Structure and Function of Chemotactic Transducer Proteins

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Introduction

Barcterial chemotaxis is a transient response of an organism in a situation where environmental homogeneity has been disturbed by certain chemical compounds. The phenomenon has been described in motile bacterial species including enteric bacteria, Gram-positives (14), Spirochaetes (6) and even Archaebacteria (8). However, most comprehensive studies have been done with *Escherichia coli* and *Salmonella typhimurium*.

Two analogies to higher eucaryotic sensory phenomena are provided by the study of bacterial chemotaxis. First, bacterial chemotaxis is similar to the stimulus-response of neuronal, immune and sperm cells. Second, studies of individual components involved in the bacterial sensory pathway can contribute to the understanding of the function of receptors, controlling signals and molecular comparators in transmembrane signalling system.

The bacterial sensory transducer, a chemoreceptor in a broad sense, is a unique entity for studying sensory function in which sonsory reception, signalling and adaptation are integrated (7, 18).

Transducers: Multifunctional Sensory Proteins

An *E. coli* cell makes net progress along chemical gradients by biasing a random walk consisting of straight line, smooth swimming punctuates by episodes of uncoordination, called tumbles, that reorient the cell in a new direction. The cell continually monitors the concentration of a few seconds previously. A difference between a past and current value results in the appropriate bias by altering the balance between counterclockwise and clockwise rotation of the bacterial flagella, corresponding to smooth swimming and tumbling, respectively. Thus *E. coli* has a rudimentary "memory" system of information storage and processing. Information about past concentrations appears to be stored in covalent modifications of transmembrane receptor proteins, called transducers. In an unchanging chemical environment, the degree to which a receptor site is occupied is reflected in the extent of carboxyl methylation of several, specific glutamyl residues in the transducer. An increase or decrease in the concentration of an attractant compound results almost immediately in a corresponding change in occupancy of the binding site. In contranst, the linked increase or decrease in methylation, catalyzed by a specific methyltransferase or a specific methylesterase, respectively, is relatively slow. Both binding site occupancy and methylation affect the excitatory domain of a transducer protein. This domain initiates a signal along an exitatory pathway linking transducers to flagellar motors. The nature of the pathway is unknown, but appears to involve diffusion of protein-sized molecules through the cytoplasm (20). The relatively slow rates of covalent modification mean that in a changing chemical environment the extent of transducer methylation does not correspond to the current level of ligand binding but instead to the level just previous; hence, the extent of methylation is a record of a past chemical environment. When the level of ligand binding and the extent of methylation are unbalanced, i.e., the current and past environments differ, the excitatory domain is induced to send the appropriate excitatory signal. However, the extent of modification is gradually adjusted to balance binding site occupancy. When the balance is achieved no further excitatory signal is generated and

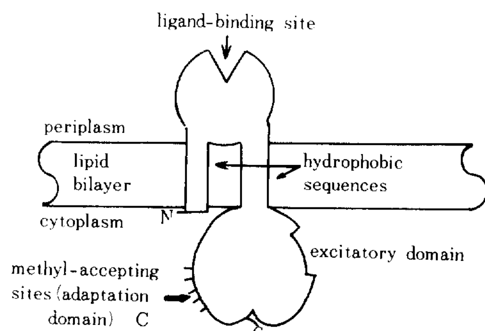

Fig. 1. Model for disposition of transducer domains across the cytoplasmic membrane.

the unstimulated behavioral pattern is restored; hence, *E. coli,* like most other sensory cells, adapts to the continued presence of stimulation.

There are four different transducers in *E. coli.* The nucleotide sequences of the corresponding genes and the respective deduced amino acid sequences (4, 5, 19) reveal a transducer gene family that codes for four homologous proteins all of approximately 60, 000 daltons. The Tsr transducer binds serine and the Tar protein recognizes aspartate. In addition, Tar also interacts with ligand-occupied, maltose-binding protein. Galactose- and ribose-binding proteins are linked in an analogous manner to the Trg transducer. The Tap transducer is linked to a putative dipeptide -binding protein (13). A number of lines of genetic and biochemical evidence lend strong support to a simple model for disposition of the transducer protein across the cytoplasmic membrane (Fig.1). The model suggests that the protein spans the membrane twice, once with a short, hydrophobic sequence near the amino-terminus and again with a short, hydrophobic stretch 40% of the way along the polypeptide chain. This places an amino-terminal, ligand-binding domain on the extra-cytoplasmic face of the membrane and a carboxy -terminal, covalent modification domain on the cytoplasmic face. The specific methyl-accepting glutamyl residues have been located in two clusters. Three or four sites (depending on the protein) in a region just past the middle of the protein and one or two sites in a region close to carboxy-terminus (10, 11, 22, 23). One or two of the glutamyl residues in the first region (again depending on the specific protein) are the result of hydrolysis of the NH2 moiety of a glutaminyl residue (a reaction we will call deamidation) to create a carboxyl group that subsequently serves as a methyl-accepting site (12, 22). The reaction is catalyzed by the *cheB* methylesterase and the rate of reaction is influenced by tactic stimuli in the same way as demethylation. The basis for this unusual sequence of events may be a requirement that newly synthesized transducers contain a balance at their modification sites between negative charge and neutral residues so that the modification domain is not at one extreme of its signaling spectrum.

Interaction of Sugar-Binding Proteins with the Trg Transducer

Our studies of the relationship of transducer structure to function have focused on Trg. One approach is to obtain and characterize mutants defective in some, but not all functions of Trg. Two *trg* mutations that eliminate tactic response to either galactose or ribose were cloned by *in vivo* recombination (15) and characterized in greater detail (16). These mutations were mapped in the amino-terminal half of the gene, thus providing support for the suggestion that amino-terminal domains of transducers contain ligand-binding sites. The mutation, *trg-19*, eliminates tactic response to galactose without a substantial effect on ribose taxis (Table 1.). The other mutation, *trg-8*, eliminates tactic response to ribose while only reducing response to galactose (Table 1.). In *trg-19*, glicine-151 is changed to an aspartate and in *trg-8*, arginine-85 is changed to a histidine (Fig. 2.). Thus two regions of the amino-terminal domain of Trg, substantially separated in the linear sequence, are identified as crucial for functional interaction with galactose-binding protein and ribose -binding protein, respectively.

These data address the issue of how two

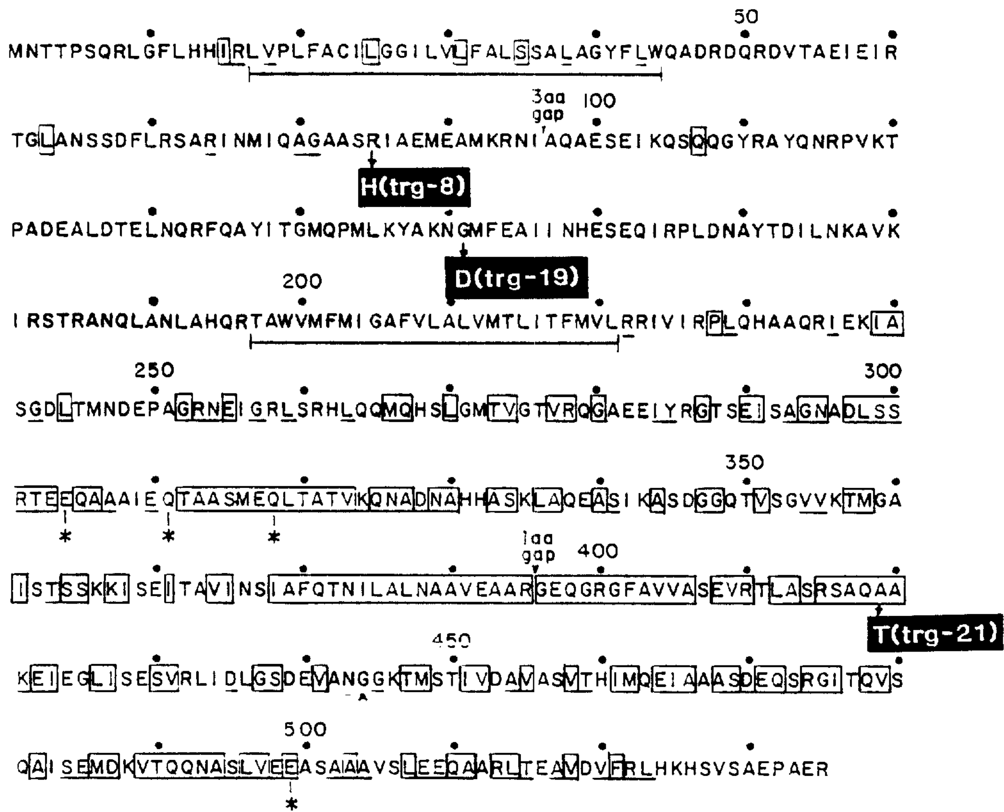

Fig. 2. Changes in Amino acid Sequences of Three *trg* Mutations. Changes were deduced from their DNA sequences. Boxes are placed around positions at which the identical amino acid is found in all four transducers from *E. coli*. Underlined positions are those at which the Trg residue matches the amino acid in two other transducer sequences. Hydrophobic regions are indicated by horizontal lines and methyl-accepting sites by vertical lines with asterisks.

Table 1. Chemotactic swarm phenotype conferred by chromosomal and plasmid-carried *trg* mutations.

Mutation	Taxis to:			
	Ribose	Galactose	Serine[a]	Aspartate or Maltose[b]
Chromosomal				
trg-8	−	(+)[c]	+	+
trg-19	+	−	+	+
trg-21	(+)	(+)	+	+
Plasmid carried				
trg-8	−	+	+	+
trg-19	+	+ −	+	+
trg-21	−	−	−	−

distinct sugar-binding proteins interact with a single kind of transducer molecule. Early work (1, 21) revealed that saturation of one binding protein with ligand interfered with tactic response mediated by the other binding protein. A model to explain this observation suggested that both proteins interacted with Trg at the same site and thus competition for occupancy resulted in the observed inhibition of tactic response (21). At the extreme, this model would require and identical site for transducer interaction on both binding proteins. Homologous sequences on galactose- and ribose-binding proteins have been investigated with the hope of identifying such a region (2). The *trg* mutants discussed here clearly establish that the interaction sites on Trg for

the two binding proteins are not identical, at least at the level of interactation with specific residues. This means that the amino acid constituents of the transducer interaction sites on the two binding proteins are not identical. Of course, competition at the level of binding could occur as the result of only a limited overlap between two sites. Alternatively, interference could result from saturation of Trg capacity at a step subsequent to the ligand binding.

A Mutation Affecting Signaling

The mutation *trg-21* was cloned and mapped by the same genetic method employed for the mutations previously described (15, 17). A single copy of the mutant gene in place of the normal chromosomal copy creates a cell that has a reduced ability to respond to stimuli of ribose or galactose (Table 1.) yet is otherwise normal in swimming behavior and tactic response to other attractants. Addition of a wild-type version of *trg* elsewhere on the chromosome restores normal response to the sugars. Thus, when present on the chromosome, *rg-21* has the properties of a leaky, recessive mutation. In striking contrast, amplification of the number of cellular copies of *trg*-21 results in a pervasive defect in tactic behavior. Otherwise wild-type cells that harbor a multicopy pUC13-*trg-21* almost never tumble and thus are defective in chemotaxis to all compounds. This means that amplification of *rg-21* creates a dominant, chemotaxis-negative phenotype. The cell can no longer respond appropriately to external chemical signals because the flagellar "gearshift" has been pushed far to the counterclockwise mode. This pattern has interesting parallels in the changes correlated with transformation of eukaryotic cells to neoplastic growth. Both specific mutations and gene amplification have been implicated in oncogenesis (3) and some oncogenes code for proteins homologous to cell surface receptors (9). Transformed cells no longer respond to extracellular signals that normally limit growth and are locked into a mode of ceaseless division. *E. coli* cells containing multiple copies of *trg-21* are unable to responde to chemical stimuli because their flagella are locked in a single direction of rotation.

Both eucaryotic receptors related to products of oncogenes and bacterial transducers are covalently modified at residues in a cytoplasmic domain. Thus, it is particularly interesting that the Trg-*21* protein is defective in covalent modification. Two methyl-accepting sites on Trg are the result of deamidation to create glutamyl from glutaminyl residues. That modification is essentially absent from the Trg-*21* protein and thus two methyl-accepting sites are eliminated. It is not clear whether this defect is the cause or effect of the dominant, chemotaxis-negative phenotype. In any case methylation and demethylation at the remaining available sites is not substantially affected.

The *rg-21* mutation substitutes threonine for alanine-419 in the transducer protein (Fig. 2.). This residue is in a part of the cytoplasmic domain, midway between the separate methyl-accepting regions, that is highly conserved among all four transducers of *E. coli*. All these proteins contain an alanine at this position. Replacement by threonine has profound effects since distant sites of covalent modification are blocked and the overall behavioral balance of the cell is deranged. It is plausible that the mutant defect is in some aspect of the signaling domain or of the linkage between the adaptation and signaling domains. This implies that the highly conserved region within which the mutational change occurs is involved in excitatory signaling to the flagella. Dominant, chemotaxis-negative mutations also occur in the *tsr* gene (18).

A stimulated transducer initiates global signals that act on two different targets. One signal shiftes the rotational bias of all flagellar motors and the other modulates activities of the entire population of modification enzymes. The degree to which the two signals are independent in either chemical nature or origin at the transducer is not known. The *trg-21* mutant provides an example in which generation of the two

signals is separated since the mutational change causes continual signalling to the flagella but has little effect on the emthylesterase. The implication is that in the normal transducer, different amino acids are involved in generation of the two global signals. It may be that the Trg-21 protein is incapable of attaining a negative excitatory mode.

Conclusions

As primary structures of more and more receptor proteins are deduced from nucleotide sequences, a few common structural motifs have emerged. One class of receptors, including bacterial transducers and eukaryotic receptors for insulin, epidermal growth factor and other polypeptide hormones (9) appears to be organized with two large domains, one extra-cytoplasmic and the other cytoplasmic, connected by a short stretch of membrane spanning sequence. Ligand binding at the extra-cytoplasmic domain results in signal generation and covalent modification at the cytoplasmic domain. The issue of how information is transferred within the proteins and across the membranes in not yet resolved. The study of bacterial transducers promises to contribute to the resolution.

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