Bacterial Flagella: Flagellar Motor

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Secondary article

The bacterial flagellar motor is a rotary molecular motor situated in the cell envelopes of bacteria that is driven by a flow of charged ions across the bacterial plasma membrane. The motor powers the rotation of helical flagellar filaments so that bacteria swim, frequently towards a more favourable location.

Introduction

Many species of bacteria actively seek out favourable conditions for growth by swimming up gradients of nutrients, oxygen, light or other attractants, or down gradients away from toxic substances (repellants). Different species employ several different modes of swimming, almost all of which are driven by the rapid rotation of helical flagellar filaments that protrude from the cell. Rotation of each filament is driven by a motor located in the cell envelope at the base of the filament. This motor is powered by the flux of ions across the plasma membrane – either hydrogen (H^+) or sodium (Na^+) ions, depending on the organism. Ions are driven directly by their electrochemical potential gradient across the membrane; hydrolysis of adenosine triphosphate (ATP) is not necessary for the motor to work. The rotating heart of the motor is a set of rings spanning the cell envelope, about 45 nm in diameter and containing several hundred molecules of about a dozen different proteins. This article covers what is known about how this remarkable protein machine generates torque, and how its control in *Escherichia coli* and *Salmonella enterica typhimurium* allows these bacteria to respond to gradients of chemical attractants, a process known as chemotaxis.

Flagellar Proteins

The products of about 50 genes are involved in producing and controlling flagellar motors in the enteric bacteria *E. coli* and *S. enterica typhimurium*, for which the workings of the bacterial flagellar motor are best understood. Of these, about a dozen are responsible for the detection and processing of chemical gradients; a further 17 or so form the physical structure of the flagellum, and the rest are needed to coordinate the assembly of workable flagella. None of the structural proteins has been crystallized, and the layout of the motor shown in **Figure 1** is based on electron microscopy (Coulton and Murray, 1978; Francis *et al*., 1989). Some uncertainty exists as to the shape and placement of both the export apparatus and the torque-generating units, and the exact site of torque generation is not known.

Export apparatus

Much of the flagellum lies outside the cytoplasmic (plasma) membrane, including the filament, hook, L and P rings, and rod. The proteins that form these structures are exported through a channel at the centre of the growing flagellum. An export apparatus located at the base of the flagellum has been postulated to drive this process, which can deliver protein monomers for addition to the distal end of a filament, which can be up to 10 μm long. The location and nature of this apparatus are unknown, but it is believed to consist of the proteins FliH, I, A, O, P, Q and R and possibly some components of the C ring.

MotA/B torque-generating units

Any rotary motor can be divided into two parts: the rotor and the stator. The rotor is the part that rotates whereas the stator is the fixed component against which the rotor rotates. In the flagellar motor the rotor consists of the C, MS, L and P rings and the rod, collectively known as the basal body. The stator consists of a circle of up to about 12–14 particles arranged around the MS and C rings in the cytoplasmic membrane, and anchored to the cell wall. If either of the genes *motA* or *motB* is deleted, the rings are no longer observed and the cells produce flagella that do not rotate, but are otherwise normal. If the missing *mot* genes are restored, the particles return and flagella begin to rotate again, starting off slowly and accelerating in up to eight equal speed increments (Blair and Berg, 1988). This evidence indicates that the particles are independent torque-generating units, perhaps two particles per unit, consisting of at least the proteins MotA and MotB. MotA is believed to consist of four membrane-spanning helices and a cytoplasmic domain that interacts with the rotor to generate torque, whereas MotB is believed to contain one membrane-spanning region and a periplasmic domain that anchors the stator units to the cell wall. Between them,

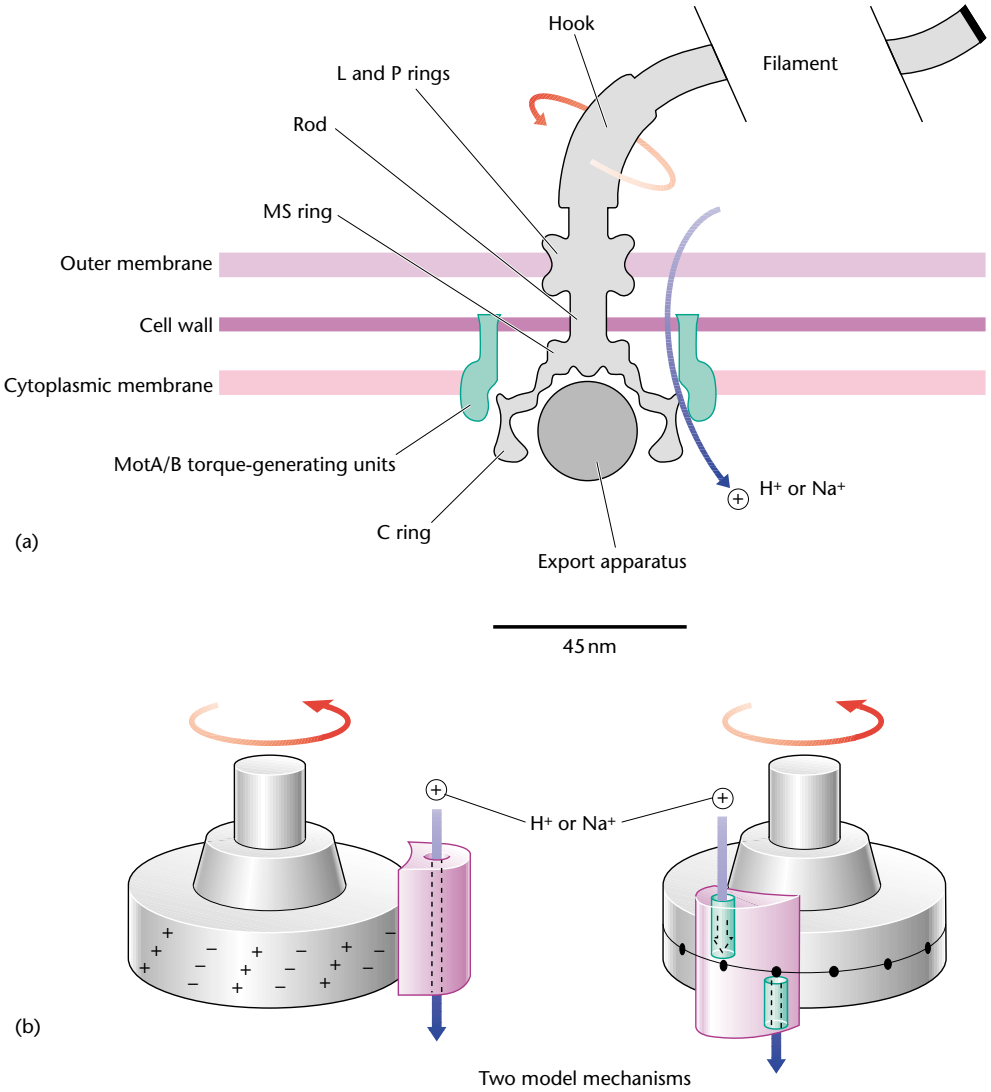

Figure 1 The bacterial flagellum. (a) The bacterial flagellar motor is a rotary motor that sits in the cell envelope of bacteria. It is driven by the flow of ions across the cytoplasmic (plasma) membrane, and its purpose is to rotate long helical filaments that protrude from the cell and propel swimming bacteria. The diagram depicts a Gram-negative envelope, typifying *Escherichia coli*; the L and P rings are associated with the outer membrane and thin peptidoglycan layer. In Gram-positive bacteria, flagellar basal bodies lack the L and P rings because of their thick cell walls. (b) Two models showing how the motor might work. The model on the left is like an electrostatic proton turbine, whereas that on the right is more like a turnstile.

MotA and MotB are also responsible for allowing the passage of protons into the cell through the motor.

C ring and MS ring

The MS ring is composed of the protein FliF and is connected by FliG to the C ring, which is composed of the proteins FliM and FliN. Torque is generated by interactions between the stator and some component of the MS and C rings, the most likely candidate being FliG. The exact nature of these interactions is unknown, but electrostatic forces are thought to be involved. The C ring also plays a role in controlling the direction of rotation of the flagellar motor, and possibly in the export of flagellar proteins.

Rod, L and P rings, hook and filament

The rod is composed of the proteins FlgB, C, F and G, and acts as the drive shaft that transmits rotation of the MS and C rings across the periplasm and out of the cell. The P and L rings are made of FlgL and FlgH respectively, and form a bushing through the outer membrane. The hook, composed of FlgE, acts as a flexible 'universal joint', allowing the filament to rotate while pointing in any direction outside the cell. The filament, composed of FliC, is by far

the largest part of the flagellum, and may extend up to 10 μm from the cell. FlgE monomers in the hook and FliC monomers in the filament are each arranged in helical lattices around a central pore, and the proteins FlgK and FlgL located at the hook–filament junction serve as adaptors between the different lattice patterns. FliD forms an end-cap to the filament and coordinates the polymerization of FliC monomers that have travelled to the end of a growing filament through the central pore.

A Reversible, Rotary, Electric, Molecular Motor

The flagellar motor can rotate in either direction

The bacterial flagellum truly rotates about its longitudinal axis. This is most clearly demonstrated by 'tethering' a single flagellum to a surface, in which case the flagellar motor causes the entire cell body to counter-rotate (**Figure 2b**). Cells that are tethered using antihook antibodies are able to rotate even without flagellar filaments, ruling out the possibility that they are swimming in tight circles about an inert point of attachment rather than rotating about a rotary motor (Silverman and Simon, 1974). A great deal has been learned about the flagellar motors of *E. coli*, *S. enterica typhimurium* and *Streptococcus* species using tethered cells. One of the most striking observations is that motors switch spontaneously between clockwise (CW) and counterclockwise (CCW) rotation. Individual switches occur at random times, but the probabilities of switching from one direction to the other are controlled in such a way as to allow the bacteria to perform chemotaxis. Tethered cells typically rotate at speeds of about 10 Hz (10 revolutions per second) in either direction.

Torque, speed and work

The motor rotates because it generates torque. Torque, or 'twisting force', is defined as the product of a pair of equal and opposite forces acting on an object and the perpendicular distance between the points at which they act. Consider, for example, unscrewing a nut with a long spanner. One force is applied by a hand on the end of the spanner, the other force is the equal and opposite reaction of the bolt on the nut, which stops the nut from moving in the direction of the first force and makes it rotate instead. The torque is defined as the product of the force exerted by the hand and the length of the spanner. If the hand moves a certain distance, this requires a fixed amount of energy, or 'work', equal to the force multiplied by the distance. The angle rotated, in radians, is defined as the distance divided by the length, so the work is also equal to the torque multiplied by the angle.

In the flagellar motor, the force is applied by the MotA/B particles to the perimeter of the rotor, and the torque is equal to this force multiplied by the radius of the rotor. In a tethered cell, this torque pushes the cell body through the water, which exerts a viscous drag torque on the cell that is proportional to the speed of rotation. The cell rotates at about 10 Hz, such that the motor torque and the viscous torque are balanced, and both are equal to about 3×10^{-18} Nm. The work done in one revolution is equal to this torque multiplied by 2π (the number of radians per revolution) or 2.0×10^{-17} J.

Protonmotive force

Unlike other molecular motors, which are driven by ATP hydrolysis, the bacterial flagellar motor is driven directly by the electrochemical gradient of protons across the plasma membrane – the 'protonmotive force' (PMF). The PMF is maintained by metabolic processes that use chemical energy to pump protons out of the cell, allowing them to flow back in again and drive various systems, including the flagellar motor. It consists of two components, the concentration gradient and the transmembrane voltage, and the total PMF is given by $kT/e \ln(C_1/C_2) + V_m$, where C_1 and C_2 are the internal and external proton concentrations, k is the Boltzmann constant, T the absolute temperature, e the proton charge, and V_m the transmembrane voltage (inside minus outside). The PMF is typically in the vicinity of -150 mV, which means that each proton that flows back into the cell through the motor can supply up to $-eV_m = 2.4 \times 10^{-20}$ J of free energy to drive the motor.

Experimental evidence indicates that the flow of approximately 1000 protons is coupled to each rotation of the motor. If this represents all the protons that flow through the motor (i.e. if no protons flow when the motor is not rotating), then the motor uses up about 2.4×10^{-17} J per revolution. This is only slightly more than the work done per revolution at 10 Hz (2.0×10^{-17} J), so the efficiency of the motor is very high in tethered cells. Torque is proportional to PMF in tethered cells, whether the PMF is in the form of a membrane voltage or a concentration gradient. This indicates that the efficiency remains constant, and is consistent with a model in which the total number of protons that flow through the motor in one revolution is fixed.

Torque versus speed

The motor rotates at about 10 Hz in a tethered cell. In swimming cells, however, the viscous drag coefficient of the rotating filament is much smaller than that of the cell body, and the motor rotates at over 100 Hz. A variety of methods

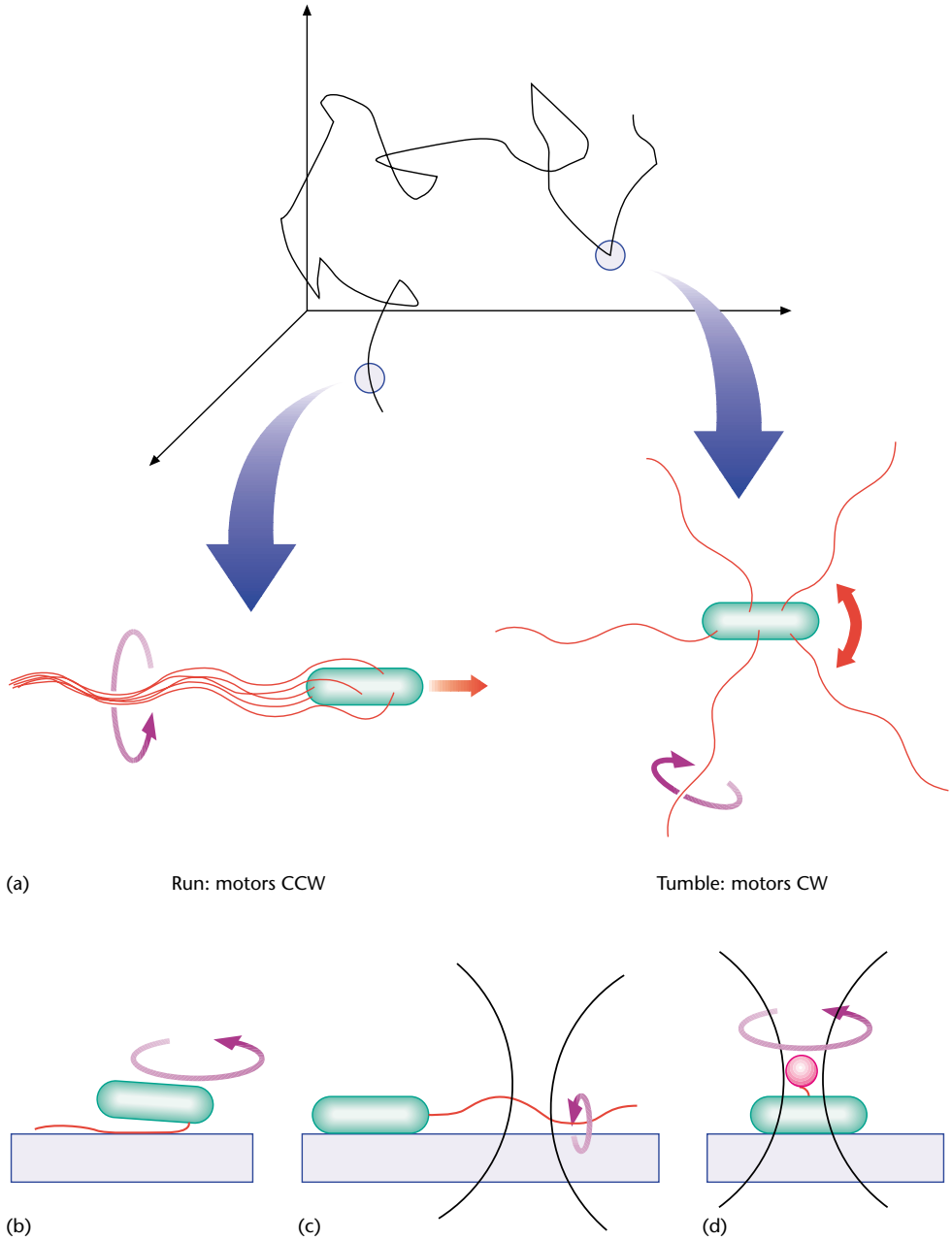

Figure 2 Swimming and flagellar rotation in *Escherichia coli*. (a) Cells of the bacterium *E. coli* swim in a series of 'runs' and 'tumbles'. In a run, flagella rotate counterclockwise (CCW) and form a bundle that propels the cell. In a tumble, flagella rotate clockwise (CW) and the bundle flies apart, causing the cell to jiggle on the spot. The rotation of individual flagellar motors can be measured using (b) tethered cells, (c) laser dark-field microscopy or (d) by attaching beads to flagella.

has been developed to measure the performance of the motor at these high speeds (see **Figure 2**). Vibrations of the cell body at the speed of the rotating flagellar bundle can be detected simply by measuring fluctuations in the light transmitted by a population of swimming cells. For more precise measurements, the rotation of flagellar filaments can be observed by laser dark-field microscopy, or small beads can be attached to truncated flagella and their rotation detected by the deviation they introduce to a laser beam (their rotation causes them to wobble from side to side). In these experiments, the speed of the motor can be varied by increasing the viscosity or by observing different sizes of bead or filament. Motor speed has also been varied by applying an external torque to a tethered cell, using

techniques such as electrorotation or 'optical tweezers' (Berry and Berg, 1997).

The relationship between the torque generated by the motor and speed is linear for speeds of up to at least 100 Hz, whether the motor is rotating forwards or being forced to rotate backwards by an external torque. Experiments using electrorotation suggest that in this range of speeds the torque is almost constant, but this result needs to be confirmed by other methods. At higher forward speeds, the torque falls more sharply with increasing speed. The motors in a swimming cell, rotating at between 100 and 200 Hz, generate about ten times less torque than those in a tethered cell rotating at 10 Hz, and if the speed is increased further the motor torque falls to zero. The zero-torque speed increases with increasing temperature, indicating that a thermally activated process within the motor becomes rate limiting at high speeds. If the motor is made to rotate still faster, the torque becomes negative and the motor resists rotation.

Models of the motor mechanism

It is not known how the motor uses the flow of ions across the membrane to generate torque. It is possible that the motor works in a similar way to ATP-driven molecular motors, such as myosin and kinesin. In this case, the MotA/B stator units would bind to the rotor, undergo a conformational change to make them exert a force on the rotor, and then detach from the rotor. Each time this cycle was repeated, some fixed number of protons would cross the membrane, and the energy they released would be used to drive the conformational changes that generate torque. Unlike other molecular motors, however, the driving force for the flagellar motor is directional in nature, with protons flowing into the cell at the periphery of the rotor. In view of this, it is possible that the motor operates on quite different principles.

Two possible mechanisms are illustrated in **Figure 1**. The mechanism on the left is like a 'proton turbine'. Charged protons flow through channels in the stator and exert electrostatic forces on helical lines of charges on the rotor. These forces will tend to keep lines of negative charges close to the positively charged proton, causing the rotor to rotate as protons flow through the stator. The mechanism on the right is more like a 'turnstile'. Protons bind the rotor, introduced from outside the cell by channels in the stator. In order to pass into the cell they must be transferred to a second set of channels by rotation of the rotor, so that rotation and proton flow are coupled. Structural studies using electron microscopy and site-directed mutagenesis, and functional studies measuring torque, speed, PMF and single molecular events on the rotation of the flagellar motor, will be needed to determine whether the motor operates by binding and conformational change or by some other mechanism, such as those in **Figure 1**.

Regulation of the Motor

Running and tumbling

E. coli swims in a series of 'runs' and 'tumbles' (**Figure 2**; Berg and Brown, 1972). During a run, the flagella rotate CCW and form a bundle that pushes the cell along. Runs are separated by tumbles during which the flagella rotate CW, causing the bundle to fly apart and the cell to jiggle on the spot. Runs typically last several seconds; tumbles are shorter. Because of its small size, the constant buffeting of the cell by water molecules (Brownian motion) prevents it from running in the same direction for more than about 3–4 s. During a tumble, the cell becomes reoriented even faster than this, as the different flagella push and pull it in all directions. The result is that each time a tumbling cell starts its next run, it heads off in a random direction. This pattern of almost-straight lines and random changes of direction forms the basis of chemotaxis.

Responses to attractants

How can a cell swim up a concentration gradient of attractant? The difference in concentration from one end of the cell to another is too small to be detected for most reasonable gradients, so the cell cannot decide which way to swim based on local information alone. Instead, cells respond to changes with time, or temporal gradients. If a cell is running up a concentration gradient, it detects the increasing concentration of attractant and delays the onset of the next tumble. Runs that do not carry the cell to higher attractant concentrations are shorter than those that do, and in this way the cell performs a biased random walk that leads it towards higher attractant concentrations.

To determine whether or not the concentration of an attractant is increasing, the cell must be able to detect the current concentration and also to remember what it was a short while before, for comparison. That cells do exactly this was demonstrated in a series of experiments in which tethered cells were exposed to changing concentrations of attractants while the direction in which they rotated was measured (Segall *et al*., 1986). In response to sudden increases in attractant concentration, the probability of CCW rotation (leading to a run) increases over the course of about 1 s and remains raised for 3–4 s before adapting (i.e. returning to its prestimulus value). Adaptation ensures that the CCW probability remains increased, and thus runs prolonged, only while the concentration continues to increase. In effect, the cell calculates whether the concentration of attractant is greater than it was a few seconds ago, and if so it increases the CCW probability of its motors. The timescale on which runs, tumbles and attractant responses occur has been optimized by evolution to match the timescale over which a cell can swim in a particular direction before being shaken off course by Brownian motion.

The Chemotaxis Pathway

The response of *E. coli* to attractants is extremely sensitive and covers a large range of concentrations. The binding of a few (possibly only a single) attractant molecule(s) to receptor molecules on the cell surface can cause measurable changes in the probability of CCW rotation, and cells are able to respond to attractant concentrations ranging from 1 μmol L^{-1} to 100 mmol L^{-1}. A great deal of work has been done to understand the biochemistry that underlies these responses, and indeed the chemotaxis pathway in *E. coli* may be the best understood network of signalling protein interactions that exists at present.

The chemotaxis pathway is summarized in **Figure 3**. The key elements of the pathway are the chemoreceptors (also known as methyl-accepting chemotaxis proteins (MCPs)), the response regulator CheY and the flagellar motor itself. Each of these elements can exist in two states. The MCPs can be either active or inactive, CheY can be phosphorylated or dephosphorylated, and the motor can rotate CW or CCW. When active, the MCPs enhance the autophosphorylation of CheA, which is bound to the MCPs in a complex with CheW. CheA transfers its phosphoryl group to CheY, and phosphorylated CheY (CheY-P) increases the probability of CW rotation of the motor (Barak and Eisenbach, 1992). Binding an attractant reduces the activity of the receptors. Thus increases in receptor occupancy lead to reduced CheA and CheY phosphorylation and more CCW rotation, extending runs, whereas reductions in receptor occupancy have the opposite effect.

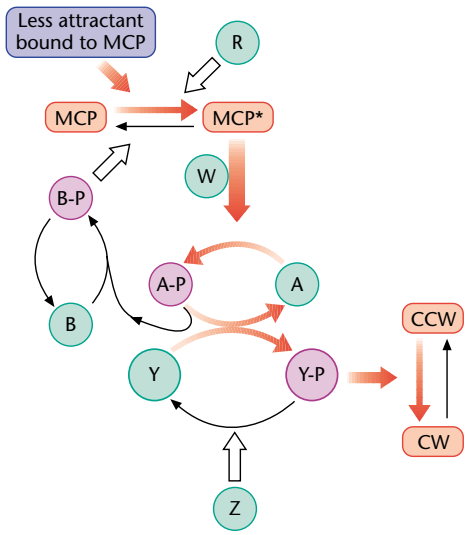

Figure 3 The chemotaxis pathway in *Escherichia coli*. Bacteria modulate the probability direction of rotation of their flagellar motors in response to changes in the concentration of chemicals in the environment. This allows them to swim towards nutrients or away from harmful chemicals, a process known as chemotaxis. The biochemical pathway that controls this response consists of membrane-bound receptors (also known as methyl-accepting chemotaxis proteins (MCPs)) and the chemotaxis proteins CheW, A, Y, Z, B and R. The curved arrows represent phosphorylation and dephosphorylation reactions; the thin straight arrows represent transitions between alternative states of the MCPs or of the flagellar motor; and the wide straight arrows represent positive influences of one element in the pathway upon a particular transition. The arrows in red highlight the sequence of events that generates an increase in the probability of clockwise (CW) rotation in response to a reduction in the amount of attractant bound to an MCP. CCW, counterclockwise.

CheR and CheB

Adaptation occurs because of negative feedback from CheA to the MCPs, via the methylesterase, CheB. Like CheY, CheB receives a phosphoryl group from CheA, and phosphorylated CheB removes methyl groups from specific sites on the MCPs, decreasing their activity. Adaptation also occurs directly at the level of the MCP. In the active conformation, the level of methylation of the MCP is reduced by changes in the activity of CheB and/or the methyltransferase, CheR, which replaces methyl groups removed by CheB. Changes in the methylation level of MCPs take place on a timescale of several seconds, and this process constitutes the short-term 'memory' that allows cells to perform the temporal comparisons of attractant concentration that are necessary for chemotaxis.

Receptors (methyl-accepting chemotaxis proteins)

There are four different receptors in *E. coli*, each of which responds to a specific set of chemicals. The receptor proteins consist of periplasmic and cytoplasmic domains connected by a membrane-spanning domain, and exist in the form of homodimers. The structure of the periplasmic domain of the aspartate receptor Tar has been determined by X-ray crystallography. Each monomer is a four-helix bundle, with two of the helices extending to cross the membrane. The long helices from the two monomers contact each other, forming another four-helix bundle. The sequences of the different receptors are homologous, most closely in the cytoplasmic domains, and less so in the periplasmic domains.

Chemical effectors can bind directly to the periplasmic domain of the receptor, or to a periplasmic protein that subsequently binds to the receptor. (For example, the Tar receptor can respond directly to aspartate, indirectly to maltose via a maltose-binding protein, and also to certain repellants.) Conformational changes that occur on ligand binding are transmitted through the membrane to the cytoplasmic signalling domain of the receptor by movements within or between the two subunits. Each receptor can be methylated at several distinct sites in the cytoplasmic domain. As well as allowing adaptation, methylation may be partly responsible for the wide range of attractant concentrations that bacteria respond to, by altering the affinity of the receptor for ligands.

CheA and CheW

In vivo, the MCPs, CheW and CheA form stable membrane-associated complexes. CheW is believed to link CheA to the MCPs, and to mediate the stimulation of CheA autophosphorylation by active MCPs. CheA autophosphorylation appears to be the most important control point in the chemotaxis pathway, with a rate that is 100-fold higher in the presence of CheW and MCPs than in the pure protein. The transfer of phosphoryl groups to CheY occurs when CheY binds to the MCP–CheW–CheA complex. Once phosphorylated, CheY-P leaves the complex, but CheW and CheA remain associated with the MCPs for many cycles of CheA autophosphorylation and phosphotransfer to CheY.

CheY, CheZ and motor switching

The transfer of phosphoryl groups between histidine residues on CheA and CheY is an example of a common signalling strategy found in bacteria and also, recently, in eukaryotes. CheA belongs to a family of 'sensory kinases' and CheY to a family of 'response regulators'. Sensory kinases consist of a sensory domain that detects some environmental stimulus (via MCPs and CheW in the case of CheA) and a transmitter domain involved in autophosphorylation and phosphotransfer to a response regulator. Response regulators have one domain for phosphotransfer and another to produce the desired environmental response, which most often involves gene regulation, but in the case of CheY is the control of motor direction. CheB is another response regulator, in which the effector domain removes methyl groups from MCPs at a rate that is enhanced 15-fold by phosphorylation of the other domain.

Until recently it was believed that CheY-P was necessary for CW rotation, since flagellar motors rotated exclusively CCW in mutants lacking CheY. It is now known that the motor is intrinsically able to rotate in either direction, but is heavily biased towards CCW rotation at room temperature and in the absence of CheY-P. The bias can be shifted towards CW rotation either by the binding of CheY-P or by reducing the temperature to close to 0°C (Turner *et al*., 1996). CheY-P binds to some component of the 'switch complex', most probably the protein FliM in the C ring, and lowers the free energy of the CW state relative to that of the CCW state, leading to an increased probability of CW rotation. The nature of the difference between these two states is unknown, but the temperature dependence of the free energies calculated from switching probabilities indicates that there is a decrease in entropy in the transition from CCW to CW states. Switching occurs very rapidly and without intermediate states of reduced speed, which suggests that a cooperative transition involving the entire rotor is responsible.

CheZ increases the rate of dephosphorylation of CheY-P. It is not clear why this is necessary, and one possibility is that the activity of CheZ is also modulated by sensory cues, providing an alternative pathway for the generation of CCW responses.

Computer modelling of the chemotaxis pathway

The chemotaxis pathway in *E. coli* is well characterized. The proteins involved are known and the rates of reactions that occur between them have been measured *in vitro*. A large number of mutations have been made in many of the chemotaxis proteins, and the effect of these mutations on the output of the pathway can easily be measured using tethered cells. This has allowed the creation of a computer model that attempts to describe in detail the entire chemotaxis pathway (Bray *et al*., 1993), which has been chosen as a representative example of the networks of interacting proteins that are everywhere in living cells. The model is able to predict many of the observed phenotypes of a large range of mutants, but perhaps it is most valuable where it fails, as this helps to focus attention on the areas where new explanations are needed.

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