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Mind your B's and R's: bacterial chemotaxis, signal transduction and protein recognition

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The crystal structures of two key regulators of the bacterial chemotaxis pathway (CheR and CheB) have been determined. These studies add further detail to the growing picture of signal transduction and attenuation in the bacterial chemotaxis pathway. The recently determined structure of the methyltransferase CheR bound to a peptide of its target receptor, provides a structural model for intermolecular receptor modification during signaling.

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Structure 15 July 1998, **6**:809–813
http://biomednet.com/elecref/0969212600600809

© Current Biology Ltd ISSN 0969-2126

Introduction

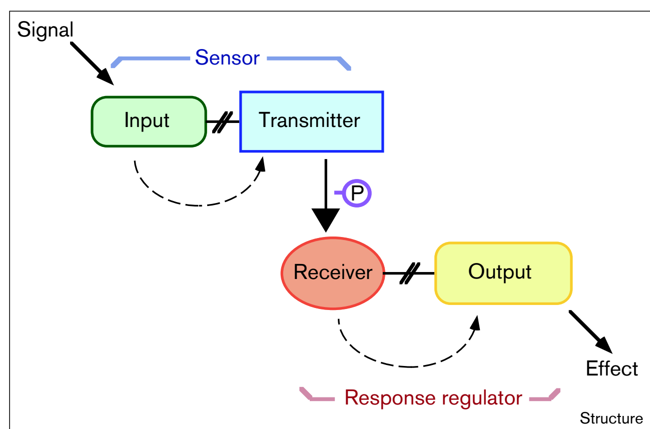
Most enteric bacteria display the ability to swim directionally in response to specific attractants and repellents in the environment. This behavior, governed by a highly efficient signal-transduction pathway, is termed 'chemotaxis' [1–4]. Directional swimming is dependent on the frequency at which bacteria throw a simple binary switch between clockwise and counterclockwise rotation of their flagella. Counterclockwise rotation results in flagella working in a concerted bundle, and bacteria move smoothly in a single direction termed 'running'. Clockwise rotation causes the flagella to fly apart in an unorganized manner, and the bacteria tumble and change direction randomly. In a stable environment the bacteria switch between running and tumbling at a frequency that allows them to make a 'random walk' through their environment. In the presence of a chemoattractant the initial rapid response of the bacteria is to suppress the frequency of flagellar reversal so that the bacteria tumble less frequently, run longer and therefore move in a biased direction. In time, if the chemoattractant concentration remains constant, the frequency of switching is reset to the random walk level so that increased concentrations of the chemotactic factor are necessary to again bias the switch for directional movement. This habituation response allows the bacteria to temporally sample their environment and respond to concentration gradients of a variety of molecules by swimming directionally. A series of recent crystallographic studies of the two enzymes that reset the chemotaxis response have greatly improved our understanding of the mechanism of signal transduction and attenuation in bacteria [5–8].

The genetic pathway of this system has been well characterized and biochemical function assigned to all the proteins encoded. The pathway can be modeled by the two-component system of phosphotransfer signal transduction (Figure 1) [9,10]. This model classifies proteins under the headings of sensors and response regulators. Sensors are composed of an input module and a transmitter module encoded as either a single polypeptide chain or as separate proteins. Response regulators consist of a receiver module coupled to an output module. Signals are communicated from the transmitter module of one protein to the receiver module of a second protein via phosphotransfer. In the chemotaxis pathway (Figure 2), a family of related transmembrane receptors act as the input module by binding either small chemotactic molecules or their periplasmic binding proteins. Once these effectors are bound the activity of a transmitter histidine kinase (CheA) that is associated with the cytosolic domain of the receptor(s) is rapidly modulated. Changes in the activity of this kinase lead to transient increases or decreases in intracellular levels of phosphorylated CheY (the response regulator) which directly affects flagellar rotation and the frequency of their reversal. Slower habituation of this response, effected at the level of receptor signaling, is induced by the reversible methylation and demethylation of a specific group of glutamate residues within predicted coiled-coil regions of the receptor cytosolic domains [11–13]. These covalent modifications are catalyzed by an *S*-adenosylmethionine-dependent methyltransferase (CheR) and a partner methylesterase (CheB) that act to respectively increase or dampen the signal. CheB, another response regulator, is also a substrate for CheA kinase activity. The protein is most active as a methylesterase in the phosphorylated form and further serves as a feedback regulator of signaling. The entire pathway is slowly being dissected at the structural level through a combination of crystallographic and nuclear magnetic resonance (NMR) studies (Table 1). In addition, the pathway is being increasingly studied and modeled through detailed computational algorithms that treat it as a complex, interconnected series of rate constants and chemical equilibria. Therefore, it may reasonably be argued that the bacterial chemotaxis pathway is currently the most well studied, complete signal-transduction system known to molecular biologists.

The methylesterase CheB

As with many response regulators, the methylesterase CheB has an N-terminal regulatory domain (receiver

Figure 1

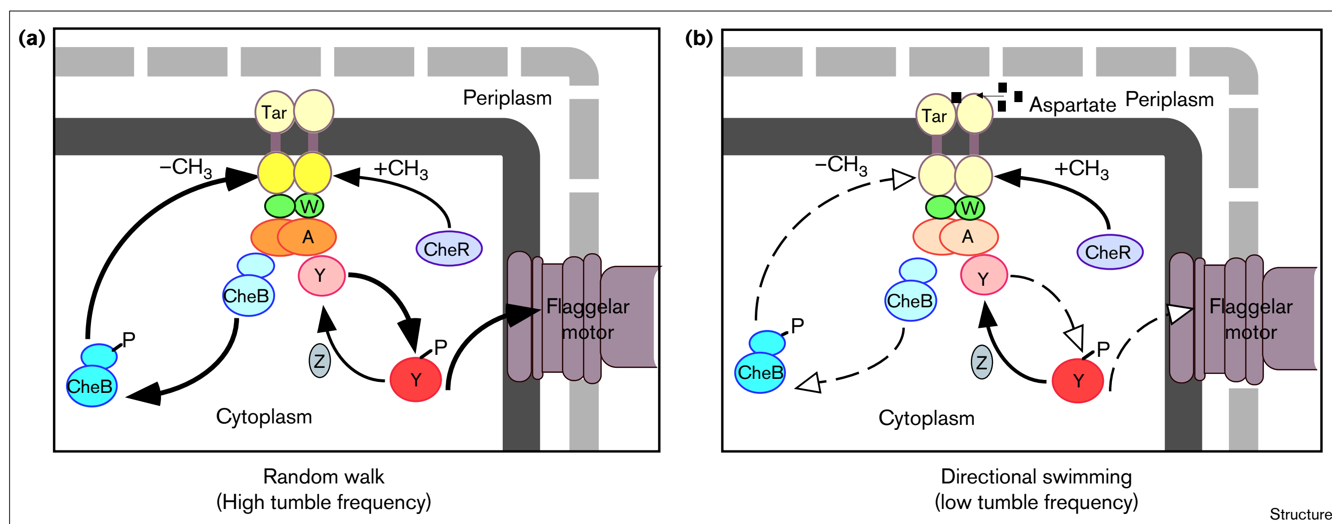


The two-component signal transduction model. The sensor component processes an environmental signal through its input module to activate the transmitter module. Phosphoryl transfer from the transmitter to the receiver module of the response regulator component activates the output module. The output module is responsible for mediating the cellular response. Diagonal lines signify that these modules may be encoded separately or as single polypeptide chains.

module) that is linked to a C-terminal effector domain (output module). CheB is activated by phosphorylation of a specific aspartate residue in its N-terminal domain. The enzyme's C-terminal domain is responsible for deamidation or demethylation of specific glutamate residues in the cytoplasmic domain of the chemoreceptors. When the C-terminal domain is expressed as a separate fragment, this activity is constitutive [14]. The recent structure of the intact CheB protein illustrates how the N-terminal domain affects the output catalytic activity of the C-terminal domain. In the unphosphorylated state, the two domains interact to create an extended interdomain interface by burying approximately 1000 Å^2 of surface area from each domain. This interface is stabilized by hydrophobic interactions at its center and by hydrogen bonds and salt bridges at its perimeter. The position of the N-terminal domain and the interdomain linker obstructs access to the active site in the C-terminal domain by creating a funnel-shaped rim around the active site. A methylated glutamate sidechain extended from an α helix (the expected substrate of the enzyme) cannot reach into the active site when this extended rim is present. Phosphorylation of Asp56 on the N-terminal domain, which is located approximately 13 Å from the interdomain interface, must lead to a conformational change at the interface that opens up access to the active site.

The available three-dimensional structures of intact bacterial response regulators also include CheY from the chemotaxis pathway (Table 1) and NarL, a transcription factor involved in nitrate regulation [15]. CheY lacks the two-domain structure found in most response regulators but is comprised of what correlates to just the N-terminal regulatory receiver module. In contrast, the structure of NarL

Figure 2



The chemotaxis pathway. **(a)** Unbound and/or highly methylated chemoreceptor (yellow) activates the kinase CheA (orange) to transfer a phosphate to the response regulators CheY (red) and CheB (blue). The subsequent high level of phosphorylated CheY increases the frequency of switching to clockwise flagellar rotation and thus tumbling. Active phosphorylated CheB slowly demethylates the receptor to reset the signaling state. **(b)** When a receptor binds ligand and/or is unmethylated CheA is inactive. The levels of phosphorylated CheY are reduced leading to more counterclockwise flagellar rotation and more running. With CheB inactive, the methyltransferase activity of CheR (purple) serves to decrease receptor sensitivity. Activated forms of the proteins are shown by darker colors.

Table 1

Structural studies of bacterial chemotaxis in the 1990s.

Protein	Complex/comments	Method	Resolution (Å)	Reference
Periplasmic binding proteins				
Maltose-binding protein	Closed form (+ maltose)	X-ray	2.3	[27]
Maltose-binding protein	Open form	X-ray	1.8	[28]
Dipeptide binding protein	Gly–Leu bound	X-ray	3.2	[29]
Glucose/galactose-binding protein	Closed form	X-ray	1.9	[30]
Receptors				
Tar ligand-binding domain	Cross-linked, + Asp + sulfate	X-ray	2.0	[31]
Tar ligand-binding domain	Cross-linked, + Asp, + metals, + aromatics	X-ray	2.2	[32]
Tar ligand-binding domain	Native, + Asp, + sulfate	X-ray	1.85	[33]
Tar ligand-binding domain	Native apo receptor	X-ray	2.0	[34]
CheY				
Wild type	Mg^{2+}-bound	X-ray	1.8	[35]
Thr87→Leu mutant	Non-signaling, phosphorylatable mutant	X-ray	2.1	[36]
Asp13→Lys mutant	Constitutive signaling, non-phosphorylatable	X-ray	2.3	[37]
CheA				
CheY-binding domain	Residues 124–257	NMR	NA	[38]
Phosphotransfer domain	Residues 1–134	NMR	NA	[39]
CheR				
Full-length protein	Complexed to *S*-Ado-Met	X-ray	2.0	[6]
CheB				
Catalytic domain	C-terminal effector domain	X-ray	1.75	[5]
Full-length protein	C-terminal effector domain + N-terminal regulatory domain	X-ray	2.4	[7]
Protein complexes				
CheY–CheY-binding domain from CheA	$K_d = 2\ \mu M$	X-ray	2.95	[40]
CheR–NWETF peptide from Tar C-terminal tail	$K_d = 2\ \mu M$	X-ray	2.2	[8]

NA, not applicable.

correlates well with that of CheB. NarL also comprises a two-domain structure and the orientation of the unphosphorylated N-terminal regulatory domain is proposed to block DNA binding, although the relative orientation of the two domains is different in the two response regulators.

The methyltransferase CheR

The methyltransferase CheR binds to a specific recognition sequence Asn-Trp-Glu-Thr-Phe-CO_2 (NWETF) located at the extreme C terminus of the Tar and Tsr chemoreceptors (sensors for aspartate and serine), with a K_d of ~2 μM for either the free peptide or the intact receptor [16]. This binding site is not near the methylation site(s) within the sequence of the receptor. In addition, the CheR recognition sequence is not found in all members of the chemoreceptor family, although all are reversibly methylated and demethylated by the same enzymes. A variety of recent biochemical studies have indicated that CheR acts during the chemotactic response by being recruited to individual receptor dimers, and from that tethered position catalyzing the intermolecular methylation of neighboring receptor dimers [16–18]. The observation that chemotaxis receptors are physically clustered in a local region of the bacterial surface is in agreement with this model [19,20]. One potential consequence of this strategy is an amplification of an initial methylation response through the localization of CheR enzyme molecules to a region of high receptor density. A separate feature of this system is the observation that the presence of CheR-binding sites is correlated with the abundance of a particular receptor type, so that high-abundance receptors such as Tar and Tsr are responsible for recruitment of methyltransferase and efficient methylation of low-abundance receptors such as Trg and Tap (sensors for sugars and dipeptides) [21].

Intermolecular reactions involving methylation by CheR can potentially be facilitated by the dynamics of the protein complex formed between CheR and the specific peptide sequence (NWETF) at the C-terminal end of the receptor. The cytosolic domain of Tar is generally acknowledged to be relatively flexible [22,23], and its C-terminal tail is best modeled in an extended conformation, as indicated by its proline-rich sequence and the structure of the final five residues bound to CheR [8].

The length of this C-terminal tail would allow bound CheR to carry out methylation of nearby receptor dimers.

The β subdomain of the CheR protein is present as an insertion within the C-terminal domain of the methyltransferase enzyme structure. This small folded motif, which consists of three β strands and a single α helix, is responsible for binding the receptor NWETF-CO_2 sequence. The structure of CheR has been determined in complex with this five amino acid receptor sequence from Tar. In this complex, the Tar peptide adopts a β conformation and forms backbone hydrogen bonds to an edge strand of the β subdomain, thus adding a fourth strand to the structure [8]. In addition to the normal pattern of backbone contacts for a β strand, the five individual sidechains of the peptide all form an excellent series of sequence-specific contacts with their binding site on CheR. The C-terminal oxygen atoms are also involved in specific hydrogen bonds, further limiting the recognition pattern to a terminal peptide of the appropriate sequence. The extension of an existing β sheet as a strategy for forming a specific protein interface is a particularly powerful method for maximizing binding specificity to a short peptide sequence, because in principle every sidechain of an edge strand on the surface of a protein is accessible for intermolecular contacts. In contrast, an α helix on the surface of a protein sequesters many of its sidechains, necessitating a longer peptide to make a similar number of contacts. A β sheet extension has been observed in many protein complexes, including the Raf–Ras complex [24], the interleukin receptor–IRS (insulin receptor substrate) complex [25], and the I-*Ppo*I homing endonuclease homodimer, which uses its C-terminal tail to donate a domain-swapped β strand as part of its dimer interface [26].

The β subdomain of CheR joins a growing list of relatively small protein domains that are used as structural 'cassettes' for the purpose of recognition and binding of specific short protein sequences. Many motifs have been proposed to act as protein–protein interaction modules. The best characterized in this role include SH2, SH3, PTB, WW and EH domains; the sequence targets of these five domains have been identified. Structural studies of all but the EH domain have probed the mechanics of molecular recognition that is the basis of signal transduction in a variety of cellular pathways. A significant difference between the CheR NWETF-binding domain and these other motifs, is the strong possibility that this insert may not be capable of folding independently of the CheR enzyme itself. The motif buries a number of hydrophobic residues against the adjacent surface of CheR, and recent studies suggest that the enzyme and the subdomain fold in a moderately cooperative manner (AM Stock, personal communication). It will be of interest to see if the structure and function of the CheR β subdomain is conserved in other signal pathways.

The future

The most important structural studies of the bacterial chemotaxis pathway will clearly now focus on the structure of the cytosolic domain of the integral membrane receptor (and of course its full-length parent), and the interactions of the receptor with its various binding partners: the modifying enzymes, CheB and CheR, and the signal transmitters, CheW coupling protein and CheA kinase. The eventual determination of these structures should provide the beginning of an extended series of studies that will eventually find common ground with the recently reported structures. A single static structure of the receptor, either alone or in complex with other protein components, will need to be followed by comparative studies of many receptor constructs: ligand-bound and unbound, methylated (or amidated) and non-methylated, activated and non-activated. For those with interest in signal transduction and receptor structure and function, this area promises to be worth watching for the immediate future and beyond.

Acknowledgements

We thank AM Stock and S Djordjevic for their comments on this review. MSJ is supported by a Molecular and Cellular Biology graduate training grant fellowship from the NIH.

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