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Forum Review

The FAD-PAS Domain as a Sensor for Behavioral Responses in *Escherichia coli*

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ABSTRACT

Aer, the aerotaxis receptor in *Escherichia coli*, is a member of a novel class of flavoproteins that act as redox sensors. The internal energy of the cell is coupled to the redox state of the electron transport system, and this status is sensed by Aer(FAD). This is a more versatile sensory response system than if *E. coli* sensed oxygen *per se*. Energy-depleting conditions that decrease electron transport also alter the redox state of the electron transport system. Aer responds by sending a signal to the flagellar motor to change direction. The output of other sensory systems that utilize redox sensors is more commonly transcriptional regulation than a behavioral response. Analysis *in silico* showed Aer to be part of a superfamily of PAS domain proteins that sense the intracellular environment. In Aer, FAD binds to the PAS domain. By using site-specific mutagenesis, residues critical for FAD binding and sensory transduction were identified in the PAS domain. The PAS domain appears to interact with a linker region in the C-terminus. The linker region is a member of a HAMP domain family, which has signal transduction roles in other systems. Antioxid. Redox Signal. 3, 867–879.

INTRODUCTION

FLAVINS function primarily as cofactors for enzyme-catalyzed oxidation–reduction reactions and electron transport. A novel role of flavins as sensory transducers that sense changes in the cellular environment was demonstrated recently. The sensory flavoproteins do not catalyze an enzyme reaction and cannot be classified by the Enzyme Nomenclature of the International Union of Biochemistry (29). Our research investigates the sensory role of flavins that are associated with a specific protein domain, the PAS domain (64). There are presently three known PAS domains that bind flavins: NifL, phototropin (Nph1), and Aer. Of these, NifL and Aer are members of a class of flavoprotein redox sensors, whereas phototropin represents a class of flavoprotein light sensors.

NifL is a flavin adenine dinucleotide (FAD)-containing sensor protein from *Azotobacter vinelandii* and *Klebsiella pneumoniae* that regulates NifA, an enhancer binding protein that controls expression of nitrogen fixation genes (27, 52). Phototropin is a blue-light phototropic receptor that is reviewed elsewhere (16, 51). The flavin mononucleotide (FMN) chromophore in phototropin is the blue-light sensor that controls plant bending toward light. Aer is a unique member of the redox sensor family (63, 64). It serves as the detector for aero-

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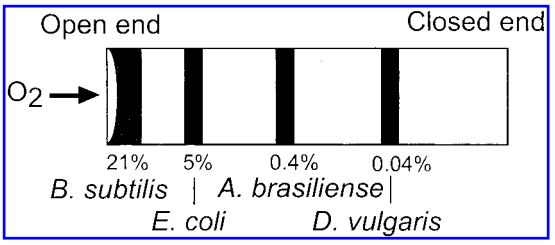

FIG. 1. Species-specific aerotaxis in an oxygen gradient. Bacterial strains in a capillary tube focus as individual bands at their preferred oxygen concentration.

taxis behavior, an early warning defense against hypoxia. Aerotaxis enables *Escherichia coli* to actively swim away from the hypoxic region, rather than waiting passively for oxygen to reach the cell by diffusion. Aer does not sense oxygen directly, but senses the decrease in internal energy that results from hypoxia (63). This review focuses on the signal transduction role of the FAD-PAS domain in sensing oxygen and redox potential in the aerotaxis transducer, Aer.

Aerotaxis enables bacteria to navigate in a spatial gradient of oxygen to a specific oxygen concentration that is optimal for energy production (35, 57, 58, 60). This can be demonstrated by placing a mixture of bacterial species in a glass capillary tube and sealing one end. Each species migrates in the gradient to the optimal oxygen concentration for the metabolic lifestyle of that species (Fig. 1). Motile anaerobes migrate away from the oxygen source to the anoxic zone (4). The obligate aerobe *Bacillus subtilis* migrates to the air–liquid interface (69). The facultative anaerobe *E. coli* bands near the meniscus (~5% oxygen) (unpublished observation), microaerophilic *Azospirillum brasiliense* migrates farther away from the meniscus (0.4% oxygen) (72), and *Desulfovibrio vulgaris*, which was previously classified as an "obligate" anaerobe, migrates to a hypoxic environment (0.04% oxygen) (32).

HISTORICAL PERSPECTIVE

Aerotaxis by bacteria has been documented for over 100 years (4, 7, 17, 19, 46). Modern studies of the molecular mechanism of aerotaxis arose out of studies of chemotaxis in *E. coli* and *Salmonella enterica* serovar Typhimurium. Aerotaxis requires an operational electron transport system (65). Environmental conditions that alter the flow of electrons through the electron transport system elicit a behavioral response (61, 66) (Fig. 2). Inhibitors of the electron transport system are repellents of *E. coli* and *S.* Typhimurium. In anoxic cells, alternative respiratory electron acceptors, such as nitrate or fumarate, can stimulate electron transport and elicit an attractant behavioral response (36, 56). Factors that change the membrane proton motive force also elicited behavioral responses (56).

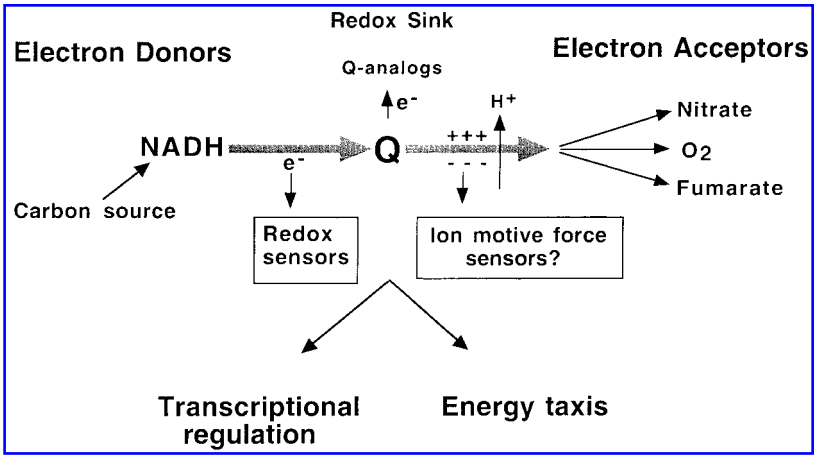

FIG. 2. Role of the electron transport system in sensing internal energy. Energy generated by the electron transport system can be sensed by redox sensors (Aer) or, in theory, ion motive force sensors. Adapted with permission, from the *Annual Review of Microbiology*, Volume 53, ©1999, by Annual Reviews *www.AnnualReviews.org* (66).

STRATEGIES FOR ENERGY SENSING

Proton motive force, electron transport, the redox states of respiratory complexes, and the ATP/(ADP + P_i) ratio are closely coupled. A bacterium could sense internal energy changes by monitoring any of these components. The proton motive force is energized to −200 mV within 5–10 ms after electron transport commences (25). Changes in ATP, on the other hand, are protracted, taking minutes, even in the presence of respiratory inhibitors (31). Hence, by monitoring changes in the redox state of electron transport system components, Aer efficiently gauges the status of energy production. Although the response time for aerotactic excitation has not been quantified, the excitation time in chemotaxis from ligand binding to the flagellar motor output is ~200 ms (30, 53), and it is likely that aerotaxis signal transduction is within this order of magnitude. As the collective aerotaxis and other electron-transport- or proton motive force-mediated behaviors are responses to the change in internal energy in *E. coli*, they were named energy taxis (63). The proposed common mechanism for energy taxis was verified with the discovery that the Aer protein is a common sensor for these behaviors (9, 48). The serine chemoreceptor, Tsr, is also an independent energy sensor for energy taxis (48).

REDOX TAXIS

Of particular interest to those who investigate oxidation–reduction reactions is the finding that bacteria in a spatial redox gradient accumulate in a sharply defined band at a specific reduction potential (8, 24). The redox response by *E. coli* occurred only in gradients of permeant redox molecules that cross the cytoplasmic membrane and interrupt electron flow by diverting electrons from the electron transport system. This results in decreased proton motive force and elicits a negative (repellent) taxis response (8). Redox taxis, like aerotaxis, can be mediated by either the Aer or Tsr protein, which monitor the redox state of the electron transport system.

BIOLOGICAL SIGNIFICANCE AND PREVALENCE OF REDOX/ENERGY TAXIS

It is advantageous for motile bacteria to have both energy taxis and chemotaxis. Bacteria respond to chemotactic stimuli and cluster around a food source at densities up to 10^9 cells/ml (60). At this high density, the bacteria will rapidly lower the oxygen level to where ATP generation is primarily via glycolysis (60). Bacteria sense a drop in intracellular energy when oxygen becomes rate-limiting for respiration (63, 66). Energy taxis then overrides chemotaxis, and the bacteria swim out of the hypoxic zone even though they are moving away from chemoattractants. This energy taxis response is akin to the "fight or flight" responses mediated by epinephrine in humans. However, if the bacteria relied solely on energy taxis, they would be attracted primarily to sugars and organic acids, and become deprived of nitrogen sources (63). Hence, when oxygen is not limiting, chemotaxis appears to be the primary determinant of bacterial behavior.

Energy taxis is often important in determining the distribution of the bacteria in the ecosystem. Magnetotactic bacteria orient and navigate by following the Earth's geomagnetic lines (11, 12). This aligns the bacteria to swim downward along the inclined geomagnetic field and away from the highly oxygenated water surface. When the preferred oxygen concentration is reached, aerotaxis reverses the direction of migration and maintains the bacteria in a band or veil at the preferred oxygen concentration (20). Energy taxis is also pivotal in the plant–microbe interactions in the rhizosphere. Aerotaxis apparently guides *Azospirillum* to regions of the root where the oxygen concentration (0.4%) is low enough to avoid oxygen inactivation of the nitrogen fixation complex (70).

ENERGY SENSORS FOR TRANSCRIPTIONAL CONTROL

Sensors such as ArcB or Fnr act at the level of transcriptional control to prepare the cell for survival in a hypoxic environment (6). Such responses are relatively slow compared with

aerotaxis. Of the pathways that are regulated by oxygen, only a few are known to sense diatomic oxygen. FixL, a histidine kinase that controls nitrogen fixation in *Sinorhizobium meliloti* and the Dos protein in *E. coli*, binds oxygen directly via a heme cofactor (18, 22). The SoxR protein in *E. coli* is thought to sense superoxide and nitric oxide radicals via an iron sulfur cofactor (26). A majority of pathways affected by oxygen likely sense the metabolic or energy change that results from an oxygen increase or decrease.

TRANSDUCERS FOR AEROTAXIS, REDOX, AND ENERGY TAXIS

The aerotaxis transducer gene was named *aer* (accession no. P50466) for aerotaxis and energy responses (9, 48). Aer is a flavoprotein that noncovalently binds one FAD per monomer (9, 49). Oxidation and reduction of FAD are thought to generate the "on" and "off" signals for aerotaxis. The FAD-bound PAS domain of Aer is postulated to interact with a component in the electron transport system and reflect the redox status of the electron transport system. A conformational change generated in the PAS domain by oxidation or reduction of FAD is transmitted to the highly conserved signaling domain in the C-terminus of Aer.

Since the discovery of Aer in *E. coli*, several apparent homologs have been identified in closely related γ-proteobacteria, including *S.* Typhimurium *S. typhi*, *Vibrio cholerae*, *Yersinia pestis*, and *Pseudomonas putida*, as well as in γ-proteobacterial species, *Bordetella pertussis* (10, 40) (Q. Ma, B. Taylor, and I. Zhulin, unpublished observation). Aer-like transducers have also been identified in *Agrobacterium tumefaciens* (γ-proteobacteria) and in the archaebacterial species, *Archaeoglobus fulgidis* and *Halobacterium salinarum*. Recently, a new class of myoglobin-like aerotaxis transducers represented by the HemAT proteins of *H. salinarum* and *B. subtilis* was discovered (28).

AER STRUCTURE AND TOPOLOGY

Aer is a 55-kDa protein that has an N-terminal PAS domain that senses redox changes

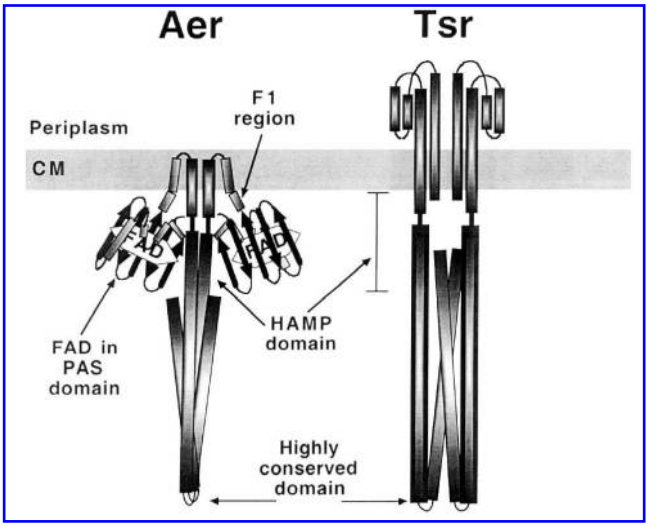

FIG. 3. Predicted topology of the Aer and Tsr proteins. The structure of Tsr is the result of crystallographic analysis and modelling (34). The putative structure of Aer incorporates the resolved structure of the photoactive yellow protein PAS domain (45) and Tsr. The PAS domain and F1 region are not drawn to scale. The transmembrane fold is a prediction. CM: cytoplasmic membrane.

(residues 1–119), a central transmembrane domain (residues 167–204), a linker/HAMP domain (residues 205–265), and a C-terminal signaling domain (residues 266–506) (Fig. 3). The predicted topology of Aer indicates that the protein is anchored in the membrane by its central hydrophobic sequence, whereas the N- and C-termini are localized in the cytoplasm (10, 48). In contrast to other bacterial chemoreceptors, Aer does not have an extended periplasmic domain for ligand binding. Several lines of evidence corroborate our predictions for Aer topology. Cytoplasmic determinants known to maintain the correct orientation of the transmembrane domain in bacterial chemoreceptors (54) are found in Aer; the hydrophobic sequence is flanked by three positively charged residues and an amphiphatic sequence at its N- and C-termini, respectively. PAS domains in all analyzed proteins are predicted to localize in the cytoplasm (64). The cytoplasmic location of PAS domains also supports their role in sensing the intracellular environment. In all membrane bound PAS proteins, PAS domains are located adjacent to the transmembrane region, leaving open the possibility that PAS domains can interact with domains of other membrane-bound proteins.

The C-terminal domain of Aer includes a se-

quence that is similar to the highly conserved signaling domains in other bacterial chemoreceptors. It is at this domain that the transduction pathways of aerotaxis and chemotaxis converge. The highly conserved domain transmits a signal to the flagellar motor via a two-component signaling circuit that involves CheW, CheA, and CheY (14, 41, 59). As aerotaxis is dependent on CheA, CheW, and CheY, the C-terminus of Aer, like the C-terminus of chemotaxis receptors, must be located in the cytoplasm (50). The hydrophobic sequence in Aer is proposed to form a hairpin loop that anchors the two cytoplasmic domains of Aer to the membrane (Fig. 3).

The HAMP domain (residues 205–265) in both Aer and chemotaxis receptors connects the C-terminal signaling domain to a transmembrane helix. The HAMP domain, also designated the F_2 domain (10), was previously considered to be a mechanical linker that connected the periplasmic and cytoplasmic domains of chemotaxis receptors (15, 42). Bioinformatic analysis in several laboratories recently determined that the "linker" domain is a widely distributed sensory transduction domain (3, 68) (Q. Ma, B. Taylor and I. Zhulin, unpublished observation). The HAMP domain is named for histidine kinases, adenylyl cyclases, methyl-accepting chemotaxis proteins, and phosphatases in which the domain is present (3). Point mutations in the HAMP domain of a chemoreceptor (Tsr) or osmosensor (EnvZ) bias the receptor output to increased clockwise or counterclockwise rotation of the motor, or lock the receptor so that it only signals either clockwise or counterclockwise rotation (1, 43). The HAMP domain is made up of two amphiphilic α-helices and between them an ordered region of undetermined secondary structure. Each of the two α-helices has a buried packing face. The carboxyl helix of HAMP domains has a coiled-coil motif that is often associated with dimerization or other protein–protein interaction (15). Together, these findings suggest a role of the HAMP domain in signal transduction through protein–protein interactions.

Aer contains three cysteines at residues 193, 203, and 253. Two of these, Cys^{203} and Cys^{193}, are in the putative transmembrane domain. Cys^{253} is in the HAMP domain. Cys^{203} crosslinks rapidly *in vivo* with the respective $Cys^{203\prime}$ from a neighboring Aer monomer after the addition of Cu^{2+} phenanthrolene (F. Roy, Q. Ma, and M. Johnson, unpublished data). This indicates that Aer is dimeric, and Cys^{203} and $Cys^{203\prime}$ residues are contiguous. Cys^{193} and $Cys^{193\prime}$ do not readily crosslink, indicating that these residues are not proximal. Truncated Aer proteins that do not bind FAD can dimerize, indicating that FAD binding is not required for dimerization. The unique sulfhydryl chemistry of cysteine residues often contributes to the redox sensing properties of proteins (55). However, replacement of the three cysteine residues in Aer did not abolish aerotaxis, indicating that the cysteines in Aer are not involved in redox sensing (10).

FAD COFACTOR AND SIGNALING

His_{6x}-tagged Aer protein can be extracted from membranes by using nonionic detergents and purified on a nickel or cobalt column (10, 49). When the Aer protein is denatured in urea, ~1 mole of FAD dissociates per mole of Aer monomer. No labile or covalently bound FMN, riboflavin, or heme cofactors were detected. The FAD in Aer is labile and easily removed. It remains to be determined whether this is due to the presence of detergent or is an intrinsic property of the native protein.

There is some evidence that suggests that the Aer protein might bind FAD more tightly *in vivo*. When Aer is overproduced in *E. coli*, there is a corresponding increase in membrane-bound FAD (9). Quantitative measurements were made of *E. coli* cells. In wild-type cells, there were ~300 copies (F. Roy, M. Johnson, and B. Taylor, unpublished data) of the membrane-bound Aer protein per cell and 2,500 molecules of FAD (49). After overexpression of Aer, there was a stoichiometric increase in membrane bound FAD to 20,000 molecules per cell. This suggests that FAD remains bound to Aer in the membrane during cell fractionation and washing of the membrane. The relationship between Aer and membrane-bound FAD is at this time the most reliable measurement of FAD binding to mutated Aer proteins (49).

The FAD-PAS domain was demonstrated to be the sensory input domain of the Aer protein

by constructing a chimera (Aesr) that fused the N-terminal PAS domain of Aer to the C-terminus of the serine chemoreceptor, Tsr (10, 49). The Tsr protein has a serine-binding N-terminal domain in the periplasm and a signaling C-terminal domain in the cytoplasm. The transmembrane sequence of Aer is incorporated into Aesr, and it is expected that the topology of Aesr is similar to the topology of Aer. The Aesr chimera had the sensory specificity of the Aer protein and restored aerotaxis when expressed in an *aer tsr* double mutant (49). Site-directed mutagenesis of Aer identified critical residues for FAD binding and sensing in the PAS domain.

STRUCTURE AND FUNCTION OF PAS DOMAINS

The N-terminal domain of Aer is a member of the PAS domain superfamily (47, 73). More than 450 PAS domains have been identified in proteins from all three kingdoms of life: Bacteria, Archaea, and Eucarya (64) (I. Zhulin, personal communication). PAS domains are found predominantly in sensory proteins and, in prokaryotes, are involved in sensing energy-related parameters such as light, oxygen, voltage, and redox (64, 71, 73). There is a positive correlation between the total number of PAS domains in a microbial genome and the number of structural genes for photosynthetic and electron transport proteins (71). PAS is an acronym formed from the names of three proteins (Per, Arnt, and Sim) in which PAS domains were first identified.

Adaptation of the PAS domain structure to sense diverse stimuli such as oxygen, ligands, light, and redox potential is present in the simplest prokaryotes, and evidence is emerging that divergent PAS domains in a single protein may be functionally differentiated to sense different stimuli. One way in which PAS domains sense different stimuli is by incorporating different cofactors into a pocket in the PAS fold. For example, photoactive yellow protein (PYP) is a photoreceptor in which photons are captured by the 4-hydroxycinnamyl chromophore in the PAS domain (13); FixL is an oxygen receptor in which oxygen binds directly to a heme in the PAS domain (23); and Aer and NifL are believed to sense redox changes via the FAD moiety in the PAS domains (10, 27, 49, 52).

Unlike most other sensor modules, PAS domains are located in the cytoplasm (64). They sense the internal environment of the cell rather than the external millieu that is sensed by other sensory input domains. As discussed above, the Aer and NifL proteins sense the internal energy of the bacteria in which they are found. Although investigations of these proteins provided the first conclusive evidence of an intracellular energy sensor, it is likely that PAS domains have an energy-sensing role in many different types of cells. This is supported by the cytoplasmic location of PAS domains and the broad distribution of PAS domains in living cells where they are most frequently associated with sensors of redox, oxygen, voltage, or light (64).

The crystal structures of three PAS domains have been resolved. They include two bacterial proteins, PYP (13) and FixL (23), and the human ether-a-go-go related gene (HERG) potassium channel (39). The overall fold resembles a left-handed glove in which the fingers enclose a pocket. The fingers of the glove are formed primarily from a β barrel, the palm from α-helical loops, and the thumb from β sheets, as described for FixL (23). The individual PAS structures can be overlaid with minimal deviation of the backbone. Most notable differences occur in the EF loop and the F-helix that form a boundary to the pocket. This unique structure of PAS domains apparently has an important function beyond the sequence of amino acids that are present. Profilin, the mammalian SH2 domain, and the PAS domain in PYP have strikingly similar three-dimensional structures, but they do not share sequence homology (13).

Although PAS domains participate in a wide range of sensory response systems and sense diverse stimuli, little is known at this time about the molecular mechanism of their role in signal transduction. Resolution of structure on a millisecond time scale identified a critical rotation of the carbonyl group of the thioester linkage to the chromophore as an intermediate in the photo response of PYP (21). However, no downstream components of a PYP sensory pathway have been identified, and it is unknown how the activated PAS domain trans-

mits a signal in *Ectothiorhodospira* bacteria. Investigations of the FAD-PAS domain in the Aer and NifL proteins, and the FMN-PAS domain in phototropin, have promise of elucidating the molecular mechanism of signal transduction in PAS domains in response systems where the transduction pathway is well defined.

MECHANISM OF AER SIGNALING

The signal for aerotaxis originates when oxygen is reduced by the terminal oxidase of the electron transport system. The identity of the precise signal is not known, but the signal is transmitted to the FAD-PAS domain of Aer (10, 49). The reduction potential of FAD in Aer is predicted to be in a range that would allow electron exchange with ubiquinone/menaquinone via the electron transport system. Candidates for interaction with the FAD-PAS domain of Aer include protein subunits of electron transport complexes, as well as diffusible cofactors such as ubiquinone or menaquinone. Aer could be in equilibrium with a respiratory component, or could form a loop with the electron transport system in which Aer is reduced by one respiratory electron donor and oxidized by a downstream electron acceptor in the electron transport system. If there is a loop, it is doubtful that Aer, with only 300 copies per cell, can substantially alter the rate of electrons through the electron transport chain by diverting reducing equivalents. In contrast, there are 1,500–3,000 copies of the ATP synthase per cell (38). As the binding of FAD to the Aer protein is labile in isolated Aer protein, the possibility cannot be excluded that there is a dynamic interaction between Aer and cytosolic FAD (10).

It is likely that oxidation/reduction of FAD triggers a conformational change that switches the Aer PAS domain from an inactive to an active form. Under conditions of limiting oxygen, it is expected that the FAD in Aer would be fully reduced ($FADH_2$), and this would elicit a repellent (tumbling) response. Oxidation to FAD would then generate an attractant (smooth) response. However, the simplest two-state model does not account for data showing a smooth response when the electron donor glycerol is added to cells that have been starved for a carbon source, nor does it account for the tumbling redox taxis response to quinone oxidants (8). In the two-state model, glycerol should increase the reduction of the electron transport system and of Aer(FAD), generating a tumbling response. Quinones should increase the oxidation of the transport system and of Aer($FADH_2$), generating a smooth response (Fig. 2). A three-state model is consistent with the data whereby the fully oxidized (FAD) and reduced ($FADH_2$) states of Aer signal a tumbling response and the semiquinone form of Aer($FADH^{\bullet}$) generates a smooth response (49). In this model, a fully functional electron transport chain maintains the cofactor in the semiquinone form and Aer in a nonsignaling (smooth) conformation, whereas an impaired, non–energy-producing electron transport chain that is deficient in a source of reducing equivalents (*e.g.*, NADH) or electron acceptors would fully oxidize or fully reduce the FAD in Aer and generate a signaling (tumbling) conformation in Aer. This model is consistent with the available data at this time, but direct demonstration of the effects of FAD oxidation and reduction are essential before such a model is proven.

The signal from the N-terminal PAS domain of Aer is transmitted to the C-terminal highly conserved domain. The highly conserved domains of Aer and bacterial chemoreceptors, in conjunction with the CheW docking protein, form a ternary complex with the input module of the CheA sensor kinase (2, 37, 44). CheA is autophosphorylated by ATP at a conserved histidine residue, and the highly conserved domain of the receptor modulates the autophosphorylation rate in the ternary complex (Fig. 4). The phosphoryl group is rapidly transferred from the CheA transmitter module to an aspartate residue in the receiver domain of the response regulator CheY. Phosphorylated CheY binds to the FliM switch protein on the flagellar motors to change the direction of flagellar rotation (5, 67). The end result of oxidation or reduction of FAD in the Aer PAS domain is to decrease or increase, respectively, the concentration of phosphorylated CheY, and thereby to control the direction of rotation of the flagellar motors.

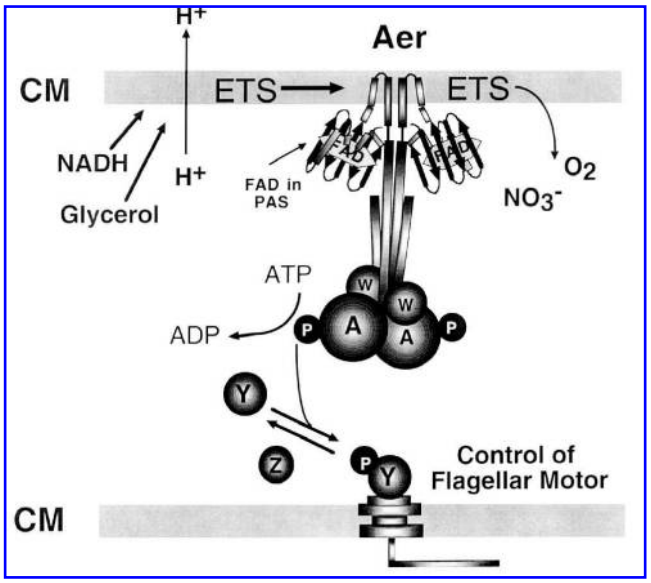

FIG. 4. Signal transduction sequence for the control of flagellar rotation in aerotaxis in *E. coli*. A decrease in electron transport is detected by the FAD-PAS domain of Aer. The signal transmitted to the c-terminal signaling domain of Aer activates autophosphorylation of CheA, followed by transphosphorylation to the CheY protein. Phospho-CheY binds to the "switch" on the flagellar motor, causing counterclockwise rotation and tumbling. *Abbreviations*: A, W, Y, Z: Che A, CheW, CheY and CheZ proteins, respectively; CM: cytoplasmic membrane; ETS: electron transport system.

PAS RESIDUES THAT ARE CRITICAL FOR SIGNALING

To explore how the redox state of the FAD cofactor might change the conformation of the PAS domain and generate a signal, we serially replaced 42 residues in the PAS domain with cysteine (49). Both conserved and nonconserved residues were mutated. Conserved residues are likely to be critical for the scaffolding that gives PAS domains their characteristic $\alpha\beta$-fold. Residues that are essential for FAD binding and the signaling function of Aer are more likely to be residues that are not conserved in all PAS domains.

Mutations at Gly^{42}, Arg^{57}, His^{58}, Asp^{60}, Asp^{68}, Trp^{79}, and Gly^{90} eliminated all responses to changes in oxygen or redox, and the Glu^{47} mutation eliminated >90% of the response to an oxygen increase (49). Five of these mutations are in conserved residues. Four of the null mutations, replacement of Arg^{57}, His^{58}, Asp^{60} and Asp^{68}, also led to the loss of FAD binding (49). Residues Arg^{57}, His^{58}, and Asp^{60} are variable residues in and near the putative EF loop (Fig. 5). Other mutant Aer proteins bound FAD with varying affinities. Further mutagenesis studies on the putative FAD-binding amino acids showed that Arg^{57} could be replaced by a lysine, and Asp^{60} could be replaced by glutamic acid without losing function. This suggests that charge is important at these two residues (49).

Mutation in Asn^{34}, Phe^{66}, and Asn^{85} transmitted a constant signal-on (tumbling) bias (49). Asn^{34} and Phe^{66} are conserved residues, and Asn^{85} is a variable residue. Replacement of Tyr^{111} inverted signaling by the Aer transducer so that positive stimuli produced negative signals and *vice versa* (49). It is clear from these results that cofactor binding and the active site for signaling are centered around the EF loop and the PAS core region (Fig. 5). This is similar to the PAS domain in PYP (45) and differs from the PAS domain in the FixL protein, in which cofactor interactions and signaling are centered around the FG loop and F helix (23). A structural model of the Aer PAS domain that used the crystal structure of PYP as template demonstrates the spatial relationships of the mutated residues (Fig. 5). Most of the residues that are involved in FAD binding and signal transduction project into an internal pocket that corresponds to the cofactor-binding site in PYP. The EF loop forms one boundary of this pocket. We propose that FAD is bound in the pocket and that residues that interact with the isoalloxazine ring of FAD transduce redox changes in FAD into a conformational change in the PAS domain. The latter conveys the aerotaxis signal to the C-terminal domain of Aer.

Interaction between Try^{111} and the isoalloxazine ring of FAD may modulate the midpoint potential of FAD as this could provide an explanation for the inverse aerotaxis responses observed in the Y111C mutant (49). A range of mutations in bacterial chemotaxis and aerotaxis are known to cause inverse responses (for reviews, see 33, 62). Possible mechanisms for the inverse responses have been proposed. The inverted aerotactic response caused by the Y111C mutation can be explained by our three-state model for Aer signaling, if the Y111C mutation shifts the redox potential of the FAD cofactor

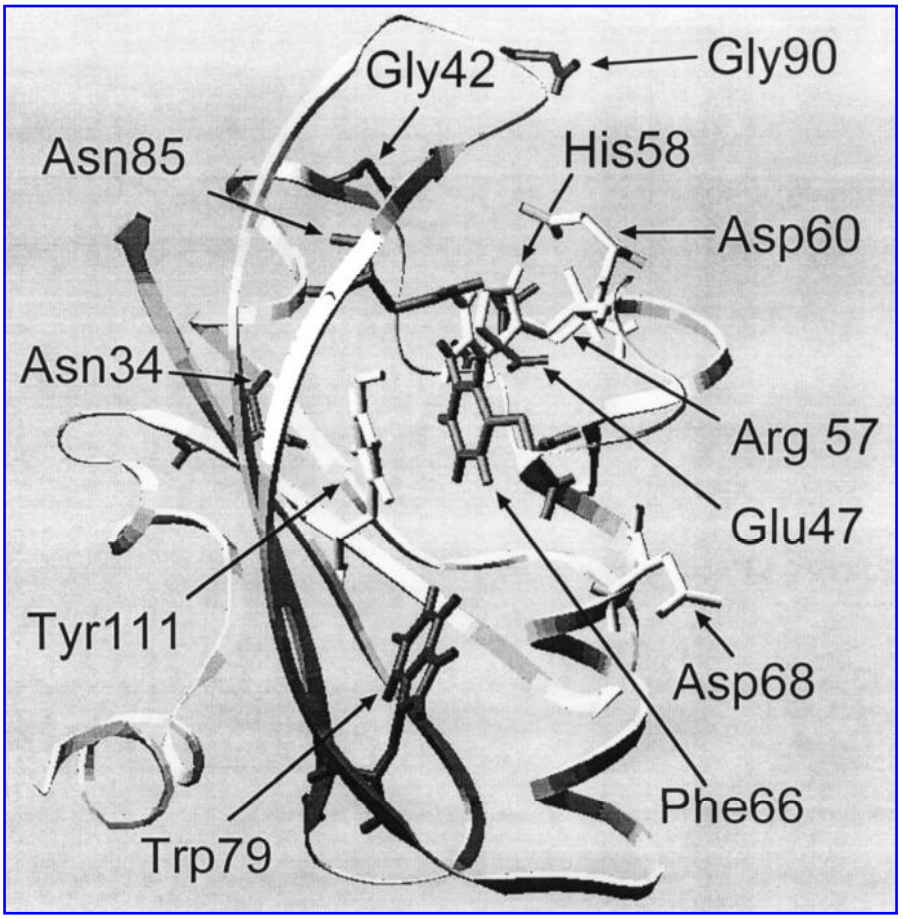

FIG. 5. Predicted topology of important residues in Aer PAS domain. A model of the Aer PAS domain showing the orientation of 12 important residues. Side chains of residues that are required for Aer signaling as well as FAD binding, and Tyr^{111}, are filled as white. Side chains of other residues that altered signaling are filled as gray. Adapted with permission from (49).

so that it is in the fully oxidized quinone form during maximal rates of electron transport. A reductive shift in the electron transport system that would normally shift the semiquinone $FADH^{\cdot}$ to the quinol state and produce a repellent signal will now shift the FAD quinone to the semiquinone state and produce an attractant signal (49).

INTERDOMAIN INTERACTIONS

The cytoplasmic N-terminal and c-terminal domains in Aer are separated by a span of 38 hydrophobic amino acids (residues 167–204) that form the hairpin loop in the membrane. The signal that is generated by a conformational change in the FAD-PAS domain could be transmitted to the signaling domain through the transmembrane segment or by physical interaction of the PAS and signaling domains.

Current evidence suggests that the two domains of Aer are in contact with each other. We prepared a series of constructs that included the PAS domain and increasing segments of Aer that are C-terminal to the PAS domain. The "soluble" constructs formed a large complex (>800 kDa) under native conditions with the GroEL chaperone (S. Herrmann, Q. Ma, M. Johnson, and B. Taylor, unpublished observation). GroEL is a large (800 kDa) 14-mer protein that assists the proper folding of ~40% of *E. coli* proteins. It encapsulates proteins that are not correctly folded and allows exposed hydrophobic residues to unfold and reorient for proper shielding before being released into the aqueous environment. As the "soluble" Aer constructs were not released, it is possible that

hydrophobic residues are exposed to the outside in these truncated constructs, and that a longer sequence is necessary for proper folding. Aer2–231 and Aer2–285, which include the transmembrane domain and a partial or complete HAMP domain, were released from GroEL. We conclude that the HAMP domain contains residues that interact with the N-terminal domain and are required for proper folding. As Aer2–285, but not Aer2-231, bound FAD, specific residues between residues 231 and 285 must be necessary for proper stabilization of the PAS domain for FAD binding (S. Herrmann, Q. Ma, M. Johnson, and B. Taylor, unpublished observation; see also ref. 10). Bibikov *et al*. designated the sequences that connect the PAS domain and the carboxyl signaling domains as the F_1 and F_2 domains, respectively (10). They predicted that the F_1 and F_2 (HAMP) domains directly or indirectly stabilize the FAD-PAS binding pocket. FAD binding in the Aer PAS domain is prevented not only by truncating the HAMP domain, but also by specific point mutations in the HAMP domain (10) (Q. Ma and B. Taylor, unpublished observation). Taken together, the data are consistent with a model whereby communication between the N- and C-termini occurs through the HAMP domain.

SUMMARY

Internal sensors: a paradigm shift in signal transduction

Sensory receptors with FAD-PAS domains constitute a novel class of flavoproteins. Investigation of the role of the FAD-PAS domain in the aerotaxis transducer, Aer, demonstrated that Aer is an internal sensor that monitors internal energy levels in *E. coli. In silico* studies revealed that the Aer protein is a prototype of a superfamily of PAS domain proteins that sense the internal environment of cells rather than the external milieu sensed by other sensory receptors. PAS domain proteins are broadly represented in the phylogenetic tree, but the greatest progress in understanding their sensory transduction mechanism has been in investigation of microbial and plant sensors.

The FAD-PAS domain of the Aer protein apparently senses cellular energy levels by sensing redox changes in the electron transport system. This provides *E. coli* with a more versatile sensory response than a system that sensed oxygen *per se*. As a consequence of this, *E. coli* is able to sense not only hypoxia, but any other unfavorable conditions that impair the activity of the electron transport system. Other organisms use similar sensory mechanisms to maintain optimal energy levels. It is more common, however, for energy-sensing mechanisms to be linked to transcriptional control of proteins for anaerobic metabolism than to a behavioral response.

The Aer protein has two cyloptasmic domains that are anchored to the membrane. The FAD-PAS domain is proposed to undergo redox changes that reflect the redox state of the electron transport system. Residues have been identified in the PAS domain that are critical for FAD binding and for signaling. Of particular interest are cysteine-replacement mutations that lock the FAD-PAS domain in a signal-on conformation and Y111C, which inverts the signaling response so that an attractant signal elicits a repellent response and vice versa.

The linker region, formally renamed the HAMP domain, is a newly recognized signal transduction domain that is essential for signal transduction in a family of sensory proteins, including Aer and chemotaxis receptors. Current evidence suggests that the aerotaxis signal is transmitted from the PAS domain to the carboxyl signaling domain in Aer via a protein–protein interaction between the PAS (or F_1) domain and the HAMP domain. As a result, the carboxyl signaling domain regulates the chemotaxis phosphorylation cascade in response to oxidation or reduction of the FAD-PAS domain.

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ABBREVIATIONS

Aer, aerotaxis and energy responses; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; HAMP, histidine kinases, adenylyl cyclases, methyl-accepting chemotaxis proteins, and phosphatases; NADH, nicotinamide adenine dinucleotide; PAS, PER-ARNT-SIM superfamily; PYP, photoactive yellow protein.

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