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GUEST COMMENTARIES

An Alternative Strategy for Adaptation in Bacterial Behavior

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In sensory systems, adaptation to incoming stimuli allows the receptor to compare the present state with the recent past and prepare the cells to respond to future changes. This record, or memory, is continually reset to optimize detection of small changes in the organism's environment. A graphic example is canine olfaction. German shepherd tracking dogs are able to distinguish between footprints laid in grass 1 s apart 20 min earlier (22). Wrong-way travel is immediately corrected by an about-face. Such ability can only be understood in light of adaptation. Rapid adaptation dulls sensing to lower concentrations and allows the full bouquet of odors to be sensed only when the canine is traveling up the concentration gradient. Bacterial chemotaxis receptors exhibit perfect adaptation, whereby the steady-state behavior is constant in a homogenous solution regardless of attractant concentration (4, 10, 25). Thus, coliform bacteria that swim into a serine-rich *Escherichia coli* heaven will soon adapt and exhibit a random bias, enabling them to respond to the depletion of oxygen by the crowd of bacteria attracted to the serine. The bacteria will swim away from a hypoxic microenvironment even when they are leaving a nutrient-rich site (18). They do this to maintain efficient energy production via aerobic metabolism. The discovery that adaptation in bacterial chemotaxis resulted from methylation of the receptor was a landmark finding because it represented the first elucidation of a molecular mechanism for adaptation and memory by a sensory system (9, 16, 17). (The discovery also gave some neurophysiologists apoplexy because they believed that a nervous system was needed for adaptation and they could not conceive of a complete sensory system in a single bacterium.) This is the context for an important paper in this issue by Bibikov, Miller, Gosink, and Parkinson entitled "Methylation-Independent Aerotaxis Mediated by the *Escherichia coli* Aer Protein" (5).

The Aer protein is a receptor-transducer in *E. coli* that senses oxygen and energy by monitoring the redox state of the electron transport system (7, 13). The C-terminal half of the Aer protein resembles the signaling domains of the chemotaxis receptors Tsr, Tar, Trg, and Tap, which are also known as methyl-accepting chemotaxis proteins (MCPs) (Fig. 1). The signaling domain of the MCP includes up to five "methylation sites" that are reversibly methylated and demethylated by the CheR and CheB proteins, respectively, to effect adaptation and a highly conserved domain that is the signal output site of the receptor (12, 15, 21). The output domain interacts with the CheW coupling protein and the CheA histidine kinase, thereby regulating the rate of autophosphorylation of CheA (8). Phosphorylation of the CheY protein by CheA-P is the signal that controls the direction of rotation of the flagellar motors. The sensing domain of the Aer receptor is in the cytoplasm, whereas the sensing domain of an MCP is in the periplasm, where it senses the external environment (Fig. 1). An earlier study, conducted before the discovery of Aer, demonstrated a methylation-independent aerotaxis response (11). An *E. coli* strain lacking the four methylation-dependent chemotaxis proteins Tsr, Tar, Trg, and Tap displayed aerotactic behavior, including adaptation. Adaptation in aerotaxis was not impaired in methylation-deficient cells, whereas all methylation-dependent adaptation was impaired. However, because the signaling domain of Aer is similar to those of the MCPs and because CheA and CheW are required for signaling by Aer, it was still possible that Aer-mediated aerotaxis would be dependent on methylation for adaptation. The paper by Bibikov et al. (5) demonstrates convincingly that Aer-mediated aerotaxis is independent of methylation. (i) The Aer protein migrated as a single band in gel electrophoresis and was not detectably methylated or demethylated by the CheR and CheB proteins. (ii) Aer promoted aerotactic migrations in semisolid agar in strains that lacked all four of the *E. coli* MCPs. CheR and CheB had no influence on the rate of aerotactic movements in those strains. (iii) The Aer methylation sites are unorthodox and do not conform to the consensus motif for CheR or CheB substrates. Amino acid mutations in critical residues at the putative Aer methylation sites generally had no deleterious effects on Aer function. In one sense, the finding that Aer-mediated aerotactic responses are methylation independent is surprising because the Aer signaling domain appears to have a common origin with that of the MCPs, but in another sense, this finding is not surprising because the biological universe is remarkably pliable and utilizes diverse strategies to achieve similar outcomes. Across the subset of bacterial species that have been studied, chemotactic mechanisms illustrate the pliable nature of living systems.

The accumulation of bacteria at the surface of pepper water observed by van Leeuwenhoek in 1676 was the first report of aerotaxis (3). Experimental investigation of aerotaxis began with Englemann and Beijerinck at the end of the nineteenth century, including the finding that motile bacterial species navigate to a concentration of oxygen that is optimal for the metabolic lifestyle of the species (3, 20, 23). Modern explora-

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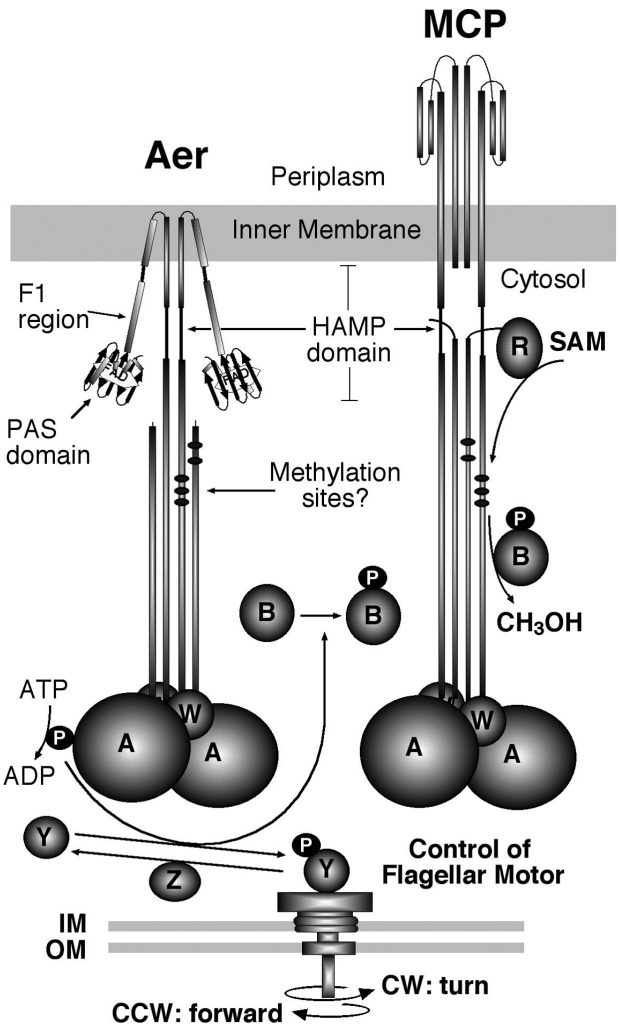

FIG. 1. Cartoon representing the *E. coli* chemotaxis pathway, including the question of Aer methylation addressed by Bibikov et al. (5). The *E. coli* MCPs Tsr, Tar, Trg, and Tap bind periplasmic ligands, whereas Aer-FAD senses changes in the redox state of the electron transport system. Repellents activate autophosphorylation of CheA, which transphosphorylates CheY, causing turns and tumbling. Attractants inhibit the cascade and prevent tumbling. MCPs containing the C-terminal pentapeptide NWEFT (Tsr and Tar) bind the CheR methyltransferase as well as the CheB methylesterase. CheR sensitizes MCP receptors by methylating glutamyl residues on attractant-inactivated receptors. CheB desensitizes receptors after being activated to the phosphorylated form by phospho-CheA. Candidate residues for Aer methylation are shown as filled ovals. Abbreviations: A, W, Y, Z, B, and R, Che proteins; SAM, *S*-adenosylmethionine; IM, inner membrane; OM, outer membrane; CW, clockwise; CCW, counterclockwise.

tion of the molecular mechanism of aerotaxis commenced with the development of new assays for aerotaxis, including a temporal assay in which oxygen gradient formation is independent of respiration (18). Bacterial chemotaxis is not dependent on metabolism of the chemoattractant molecule (1). Conversely, aerotaxis is a response to the change in respiratory electron transport elicited by a change in the concentration of oxygen at the terminal oxidase (20). In *E. coli*, the transducers for aerotaxis, Aer and the serine chemoreceptor Tsr, do not monitor oxygen concentration directly but monitor changes in electron transport. The signaling pathway by which Aer and Tsr receive information on the state of the electron transport system has not been determined. Most of the current investigations of aerotaxis, such as those of Bibikov et al. (5), focus on signaling within the Aer protein.

Having excluded the possibility that Aer methylation had a role in aerotaxis, Bibikov et al. were able to address the implications for signal transduction in Aer. A chimeric aerotaxis receptor, Aear, was constructed, and this receptor included the PAS and HAMP domains (residues 1 to 269) from Aer and the C-terminal signaling domain and methylation sites from the aspartate chemoreceptor, Tar, which is an orthodox MCP (5). Aear exhibited both methylation-dependent and methylation-independent aerotactic behavior. A surprising conclusion based on this finding is that methylation-independent adaptation does not emanate from the Aer signaling domain. This conclusion suggests that adaptation in Aer involves the PAS and HAMP domains (Fig. 1). Another possibility raised by Bibikov et al. is 'adaptation' of the redox signal itself. That is, the cellular redox changes that trigger Aer-promoted aerotactic responses may be transient and quickly return to prestimulus levels, ending the aerotaxis response. However, there is as yet no evidence to support such a model. Although Bibikov et al. were able to infer bacterial behavior or bias by observing the spreading of bacteria in semisolid agar, it would be reassuring to have real-time observations of the behavior of individual cells, including bias of the flagellar motors.

The sensing domain of Aer is the PAS domain, which has a noncovalently bound flavin adenine dinucleotide (FAD) cofactor (6, 14) (Fig. 1). A superfamily of PAS domains has been identified in sensory proteins from bacteria to humans, including histidine and serine/threonine kinases, chemoreceptors and photoreceptors for taxis and tropism, circadian clock proteins, voltage-activated ion channels, cyclic nucleotide phosphodiesterases, and proteins involved in regulation of responses to hypoxia (19). PAS domains invariably sense a cell's internal environment rather than its external environment. Although it appears likely that sensing by the Aer PAS domain involves oxidation-reduction of FAD, the source of the redox changes is unknown. Aer could interact with a component of the electron transport system, for example, or Aer-bound FAD could exchange with a pool of free FAD/FADH that mirrors the respiratory redox state. The advantage of the Aer PAS domain sensing redox status and not oxygen per se is that respiration and energy levels (proton motive force) are intertwined and that *E. coli* is able to escape not only hypoxia but also a variety of other situations that threaten cellular energy levels (20). A newly recognized signaling domain in Aer is the HAMP domain that links the membrane sequence to the signaling domain (Fig. 1). HAMP domains are also found in histidine kinases, adenylyl cyclases, MCPs, and phosphatases (2). A signal transduction role for HAMP domains is indicated by genetic analysis of receptors in which HAMP mutations bias the signal transmitted by the protein (24). Additional mutations that prevent FAD binding to the PAS domain have been identified in the Aer HAMP domain (6, 14). This finding suggests that contact between the HAMP and PAS domains may stabilize a conformation of the PAS domain that binds FAD. If so, the sensory signal that originates in the PAS domain could be transmitted to the signaling domain across the contact in-

terface between the PAS and HAMP domains. The HAMP domain is contiguous with the signaling domain. The Aear chimera constructed by Bibikov et al. (5) has promise for in vitro and in vivo studies of aerotaxis signal transduction. *E. coli* cells in which Aer is the only chemotaxis transducer have a smooth swimming bias unless Aer is heavily overexpressed. Bibikov et al. propose that when Aer is replaced by Aear-269, the bias is reset for normal tumbling frequency by methylation of the Aear signaling domain. However, that finding is inferred and definitive information on Aear adaptation must await quantitative analysis of the behavior of individual bacteria. Nevertheless, the Aear chimera holds great promise for the future investigation of Aer signal transduction and may prove to be a most important contribution of the study of Bibikov et al.

The analysis of the Aer protein has clearly established that an MCP-like receptor can utilize methylation-independent adaptation to stimuli. How important are the Aer-type behaviors in the microbial world? Bioinformatic analysis of bacterial genomes revealed that only a minority of bacterial species have aerotaxis receptors that resemble Aer. Other species utilize different strategies for aerotaxis, and many of the aerotaxis strategies remain to be identified (I. B. Zhulin, personal communication). Earlier speculation that Aer might be a pro-MCP is now considered to be wrong because the in silico analysis indicated that it is more likely that the Aer signaling domain originated from an MCP and subsequently lost its methylation capabilities (I. B. Zhulin, personal communication). An aerotaxis response is universal in motile bacteria. It is not one of the strong responses, and bacteria probably are guided mostly by chemotaxis receptors in the search for nutrients, but in a microenvironment that stresses bacterial energy levels, aerotaxis and energy taxis behaviors dominate over strong chemotactic responses to initiate an emergency response (20). As a result, *E. coli* is not trapped in a serine gradient by hypoxia. The Aer-mediated responses may be viewed as avoidance responses that have a role in bacteria similar to that of the "fright and flight" responses mediated by adrenalin in humans.

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