

Bacterial chemotaxis: The five sensors of a bacterium

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The components of the *Escherichia coli* chemosensory system have been identified and their activities characterized, but how sensory information is processed to give an integrated response remains an open question.

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Humans use five senses to obtain information about their environment and plan their actions. Now that the *Escherichia coli* genome has been sequenced, it is clear that biology's best understood bug sports the same number of chemotaxis sensors: Trg, for ribose and galactose; Tar, for aspartate; Tsr, for serine; Tap, for peptides; and the newly discovered Aer, which may be a redox detector.

Research into bacterial chemosensation started in the 1880s, when Engelmann and Pfeffer discovered that bacteria are attracted by some compounds and repelled by others (see [1]). Bacteria were observed gathering around oxygen-producing chloroplasts, swimming into capillaries filled with meat extract, and escaping from capillaries filled with noxious acids. Now, almost 120 years later, the molecular mechanisms that underlie these behavioral responses are beginning to be understood [2,3]. Bacterial chemoreceptors are transmembrane proteins that detect chemical stimuli in the environment and relay these sensations to a cytoplasmic 'two-component' signal transduction system, consisting of a kinase (CheA) and a response regulator (CheY).

Binding of repellents to the receptors activates the CheA kinase, resulting in an increase in phosphorylation of the CheY response regulator. Phosphorylation induces a conformational change in the response regulator that allows it to bind to switching proteins at the flagellar motor, causing a repellent response. Attractants have the opposite effect, inhibiting the kinase, preventing response regulator phosphorylation and thereby blocking the interaction between response regulator and motor. *E. coli* cells respond to changes in the concentrations of attractant or repellent, rather than their absolute levels [4]. Immediately after a cell is exposed to a repellent stimulus, its receptors send a strong signal to activate the kinase and effect a repellent motor response. After only a few seconds, however, the cells return exactly to their prestimulus swimming behavior, despite the continued presence

of the repellent. This is accomplished by a complex adaptation mechanism that involves changes in methyl esterification of glutamate sidechains in the receptor proteins [5].

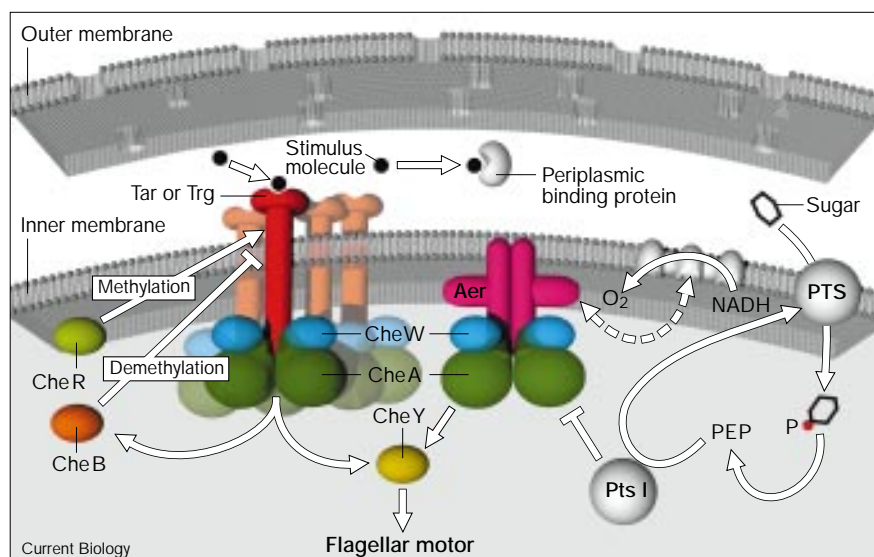
Specific glutamate residues in the conserved signaling domains of each of the receptors are subject to methylation by a specific methyltransferase, CheR, and demethylation by a specific methylesterase, CheB. The demethylating activity has a regulatory domain that is homologous to the response regulator CheY, and it is phosphorylated and activated by the kinase CheA. Demethylation forces the receptor into a state that tends to inactivate the kinase, so the demethylation reaction acts as a negative feedback mechanism to cause adaptation to repellents. Conversely, attractants cause an increase in receptor methylation that activates the kinase and causes adaptation to attractants. Methylation provides a robust mechanism that maintains a constant steady-state swimming behavior under a wide range of different environmental conditions [6]. To date, the reversible methylation of glutamates appears to be unique to bacterial chemoreceptors; this family of receptors has therefore been termed methyl-accepting chemotaxis proteins (MCPs).

Receptor signaling in bacterial chemotaxis occurs within stable complexes between membrane receptors and the CheA kinase. As both receptors [8] and the CheA kinase [9] have been isolated as dimers, it was originally assumed that transmembrane signaling involved a pairwise dimer–dimer interaction. Recent evidence, however, indicates that higher-order receptor–kinase complexes may be involved. Immuno-electron microscopy has shown that receptor–kinase complexes tend to cluster in a few dense patches localized to the poles of the *E. coli* cell envelope [10]. Arrays of Trg reconstituted into phospholipid bilayers have been shown to be organized with a tetrameric unit cell [11], as has also been suggested from crosslinking studies of Tar and Tsr [7], and active Tar signaling domain–kinase complexes have been shown to be large assemblies with about 14 receptor signaling domains per unit cell [12].

The observation that mutant forms of Tar, expected to form dimers with only a single signaling domain, can still function in chemotaxis [13,14] can also be readily understood in terms of signal transduction through higher-order receptor oligomers, rather than independent dimers. It thus seems likely that individual dimers are not the relevant signaling unit. Rather, the combined effects of stimulus-induced changes to packing interactions between numerous individual receptor subunits may act to control

Figure 1

The *E. coli* chemotaxis system. Stimulus molecules diffuse through the outer membrane into the periplasm, where they bind to their respective receptors either directly or indirectly via periplasmic binding proteins. The chemotaxis receptors signal through the inner membrane where they interact with the adaptor protein CheW and the kinase CheA. The aerotaxis receptor, Aer, apparently receives intracellular redox signals through its flavin-binding domain, and is thought to interact with the electron transport chain. The chemotaxis system can adapt to changes in attractant or repellent concentrations by covalently modifying the membrane receptors. The methyltransferase CheR transfers methyl groups from S-adenosylmethionine (AdoMet) to conserved glutamate residues on the cytoplasmic signaling domains of the chemotaxis receptors. The methyl-erastase CheB removes these groups when activated through phosphorylation by CheA. Enzyme I (Pts I) of the phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) binds and



inhibits CheA when a variety of hexoses are transported into the cell. All chemotaxis signals are ultimately integrated at the level of

phosphorylation of the response regulator CheY, which directly interacts with the flagellar motor.

the kinase activity within relatively large signaling arrays at the membrane–cytoplasm interface [12].

With the basic components of the chemotaxis system laid out (Figure 1), one of the questions that still awaits resolution is how information from different receptors is processed to provide a single motor output. Does each chemoreceptor act independently, or are the receptors organized into signal-processing clusters that integrate sensory information? The two major receptors in *E. coli*, Tar and Tsr, can each sum the signals from several different, and apparently unrelated, stimuli to generate an integrated output. For example, responses to both the attractant serine and the repellent acid are mediated by Tsr, with serine tending to counteract the effects of acid [15], whereas the two attractants aspartate and maltose give additive responses mediated by Tar [16]. Crosslinking studies have clearly shown that Tsr and Tar both form higher-order homo-oligomers [7], but no comparable Tsr–Tar heteromers could be detected. There is no evidence to support the idea that Tar and Tsr communicate directly with one another.

When *E. coli* cells are exposed to attractant and repellent stimuli, one mediated by Tar and the other by Tsr, the response can be biphasic, with Tsr mediating a fast repellent response, followed by a relatively slow attractant response through Tar [15]. This and the above-mentioned results support the idea that Tsr and Tar act independently. This view fits the long-standing observation that changes in Tar and Tsr methylation are specific — for

example, aspartate and maltose both cause increases in Tar methylation but do not affect Tsr, whereas serine causes an increase and indole a decrease in Tsr methylation without affecting Tar. Conceptually, there is no requirement for a preliminary integration step before chemotaxis signals reach the cytoplasm. All the integration could be performed at the level of the kinase CheA, with each receptor controlling an independent pool of associated kinase molecules.

What about the other chemoreceptors, Trg, Tap and Aer? Although the intensities and durations of the responses mediated by these so-called minor receptors are quantitatively similar to those mediated by Tar and Tsr, the proteins are present at only about one-tenth the level of Tar or Tsr. Over the past few years, results from several different groups have provided evidence that signaling through the minor receptors may at least in part depend on their interactions with Tar and Tsr signaling complexes. Although all *E. coli* chemoreceptors are subject to adaptational methylation and demethylation, only Tar and Tsr have a specific pentapeptide methyltransferase binding site. Apparently, the transferase methylates target glutamate residues in Trg, Tap, and presumably Aer, *in trans* while bound to neighboring Tsr and/or Tar subunits [17,18]. Trg cannot function in the absence of Tsr and Tar [19]. An intimate association between major and minor receptors, at least in terms of the methylation/adaptation system, is also suggested by the observation that a mutant form of Trg lacking methylatable sites can still support adaptive chemotaxis responses [20]. Presumably, Trg can

induce changes in Tar and/or Tsr methylation sufficient to cause adaptation. It should be noted that, whereas receptor specificity has been demonstrated for stimuli-induced changes in Tar and Tsr methylation, this has never been shown to apply to minor receptors such as Trg.

How does the newly discovered aerotaxis receptor, Aer [21,22], fit into this scheme? Aer has a cytoplasmic signaling domain similar to those of the other chemoreceptors. It has been predicted to have two transmembrane helices, possibly for dimerization, but it has no periplasmic sensing domain. Although, from its sequence, Aer appears to have target sites for methylation and demethylation, there has been some question as to the role of methylation in adaptation to O₂. It should be emphasized that Aer, despite its name, is not a direct receptor for O₂ — the Aer sensing domain appears to be a flavin-binding protein, and it seems likely that the redox state of a bound flavin controls Aer's signaling activity.

There is no evidence as to the nature of the electron donor/acceptor that controls the redox state of Aer, and it is not at all clear what relationship this putative donor/acceptor would have to the O₂ levels in the cell's surroundings. Moreover, it is known that Tsr also participates in responses to O₂. This response also appears to be indirect, however, perhaps involving O₂-induced changes in transmembrane proton gradients or intracellular pH. One would expect that internal signals, such as cytoplasmic pH or intracellular redox potential, would be subject to homeostatic regulatory mechanisms that are unrelated to chemotaxis but could lead to nonspecific, methylation-independent adaptive effects. Aer appears to be a novel receptor in *E. coli*, in that it is specialized to detect intracellular, rather than extracellular, sensory inputs.

In addition to the transmembrane chemoreceptors, there is at least one further nutrient sensor that feeds its information into the chemotaxis signaling network. Enzyme I of the phosphoenolpyruvate (PEP)-dependent-phosphotransferase system binds to the chemotaxis kinase CheA to control CheY phosphorylation in response to changes in sugar transport and metabolism. In *E. coli*, glucose and several other hexoses are transported and phosphorylated by the phosphotransferase system. Phosphoryl groups are first transferred from PEP to a histidine residue in Enzyme I and then, via transfer through additional phosphoprotein intermediates, to incoming sugar molecules to form sugar phosphates. The sugar phosphates are in turn converted, through glycolysis, back to PEP. The dephosphorylated form of Enzyme I binds and inhibits the kinase CheA [23].

Increases in extracellular hexoses thus cause a transient attractant response by increasing the rate of phosphotransfer from phospho-Enzyme I to sugar. Homeostatic

mechanisms that regulate hexose metabolism and the intracellular PEP level would be expected to be involved in adaptation. The role of methylation is unclear in this case, as the effect of Enzyme I on receptor-kinase complexes has not been examined. It should be noted that metabolic perturbations that significantly alter respiration or glycolysis would also be expected to cause changes in levels of the methyl donor S-adenosylmethionine. In such a case, the methylation adaptation system would be expected to act as an internal sensor, the effects of which would be mediated primarily by the major receptors, Tar and Tsr.

The *E. coli* chemotaxis signal transduction system monitors a wide range of internal and external signals. Cells perceive extracellular nutrients, intracellular redox potential, pH, temperature and a spectrum of repellents using only five membrane receptors and the phosphotransferase system. The various, and often conflicting, information generated within the chemosensing network must be integrated to produce a single motor output. Information appears to be processed at all levels within the chemotaxis signal transduction network. The major receptors, Tar and Tsr, appear to provide independent parallel processing systems into which the minor receptors seem to connect. How much integration can be performed within interacting clusters of receptor dimers is at present not clear, and we are only just beginning to understand how metabolic signals produce sensory responses and how related global homeostatic mechanisms might operate to modulate stimulus-response coupling.

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