THE SENSORY TRANSDUCTION PATHWAYS
IN BACTERIAL CHEMOTAXIS

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Bacterial chemotaxis is the least complex behavioral response and will probably be the first behavioral system in which the entire sensory transduction pathway from stimulus to response can be described in terms of a sequence of biochemical and physical events. As such, it is a useful model for understanding how more complex cells and organisms respond to changes in their surroundings. Chemotaxis is the name given to the movement of motile bacteria toward a source of nutrients and away from harmful substances, thereby enhancing their chances of survival. Bacteria also respond to a variety of other sensory stimuli (Koshland, 1988; Macnab, 1987b; Taylor, 1983a).

Escherichia coli and Salmonella typhimurium, the bacteria most commonly investigated, swim by rotating four to nine flagella per cell. The flagellar filament, which is composed of a single type of protein, is like a flexible corkscrew with a left-handed helix (Macnab, 1987a). The flagellar motor is embedded in the plasma membrane and anchored to the peptidoglycan and outer membrane. The rod, which is the shaft of the motor, is connected to the filament by a universal joint known as the hook.

If the flagellar motors rotate in a counterclockwise direction, hydrodynamic forces collect the flagella into a bundle which has a synchronized wave propagation that propels the bacterium forward (Macnab, 1987b). When the motors briefly reverse and rotate the flagella in a clockwise direction, the flagella bundle flies apart causing a chaotic tumbling motion that reorients the bacterium. When counterclockwise rotation is resumed, the bacterium swims off in a different direction. The net result of the random alternation between counterclockwise and clockwise rotation is a random walk type of motion (Berg and Brown, 1972).

A temporal sensing mechanism is utilized by the bacteria to continuously sample attractants and repellents in the environment and to compare the present environment with the environment that the bacterium has just left (Macnab and Koshland, 1972). If the difference is favorable, tumbling is suppressed and the bacterium continues in the favorable direction. If the difference is unfavorable, the probability of tumbling increases thereby ensuring that the bacterium will change direction. The net effect is to bias the random walk motility so that the bacteria migrate to a favorable environment. A central goal of research into chemotaxis is to determine the pathway by which external stimuli modulate the probability of clockwise rotation of the flagellar motors.

The strongest attractants for E. coli and S. typhimurium are the amino acids serine and aspartate (Macnab, 1987b). Other chemical attractants include some of the other amino acids, sugars and sugar alcohols. Chemical repellents include short-chain fatty acids and alcohols, some hydrophobic amino acids, indole, benzoate, sodium sulfide and the divalent cations Co^{2+} and Ni^{2+}. Other

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tactic stimuli include oxygen, temperature, pH and osmotic strength. Many species of phototrophic bacteria are phototactic.

The methylation-dependent pathways for bacterial chemotaxis are represented in Figure 1. Chemoattractants either bind directly to a specific membrane-spanning transducing protein or activate a soluble binding protein that subsequently binds to a transducing protein (Koshland, 1988; Macnab, 1987b). No specific receptors for repellents have been unequivocally identified; repellents may act by perturbing the membrane domain that surrounds the transducing protein. The four transducing proteins in E. coli that have been identified are the products of the tsr, tar, trg and trp genes. Each consists of a periplasmic domain, two membrane-spanning sequences and a cytoplasmic domain (Koshland, 1988; Krikos et al, 1983).

There is a high degree of sequence identity in the cytoplasmic domains of the four transducing proteins (Krikos et al, 1983). Two conserved regions contain the sites that are methylated during adaptation (see below). Another highly conserved region is believed to be involved in transmitting chemotactic signals to the flagellar motors. This assignment is made on the basis of signaling-deficient bacteria that have mutations in the conserved region. Specificity is conferred on the transducing proteins by the variable binding domains in the periplasmic portion of the protein. This has been verified using chimeric constructs of the tsr and tar genes that consist of a 5' region coding for the N terminus of one transducing protein and the 3' region coding for the C terminus of the other transducing protein (Krikos et al, 1985). Receptor specificity of the chimeric protein is similar to that of the Tsr or Tar transducer that has the same N terminus.

Until recently little progress had been made in identifying the post-transducer events in signal transduction. The application of three experimental strategies has now revealed at least the skeleton of the transduction pathway. A novel method for depleting S. typhimurium of ATP was used by Junichi Shioi in my laboratory to demonstrate a requirement for ATP in chemotaxis (Shioi et al, 1982). So called "gutted" strains of E. coli that were depleted of chemotaxis genes but had normal flagellar motors were used to study the effect on chemotaxis of restoring a single chemotaxis gene or a combination of genes to the gutted strain (Wolfe et al, 1987). A comparison of the sequence of three chemotaxis genes, cheA, cheY and cheB, with gene sequences available in gene banks revealed a striking similarity with the structural genes for a family of bacterial regulatory proteins (Stock, 1987). The ntrB and ntrC genes involved in nitrogen assimilation in E. coli are the most studied members of this family.

In the gutted strain the motor rotates only in a counterclockwise direction (Parkinson and Houts, 1982). Investigations in the laboratory of Daniel Koshland, Jr. demonstrated that introduction of the CheY protein restored clockwise rotation (Clegg and Koshland, 1984). The probability of clockwise rotation was a hyperbolic function of the concentration of CheY indicating that the binding of CheY to the switch was the signal for clockwise rotation (Kuo and Koshland, 1987). Subsequent studies in our laboratory established that an active form of CheY causes clockwise rotation and ATP is essential to activate CheY (Smith et al, 1988). This and the similarity of the Che and Ntr regulatory proteins suggested that the CheY protein was activated by phosphorylation of the protein.

Wolfe, Conley, Kramer and Berg (1987) discovered that the minimal additions to the gutted strain required for a chemotaxis signal from the Tar

transducing protein to reach the motor were the cheA, cheW and cheY genes. Hess, Oosawa, Matsumura and Simon (1987) found that the CheA protein is autophosphorylated in vitro by ATP and then transfers the phosphate moiety to the CheY protein. It is assumed, but not yet proven, that in vivo the transducing proteins control either the phosphorylation of CheA or the transfer of phosphate from CheA to CheY.

In addition to responding to chemotactic stimuli, bacteria adapt to such stimuli. This was first demonstrated when Macnab and Koshland (1972) used a rapid-mixing device to add attractant to a culture of S. typhimurium. The cells suppressed all tumbling and swam smoothly for a short interval, then adapted to the attractant and returned to a random motility pattern. At the molecular level, adaptation to an attractant occurs when the transducing protein is multiply methylated by a protein methyltransferase that is the product of the cheR gene (Springer et al, 1979; Springer and Koshland, 1977). The methyl donor in this reaction is S-adenosylmethionine. Methylation precisely cancels the signal generated by the attractant. If the attractant is subsequently removed or if a repellent is added, the cells tumble continuously then adapt when some of the methyl esters on the transducing proteins are hydrolyzed by the esterase activity of the cheB gene product (Stock and Koshland, 1978).

The methylation-dependent pathways are the major chemotactic pathways and are utilized in responding to most stimuli. However, Mitsuru Niwano working in my laboratory discovered that adaption to oxygen and to most sugars is independent of transducer methylation (Niwano and Taylor, 1982). The major focus of our research has been these methylation-independent pathways.

The attraction of E. coli or S. typhimurium to oxygen is readily observed in the accumulation of these bacteria around a trapped air bubble in a drop of culture beneath a cover slip (Taylor, 1983a). Some other species behave differently in the presence of a gradient of oxygen. Beijerinck (1893) observed in the last century that aerobic bacteria beneath a coverglass form a band near the air-liquid interface. Microaerophilic bacteria accumulate in a band that is some distance from the interface and anaerobic bacteria accumulate in the center of the cover slip. This suggests that oxygen is both an attractant and a repellent and that bacteria migrate to where the oxygen concentration is optimal for their metabolic lifestyle (Taylor, 1983a,b). This is not surprising in view of the toxicity of some oxygen derivatives.

To distinguish between the responses of enteric bacteria to high ($K_{0.5}$ = 1.0 mM) and low ($K_{0.5}$ = 0.7 μM) concentrations of oxygen, the responses will be referred to as the oxygen repellent and oxygen attractant responses, respectively (Laszlo et al, 1984; Shioi et al, 1987). We found that the attractant response to oxygen is mediated by the proton motive force (Laszlo and Taylor, 1981; Shioi and Taylor, 1984). Oxygen binding to the terminal oxidase of the respiratory chain increases the rate of electron transport which is coupled to translocation of protons across the inner membrane. E. coli and S. typhimurium sense and respond to changes in the proton motive force. This is also the basis of the phototactic response in photosynthetic bacteria (Harayama and Iino, 1977). We have shown that tactic responses result from a wide variety of phenomenon that perturb the proton motive force (Taylor, 1983a,b).

Ongoing studies in our laboratory are looking at the convergence of the methylation-dependent and methylation-independent pathways for chemotaxis. The gutted strain of E. coli with a functional flagellar motor did

not respond to oxygen or to the sugar mannose which acts via the phosphotransferase system, another methylation-independent pathway (Rowsell et al, 1988; Taylor et al, 1988). The addition of various chemotaxis genes showed that a normal response to oxygen or mannose was not observed unless the cheA, cheW and cheY genes were present. This indicates that the methylation-independent and methylation-dependent pathways converge at or before the cheA protein. It remains to be determined how the methylation-independent pathways modulate the phosphorylation of the cheY protein.

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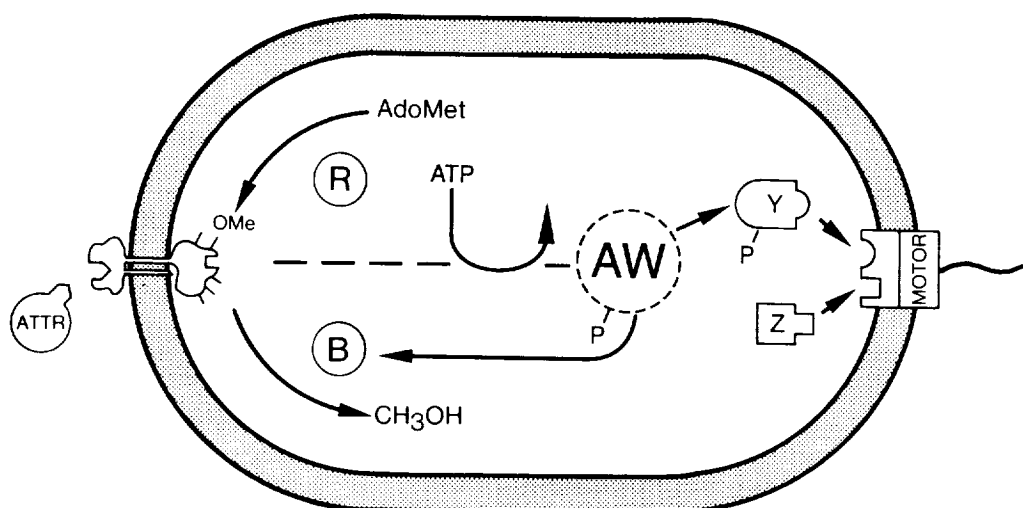
Figure 1.   Scheme for sensory transduction in methylation-dependent chemotaxis in E. coli and S. typhimurium. R, B, A, W, Y and Z represent the product of the che genes with the same letter designation.   Attr, attractant; AdoMet, S-adenosylmethionine; OMe, γ-glutamyl carboxymethyl ester;   ---> order of reactions is tentative.