Primer

Bacterial chemotaxis

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Chemotaxis is the directed motion of an organism toward environmental conditions it deems attractive and/or away from surroundings it finds repellent. Movement of flagellated bacteria such as *Escherichia coli* can be characterized as a sequence of smooth-swimming runs punctuated by intermittent tumbles. Tumbles last only a fraction of a second, which is sufficient to effectively randomize the direction of the next run. Runs tend to be variable in length extending from a fraction of a second to several minutes.

As *E. coli* cells are only a few microns long, they behave essentially as point sensors, unable to measure gradients by comparing head-to-tail concentration differences. Instead, they possess a kind of memory that allows them to compare current and past chemical environments. The probability that a smooth swimming *E. coli* cell will stop its run and tumble is dictated by the chemistry of its immediate surroundings compared to the chemistry it encountered a few seconds previously.

The tendency to tumble is enhanced when the bacterium perceives conditions to be worsening — when attractant concentrations decrease or repellent concentrations increase. Conversely, tumbling is suppressed and cells keep running when they detect that conditions are improving. Thus, when a bacterium runs up a gradient of attractants or down a gradient of repellents it tends to continue on course (Figure 1).

Biochemistry of signal transduction

How is this type of behavior achieved? It was observed over a hundred years ago that bacteria tend to congregate in a capillary filled with meat extract, while staying away from a similar tube containing poison. But the molecular details underlying this behavior have only recently been determined.

The central mechanism of signal transduction involves two families of proteins found in microorganisms and plants which work in pair-wise fashion to mediate chemotaxis, as well as other regulatory processes ranging from cell differentiation and development to antibiotic resistance and fruiting. *E. coli* alone has over 30 different examples of these so-called 'two-component' regulatory systems.

One of the families of proteins that mediate two-component signaling consists of histidine protein kinases, which catalyse the transfer of γ-phosphoryl groups from ATP to one of their own histidine residues. The other family consists of 'response regulator' proteins, which are activated by the transfer of phosphoryl groups from the kinase phosphohistidines to one of their own aspartic acid residues.

Most histidine protein kinases are transmembrane receptors with a variable external sensing domain connected *via* hydrophobic membrane spanning sequences to a highly conserved autophosphorylating kinase domain in the cytoplasm. Stimulatory ligands interact with the receptor's external sensing domain to control the rate of kinase autophosphorylation and hence the rate of response regulator phosphorylation in the cell's interior.

The response regulators are generally free to diffuse around the cytoplasm, and aspartate phosphorylation generally enhances the ability of a regulator to bind to DNA, or in the case of the chemotaxis response regulator, to bind to motor proteins and regulate the probability of a tumble.

The histidine protein kinase that mediates chemotaxis responses is called CheA and the chemotaxis response regulator is CheY. CheA differs from most histidine kinases in that it is not an integral membrane protein. Instead, CheA is tightly associated with, and regulated by, several different

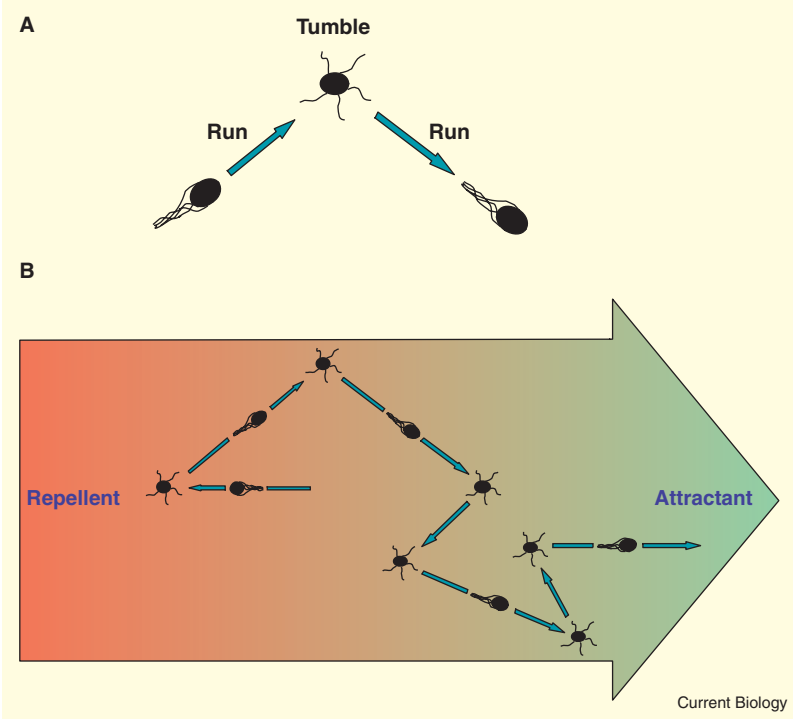

Figure 1. Chemotaxis —migration towards attractants and away from repellents. (A) Bacteria such as *E. coli* exhibit two modes of swimming: runs and tumbles. (B) Cells tend to continue on course when running towards attractants; when swimming away from attractants they tend to tumble and change direction.

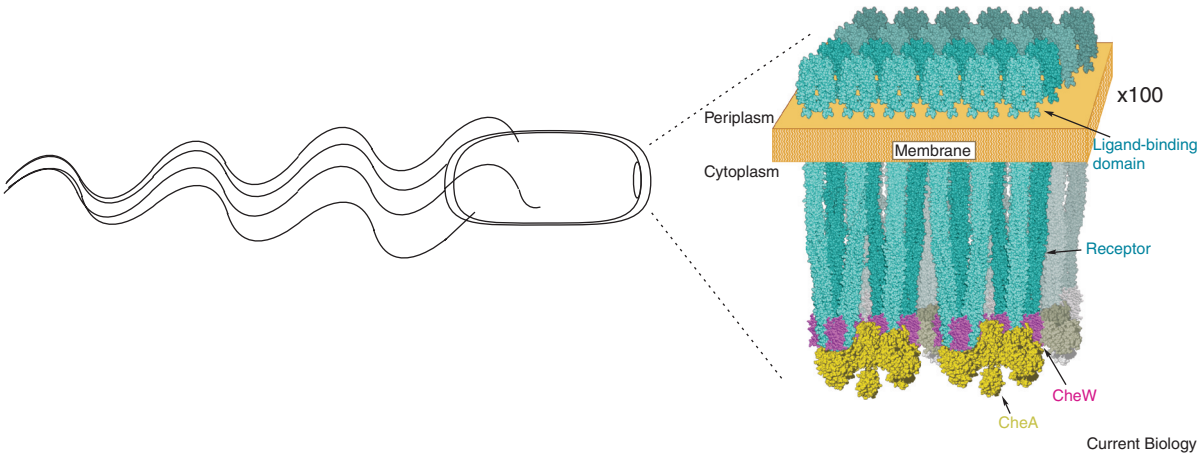

Figure 2. The bacterial nanobrain. The chemotaxis sensory array is located at the cell pole. A conceptualization of a small segment of this network of proteins is shown in the expanded view. (Adapted from Levit, Grebe, and Stock, 2002.)

transmembrane chemotaxis receptors, each of which functions to detect a different class of attractant and repellent chemicals. These receptors transmit a signal that increases CheA autophosphorylation when attractants are absent or repellents are present. Increased CheA phosphorylation leads to an increase in the level of phosphorylated CheY.

Phospho-CheY diffuses from CheA freely through the cell, and when it encounters a flagellar motor it binds to a flagellar protein called FliM. Phospho-CheY bound to FliM induces tumbling by causing a change in the sense of flagellar rotation from counterclockwise to clockwise, as viewed from behind. The six to eight flagella scattered over the cell surface rotate coordinately to form a bundle during smooth swimming. This bundle is suddenly thrown into disarray when one or several of the motors reverse direction, causing the characteristic tumble that randomizes the direction of the next period of coordinated smooth swimming. Whereas the receptor–CheA complex controls the rate of CheY phosphorylation, a phosphatase termed CheZ is responsible for phospho-CheY dephosphorylation.

Chemotaxis receptors, methylation and memory

The *E. coli* chemotaxis system has five different transmembrane proteins. Tar mediates responses to aspartate, glutamate and maltose; Tsr mediates responses to serine; Trg mediates responses to ribose and galactose; Tap mediates responses to dipeptides; and Aer mediates responses to oxygen. Aspartate and serine bind directly to Tar and Tsr, respectively. Tar senses maltose through the periplasmic maltose binding protein; and Trg and Tap sense ribose, galactose and dipeptides *via* periplasmic binding proteins for these nutrients. Aer senses O_2 through the redox state of a bound flavin.

Although the sensory domains of these five receptor proteins are variable in structure and ligand-binding specificity, their cytoplasmic domains are highly conserved and are to some degree interchangeable. Functional receptor chimeras can be made by joining the sensing domains of one receptor to the cytoplasmic signaling domain of another. As expected, the stimulus specificity of these constructs is determined by the ligand-binding specificity of the sensory domain independent of the origin of the cytoplasmic signaling domain.

Chemotaxis receptor proteins were originally termed methyl-accepting chemotaxis proteins or MCPs. The five MCPs encoded in the *E. coli* genome are actually a rather small number: *Caulobacter crescentus* has 18 and *Vibrio cholerae* at least 46. Across bacterial species, the sensory domains of MCPs are extremely diverse and the cytoplasmic signaling domains highly conserved, as is the chemotaxis histidine kinase, CheA, and the response regulator, CheY.

The conserved cytoplasmic MCP signaling domains are long α-helical coiled-coils with four or more specific glutamyl residues that are methylated by a conserved S-adenosylmethionine-dependent methyltransferase, CheR, and demethylated by a conserved methyl esterase, CheB. Methylation and demethylation provide a mechanism for sensory adaptation.

The activity of the kinase CheA is dependent on the methylation state of the MCPs with which it is associated. Low levels of MCP methylation are associated with low CheA kinase activity, and elevated methylation levels have the opposite effect.

Increased concentrations of attractants act *via* their MCP receptors to cause an immediate inhibition of CheA kinase activity. The same changes in MCP conformation that inhibit CheA lead to relatively slow increases in MCP methylation by CheR, so that despite the continued presence of attractant, CheA activity is eventually restored to the same value it had in the absence of attractant. Conversely, CheB acts to demethylate the MCPs under conditions that cause elevated CheA activity. Methylation and demethylation occur much more

slowly than phosphorylation of CheA and CheY. The methylation state of the MCPs can thereby provide a memory mechanism that allows a cell to compare its present situation to its recent past.

The receptor–kinase signaling array

The regulatory interaction between MCPs and the central CheA–CheY two component signaling system is complex. Virtually all of the MCPs in the cell, together with CheA and an activator protein termed CheW, are clustered together in a large assembly at one end of the cell (Figure 2). In *E. coli*, there are a total of about 10,000 MCP monomers in this array, with the Tar and Tsr receptors being the most abundant, and much lower levels of Tap, Trg, and Aer.

At any instant in time, each MCP may or may not be associated with a stimulatory ligand or periplasmic binding protein, and each MCP can have 16 possible patterns of methylation (assuming four methylation sites). This results in a staggering number of possible states for 10,000 of these types of receptors. Given this complexity it is not surprising that no two bacteria respond in precisely the same way to attractant and repellent stimuli.

The individuality exhibited by genetically identical bacteria within a population is just one intriguing aspect of the function of chemotaxis receptor arrays that reflects the overall lack of any simple, fixed relationship between stimulus and response. This can be seen most clearly when the methylation state of the MCPs is altered. For instance, under conditions where Tsr is demethylated, just a few serine molecules can effectively inhibit the total CheA activity in the cell. But under conditions where Tsr is highly methylated, thousands of serine molecules are required to inhibit CheA. Thus, when cells are in media where serine is at a very low concentration and methylation is low, they are attracted to even small sources of serine. But as serine causes an increase in Tsr methylation, under conditions where ambient concentrations of serine are high, cells respond only to correspondingly large changes in serine concentration. In general it is apparent that the change in stimulus concentration that a bacterium can detect is a constant fraction of the background stimulus intensity. This relationship, known as Weber's Law of psychophysics, is a general feature of animal sensory systems. It is interesting that it seems to apply as well to bacteria.

A bacterial nanobrain?

In many ways, bacterial chemotaxis receptor complexes seem to function as rudimentary brains. In view of the fact that they are only a few hundred nanometers in diameter, we have termed them nanobrains. This raises a question as to what is a brain. If a brain is an organ that uses sensory information to control motor activity, then the bacterial nanobrain would fit the definition.

Neurobiologists have difficulty with this concept, however. They argue that bacteria are too small and too primitive to have brains — brains are relatively large, complex, multicellular assemblies with neurons. On the other hand, neurobiologists have no problems with the concept of artificial intelligence and machines that function as brains. If one considers the evolution of machine intelligence, it is obvious that size and apparent complexity are a poor measure of processing capacity. After all, the small computers of today are much more powerful than their larger and superficially more complex predecessors.

The idea that bacteria are primitive is also a false notion; perhaps derived from the same source that leads one to believe that big is better where brains are concerned. Bacteria have been evolving for billions of years longer than animals, and with their short generation times and enormous population sizes, bacterial systems are likely much more highly evolved than anything the animal kingdom has to offer.

In attempting to assess bacterial intelligence one runs up against fundamental questions of individual versus population behavior. One normally only considers average behaviors. Because of the huge range of non-genetic individuality in bacterial populations, however, among hundreds of bacteria swimming up an attractant gradient, some swim continuously in the preferred direction. Are these individuals making all the right moves by accident? And what about the few that swim in the 'wrong' direction, down the attractant gradient? Furthermore, besides being attracted to nutrients in their environments, bacteria secrete signaling molecules so that they tend to associate into multicellular assemblies wherein there are further social interactions leading to processes such as biofilm formation and pathogenesis.

Though well characterized with respect to its individual components, the intricacies of the interactions among the components of the chemotaxis system have only just begun to be considered and appreciated. It seems prudent at this time to leave open the question of how smart bacteria really are until we have a more thorough understanding of what they might be thinking, and how much they might be talking with one another.

Key references

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