9
Behavioral Hardware

Components

Suppose you discovered a computerized factory turning out small cars, and you wanted to know how those cars were assembled and how they functioned. One way to identify essential components would be to remove those components one at a time and then characterize the resulting defects. For example, if you removed the drive shaft, the engine would run but the drive wheels would not turn, so the car would be paralyzed. If you knew the computer program, you could do this at will by removing the instructions for fabrication or assembly of drive shafts. If you did not know those instructions, or indeed even what a car might be, you could still learn a great deal by changing the program at random (e.g., by making mutants). This is how things proceeded in the early days of bacterial chemotaxis. One mutagenized cells, isolated mutants with interesting defects (e.g., cells with flagella that failed to spin), and then mapped the gene. Given the gene, one could identify the gene product. Now things are much easier. The genetic program is known in detail, and one can modify it in any way that one desires. For example, one can amplify a specific gene by using the polymerase chain reaction (PCR), change its sequence at will, and put it back into the chromosome by homologous recombination. Or one can paste the gene into a multicopy plasmid behind a strong promoter and express the gene product at high concentrations. The techniques for doing these things are straightforward, but outside the scope of this book. The essential point is that one can use genetics to identify and manipulate components (proteins) involved in any cellular process, including bacterial chemotaxis. The parts required for motility and chemotaxis are described in this chapter. The way in which the genetic map is read and these gene products are assembled is described in the next chapter.

Mutations affecting chemotaxis have specific phenotypes (behavioral defects), and genes tend to be named for those defects. In some cases, where the gene was identified first in another context (e.g., the gene for the maltose binding protein, *malE*, involved in maltose transport), the name is foreign to chemotaxis. In most cases, however, the abbreviation is closer to home; for example, *trg*, for taxis toward ribose or galactose; or *cheA*, for the first gene identified with a generally nonchemotactic phenotype—*che* cells swim but do not make chemotactic rings or respond in the capillary assay; or *motB*, for the second gene identified with a defective motility phenotype—*mot* cells make flagella, but these flagella fail to spin; or *fliF*, for a gene required for flagellar synthesis. The early flagellar mutants were named *flaA*, *flaB*, etc., but the alphabet proved too short, so now they are called *flg*, *flh*, *fli*, and *flj*, depending on their location on the chromosome (Iino et al., 1988). When one refers to the gene product, that is, the protein specified rather than the gene, the first letter is capitalized and italics are not used. Names appear in this form in the parts lists given in the appendix. Table A.1 in the appendix lists components involved in chemoreception, Table A.2 lists components involved in signal processing, and Table A.3 lists components involved in motor output. Components of different types or subtypes are listed alphabetically.

Signaling Pathway

The sensory transduction pathway is shown schematically in Fig. 9.1, where the information flow is from left to right. The same system is depicted four times: each row of the figure illuminates a different aspect of the mechanism, as explained in the figure legend. The basic scheme, shown in row 1, is typical of a number of so-called two-component signaling pathways in bacteria, in which information, embodied by a phosphate group, is passed from a histidine kinase to an aspartate kinase (Parkinson and Kofoid, 1992). These components are named for the amino acid residues that carry the phosphate. The histidine kinase is coupled to a sensor, and the aspartate kinase (also called a response regulator) is coupled to an effector. In pathways involving gene regulation, the effector interacts with a particular transcriptional control element. In chemotaxis, there are two effectors, the rotary motor and a methylesterase, an enzyme that demethylates the

receptor, as shown in rows 2 and 3. The response regulator that interacts with the motor diffuses to its base, where, if phosphorylated, it binds and increases the probability of clockwise (CW) rotation. The response regulator that activates the methylesterase comprises the N-terminal domain of the same protein; if phosphorylated, it activates the C-terminal domain, which carries the catalytic site. During adaptation to rising concentrations of attractants, the receptor is methylated by a methyltransferase; during adaptation to falling concentrations of attractants, the receptor is demethylated by the methylesterase. The different proteins that make up this system are named in row 4, where the example given is for taxis toward the sugar maltose and the amino acid aspartate.

It is worth noting the location of this hardware within the cell plan shown in Fig. 8.1. Tar spans the inner membrane. Aspartate or MalE binds transiently at its periplasmic end. Aspartate finds Tar and maltose finds MalE by diffusing through the porins in the outer membrane. MalE is confined to the periplasmic space. CheR binds transiently to the C-terminal end of Tar, within the cytoplasm at a site located in between the inner membrane and the innermost end of the receptor. CheW and CheA bind at the innermost end of the receptor to form a stable complex. Che B and CheY bind to CheA until phosphorylated, and then they diffuse freely within the cytoplasm. FliM is a component at the cytoplasmic face of the flagellar motor. There are several motors distributed at random along the sides of the cell, each of which penetrates the cell wall.

So, we have two kinds of sophisticated protein machines, both embedded in the inner membrane: the receptor complex and the flagellar motor. They are coupled by diffusion of a small cytoplasmic protein, activated by phosphorylation.

Receptor Complex

The receptor named in row 4 of Fig. 9.1, Tar (for taxis toward aspartate or away from certain repellents), is in a class of receptors known as methyl-accepting chemotaxis proteins (MCPs), all of which span the cytoplasmic membrane (see Table A.1). Another class of membrane receptors (not shown in the figure) phosphorylate their substrates and transport the derivatives. They are part of the phosphotransferase system (PTS). A novel receptor, Aer, related to the MCPs, carries a flavin adenine dinucleotide

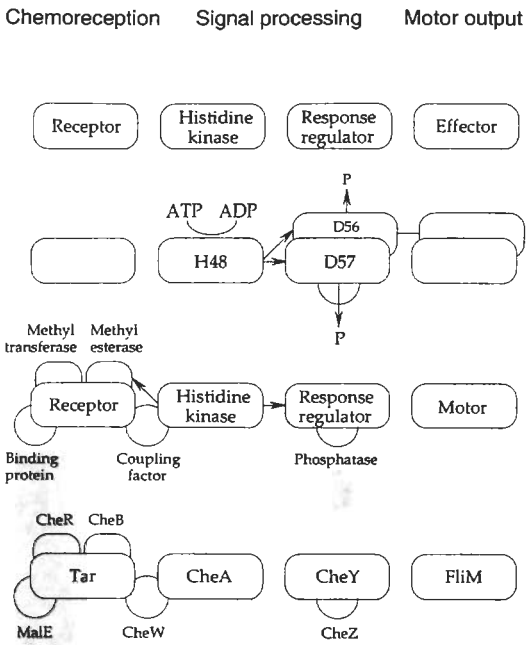

FIGURE 9.1. The sensory transduction pathway, shown in block form, repeated four times. Information flows from left to right.
Row 1: *Basic scheme*. An attractant molecule (the ligand) binds to a receptor at the outer surface of the inner membrane (in the periplasmic space). This changes the level of activity of a cytoplasmic histidine kinase that phosphorylates two response regulators (also called aspartate kinases). These, in turn, act on two effectors. The effector for the first response regulator is the flagellar motor. The effector for the second response regulator is an enzyme (a methylesterase) that targets receptor methyl groups. Interactions between the response regulators and their effectors change the probabililty that the motor spins clockwise and the activity of the methylesterase, respectively.
Row 2: *Phosphate flow*. The histidine kinase catalyzes the transfer of inorganic phosphate from adenosine triphosphate (ATP) to its own histidine-48 (H48), leaving ATP as the diphosphate (ADP). The first response regulator (shown in front) catalyzes the transfer of phosphate from H48 to its own aspartate-57 (D57), and the second response regulator (shown in the back) catalyzes the transfer of phosphate from H48 to its own aspartate-56 (D56). Hydrolysis of D57-P (removal of the phosphate) is accelerated by another enzyme (a phosphatase). Hydrolysis of D56-P occurs spontaneously; it is not catalyzed by a phosphatase. The effector for the second response regulator (the methylesterase) is the C-terminal domain of the same protein, so it is shown connected to the response regulator by a horizontal line.

that serves as a redox sensor; however, this receptor is not methylated (Taylor et al., 1999).

As noted above, Tar, CheW, and CheA form a complex, a supramolecular machine, shown schematically in Fig. 9.2. Early studies of isolated components suggested that each complex comprises two molecules of Tar, two molecules of CheW, and two molecules of CheA (or possibly one molecule of $CheA_S$ and one of $CheA_L$; see Table A.2, note c); however, the exact stoichiometry is still a matter of debate. CheA and CheW bind at the extreme intracellular end of the Tar dimer, and CheR binds to a pentapeptide at the Tar C-terminus. CheB binds to a domain in CheA downstream of H48, as does CheY.

Tar is made up of a string of α-helical segments, denoted $\alpha 1$ through $\alpha 9$ (Kim et al., 1999). Helix $\alpha 1$ (also called TM1, for transmembrane 1) starts at the inner face of the cytoplasmic membrane, crosses this membrane, and extends into the periplasm, where with helices $\alpha 2$ to $\alpha 4$ it forms an antiparallel 4-helix bundle. Helix $\alpha 4$ (also called TM2) goes back through the membrane and is connected by a linker region that includes $\alpha 5$, to the remaining helices, $\alpha 6$ to $\alpha 9$. These fold back onto one another and with helices $\alpha 6$ to $\alpha 9$ of the other copy of Tar form a second antiparallel 4-helix bundle. Helices $\alpha 6$ and $\alpha 9$ and their

Row 3: *Additional components*. The response regulator/methylesterase has been redrawn as a single component at the left, in contact with the receptor, with which it interacts. The arrows from the histidine kinase indicate phosphate transfer from H48 to D57 and D56, as before. Additional components include periplasmic binding proteins, required for chemotaxis toward certain sugars or dipeptides and away from nickel, a coupling factor required for activation of the histidine kinase, and a methyltransferase that methylates the receptor. The phosphatase, shown earlier, is now labeled as such.
Row 4: *Complete system*. This is shown for chemotaxis toward the disaccharide maltose and the amino acid aspartate. The receptor Tar binds aspartate and the maltose binding protein when the latter carries maltose. It also binds the nickel binding protein when it carries Ni^{2+} (not shown). CheR is the methyltransferase, CheB the methylesterase (both domains), CheW the coupling factor, CheA the histidine kinase, CheY the response regulator that when phosphorylated binds to the flagellar motor, CheZ the phosphatase that accelerates the dephosphorylation of CheY-P, and FliM the component at the base of the flagellar motor to which CheY-P binds.

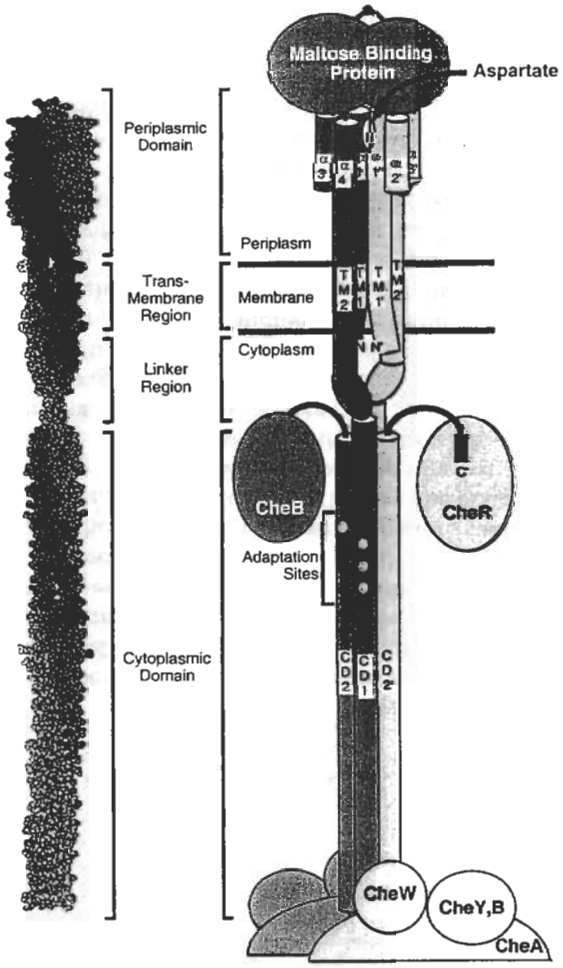

FIGURE 9.2. *Left*: A space-filling model of the Tar receptor dimer. It is 38 nm long by 2.5 nm in diameter (at the intracellular end). *Right*: A cartoon of the Tar receptor complex, including CheB, CheR, CheA, and CheW, drawn to the same scale. The dimeric association is stable; one set of components is more darkly shaded. Aspartate and the maltose binding protein are in rapid association-dissociation equilibrium with Tar. Aspartate binds in a cleft between α-helices $\alpha 1$ and $\alpha 1'$. The maltose binding protein has two domains connected by a hinge. The hinge closes when maltose binds, and then the protein binds at the periplasmic tip of the Tar dimer. Che R, CheB, and CheY also come and go, with the affinity between CheA and the phosphorylated products, CheY-P and CheB-P, substantially reduced. CheR binds to a short peptide, part of a flexible

homologs form a methylation domain—the sites of methylation, called adaptation sites in Fig. 9.2, are glutatmate side chains—while helices $\alpha 7$ and $\alpha 8$ and their homologs form a signaling domain. These domains are highly conserved between members of the MCP class.

Figure 9.2 shows the maltose binding protein and aspartate interacting with this receptor complex. The maltose binding protein has two domains connected by a multistrand hinge, as indicated in the figure. Maltose binds in a cleft between the two domains, and the hinge closes. Following this event, the protein binds at the extreme extracellular end of the Tar receptor complex. Aspartate binds in a cleft between $\alpha 1$ and its homolog $\alpha 1'$. Two binding sites are possible, but binding at one site markedly reduces the affinity of binding at the other. A great deal of work has gone into determining the change in structure that carries information about binding across the cytoplasmic membrane. The majority view is that it is a surprisingly small (0.16 nm) piston movement (toward the cytoplasm) of helix $\alpha 4$ of only one of the Tar subunits (Falke and Hazelbauer, 2001). But not all agree (Kim et al., 2002).

In any event, when ligand binding occurs, the activity of CheA is reduced, and the rate of CheY-P production falls. Since CheY-P is hydrolyzed, its concentration falls, and less CheY-P binds to the base of the flagellar motor (to FliM). Therefore, the motor is more likely to spin counterclockwise (CCW), and runs are extended. In addition, the change of structure in the methylation domain increases the activity of the methyltransferase, CheR, and the reduced activity of CheA decreases the concentration of CheB-P, the active form of the methylesterase. Therefore, more glutamate side chains are methylated. This acts like a volume control to compensate for the effect of chemoattractant binding, and the activity of CheA returns to its initial value. Thus, if cells are exposed to a step-change in the concentration of maltose or

chain at the C-terminus of Tar, in a position where it can reach the methylation sites. These are shown as lighter gray dots (one set of four, labeled Adaptation Sites). CheB was thought to bind in a similar way (as shown) but is now known to bind more tightly to CheA. TM, transmembrane helix; CD, cytoplasmic domain. Proteins other than Tar are shown as ellipsoids, with CheA truncated to save space. [Courtesy of Joseph Falke, who used the space-filling model of Kim et al. (1999).]

aspartate, they eventually adapt. Addition of methyl groups is a relatively slow process, regulated by the shape of the Tar substrate. Removal of methyl groups, on the other hand, is a relatively fast process, catalyzed by CheB-P and regulated by CheA. At steady state, the rates of methylation and demethylation balance, and methylation levels are constant.

The receptor complex shown in Fig. 9.2 is a remarkable system that acts as a comparator. The output of this comparator (the kinase activity) depends on the difference between the time-average occupancies of the receptor binding sites and the level of methylation. The kinase is activated if the methylation level is relatively high and inactivated if it is relatively low. Changes in the occupancies of the receptor binding sites are very fast, and reflect the present concentrations of ligands. Changes in the levels of receptor methylation, on the other hand, are relatively slow, and reflect the past concentrations of ligands. Thus, the cell is able to make temporal comparisons. If the concentration of attractant increases steadily with time, for example, as it does when a cell swims up a spatial gradient of aspartate, the receptor occupancy rises accordingly, and the system goes out of balance. The methylation level lags behind receptor occupancy, and the kinase is slightly inactivated. Therefore, favorable runs are extended. When the cell swims down a spatial gradient of attractant, the receptor occupancy falls accordingly. But now, since demethylation is rapid, the methylation level drops rapidly, as well, and the system remains more closely in balance. Thus, the cell tends to tumble as often as it does in the absence of a stimulus. (But this is not the whole story, because, as discussed earlier, there is a threshold for repellent stimuli below which no behavioral changes can be detected.)

CheY

The structures of all of the components shown in Fig. 9.1, row 4 (except for the N-terminal domain of FliM) have been determined by x-ray diffraction or nuclear magnetic resonance. For a review of some of this work, see Falke et al. (1997). We already have seen one example taken from that source: Fig. 8.3 shows a ribbon diagram of CheY. The autocatalytic aspartate kinase pocket is at the top, formed by loops at the end of the β-sheet, with aspartate-57 shown in space-filling spheres. Overlapping domains of the

surface of the molecule interact specifically with other components of the transduction system, with CheA on the left, CheZ on the right, and FliM in the middle. This is one of the smallest components of the chemotaxis system (molecular weight 14,000), a protein optimized for diffusion. Since CheY-P is unstable, its structural analysis has required major feats. The structure of activated CheY bound to the N-terminal 16 residues of its target, FliM, has been determined by x-ray diffraction of a stable beryllium fluoride derivative (Lee et al., 2001). As for Tar, the differences in structure between inactive and active forms appear to be subtle.

Flagellar Motor

The flagellar motor is shown schematically in Fig. 9.3. The electron micrograph is of the part of the motor attached to the hook that survives extraction with neutral detergents. The image has been rotationally averaged: it is what you would see if you could look through this part of the motor as it rotates. Structures outside the cell wall include the filament (the propeller), which can be up to about 10 μm long, and the hook (a flexible coupling, or universal joint). Structures embedded in the cell wall comprise the basal body and include several rings and a rod. The outer pair of rings (FlgH, called the L-ring, for lipopolysaccharide, and FlgI, called the P-ring, for peptidoglycan), is thought to serve as a bushing that gets the rod (FlgB, FlgC, FlgF, and FlgG) through the outer membrane. The rod serves as the drive shaft. Other bacteria that do not have an outer membrane, so-called gram-positive cells, do not have the outer pair of rings. And mutants of *E. coli* in which these rings are missing are motile, provided the hook protein (FlgE) is overproduced. Therefore, the L and P rings are not involved in torque generation. The inner pair of rings, formerly called M (for membranous) and S (for supramembranous) are now called MS, because they are the product of a single gene, *fliF*. An additional ring (called the C-ring, for cytoplasmic) comprises part of a switch complex (FliG, FliM, and FliN) that controls the direction of flagellar rotation. These components are also implicated in torque generation. The interaction of CheY-P with FliM stabilizes the state in which the filament, viewed along its helical axis looking toward the cell, spins CW. At room temperature, the null state is CCW.

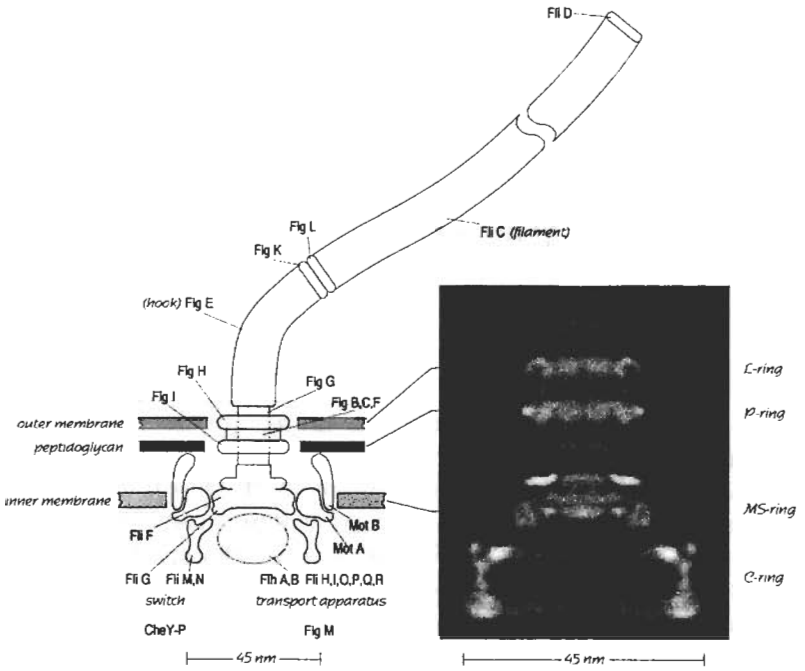

FIGURE 9.3. A schematic diagram of the flagellar rotary motor, drawn to scale. Inset: Rotationally averaged reconstruction of electron micrographs of purified hook-basal bodies. Compare Table A.3. The signaling molecule CheY-P, which binds FliM, is shown at the lower left. FlgM (lower right) blocks the activity of a sigma-factor that activates late genes. FlgM is pumped out of the cell via the transport apparatus once the basal part of the motor is complete. (Image reconstruction courtesy of David DeRosier, Brandeis University.)

It is not clear to me why the apparatus that controls the direction of rotation is called a switch. I think of a switch as something that turns an electric motor on and off, although one could have a switch that changes the sign of the current flow in its windings and, thus, its direction of rotation. Gear shift might be more appropriate, but there are no gears or transmission. However, the flagellar motor is driven by an electric current: in *E. coli*, this is a flow of protons down an electrochemical gradient, from the outside to the inside of the cell. In marine bacteria or certain bacteria that live at high pH (where protons are scarce), it is a flow of sodium ions. It is thought that protons travel from the periplasm to the C-ring/MS-ring complex via a channel that crosses the cytoplasmic membrane, mostly in MotA, causing the cytoplasmic part of MotA to pull on FliG at the periphery of the MS-ring. MotB links MotA to the peptidoglycan layer, that is, to the rigid framework of the cell wall. If this view is correct, then the C-ring/MS-ring complex serves as the rotor and MotA/MotB serves as the stator. More will be said about this in Chapter 12.

Flagellar Filament

Flagellar filaments are polymers of identical subunits, molecules of FliC, also called flagellin (named by Astbury et al., 1955). This protein can be obtained in monomeric form by removing filaments from cells mechanically, suspending them in physiological saline, and heating to 60°C. And filaments can be reconstituted from such a solution (Abram and Koffler, 1964; Asakura et al., 1964). The flagellum was recognized as an organelle of locomotion early on (see Chapter 2). Its filament scatters enough light to be seen in the light microscope (Reichert, 1909) and is readily resolved in the electron microscope (Piekarski and Ruska, 1939). Recall the electron micrograph of Fig. 2.5, and the fluorescence images of Figs. 5.4 and 5.5. Originally, the filament was thought to be a kind of primitive muscle, either a bending machine or a device that could propagate spiral waves. Later, it proved to be simply a propeller (Berg and Anderson, 1973; Silverman and Simon, 1974). The flagellin subunits are arranged on the surface of a cylinder in two different ways, as illustrated in Fig. 5.3. Their arrangement is hexagonal, with 1-, 5-, 6-, and 11-start helices. The 11-start helices appear as protofilaments that are nearly longitudinal. As discussed in Chapter 5, the subunits in one form (L-type, left) are farther apart, and in the other form (R-type, right) they are closer together. If filaments are constructed of only one type of protofilament, as shown in the figure, they are straight, with a left-handed or right-handed twist, respectively. If they are constructed of both types of protofilament, they are helical, with curvature as well as twist. On the assumption that the elastic strain energy is minimized when protofilaments of the same type are adjacent to one another, 12 different forms are predicted (two straight and 10 helical, with 1, 2, . . . , 9, or 10 protofilaments in the R form, respectively; see Calladine, 1978). For the helices shown in Fig. 5.2, 2, 4, 5, or 6 of the protofilaments are in the R form, respectively. In solution, the

flagellin molecule is disordered at both its N- and C-termini. The ends of the molecule become ordered as subunits polymerize, forming α-helical coiled coils in two cylindrical shells near the core of the filament, surrounding a 3 nm pore (see Namba and Vonderviszt, 1997). The central part of the flagellin molecule ends up on the outside of the filament and tolerates large structural modification. A truncated form of flagellin, formed by clipping off peptides from either end of the molecule, has been crystallized, yielding a structure for the R-type subunit. When this structure is stretched via computer simulation, it snaps into a putative L-type form (Samatey et al., 2001). Complete atomic models of both the R-type and L-type filaments should be available soon.

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