A Hitchhiker's guide through advances and conceptual changes in chemotaxis

ARTICLE *in* JOURNAL OF CELLULAR PHYSIOLOGY · DECEMBER 2007

Impact Factor: 3.84 · DOI: 10.1002/jcp.21238 · Source: PubMed

1 AUTHOR:

Michael Eisenbach
Weizmann Institute of Science
126 PUBLICATIONS **4,557** CITATIONS

SEE PROFILE

Journal of Cellular Physiology

A Hitchhiker's Guide Through Advances and Conceptual Changes in Chemotaxis

MICHAEL EISENBACH*

Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot, Israel

Chemotaxis is a basic recognition process, governed by protein network that translates molecular-based information on the surrounding environment into a guided motional response of the recipient cell or organism. This process is prevalent from bacteria to human beings. Some of the chemotaxis systems—like that of the bacterium *Escherichia coli*—are well established; others—like that of mammalian sperm cells—are at their relatively early stages of research. In contrast to mammalian sperm chemotaxis, where studies have so far been limited to the phenomenological level primarily, the model of bacterial chemotaxis is known down to the angstrom resolution. Despite this difference in depth of understanding, many fundamental questions are open not only in the new but also in the old chemotaxis fields of research, and recent advances in them are raising additional intriguing questions. This review summarizes some of these surprises and previously unasked or overlooked questions, and as such it offers a guided tour through conceptual changes in chemotaxis.

J. Cell. Physiol. 213: 574–580, 2007.

Chemotaxis is the active movement of a cell or an organism toward or away from a chemical source, the chemical defined as chemoattractant or chemorepellent, respectively. A large variety of species and cells employ chemotaxis to guide them to their targets [see reference Eisenbach (2004) for an extensive review]. Thus, bacteria reach beneficial environments and avoid noxious ones by chemotaxis; butterflies, bees, and other insects find food sources by chemotaxis; hunting mammals follow smells of their prey by chemotaxis; and animals, in general, may be attracted to the other gender by following pheromones secreted from the latter. Within the body, chemotaxis is used, for example, to guide white blood cells, nerve cells, metastatic tumor cells, and sperm cells to their respective targets.

The first and the most thorough studies of the molecular mechanism of chemotaxis have been carried out with bacteria (Adler, 1966, 1969). Today bacterial chemotaxis is considered as the most understood signaling system at the molecular level. Since the discovery of this process in bacteria, chemotaxis has been discovered and investigated at the molecular level in an increasing number of biological systems (Eisenbach, 2004). The most recently discovered system—chemotaxis of mammalian sperm cells—even broke the prevailing dogma that, in mammals, sperm cells are not guided to the oocyte but rather reach it by coincidence [see reference Eisenbach and Giojalas (2006) for a review].

Unconventionally, this mini review does not summarize the current knowledge in chemotaxis. Instead, it subjectively and selectively reviews results, obtained primarily in my group, which changed concepts in the best understood chemotaxis system (that of the bacterium *Escherichia coli*) and raised intriguing questions in the apparently least understood chemotaxis system (that of mammalian sperm cells). Thus, it summarizes studies showing that *E. coli* possesses an additional signaling system, that the switch of the flagellar motor contains a component not known to be associated with it and, therefore, is more complex than previously realized, and that the signaling molecule CheY in vivo is covalently modified by acetylation. It further indicates open dilemmas in chemotaxis of mammalian sperm cells and summarizes first results on the swimming behavior of these cells in a chemoattractant gradient.

Bacterial Chemotaxis

Introduction

The bacterium *E. coli* possesses two types of supramolecular complexes: a receptor supramolecular complex at the pole(s) of the cell, and a number of flagellar–motor supramolecular complexes, randomly distributed around the cell and embedded within the cell membrane [see reference Eisenbach (2004) for a review]. The receptor complex includes the chemotaxis receptors as well as enzymes, which modulate the receptor activities, and enzymes regulated by the receptors. The flagellar–motor complex includes a rotary motor, driven by a flux of protons, and a gearbox, termed "switch" (Fig. 1) [see references Berg (2003) and Kojima and Blair (2004) for reviews]. The switch is a ring of three proteins—FliM, FliN, and FliG—at the base of the flagellar motor. The communication between the receptor and flagellar–motor complexes is done by a 14 kDa protein, CheY, which shuttles back and forth between the two complexes and transduces sensory information between them. When CheY is non-phosphorylated, it is bound to the receptor supramolecular complex via its histidine kinase, CheA. When CheY becomes phosphorylated by this kinase in response to a change in the

M.E. is an incumbent of the Jack and Simon Djanogly Professorial Chair in Biochemistry.

Contract grant sponsor: US-Israel Binational Science Foundation;
Contract grant number: 2005199.
Contract grant sponsor: Israel Science Foundation;
Contract grant number: 290/06.
Contract grant sponsor: Minerva Foundation.
Contract grant sponsor: Dr. Josef Cohn Minerva Center for Biomembrane Research.

*Correspondence to: Michael Eisenbach, Department of Biological Chemistry, The Weizmann Institute of Science, 76100 Rehovot, Israel. E-mail: m.eisenbach@weizmann.ac.il

Received 3 July 2007; Accepted 5 July 2007

DOI: 10.1002/jcp.21238

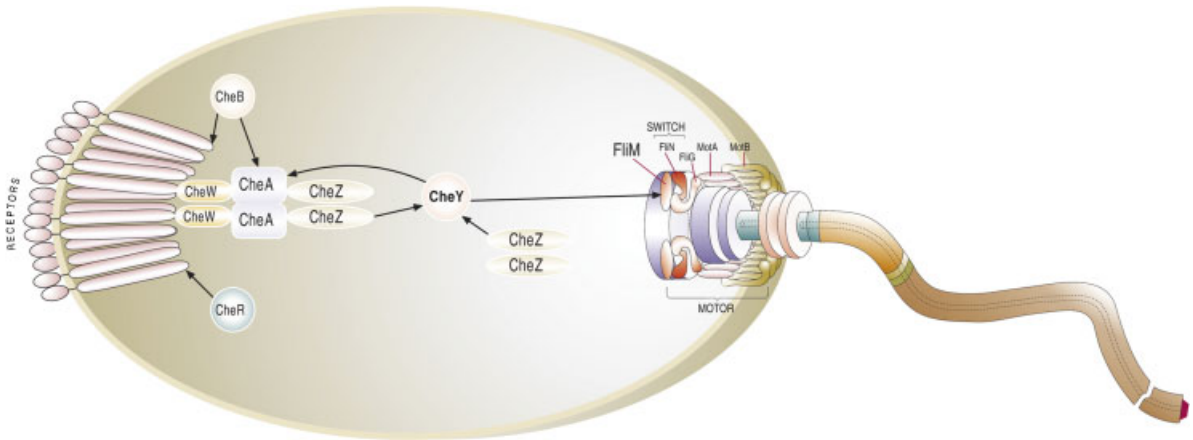

Fig. 1. Simplified scheme of protein–protein interactions that transduce the sensory signal from the receptor supramolecular complex to the flagellar–motor supramolecular complex in *E. coli* chemotaxis. The scheme is not drawn to scale. Black arrows stand for regulated interactions. CheA is a histidine kinase that phosphorylates CheB and CheY, CheB is a specific methylesterase that demethylates the chemotaxis receptors, CheR is a specific methyltransferase that methylates the chemotaxis receptors, CheW is a scaffolding protein that couples CheA to the receptors, CheY is the key response regulator in chemotaxis of *E. coli*, and CheZ is a phosphatase that enhances the spontaneous dephosphorylation of CheY. [Modified with permission from Bren and Eisenbach (2000).] [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

receptor occupancy, its affinity for the kinase decreases and, consequently, it dissociates from the receptor supramolecular complex. At the same time, the affinities of CheY for the N-terminus of the switch protein FliM and for the C-terminus of the phosphatase CheZ increase and, consequently, CheY binds to the switch and shifts the rotation of the motor from its default direction, counterclockwise, to increase the probability of clockwise. While the effect of CheY–FliM binding on flagellar rotation is instantaneous, the effect of CheY binding to CheZ activates the phosphatase and terminates the clockwise signal only after a delay.

The direction of flagellar rotation affects the mode of swimming of *E. coli*. When all the flagella rotate counterclockwise, they form a bundle that pushes the cell in a rather straight line forward (a run). When one or more of the flagella rotate clockwise, the cell turns or tumbles, the vigor of the turn being dependent on the number of flagella that shift to clockwise rotation. Under physiological conditions, the clockwise period and, consequently, the turn are usually brief, after which the cell continues to swim in a straight line. The outcome of these two modes of swimming is a random walk, composed of relatively long runs with occasional, brief periods of moderate or abrupt turning (Berg and Brown, 1972; Macnab and Koshland, 1972; Turner et al., 2000). In a chemical gradient, the random walk is biased toward the chemoattractant or away from the chemorepellent.

From this simplified description of the sequence of events, it may seem that the mechanism of *E. coli* chemotaxis is almost entirely understood. I wish to bring here three surprising findings, which, on the one hand, suggest that this mechanism is far from being fully known, but, on the other hand, open exciting new avenues of investigation.

Surprise no. 1: *E. coli* has an additional, yet unknown, chemotaxis signaling system

One of these findings, perhaps the most surprising one, was that *E. coli* appears to have at least two signaling systems for chemotaxis. The story started a number of years ago, when Barak and Eisenbach (1999) overexpressed CheY from a plasmid in a "gutted" *E. coli* strain, lacking the chemotaxis proteins and some of the chemotaxis receptors, and found that this strain has some chemotactic activity in spite of being devoid of the chemotaxis machinery. Indeed, the response was not as efficient as the response of wild-type cells, but a considerable chemotactic activity remained. Unpredictably, the response appeared to be more sensitive than the conventional response, as the peak response was at orders of magnitude lower chemoattractant concentrations. This suggested that an additional signal-transduction pathway, other than the conventional one, is involved in chemotaxis of *E. coli*. So far, nothing is known on the identity of this pathway.

This finding raised several intriguing questions. The most stimulating one is, perhaps, the identity of the molecular components involved in this non-conventional signaling pathway. Future studies should determine whether the receptors are substrate-recognition components of corresponding transport systems, as proposed (Barak and Eisenbach, 1999), thus having a dual function, how the receptors transduce the signal to the flagellar motor, and whether CheY is involved in this signaling. Another question is what advantage has a cell, which possesses two signaling systems of chemotaxis, over a cell that has only a single system. The non-conventional pathway might be, in wild-type cells, a back-up system that detects low concentrations of stimuli under certain conditions, for example, in stationary phase or under anaerobic conditions. It might also be a primitive mechanism, which, during evolution, became masked by a better, more efficient system.

The following two examples of major surprising findings relate to the conventional signaling system of *E. coli* chemotaxis. Both of them have roots in the early days, when nothing was known about the signaling pathway and a number of research groups invested much effort to investigate how the direction of flagellar rotation is controlled.

Surprise no. 2: fumarate reductase is an integral component of flagellar switching and assembly

One of the very early approaches of my group to reveal the molecular mechanism of bacterial chemotaxis was to prepare cytoplasm-free envelopes of *E. coli* and *Salmonella enterica* serovar Typhimurium, having functional flagella, by sequential

penicillin treatment and osmotic lysis of intact cells (Eisenbach and Adler, 1981), and examine what is needed for the generation of clockwise rotation. The observations that these envelopes, when externally energized, rotate their flagella exclusively counterclockwise (Ravid and Eisenbach, 1984) unless they contain CheY, in which case some of the envelopes rotate their flagella clockwise (Ravid et al., 1986), provided one of the first lines of evidence that the default direction of flagellar rotation is counterclockwise and that CheY is required for clockwise rotation. An unexpected finding made then was that none of the CheY-containing envelopes could switch the direction of flagellar rotation; their flagella rotated either counterclockwise or clockwise, but they never switched directions (Ravid et al., 1986). Later, being inspired by the finding of Marwan et al. (1990) that fumarate is a factor needed for the archeal *Halobacterium salinarium* to swim back and forth, we found that fumarate is the missing link to enable switching in *E. coli* envelopes (Barak and Eisenbach, 1992b; Barak et al., 1996). Subsequent studies found that fumarate has a similar effect in intact *E. coli* cells (Montrone et al., 1998; Prasad et al., 1998), that fumarate is effective independently of CheY (indicating that the target of fumarate is the switch, not CheY), and that one of its acts is to lower the standard free energy of the clockwise state relative to that of the counterclockwise state (Prasad et al., 1998). However, the mechanism by which this dicarboxylate molecule affects the switch has remained an enigma.

A straightforward approach to start resolving this enigma is to identify the switch component with which fumarate interacts. Surprisingly, however, we found that none of the switch proteins, either in purified form (individually or together) or within the isolated switch–motor complex, bound fumarate (Cohen-Ben-Lulu et al., submitted). This suggested that fumarate might bind to another protein that transmits it to the switch. This protein should be membrane bound because fumarate is effective even in cytoplasm-free envelopes (Barak and Eisenbach, 1992b; Barak et al., 1996). Two *E. coli* proteins fulfill the requirements of being membrane bound and capable of fumarate binding: succinate dehydrogenase (SDH) and fumarate reductase (FRD)—enzymes that are similar in their amino-acid sequence, 3D structure, cofactors, and mechanisms of function (so much so that, functionally, they can replace each other) (Cecchini et al., 2002). By a combination of multiple biochemical approaches as well as in vitro and in vivo immuno-electron-microscopical studies, we demonstrated that these proteins indeed bind to the switch, the docking protein being FliG (Cohen-Ben-Lulu et al., submitted). Nevertheless, FRD and SDH were functionally different: while *frd* deletion caused loss of the fumarate effect in envelopes (Cohen-Ben-Lulu et al., submitted), *sdh* deletion did not have any effect in envelopes (Barak et al., 1996).

The docking protein FliG is central to the function of the flagellar motor. It is known to be directly involved in motor assembly, torque generation, and switching the direction of rotation (Kojima and Blair, 2004). Consequently, defects in this protein may lead to loss of flagella, paralysis, or biased direction of flagellar rotation. If the interaction of FliG with SDH or FRD is required for its function, absence of SDH or FRD could lead to any one of these phenotypes, assigned to FliG. When examining this prediction, we obtained results that were both expected and surprising. The Δ*sdh* mutant was not different from its wild-type parent with respect to motility. Surprisingly, however, the Δ*frd* mutant was barely motile: many cells did not swim at all, others swam slower than usually and, in most of these latter cases, the movement was wobbly. This behavior reflected a deficiency of flagella: while the wild-type parent had five to six flagella/cell at the average, the Δ*frd* mutant had one to two flagella per cell, with many cells having no flagella at all. Complementing the *frd* deletion with a plasmid producing a single copy of FRD under its native promoter, restored, at least partially, the number of flagella and, consequently, the fraction of motile cells (Cohen-Ben-Lulu et al., submitted). This discovery was surprising for three main reasons. First, FRD expression or function under aerobic conditions has not been recognized hitherto (Cecchini et al., 2002). Second, FRD and SDH are so similar, and both of them bind to FliG; nevertheless, only FRD seemed to be required for flagellar assembly. Third, FRD, like SDH, is known to be involved in the energy-conversion system, but it has never been thought to be associated, in any way, with flagellar function or with the motility system, in general.

But the effects of FRD absence on flagellar assembly in intact cells and on fumarate-enabled switching in envelopes were not the only defects observed. Even the few remaining flagella in intact Δ*frd* cells were affected by the absence of FRD: their rotation was almost exclusively counterclockwise, with no switching events. In response to a repellent, these flagella hardly shifted to clockwise rotation, only making futile attempts to switch (Cohen-Ben-Lulu et al., submitted). This indicated that FRD (but not SDH) is needed for both flagellar assembly and ability to switch to clockwise rotation.

These rather unexpected findings raise a number of intriguing questions, both at the mechanistic and functional levels. For example: FliG is part of the rotor; does FRD, as a whole, rotate together with FliG within the membrane, or is the rotation done by a cytoplasmic subunit of FRD? How is FRD involved in flagellation and switching? Has it a function at the switch beyond fumarate binding? Are other energy-linked enzymes concentrated near the switch–motor complex? If so, for what purpose?

Surprise no. 3: the chemotaxis response regulator, CheY, is acetylated in vivo

The other surprising finding made with the system of cytoplasm-free envelopes was that phosphorylation of CheY within the envelopes did not generate more clockwise rotation, as was anticipated, only doing so in the presence of cytoplasmic constituents other than chemotaxis proteins (Barak and Eisenbach, 1992a). This suggested that the enhancement of CheY binding to the switch by phosphorylation is insufficient to increase the probability of flagellar rotation in the clockwise direction. While trying to identify what else is required for clockwise generation, we found that phosphorylation is not the only chemical modification that CheY undergoes and not the only one that activates the protein. By means of mass spectrometry, Western blots with an anti-acetyl-lysine antibody, and radioactive assays, we demonstrated that CheY undergoes N-acetylation (Barak et al., 1992; Barak et al., 2004) (Fig. 2), and that this acetylation activates the protein to generate clockwise rotation both in envelopes (Barak et al., 1992) and in intact cells (Barak et al., 1998). We found that this acetylation is accomplished by at least two mechanisms: acetylation by acetate, mediated by the enzyme acetyl-CoA synthetase (Acs) (Barak et al., 2004), and autoacetylation with AcCoA as an acetyl donor (Barak et al., 2006). We further found that the acetylation sites of CheY are the same in both mechanisms—up to six lysine residues at the C-terminus of the protein (Fig. 2) (Ramakrishnan et al., 1998; Barak et al., 2004, 2006). Because all the acetylation sites are clustered at the CheY surface that binds CheA, CheZ, and FliM, it was reasonable to assume that acetylation would affect the binding, but this was not the case, at least with respect to FliM (Ramakrishnan et al., 1998; Liarzi and Eisenbach, unpublished data).

CheY is a remarkable protein: although it is rather small, it binds to three different proteins and it catalyzes its own phosphorylation and acetylation. Obvious questions are

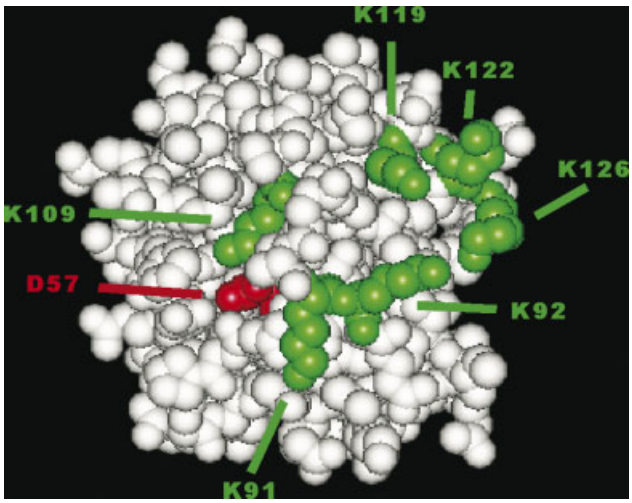

Fig. 2. Space-filling presentation of CheY, demonstrating the distribution of its acetylation sites (lysine residues shown in green) and the location of its phosphorylation site (an aspartate residue shown in red). [Modified from Barak et al. (2004).] [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

whether the activation of CheY by acetylation is involved in chemotaxis and, if so, what its function is, and why two different covalent modifications are used to activate this protein. The observations that mutants lacking the acetylating enzyme Acs or missing an acetylation site are chemotaxis defective suggest that CheY acetylation has a function in chemotaxis (Barak and Eisenbach, 2001). Although it is not yet known what this function is, it is clear that both covalent modifications of CheY mutually affect each other (Barak and Eisenbach, 2004). Thus, each of the phosphodonors of CheY, the histidine kinase CheA and acetyl phosphate, inhibits the acetylation of CheY. Conversely, the phosphatase CheZ enhances CheY acetylation. The presence of the acetylating enzyme Acs elevates the phosphorylation levels of both CheA and CheY, and the acetyl donor, acetate, represses this stimulation. We proposed that these mutual effects might play a role in the robustness of chemotaxis (Alon et al., 1999; Løvdok et al., 2007), serving as a tuning mechanism that compensates for cell-to-cell variations in the intracellular concentrations of CheA and CheZ (Barak and Eisenbach, 2004). However, CheY acetylation probably has an additional function, not yet revealed.

Recently it became clear that, in vivo, the level of CheY acetylation is much higher than that of purified CheY in vitro, with almost every molecule of CheY being acetylated at the average (Barak et al., submitted). This finding made the question of what the function of CheY acetylation is even more intriguing because all studies of the function–structure relationship of CheY were carried out with purified, non-acetylated CheY. If, in vivo, CheY is acetylated, its physiological structure and the structural changes responsible for its activation may be different from those believed hitherto.

Conclusion

The examples of unexpected findings, summarized above, reflect important advances made in revealing the molecular mechanism of bacterial chemotaxis, which, as already mentioned, is regarded as the best understood signal-transduction system. Thus, these examples showed that *E. coli* possesses an additional signal-transduction pathway for chemotaxis, that the enzyme FRD—hitherto unknown to be related to motility or chemotaxis and to be functional under aerobic conditions—is tightly associated with the flagellar–switch complex and is involved in its function, and that CheY—the central signaling protein in bacterial chemotaxis—is acetylated in vivo. Future lines of research should reveal the additional signal-transduction pathway in bacterial chemotaxis and its function. Among others, they should also provide answers to how the FliG–FRD interaction affects each of these proteins, how fumarate affects this interaction, whether this interaction is unique to *E. coli* or common to all motor switches, and, if so, what protein(s) substitutes for FRD in species lacking this protein. Future investigations should also find out the advantage given to the cell by having two covalent modifications of the very same protein, each of which can generate clockwise rotation. I wish to point out that there are many additional exciting questions and lines of investigation in different aspects of bacterial chemotaxis, resulting from studies of other research groups. These include, among others, the mechanisms by which the chemotactic signal is amplified by two orders of magnitude [for reviews, see references Bray (2002); Sourjik (2004); Webre et al. (2004); and references therein], understanding why and how all the chemotaxis receptors are clustered together at the poles [see references Parkinson et al. (2005) and Kentner and Sourjik (2006) for reviews], and the mechanisms of function of the flagellar switch and motor [see references Eisenbach and Caplan (1998); Berg (2003); and Kojima and Blair (2004) for reviews].

Mammalian Sperm Chemotaxis

Introduction

For fertilization to occur in mammals, ejaculated sperm cells must reach the oocyte, which, following ovulation, has moved from the ovary into the Fallopian tube. Only small numbers of the ejaculated sperm cells enter the oviduct (Fallopian tube), attach to the oviductal epithelium at the isthmus (the part closer to the uterus), thus forming there a sperm storage site (Suarez, 2002). Sperm cells at this site, a few at a time (Cohen-Dayag et al., 1995; Eisenbach, 1999a), are thought to undergo capacitation, that is, to acquire a state of readiness for fertilizing the oocyte [cells at this state are termed capacitated—see reference Jaiswal and Eisenbach (2002) for a review]. These few are released from the storage site and must be guided in order to make the remaining long, obstructed way to the oocyte [see reference Eisenbach and Giojalas (2006) for a recent review]. This current understanding challenged the dogmatic belief that, in mammals, where so many sperm cells are ejaculated directly into the female reproductive tract, large numbers reach the oocyte even without a guidance mechanism. By extensive work for over a decade we, followed by other research groups, showed that this is not the case and that mammalian sperm cells possess at least two guidance mechanisms: chemotaxis (Eisenbach, 1999b) and thermotaxis (Bahat and Eisenbach, 2006). The discovery of sperm chemotaxis in mammals is a remarkable story, which started from a situation in which we faced much skepticism, not to say disbelief, and which developed into an increasing, lively field, studied by a number of groups in four continents.

Not only the dogma was a reason for the initial disbelief, but also the characteristics of mammalian sperm chemotaxis contributed to this situation, primarily the fact that only capacitated sperm cells are chemotactically responsive (Cohen-Dayag et al., 1995; Eisenbach, 1999a; Fabro et al., 2002; Giojalas et al., 2004). The life span of the capacitated state is rather short [only 1–4 h in humans (Cohen-Dayag et al., 1995)]. The outcome is that the fraction of capacitated, chemotactically responsive cells is very small [~10% in humans (Cohen-Dayag

et al., 1995) and mice (Oliveira et al., 1999)] and, consequently, the signal-to-noise ratio in chemotaxis assays is poor (Eisenbach, 1999b; Ralt et al., 1994). The small fraction of chemotactically responsive cells not only posed technical difficulties, but also evoked the question of scientific credibility. This is because scientists naturally trust strong signals and large responses. Yet, the reader should bear in mind that specific signals in nature are often the outcome of very delicate balance, hard to be detected experimentally. Therefore, unless intentionally amplified by the experimentalist, a small signal should not be disregarded as irrelevant or non-specific. Efforts in my laboratory to increase the fraction of capacitated sperm cells and, thereby, the chemotactic response, were heretofore unsatisfactory.

This difficulty is especially prominent while trying to distinguish, in a chemoattractant gradient, a responsive sperm cell from non-responsive ones. This is because non-responsive sperm cells constitute the majority of the population and they do not differ in their noticeable swimming characteristics from the responsive ones. This section summarizes first attempts to reveal the swimming response of mammalian sperm cells to chemoattractants.

Swimming behavior of sperm cells in a chemoattractant gradient

The swimming behavior of sperm cells is relatively well characterized in marine species. One of the latest examples is of sea urchin sperm cells, which, in the absence of a chemoattractant gradient, move in circles as a result of slight and permanent flagellar asymmetry. In a chemoattractant gradient, the center of the circles moves on a curved trajectory toward the chemoattractant source (Böhmer et al., 2005).

The only information available for mammalian sperm cells is for humans. Human sperm cells in an ascending gradient of the chemoattractant bourgeonal were reported to beat their flagella symmetrically and reach the chemoattractant source by maintaining the same swimming direction. Concomitantly, their swimming speed increases (a phenomenon known as chemokinesis) due to increased flagellar beat frequency. Sperm cells in a descending gradient of bourgeonal appear to turn abruptly due to asymmetrical flagellar beats and to reorient toward the source of bourgeonal (Spehr et al., 2004). This clear-cut observation is rather amazing in view of the fact that, even in bacteria, where the whole population responds, the change in swimming toward the chemoattractant is difficult to detect. In mammalian sperm cells, the detection of changes in swimming direction is expected to be much more difficult because of the small fraction of responsive cells, observed with all chemoattractants tested—follicular fluid (Ralt et al., 1994), egg- and cumulus-conditioned media (Sun et al., 2003, 2005), bourgeonal (Spehr et al., 2006), and progesterone (Teves et al., 2006).

Detection of a temporal versus spatial chemoattractant gradient

In principle, sperm cells may either sense a temporal gradient of the chemoattractant, comparing the occupancy of its receptors at different time points [as do bacteria (Macnab and Koshland, 1972)], or they may detect a spatial gradient, comparing the occupancy of receptors at different locations along their body [as do leukocytes (Devreotes and Zigmond, 1988)]. Sea-urchin sperm cells sense a temporal gradient (Kaupp et al., 2003), and respond to it with a transient increase in flagellar asymmetry. The outcome is a turn in the swimming path, followed by a period of straight swimming (Kaupp et al., 2003), leading to the observed epicycloid-like movements directed toward the chemoattractant source (Böhmer et al., 2005).

Human sperm cells, too, appear to sense a temporal gradient. Mixing them with the chemoattractant progesterone or bourgeonal results in a transient increase in swimming velocity and lateral head displacement, leading to a transient decrease in the linearity of swimming (Gakamsky, 2006). When the added chemoattractant concentration is high, the sperm cells exhibit hyperactivated-like motility. [Hyperactivated motility is characterized by increased velocity, decreased linearity, increased amplitude of lateral head displacement, and flagellar whiplash movement (Suarez and Ho, 2003).] Likewise, photorelease of cyclic nucleotides, considered as putative second messengers of the chemotactic pathway(s) (Kaupp et al., 2003; Spehr et al., 2004), from their caged compounds caused a delayed decrease in the linearity of swimming (Gakamsky, 2006).

Hyperactivated motility of mammalian spermatozoa resembles, to some extent, the bacterial tumbling motion, and, like sperm chemotaxis, is thought to be restricted to capacitated cells [see references Suarez (1996) and Jaiswal and Eisenbach (2002) for reviews]. We suspect that hyperactivated motility may be a part of the chemotactic response. Just like bacteria that run and tumble even without a stimulus, capacitated sperm cells exhibit runs and episodes of hyperactivated motility. It is reasonable that these episodes, like tumbles in the case of bacteria, are responsible for the directional changes, resulting in swimming along the chemoattractant gradient.

Projection of the response in a temporal gradient onto that in a continuous spatial gradient

The observations, summarized above, are consistent with the possibility that human sperm cells detect a temporal chemoattractant gradient rather than a spatial gradient. Can we deduce from the response to a temporal gradient how sperm cells behave when they swim and sense a gradual, continuous change in the chemoattractant concentration? The most pronounced characteristic of the response to a temporal gradient is that it is composed of two phases: a delay (i.e., no change in the motility parameters) followed by a decrease in the linearity of swimming (Gakamsky, 2006). When a human sperm cell swims up the concentration gradient and its chemotaxis receptors continuously become more and more occupied, the cell is repeatedly stimulated. Therefore, a reasonable model is that only the first phase would take place (because the cell is re-stimulated before it has a chance to acquire the second phase) and the cell would maintain the same swimming direction with no change in the motility parameters. When the cell happens to swim in a direction in which it ceases to sense a concentration increase, the second phase would dominate and the cell would turn. Obviously, the temporal concentration changes sensed by sperm cells swimming in a spatial gradient are orders of magnitude lower than those sensed in temporal assays. Therefore, the turns in the swimming path would be much more subtle and rare. These subtle turns would continue until the cell happens to swim up the gradient, at which stage the first segment of the temporal response would dominate and the cell would continue swimming up the gradient.

This model does not assume that the chemoattractant concentration should decrease to stimulate a turn. Cessation of sensing an increase in the chemoattractant concentration is sufficient. This distinction is important in view of the possibility that some chemoattractants with very low K_d values [e.g., resact in the case of sea-urchin sperm cells (Kaupp et al., 2006) and progesterone in the case of mammalian sperm cells (Eisenbach and Giojalas, 2006), but not bourgeonal, to whom the affinity of the sperm receptors is not high (Spehr et al., 2003)] may bind irreversibly to their cognate receptors, suggesting that sperm cells cannot sense decreasing gradients of

these chemoattractants. According to the model, swimming down the chemoattractant gradient would stimulate a turn independently of whether the cells sense a decrease in the chemoattractant concentration (as in the case of bourgeonal) or they just stop sensing an increase in the concentration (as in the case of progesterone).

Lack of correlation between the response to a temporal gradient and the level of capacitated cells

An unexpected observation in the temporal assays was that most of the cells in the observation field responded, indicating lack of correlation with the fraction of capacitated sperm cells (Gakamsky, 2006). Initially, this observation was bothering because, as mentioned above, only capacitated sperm cells are chemotactically responsive (Cohen-Dayag et al., 1995; Eisenbach, 1999a; Giojalas et al., 2004), suggesting, perhaps, that the observed behavioral changes are not related to chemotaxis. However, capacitation is a multi-process event, with many of the processes occurring at the cell surface level, and it is not known which of these processes are obligatory for the occurrence of chemotaxis and what makes a capacitated cell chemotactically responsive (Jaiswal and Eisenbach, 2002). We propose that the main difference between capacitated and non-capacitated sperm cells with respect to chemotaxis is the chemoattractant detection threshold. It is reasonable to suppose that the threshold is significantly lower in capacitated cells, that is, they have higher sensitivity. Thus, in temporal assays, where the changes in the chemoattractant and cyclic nucleotide concentrations are high, most of the cells can sense the changes and respond. In a continuous gradient over space, where the concentration changes are orders of magnitude lower, only capacitated sperm cells can sense the changes and respond. It is generally true that temporal changes should be relatively large to observe a behavioral response, and that behavioral responses in a continuous gradient over space are hard to detect. [This holds even in the case of bacteria, where all the cells are chemotactically responsive. Thus, when *E. coli* cells are exposed to a temporal chemoattractant or chemorepellent gradient, achieved by rapid addition of the stimulant (Macnab and Koshland, 1972) or by its photorelease from a caged compound (Khan et al., 1993), the runs are elongated or the tumbling frequency increases, respectively. However, when the changes in stimulant concentration are subtle as happens when a bacterium swims in a continuous gradient over space, the changes in swimming direction are very slight and difficult to detect (Block et al., 1982).]

Conclusion

Evidently, the understanding of the behavioral response of mammalian sperm cells to chemoattractants is at its initial stages. This is just one example out of many, in all of which the level of understanding is very limited, including the molecular mechanism of sperm chemotaxis. One of the questions, which most intrigue me, is why there are so many chemoattractants. For example, at least five different chemoattractants for human sperm cells have been identified (Eisenbach and Giojalas, 2006). In the simplest model for sperm guidance in the female genital tract, two chemoattractants are sufficient: one secreted from the oocyte and one from the surrounding cumulus cells (Eisenbach and Giojalas, 2006). Does this multiplicity of chemoattractants reflect sperm guidance in regions other than in the vicinity of the oocyte–cumulus complex? Or does it indicate that the guidance mechanism is much more complex, with a repertoire of chemoreceptors allowing the detection of subtle changes in the concentrations of all the chemoattractants and, consequently, accurate location determination and navigation? On the basis of these and other highly intriguing questions, it seems that the next few years of exploring mammalian sperm chemotaxis will be at least as exciting and revealing as those in the last decade or so.

Acknowledgments

Special thanks are due to P. Reuven for his excellent suggestions and comments on the article. I also wish to thank A. Bahat, R. Barak, S.R. Caplan, G. Cohen Ben-Lulu, and A. Gakamsky for their critical reading of the article. Studies in the author's laboratory were supported by a US-Israel Binational Science Foundation research grant no. 2005199, by grant no. 290/06 from the Israel Science Foundation, by the Minerva Foundation, Germany, and by the Dr. Josef Cohn Minerva Center for Biomembrane Research.

Literature Cited

Adler J. 1966. Chemotaxis in bacteria. Science 153:708–716.
Adler J. 1969. Chemoreceptors in bacteria. Science 166:1588–1597.
Alon U, Surette MG, Barkai N, Leibler S. 1999. Robustness in bacterial chemotaxis. Nature 397:168–171.
Bahat A, Eisenbach M. 2006. Sperm thermotaxis. Mol Cell Endocrinol 252:115–119.
Barak R, Eisenbach M. 1992a. Correlation between phosphorylation of the chemotaxis protein CheY and its activity at the flagellar motor. Biochemistry 31:1821–1826.
Barak R, Eisenbach M. 1992b. Fumarate or a fumarate metabolite restores switching ability to rotating flagella of bacterial envelopes. J Bacteriol 174:643–645.
Barak R, Eisenbach M. 1999. Chemotactic-like response of *Escherichia coli* cells lacking the known chemotaxis machinery but containing overexpressed CheY. Mol Microbiol 31:1125–1137.
Barak R, Eisenbach M. 2001. Acetylation of the response regulator, CheY, is involved in bacterial chemotaxis. Mol Microbiol 40:731–743.
Barak R, Eisenbach M. 2004. Co-regulation of acetylation and phosphorylation of CheY, a response regulator in chemotaxis of *Escherichia coli*. J Mol Biol 342:375–381.
Barak R, Welch M, Yanovsky A, Oosawa K, Eisenbach M. 1992. Acetyladenylate or its derivative acetylates the chemotaxis protein CheY in vitro and increases its activity at the flagellar switch. Biochemistry 31:10099–10107.
Barak R, Giebel I, Eisenbach M. 1996. The specificity of fumarate as a switching factor of the bacterial flagellar motor. Mol Microbiol 19:139–144.
Barak R, Abouhamad WN, Eisenbach M. 1998. Both acetate kinase and acetyl coenzyme A synthetase are involved in acetate-stimulated change in the direction of flagellar rotation in *Escherichia coli*. J Bacteriol 180:985–988.
Barak R, Prasad K, Shainskaya A, Wolfe AJ, Eisenbach M. 2004. Acetylation of the chemotaxis response regulator CheY by acetyl-CoA synthetase from *Escherichia coli*. J Mol Biol 342:383–401.
Barak R, Yan J, Shainskaya A, Eisenbach M. 2006. The chemotaxis response regulator CheY can catalyze its own acetylation. J Mol Biol 359:251–265.
Berg HC. 2003. The rotary motor of bacterial flagella. Annu Rev Biochem 72:19–54.
Berg HC, Brown DA. 1972. Chemotaxis in *Escherichia coli* analysed by three-dimensional tracking. Nature 239:500–504.
Block SM, Segall JE, Berg HC. 1982. Impulse responses in bacterial chemotaxis. Cell 31:215–226.
Böhmer M, Van Q, Weyand I, Hagen V, Beyermann M, Matsumoto M, Hoshi M, Hildebrand E, Kaupp UB. 2005. Ca^{2+} spikes in the flagellum control chemotactic behavior of sperm. EMBO J 24:2741–2752.
Bray D. 2002. Bacterial chemotaxis and the question of gain. Proc Natl Acad Sci USA 99:7–9.
Bren A, Eisenbach M. 2000. How signals are heard during bacterial chemotaxis: Protein-protein interactions in sensory signal propagation. J Bacteriol 182:6865–6873.
Cecchini G, Schroder I, Gunsalus RP, Maklashina E. 2002. Succinate dehydrogenase and fumarate reductase from *Escherichia coli*. Biochim Biophys Acta 1553:140–157.
Cohen-Dayag A, Tur-Kaspa I, Dor J, Mashiach S, Eisenbach M. 1995. Sperm capacitation in humans is transient and correlates with chemotactic responsiveness to follicular factors. Proc Natl Acad Sci USA 92:11039–11043.
Devreotes PN, Zigmond SH. 1988. Chemotaxis in eukaryotic cells: A focus on leukocytes and dictyostelium. Annu Rev Cell Biol 4:649–686.
Eisenbach M. 1999a. Mammalian sperm chemotaxis and its association with capacitation. Dev Genet 25:87–94.
Eisenbach M. 1999b. Sperm chemotaxis. Rev Reprod 4:56–66.
Eisenbach M. 2004. Chemotaxis. London: Imperial College Press. 515 p.
Eisenbach M, Adler J. 1981. Bacterial cell envelopes with functional flagella. J Biol Chem 256:8807–8814.
Eisenbach M, Caplan SR. 1998. Bacterial chemotaxis: Unsolved mystery of the flagellar switch. Curr Biol 8:R444–R446.
Eisenbach M, Giojalas LC. 2006. Sperm guidance in mammals—an unpaved road to the egg. Nat Rev Mol Cell Biol 7:276–285.
Fabro G, Rovasio RA, Civalero S, Frenkel A, Caplan SR, Eisenbach M, Giojalas LC. 2002. Chemotaxis of capacitated rabbit spermatozoa to follicular fluid revealed by a novel directionality-based assay. Biol Reprod 67:1565–1571.
Gakamsky A. 2006. Gradient sensing mechanism and behavioral response of mammalian sperm chemotaxis [Ph.D. thesis]. Rehovot, Israel: Weizmann Institute of Science.
Giojalas LC, Rovasio RA, Fabro G, Gakamsky A, Eisenbach M. 2004. Timing of sperm capacitation appears to be programmed according to egg availability in the female genital tract. Fertil Steril 82:247–249.
Jaiswal BS, Eisenbach M. 2002. Capacitation. In: Hardy DM, editor. Fertilization. San Diego: Academic Press. pp 57–117.
Kaupp UB, Solzin J, Hildebrand E, Brown JE, Helbig A, Hagen V, Beyermann M, Pampaloni F, Weyand I. 2003. The signal flow and motor response controlling chemotaxis of sea urchin sperm. Nat Cell Biol 5:109–117.
Kaupp UB, Hildebrand E, Weyand I. 2006. Sperm chemotaxis in marine invertebrates—molecules and mechanism. J Cell Physiol 208:487–494.
Kentner D, Sourjik V. 2006. Spatial organization of the bacterial chemotaxis system. Curr Opin Microbiol 9:619–624.

Khan S, Castellano F, Spudich JL, McCray JA, Goody RS, Reid GP, Trentham DR. 1993. Excitatory signaling in bacteria probed by caged chemoeffectors. Biophys J 65:2368–2382.
Kojima S, Blair DF. 2004. The bacterial flagellar motor: Structure and function of a complex molecular machine. Int Rev Cytol 233:93–134.
Løvdok L, Kollmann M, Sourjik V. 2007. Co-expression of signaling proteins improves robustness of the bacterial chemotaxis pathway. J Biotechnol 129:173–180.
Macnab RM, Koshland DE. 1972. The gradient-sensing mechanism in bacterial chemotaxis. Proc Natl Acad Sci USA 69:2509–2512.
Marwan W, Schafer W, Oesterhelt D. 1990. Signal transduction in *Halobacterium* depends on fumarate. EMBO J 9:355–362.
Montrone M, Eisenbach M, Oesterhelt D, Marwan W. 1998. Regulation of switching frequency and bias of the bacterial flagellar motor by CheY and fumarate. J Bacteriol 180:3375–3380.
Oliveira RG, Tomasi L, Rovasio RA, Giojalas LC. 1999. Increased velocity and induction of chemotactic response in mouse spermatozoa by follicular and oviductal fluids. J Reprod Fertil 115:23–27.
Parkinson JS, Ames P, Studdert CA. 2005. Collaborative signaling by bacterial chemoreceptors. Curr Opin Microbiol 8:116–121.
Prasad K, Caplan SR, Eisenbach M. 1998. Fumarate modulates bacterial flagellar rotation by lowering the free energy difference between the clockwise and counterclockwise states of the motor. J Mol Biol 280:821–828.
Ralt D, Manor M, Cohen-Dayag A, Tur-Kaspa I, Makler A, Yuli I, Dor J, Blumberg S, Mashiach S, Eisenbach M. 1994. Chemotaxis and chemokinesis of human spermatozoa to follicular factors. Biol Reprod 50:774–785.
Ramakrishnan R, Schuster M, Bourret RB. 1998. Acetylation at Lys-92 enhances signaling by the chemotaxis response regulator protein CheY. Proc Natl Acad Sci USA 95:4918–4923.
Ravid S, Eisenbach M. 1984. Direction of flagellar rotation in bacterial cell envelopes. J Bacteriol 158:222–230 [Erratum: 159 (1984) 1433].
Ravid S, Matsumura P, Eisenbach M. 1986. Restoration of flagellar clockwise rotation in bacterial envelopes by insertion of the chemotaxis protein CheY. Proc Natl Acad Sci USA 83:7157–7161.
Sourjik V. 2004. Receptor clustering and signal processing in *E. coli* chemotaxis. Trends Microbiol 12:569–576.
Spehr M, Gisselmann G, Poplawski A, Riffell JA, Wetzel CH, Zimmer RK, Hatt H. 2003. Identification of a testicular odorant receptor mediating human sperm chemotaxis. Science 299:2054–2058.
Spehr M, Schwane K, Riffell JA, Barbour J, Zimmer RK, Neuhaus EM, Hatt H. 2004. Particulate adenylate cyclase plays a key role in human sperm olfactory receptor-mediated chemotaxis. J Biol Chem 279:40194–40203.
Spehr M, Schwane K, Riffell JA, Zimmer RK, Hatt H. 2006. Odorant receptors and olfactory-like signaling mechanisms in mammalian sperm. Mol Cell Endocrinol 250:128–136.
Suarez SS. 1996. Hyperactivated motility in sperm. J Androl 17:331–335.
Suarez SS. 2002. Gamete transport. In: Hardy DM, editor. Fertilization. San Diego: Academic Press. pp 3–28.
Suarez S, Ho HC. 2003. Hyperactivated motility in sperm. Reprod Domest Anim 38:119–124.
Sun F, Giojalas LC, Rovasio RA, Tur-Kaspa I, Sanchez R, Eisenbach M. 2003. Lack of species-specificity in mammalian sperm chemotaxis. Dev Biol 255:423–427.
Sun F, Bahat A, Gakamsky A, Girsh E, Katz N, Giojalas LC, Tur-Kaspa I, Eisenbach M. 2005. Human sperm chemotaxis: Both the oocyte and its surrounding cumulus cells secrete sperm chemoattractants. Hum Reprod 20:761–767.
Teves ME, Barbano F, Guidobaldi HA, Sanchez R, Miska W, Giojalas LC. 2006. Progesterone at the picomolar range is a chemoattractant for mammalian spermatozoa. Fertil Steril 86:745–749.
Turner L, Ryu WS, Berg HC. 2000. Real-time imaging of fluorescent flagellar filaments. J Bacteriol 182:2793–2801.
Webre DJ, Wolanin PM, Stock JB. 2004. Modulated receptor interactions in bacterial transmembrane signaling. Trends Cell Biol 14:478–482.