (via $X_2^*, \ldots, X_m^*$) for spontaneous as well as for stimulated reversals. In particular, the signal $s(t)$ should modulate the properties of the switch complex at equivalent sites. For these reasons, I shall assume that the kinetics of transitions between the switch states is effectively the same in the CW and CCW states and restrict myself to the discussion of CW intervals.

Two basically different reactivation mechanisms of the switch complex were shown to be consistent with the observed spontaneous swimming behavior (Naber 1996). In the following, I shall focus on that model in which reactivation is interpreted as a multi-step process leading to a considerable idle time of the switch complex with respect to its ability to switch the flagellar motor. In the unstimulated case, each transition probability per unit time (or transition rate) between the states of the switch complex is operationally constant. This is in contrast with the alternative model (cf. discussion) in which, even in the unstimulated case, reactivation is triggered by an oscillating subsystem of the cell leading to effectively time-dependent transition rates.

As in the case of spontaneous motor switchings, it is assumed that the duration of a swimming interval does not depend on the duration of the preceding one (Naber 1996). Therefore, the frequency distribution of swimming interval lengths is equal to the first-passage-time (fpt) distribution $\rho(t)$ for the sequence of steps

$$X_1 \underset{\mu_2}{\overset{\lambda_1}{\rightleftarrows}} X_2 \underset{\mu_3}{\overset{\lambda_2}{\rightleftarrows}} \cdots \underset{\mu_n}{\overset{\lambda_{n-1}}{\rightleftarrows}} X_n \xrightarrow{\lambda_n} X_1^* , \qquad (1)$$

where λ_i and μ_i denote the transition rates between the states of the switch complex. Immediately after a switching event at time $t=0$, the system is in state X_1 with probability 1 and then runs through the states $X_2, \ldots, X_1^*$. Because in Eq. (1) X_1^* is an absorbing state, the probability $p_1^*(t)$ to find the system in state X_1^* at time t equals the probability that the first-passage time τ (i.e., the time the switch needs to run from $X_1(t=0)$ to $X_1^*(t=\tau)$) is smaller than t,

$$p_1^*(t) = \mathrm{Prob}\{\tau < t\} = \int_0^t \rho(t')\,\mathrm{d}t' , \qquad (2)$$

and thus

$$\rho(t) = \frac{\mathrm{d}p_1^*(t)}{\mathrm{d}t} . \qquad (3)$$

The temporal evolution of the system is described by a Master equation (van Kampen 1981), which reads ($i=2, \ldots, n-1$)

$$\begin{aligned}
\dot{p}_1 &= -\lambda_1 p_1 + \mu_2 p_2 \\
\dot{p}_i &= \lambda_{i-1} p_{i-1} - (\lambda_i + \mu_i) p_i + \mu_{i+1} p_{i+1} \\
\dot{p}_n &= \lambda_{n-1} p_{n-1} - (\lambda_n + \mu_n) p_n \\
p_1^* &= \lambda_n p_n \,. \qquad (4)
\end{aligned}$$

In order to calculate $\rho(t)$, it has to be solved with initial conditions $p_1(0)=1$, $p_2(0)=\cdots=p_1^*(0)=0$. Simulations of trajectories of the model are done using standard methods (for details, see Naber 1993).

3.3 The effect of external stimulation

External stimulation results in a transient modulation of the switching behavior. This means that at least one transition rate $\alpha \in \{\lambda_1, \ldots, \lambda_n, \mu_2, \ldots, \mu_n\}$ becomes explicitly time-dependent:

$$\alpha(t) = \alpha_0 \, s(t) \qquad (5)$$

where α_0 is the value of α if the bacteria are adapted to their environment. Therefore, necessary requirements on the signal $s(t)$ are

$$s(t)=1 \quad \text{if} \quad t \le t_s \quad \text{and} \quad \lim_{t\to\infty} s(t) = 1, \qquad (6)$$

where t_s is the moment when the stimulus is applied[2]. Because α denotes a rate, $s(t)$ has furthermore to be non-negative. The general form of $s(t)$ is

$$s(t) = \begin{cases} 1 & \text{if} \quad t \le t_s + t_p \\ \sigma(t - t_s - t_p) & \text{else} \end{cases}, \qquad (7)$$

where t_p is an additional delay due to stimulus processing. The function $\sigma(t)$ describes the action of the stimulus. It is assumed to be independent of t_s, reflecting the assumption of constant sensitivity during a swimming interval. Because experimental phase-response plots for strong stimuli show a clearly discernible structure, fluctuation of $\sigma(t)$ will not be considered.

Even repellent stimuli that are applied when there is a high probability of the occurrence of a spontaneous switching event (e.g. $t_s \approx 10$ s) may induce a reversal within 2 s (Hildebrand and Schimz 1987, Krohs 1995). I therefore assume that it is the switching rate λ_n that is modulated by the stimulus. For the present, this may be the only parameter affected by the stimulus. Furthermore, I assume that attractant and repellent stimuli affect the same parameter. At present, this assumption does not contradict experimental results. However, one has to bear in mind that there are two factors that are necessary for the occurrence of motor switching events, phosphorylated CheY and fumarate. So there might be two independent targets for light stimuli.

[2] In the following, unless otherwise provided, $t=0$ refers to the moment of the switching event relative to which the stimulus is applied

4 Results

4.1 General properties of the model

If each transition rate in Eq. (1) is constant the model describes spontaneous behavior. In this case, it has been proved that the variability of the model with respect to the fpt-distribution $\rho(t)$ is retained even if all backward rates $\mu_2, \ldots, \mu_n$ are set zero (Naber 1996). This simplification considerably reduces the number of free parameters and will also be used in the analysis of stimulated behavior. The price one has to pay for it is that the remaining parameters $\lambda_1, \ldots, \lambda_{n-1}$ in general cannot be interpreted as measurable transition rates[3]. They approximate measurable rates if the respective steps are operationally irreversible.

With this simplification a quantitative least square fit of the fpt-distribution for sequence Eq. (1) to frequency distributions of spontaneous swimming interval lengths is possible if the following requirements are full-filled: (1) There is a sufficient number of steps between every two switching events acting as a simple retardation mechanism; (2) the transition rate of one of these steps is of the order of the rate of exponential decrease of the interval distribution; (3) the remaining steps occur on a faster time scale with approximately equal transition rates (Naber 1996). About 16 steps are necessary to describe the low probability of short spontaneous swimming intervals and the subsequent rapid increase of the distribution to its maximum. A single step (say step no. k) occurring on a slower time scale than the remaining steps ($\lambda_k \sim 0.2\ \mathrm{s}^{-1}$ vs. $\lambda_{i\neq k} \sim 2\ \mathrm{s}^{-1}$ at a temperature of 23 °C) ensures that the distribution falls off approximately exponentially from its maximum. A quantitative description of experimental data is equally well possible with moderate ratios of backward and forward rates ($\mu_{i+1}/\lambda_i \lesssim 0.1$) and forward rates λ_i of the same order of magnitude as obtained by the least square fits (unpublished results by the author).

In the spontaneous case, $\rho(t)$ is independent of a specific succession of steps (Naber 1996). Modelling stimulated behavior, I assume that the smallest transition rate is associated with switching, i.e. the modulated step, because in this case repellent stimuli are most effective. The transition rates for the first $n-1$ steps are set equal according to the results of the analysis of spontaneous switching events.

These considerations lead to the following choice of parameters:

$$\begin{aligned}
&\lambda_1 = \ldots = \lambda_{n-1} = \lambda' \\
&\mu_2 = \ldots = \mu_n = 0 \\
&\lambda_n = \lambda \, s(t) \,, \qquad (8)
\end{aligned}$$

where n, λ, and λ' are chosen by fitting the model to an empirical distribution of spontaneous interval lengths. Because it is explicitly time-dependent, the resulting Master equation (4) is effectively nonlinear and can in general not

[3] For the sake of simplicity I shall adhere to the term "rate", though

be solved in closed form. Formally, $\rho(t)$ is the distribution of the sum of n independent random variables, $\tau_1, \ldots, \tau_n$, that are the dwell-times in the respective states $X_1, \ldots, X_n$. The first $n-1$ steps corresponding to reactivation of the switch complex are Poisson processes with transition rate λ'. The distribution of τ_n, the time for the switching process itself, can be calculated by solving the Master equation for a single step with transition rate $\lambda s(t)$. One thus finds

$$\rho(t) = \int_0^t \left[\lambda' \frac{(\lambda' t')^{n-2}}{(n-2)!} \exp(-\lambda' t') \times \lambda s(t) \exp\left\{ -\lambda \int_0^{t-t'} s(t''+t')\,\mathrm{d}t'' \right\} \right] \mathrm{d}t' . \qquad (9)$$

4.2 Response to attractant stimuli

The average time to reversal $\langle \tau^* \rangle = \langle \tau \rangle - t_s$ after a single attractant stimulus is constant for saturating stimuli, but decreases as a function of t_s for sub-maximal stimuli (Krohs 1994b). This phenomenon is studied in the model using the step-like signal

$$s(t) = \begin{cases} 0 & \text{if } t_s < t \le t_s + u \\ 1 & \text{else} \end{cases} , \qquad (10)$$

which obviously delays switching events and therefore causes an attractant response. According to experimental results, the stimulus strength is reflected in the duration u of reversal suppression. For simplicity, a signal processing time t_p has been omitted, because it is equivalent to a shift of t_s.

A discontinous signal is certainly unrealistic, but it is adequate to analyse the behavior of the model, because transient reversal suppression is the significant effect of an attractant stimulus (Krohs 1994b), and because $\langle \tau^* \rangle$ can be calculated analytically with this choice of $s(t)$ giving the opportunity for a sound interpretation of the result.

Because $s(t)$ is piece-wise constant, the last step in Eq. (1) can also be described in terms of a Poisson process. The average time to reversal $\langle \tau^* \rangle$ as a function of t_s and u is calculated via the Laplace transform of the Master equation (4). One finds (see appendix)

$$\langle \tau^* \rangle = u + \frac{1}{\lambda} + [n - \langle k \rangle] \frac{1}{\lambda'} , \qquad (11)$$

where $\langle k \rangle$ denotes the state X_k that is on the average occupied by the switch complex at time $t_s + u$. The average is restricted to bacteria that did not reverse spontaneously before the stimulus was applied.

This result is interpreted by noticing that for a Poisson process the average transition time is equal to the inverse transition rate. No switching event at all can occur in the time intervall $t_s < t \le t_s + u$. Afterwards, at least the last step with transition rate λ has to be performed. The average number $n - \langle k \rangle$ of λ' steps that remain to occur for $t > t_s + u$ depends on the distribution of switch states $p_1(t), \ldots, p_n(t)$ at $t = t_s + u$. If a weak stimulus (small u) is applied shortly after a reversal, the probability that early switch states are still occupied at $t = t_s + u$ is significantly higher than if the same stimulus is applied late after a reversal. Therefore, $\langle \tau^* \rangle$ increases with decreasing t_s (Fig. 3). For a very strong stimulus, early switch states are depleted while switching events are suppressed. The probability to find the switch in state X_n when switching ability is recovered is close to one, the time to reversal is (approximately) constant (Fig. 3).

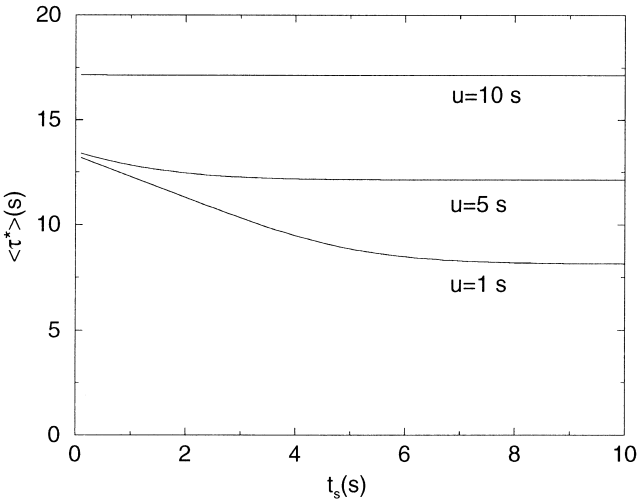

Fig. 3 Average time to reversal $\langle \tau^* \rangle$ Eq. (11) after a step-like attractant signal Eq. (10), computed by numerically integrating the Master equation (4). Parameters: $n=16$, $\lambda=0.14\ \mathrm{s}^{-1}$, $\lambda'=2.44\ \mathrm{s}^{-1}$, u as indicated

Going beyond a qualitative description, the model also fits the experimental results quantitatively. For saturating attractant stimuli, when a constant time to reversal is observed, the period of reversal suppression lasts approximately 10 s (Krohs 1994a). In the model, the probability that the switch complex runs through states X_1 to X_n within this period is close to one for a value of λ' which is chosen by a fit of the model to an empirical distribution of spontaneous swimming intervals. This shows that it is reasonable to assume that an attractant stimulus modulates the slow switching step, but leaves reactivation of the switch complex unaffected.

4.3 Response to repellent stimuli

The frequency distribution of swimming intervals lengths observed after repellent stimulation shows two maxima if the stimulus was applied within approximately 2 s after a spontaneous reversal, but only one maximum if the same stimulus was applied later (cf. Fig. 1b).

In the model, this phenomenon is analysed using two different kinds of repellent signals. The first is based on the assumption that a repellent signal is simply inverse to an attractant signal, i.e. transiently increases the switching

rate. The second refers to the proposition that a repellent stimulus modulates the switching rate in an oscillatory manner (Krohs 1995).

Both signals are modelled by two simple functions $\sigma(t)$ [cf. Eq. (7)], which combine a slow increase of the switching rate with a steady return to its prestimulus value and are thus more realistic than the step-like signal Eq. (10). They will also be used in the simulations of phase-response plots below. In particular, I choose

$$\sigma(t) = \max\{\varepsilon;\ 1+\mu\, k_a t \exp(-k_a t+1)\} \tag{12}$$

and

$$\sigma(t) = \max\{\varepsilon;\ 1+\mu \sin(2\pi t/T) \exp(-k_a t)\} \tag{13}$$

respectively. Here, μ corresponds to the stimulus strength, k_a determines how fast the bacteria adapt to the new light intensity, and T is the period of the oscillatory signal. The lower bound $\varepsilon \in [0,1]$ of $\sigma(t)$ has been introduced to ensure $\sigma(t) \geq 0$ even if $\mu > 1$. Furthermore, choosing ε appropriately allows one do discriminate whether a repellent stimulus only increases the switching rate ($\varepsilon = 1$) or whether there is intermittently also a depression of reversals compared to the unstimulated case ($\varepsilon < 1$).

Within the framework of the model presented here, only the assumption of an oscillatory repellent signal leads to a behavior which is in accordance with the experimental findings, i.e. leads to a swimming interval distribution with two or more maxima, whose relative heights depend on the time of stimulation (Fig. 4). This can be understood as follows. A repellent stimulus is effective, that is, is able to induce a reversal, only if the switch complex is in its active state. Because reactivation is rather slow, shortly after a reversal sometimes the first and sometimes the second maximum of $s(t)$ induces the following reversal. This leads to a bi-modal distribution of swimming interval lengths. If stimulated late after a reversal a cell nearly always responds to the first maximum of $s(t)$.

A non-oscillatory repellent signal at best reduces the length of a swimming interval to the time necessary for reactivation of the switch complex, but does not yield bi-modal interval distributions. A period of inactivity of the switching mechanism is necessary but not sufficient to describe the experimental data. To explain bi-modal interval distributions after strong repellent stimulation either the signal chain has to exhibit more complex dynamics, as proposed here, or the reactivation of the switch complex has to be governed by a non-trivial dynamical system (cf. discussion).

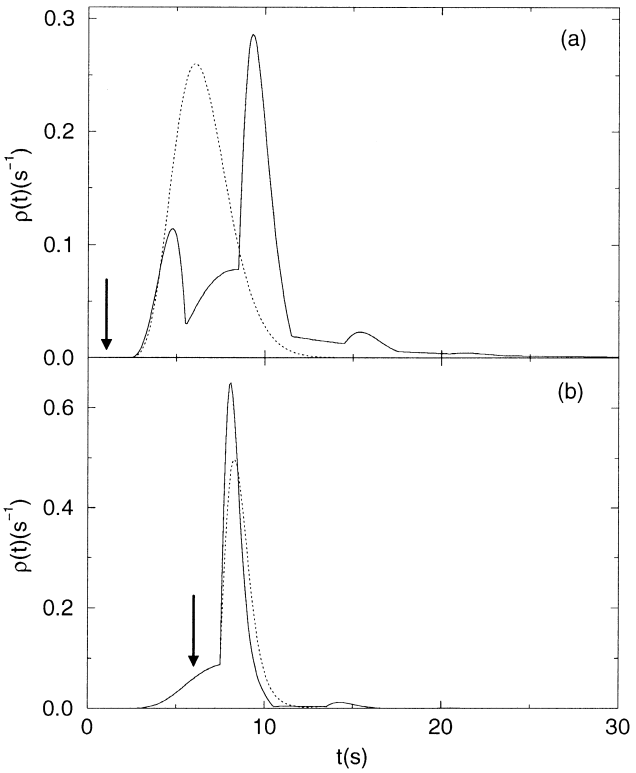

Fig. 4a, b First-passage-time distribution $\rho(t)$ Eq. (9) for repellent signals Eq. (12) (*dotted lines*) and Eq. (13) (*solid lines*). The *arrows* mark the stimulus, (**a**) $t_s = 1$ s and (**b**) $t_s = 6$ s. $\rho(t)$ was calculated by numerically solving the Master equation (4). Parameters of the switch: $n = 16$, $\lambda = 0.14$ s^{-1}, $\lambda' = 2.44$ s^{-1}. Parameters of the repellent signal: $t_p = 1.5$ s, $\mu = 20$, $\varepsilon = 1$, $k_a = 0.2$ s^{-1}, $T = 6$ s

4.4 Stimulations of phase-response plots

A stochastic simulation of the temporal evolution of the model allows for a direct comparison of the effect of stimulation in the biological and the model system. Therefore, simulation is helpful to predict additional characteristics of how light stimuli modulate the internal state of a cell.

It was shown in the previous sections that the model qualitatively reproduces the experimental observations. This result is confirmed by simulations of phase-response plots using Eqs. (12) and (13) to model the effect of single attractant and repellent stimuli, respectively (Fig. 5). The parameters T and t_p can be directly estimated from the empirical phase-response plots, The "decay-rates" k_a of the intracellular signals have been chosen such that a good correspondence between empirical and simulated data was obtained. For both, attractant and repellent stimuli, k_a mainly influences the width of the distribution of intervals which have been influenced by the stimulus. In this respect, there is some variation in the biological system too, depending on the stimulated photosystem (Krohs 1994b, 1995). The particular value of k_a has little effect on the qualitative features of the simulated phase-response plots.

The correspondence between simulated attractant responses and the data is generally very good if the switching rate is zero (or at least very small) for a period approximately equal to the experimentally observed period of reversal suppression (compare Fig. 5a and Fig. 1a). The stimulation of repellent responses, however, shows some differences compared to the experimental data. The probability that the second maximum of the repellent signal Eq. (13) induces the first reversal after stimulation if $t_s > 2$ s is much higher than it appears in the experiments (compare Fig. 5b and Fig. 1b). Furthermore, the separation of both

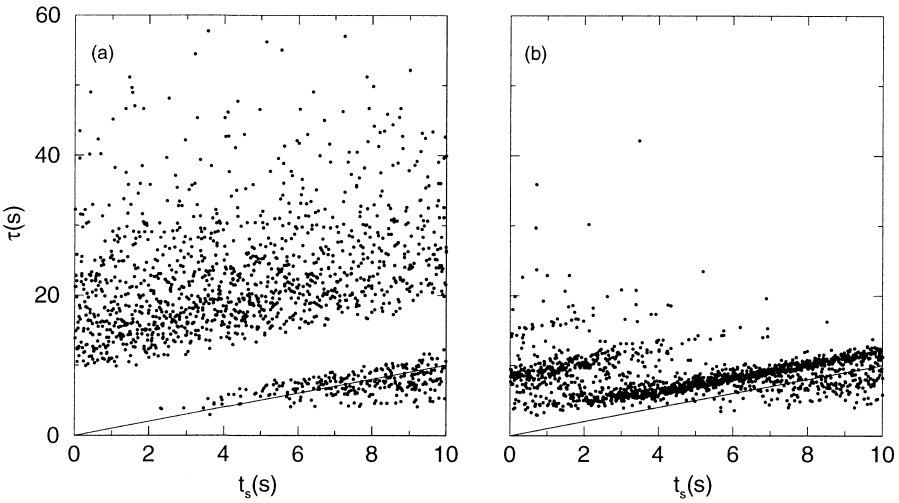

Fig. 5 Simulation of phase-response plots for attractant stimuli (**a**) and repellent (**b**) stimuli. t_s has been varied in steps of 20 ms and for each value three switching events have been simulated. The straight line ($\tau=t_s$) in both plots separates switching events which have occurred before and after stimulation. Parameters: $n=16$, $\lambda=0.14\ s^{-1}$, $\lambda'=2.44\ s^{-1}$, $t_p=1.5$ s; (**a**) $\mu=-1.5$, $\varepsilon=0$, $k_a=0.33\ s^{-1}$; (**b**) $\mu=20$, $\varepsilon=1$, $k_a=0.2\ s^{-1}$, $T=6$ s

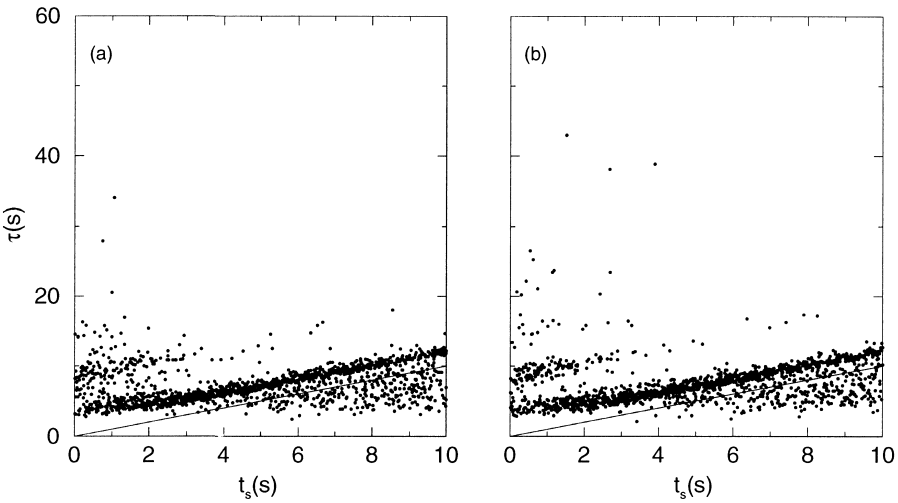

Fig. 6a, b Simulations of phase-response plots for repellent stimuli. Also reactivation of the switch complex has been modulated, as described in the text. In panel (**b**), it was furthermore assumed that a repellent stimulus slightly delays reversals between the periods of reversal induction. Parameters: $r=1.5$, (**a**) $\varepsilon=1$, (**b**) $\varepsilon=0.5$, others as in Fig. 5

populations of dots is not so clear. More satisfying simulations are obtained if it is assumed that a repellent stimulus also accelerates reactivation of the switch complex. In Fig. 6a, λ' has been increased by a factor $r=1.5$ for stimulus-induced events:

$$\lambda_i=\begin{cases}\lambda' & \text{if } t<t_s+t_p\\ r\lambda' & \text{else}\end{cases},\quad i\neq n. \tag{14}$$

This assumption is in accordance with the experimental finding that the frequency of short swimming intervals upon repellent stimulation is significantly increased compared to the frequency of short spontaneous intervals (Schimz and Hildebrand 1985, Krohs 1995). For simplicity, adaptation concerning this effect has been neglected. Moreover, it would not significantly influence the result. Of course, this simplification is not allowed if more than one reversal after stimulation is considered.

An oscillating signal $s(t)$ upon strong repellent stimulation may be caused by a feed-back loop localized in an early step of the signal chain, that also allows the cells to quickly adapt to their environment (Krohs 1995). This suggests that λ_n varies about its spontaneous level [$\varepsilon<1$ in Eq. (13)], which means that a repellent stimulus delays reversals between the periods of reversal induction. This would be a plain explanation for a clear separation of both populations of reversals for early repellent stimuli. In fact, best correspondence between simulated and experimental data was obtained with this additional assumption (Fig. 6b). Also the clear separation of populations of first and second reversals after repellent stimulation (Krohs 1995) could be reproduced (unpublished results by the author).

5 Discussion

The close relationship of the molecular mechanisms of signal transduction in *E. coli* and *Halobacterium* suggests the possibility to extend the comparative discussion to the mo-

tor apparatus, although there are basic behavioral differences. Common to both is the notion of a "motor switch", which is viewed as a substructure of the flagellar motor apparatus determining its direction of rotation. It has been proposed that between every two switching events the switch complex undergoes several changes of state that are correlated with the reactivation of the switch followed by the switching step itself. To describe the reactivation of the switch complex correctly, a fairly large number of discrete steps was necessary. Candidates for such steps are binding and release of fumarate and phosphorylated CheY or conformational changes of subunits of the switch complex. In fact, Kuo and Koshland (1989) found that in *E. coli* the behavioral response of a single flagellar motor to different concentrations of CheY is sigmoidal with an apparent Hill-coefficient >5, which may be explained by a cooperative binding of several molecules of CheY. By structural analysis, it has been shown that the flagellar motor of *Salmonella typhimurium* and *E. coli* is composed of a large number of different proteins, each of which is present in multiple copies constituting ring-like structures. For example, the hook-basal body complex consists of about 26 copies of its protein components (Sosinsky et al. 1992), while there are probably about 12 Mot-protein complexes (Khan et al. 1988). It is likely that such structural features are also characteristic for the flagellar motor in halobacteria, although this has not yet been proven (see Jaschke et al. 1994, Kupper et al. 1994, for recent results). They form the basis for a microscopic interpretation of the model. If many microscopic events such as ligand binding and conformational changes occur on a comparatively slow time-scale, they may well be reflected in a low frequency of short swimming intervals. In this context, it has to be mentioned that also in *E. coli* the shortest spontaneous swimming intervals are not the most frequent ones (Segall et al. 1982). However, with respect to the exponentially decreasing part of the distribution the period of low reversal frequency just after a switching event is much shorter than in halobacteria. On the basis of the model, this difference could easily be explained by different ratios of large and small transition rates between the states of the switch complex in either species.

To describe the effect of stimulation, it has been assumed that light stimuli in the first place modulate the spontaneous switching rate. The features of behavioral responses are consistent with the assumption of constant sensitivity to light stimuli during a swimming interval, which is reflected in the structure of the model that proposes a uni-directional coupling between sensory system and flagellar motor apparatus. Systematic variations of the time to reversal after a stimulus as a function of the time of stimulation relative to the preceding switching event can be understood as a consequence of the slow reactivation of the switch complex. However, experiment and theory reveal some asymmetry regarding the intracellular processing of attractant and repellent stimuli. First, the effects of repellent and attractant stimuli are not simply inverse to each other. On the basis of the model, attractant responses can be understood merely by assuming a transient suppression of switching events. Repellent responses, however, are explained by a stimulus-evoked oscillatory signal. Even if only one reversal after stimulation is considered the experimental data cannot be explained by the fact that the flagellar motor cannot switch at arbitrarily short interals. Second, for a satisfying description of attractant responses it is sufficient to consider a single modulation site, whereas for repellent responses the analysis suggests that reactivation of the switch complex is modulated as well. A speculative microscopic explanation for this finding would be that repellent stimuli affect the kinetics of two "switch factors" (fumarate and phosphorylated CheY), while attractant stimuli modulate one factor only.

Analysing sequences of successive spontaneous switching events, it was shown that spontaneous swimming behavior of *Halobacterium* is consistent with an alternative reactivation mechanism of the switch complex (Naber 1996). This model was based on the hypothesis that there is an intracellular biochemical oscillator which controls the occurrence of switching events even in the absence of chemical or light stimuli (Schimz and Hildebrand 1985). It was assumed that the oscillator triggers transitions between the switch states if the concentration of one of its components reaches some threshold. Therefore, the interval between two successive switching events is correlated with the oscillation period. This mechanism was used as an alternative to the retardation mechanism of the model discussed here to account for the low probability of short swimming intervals. The oscillation was supposed to be self-sustained. It must be clearly distinguished from the damped oscillation of the intracellular signal $s(t)$, which has been postulated to occur in an early step of the signal chain after strong repellent stimulation. In contrast to the reactivation mechanism which was discussed in this paper, the alternative mechanism did not yield satisfying results when the effect of stimulation was considered, regardless of whether it was assumed that a stimulus modulates the temporal evolution of the oscillator or the properties of the switch complex. Only in the case of attractant stimulation was a good correspondence between stimulated and experimental data obtained (Naber 1993). Bi-modal interval distributions after repellent stimuli applied within a few seconds after a reversal are possible if the action of the stimulus is described by Eq. (12). However, the correspondence between simulated and experimental data was much worse than for the model presented here (Naber 1993). The assumption that in addition to the persistent oscillation there is also an oscillatory repellent signal led to bi-modal interval distributions as well. But the results differed from experiments with respect to late stimuli (unpublished results by the author).

From the point of view of these results, future analysis of the light-controlled swimming behavior of *Halobacterium* could be based on the model presented in this paper. It represents a sound working hypothesis to explain the characteristics of sequences of spontaneous switching events as well as to understand the interplay between sensory systems and flagellar motor apparatus.

Acknowledgement I am grateful to E. Hildebrand, U. Krohs, and A. Schimz for enlightening discussions and critical reading of the manuscript.

Appendix

In this appendix, the average time to reversal $\langle\tau^*\rangle$ after an attractant stimulus is derived in the case of a step-like attractant signal. The calculation is based on the fact that for $s(t)$ given by Eq. (10) the Master equation (4) can be solved by piece-wise integration. In principle, the following first-passage-time problem has to be solved: Given a population of bacteria all of which have reversed their swimming direction at time $t=0$. To calculate $\langle\tau^*\rangle$, only those bacteria have to be considered that do not reverse spontaneously before stimulation at $t=t_s$. At time $t=t_s+u$, when switching ability is regained, this subpopulation is characterized by the fractions q_k, $k=1, \ldots, n$, of bacteria, whose switch is in state X_k. Then, $\langle\tau^*\rangle-u$ is equal to the mean first-passage time $\langle\tau\rangle$ for the sequence of states Eq. (1), where $X_1, \ldots, X_1^*$ are initially occupied with probabilities

$$\begin{aligned} &p_k(0) = q_k\,, \quad k = 1, \ldots, n \\ &p_1^*(0) = 0\,. \end{aligned} \tag{15}$$

In other words, Master equation (4) has to be solved with this initial condition and $s(t)\equiv 1$, where the q_k are related to the solution with initial condition $p_1(0)=1$, $p_2(0)=\cdots=p_1^*(0)=0$ through

$$q_k = p_k(t_s+u) \Big/ \sum_{i=1}^{n} p_i(t_s+u)\,. \tag{16}$$

Because p_1^* does not couple into the equations for $p_1, \ldots, p_n$, it is sufficient to consider the first n equations. By means of the Laplace transformation these are transformed to the linear algebraic equation system for the Laplace transforms $\tilde{p}_k(s)$ of $p_k(t)$:

$$\begin{aligned} (-\lambda' - s)\,\tilde{p}_1 &= -q_1 \\ \lambda'\,\tilde{p}_{k-1} - (\lambda' + s)\,\tilde{p}_k &= -q_k\,, \quad k = 2, \ldots, n-1 \\ \lambda'\,\tilde{p}_{n-1} - (\lambda + s)\,\tilde{p}_n &= -q_n\,, \end{aligned} \tag{17}$$

where initial condition Eq. (15) has been inserted. The system Eq. (17) is easily solved iteratively for $\tilde{p}_n$. One finds

$$\tilde{p}_n(s) = \sum_{k=1}^{n} q_k \,\frac{1}{\lambda + s}\left(\frac{\lambda'}{\lambda' + s}\right)^{n-k}. \tag{18}$$

Using the identities $\langle\tau^*\rangle=\langle\tau\rangle+u$, $\langle\tau\rangle=-\tilde{\rho}'(0)$, which connects the mean first-passage time $\langle\tau\rangle$ with the derivative of the Laplace transform $\tilde{\rho}(s)$ of its distribution, and $\tilde{\rho}(s)=\lambda\tilde{p}_n(s)$ [Eqs. (3) and (4)], one ends up with

$$\begin{aligned} \langle\tau^*\rangle &= u + \sum_{k=1}^{n} q_k \left[\frac{1}{\lambda} + (n-k)\,\frac{1}{\lambda'}\right] \\ &= u + \frac{1}{\lambda} + \left[n - \langle k\rangle\right]\frac{1}{\lambda'}\,, \end{aligned} \tag{19}$$

where $\langle k\rangle=\sum_{k=1}^{n} k q_k$ denotes the state X_k that is on the average occupied by the switch complex at time $t=t_s+u$. It is obvious from the structure of the process that $\langle k\rangle$ tends to n for large t_s+u. Therefore, $\langle\tau^*\rangle$ is (approximately) constant for late or strong stimuli.

References

Alam M, Oesterhelt D (1984) Morphology, function and isolation of halobacterial flagella. J Mol Biol 176:459–475

Barak R, Eisenbach M (1992) Fumarate or a fumarate metabolite restores switching ability to rotating flagella of bacterial envelopes. J Bacteriol 174:643–645

Eisenbach M (1991) Signal transduction in bacterial chemotaxis. In: Spudich JL, Satir BH (eds) Sensory receptors and signal transduction. Wiley-Liss, New York, pp 137–208

Francis NR, Sosinsky GE, Thomas D, DeRosier DJ (1994) Isolation, characterization and structure of bacterial flagellar motors containing the switch complex. J Mol Biol 235:1261–1270

Hildebrand E, Schimz A (1985) Behavioral pattern and its photosensory control in *Halobacterium halobium*. In: Eisenbach M, Balaban M (eds) Sensing and response in microorganisms. Elsevier, Amsterdam, pp 129–142

Hildebrand E, Schimz A (1987) Role of the response oscillator in inverse responses of *Halobacterium halobium* to weak light stimuli. J Bacteriol 169:254–259

Jaschke M, Butt HJ, Wolff EK (1994) Imaging flagella of halobacteria by atomic force microscopy. Analyst 119:1943–1946

Khan S, Dapice M, Reese TS (1988) Effects of *mot* gene expression on the structure of the flagellar motor. J Mol Biol 202:575–584

Krohs U (1994a) Das Verhalten von *Halobacterium salinarium* nach stufenförmigen Lichtreizen. PhD thesis, University of Technology, Aachen, Germany

Krohs U (1994b) Sensitivity of *Halobacterium salinarium* to attractant light stimuli does not change periodically. FEBS Lett 351:133–136

Krohs U (1995) Damped oscillations in photosensory transduction of *Halobacterium salinarium* induced by repellent light stimuli. J Bacteriol 177:3067–3070

Kuo SC, Koshland Jr DE (1989) Multiple kinetic states for the flagellar motor switch. J Bacteriol 171:6279–6287

Kupper J, Marwan W, Typke D, Grünberg H, Uwer U, Gluch M, Oesterhelt D (1994) The flagellar bundle of *Halobacterium salinarium* is inserted into a distinct polar cap structure. J Bacteriol 176:5184–5187

Lanyi JK (1984) Bacteriorhodopsin and related light-energy converters. In: Ernster L (ed) Bioenergetics. Elsevier, Amsterdam, pp 315–335

Marwan W, Alam M, Oesterhelt D (1991) Rotation and switching of the flagellar motor assembly in *Halobacterium halobium*. J Bacteriol 173:1971–1977

Marwan W, Bibikov SI, Montrone M, Oesterhelt D (1995) Mechanism of photosensory adaptation in *Halobacterium salinarium*. J Mol Biol 246:493–499

Marwan W, Oesterhelt D (1987) Signal formation in the halobacterial photophobic response mediated by a fourth retinal protein (P_{480}). J Mol Biol 195:333–342

Marwan W, Oesterhelt D (1990) Signal transduction in *Halobacterium* depends on fumarate. EMBO J 9:355–362

McCain DA, Amici LA, Spudich JL (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

Montrone M, Marwan W, Grünberg H, Mußeleck S, Starostzik C (1993) Sensory rhodopsin-controlled release of the switch factor fumarate in *Halobacterium salinarium*. Mol Microbiol 10:1077–1085

Naber H (1993) Stochastische Modelle zur Beschreibung zeitlicher Strukturen im lichtgesteuerten Schwimmverhalten von *Halobacterium halobium*. PhD thesis, University of Technology, Aachen, Germany

Naber H (1996) Two alternative models for spontaneous flagellar motor switching in *Halobacterium salinarium*. J theor Biol 181: 343–358

Petracchi D, Lucia S, Cercignani G (1994) Photobehaviour of *Halobacterium halobium:* proposed models for signal transduction and motor switching. J Photochem Photobiol B Biol 24:75–99

Rudolph J, Oesterhelt D (1995) Chemotaxis and phototaxis require a CheA histidine kinase in the archaeon *Halobacterium salinarium*. EMBO J 14:667–673

Rudolph J, Oesterhelt D (1996) Deletion analysis of the *che* operon in the archaeon *Halobacterium salinarium*. J Mol Biol 258:548–554

Rudolph J, Tolliday N, Schmitt C, Schuster SC, Oesterhelt D (1995) Phosphorylation in halobacterial signal transduction. EMBO J 14:4249–4257

Schimz A, Hildebrand E (1985) Response regulation and sensory control in *Halobacterium halobium* based on an oscillator. Nature 317:641–643

Segall JE, Manson MD, Berg HC (1982) Signal processing times in bacterial chemotaxis. Nature 296:855–857

Sosinsky GE, Francis NR, DeRosier DJ, Wall JS, Simon MN, Hainfeld J (1992) Mass determination and estimation of subunit stoichiometry of the hook-basal body flagellar complex of *Salmonella typhimurium* by scanning transmission electron microscopy. Proc Natl Acad Sci USA 89:4801–4805

Spudich JL, Bogomolni RA (1988) Sensory rhodopsins in halobacteria. Ann Rev Biophys Biophys Chem 17:193–215

Spudich JL, Stoeckenius W (1979) Photosensory and chemosensory behavior of *Halobacterium halobium*. Photobiochem Photobiophys 1:43–53

Sundberg SA, Alam M, Spudich JL (1986) Excitation signal processing times in *Halobacterium halobium* phototaxis. Biophys J 50: 895–900

van Kampen NG (1981) Stochastic processes in physics and chemistry. Elsevier, Amsterdam

Wolfe AJ, Conley MP, Kramer TJ, Berg HC (1987) Reconstitution of signaling in bacterial chemotaxis. J Bacteriol 169:1878–1885

Yao VJ, Spudich JL (1992) Primary structure of an archaebacterial transducer, a methyl-accepting protein associated with sensory rhodopsin I. Proc Natl Acad Sci USA 89:11915–11919