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BACTERIAL FLAGELLA

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INTRODUCTION

Flagella are responsible for bacterial motility and chemotaxis. They are subcellular organelles that originate in the membrane of the cell and extend 15–20 μm from the cell surface. The bacterial flagellar system has been studied from a variety of points of view, including as a model for the regulation of organelle formation and morphogenesis, as a system that transduces chemical energy into motion, and as the basic effector involved in chemotaxis. To frame precise questions in each of these areas, it is necessary that a great deal be known about the nature of the components and the behavior of the system. Recent work has led to a clearer characterization of structure and function of bacterial flagella in *Escherichia coli* and in *Salmonella*. Our objective in this review is to summarize the status of current work, as well as to identify some of the new questions this work raises. We are selective, particularly in the area of chemotaxis since comprehensive reviews of recent (4, 25, 85, 101) as well as earlier work (24) in this area have appeared. Flagellar assembly and the

regulation of flagella formation have received somewhat less attention. The most recent extensive reviews were by Iino (63) and Doetsch & Hageage (47).

In this article, we describe the structure of the organelle and the genetic basis for flagellar formation and function, summarize information relating to the gene products and the mechanisms involved in regulating their synthesis, and discuss flagellar function. The discussion is limited to the *E. coli* and *Salmonella* systems.

FLAGELLAR STRUCTURES

Flagellar organelles found on intact cells and in cell lysates have been studied extensively by electron microscopy (2, 3, 35, 98). In most bacteria, the organelle was found to be composed of three morphologically distinct substructures: the filament, the hook, and the basal structure. The purification of apparently intact organelles from *E. coli* (41) and *Bacillus subtilis* (45) has resulted in a clearer picture of the structures involved in forming the base of the organelle and has revealed the relationships of the basal elements to the cell membrane.

The Filament

The filament is generally made up of a single protein subunit component, flagellin. This polypeptide is readily isolated and has been purified and characterized from a variety of strains (78). The structure of the subunit is responsible for the antigenic activity of the flagellar filament, as well as for the overall shape of the filament. This has been clearly demonstrated by the isolation of a large number of mutants that result in changes in antigenic specificity and flagellar shape (62, 67, 138). In *Salmonella,* many of these mutations have been mapped and appear to be localized in the central and terminal regions of the structural gene that codes for flagellin (58). Amino acid sequences have been determined with a view to understanding the relationship between the sequence and flagellar antigenic specificity and shape. The complete sequence of *B. subtilis* flagellin (33, 39, 110) and partial sequences of *Salmonella* flagellins have been reported (73). Although some of the peptide changes that may be involved in changing antigenic specificity have been identified, a clear-cut definition of the antigenic determinants has not been made. Nonetheless, the great serological diversity among flagellar antigens has been used for many years to type *Salmonella* and *E. coli* strains. It has been suggested that a similar system could be useful in the classification of *Bacillus* strains (49).

The flagellin subunits are capable of reassembling in vitro to form flagellar filaments that show many of the same characteristics as the filament portion of the intact organelle (1, 17). In a series of very elegant experiments, by using antibody labeling and electron microscopy, it was shown that the filament grows in a polar fashion. Filaments are elongated by adding subunits to one end (18). The same polarity of elongation was observed in vivo, i.e. the flagellar filament grows by adding subunits to its distal end. This was demonstrated in *Salmonella* by studying the incorporation of amino acid analogues, which change the shape of the flagellar filament (64). A pulse of incorporated analogue caused the shape change to occur at the tip of the filament. In experiments with *B. subtilis,* pulse labeling with

radioactive leucine followed by electron microscope radioautography was used to show that the label was incorporated at the flagellar tip (50). The most reasonable hypothesis to explain this growth is that the subunits are synthesized inside the cell and then transported down the length of the flagellar filament through a central cavity. They are then incorporated into the growing tip of the organelle. This model receives support from a number of observations. First, in all situations where flagellar filament elongation has been observed, relatively high concentrations of flagellin are required (17). However, in cultures of bacteria, little or no flagellin subunit protein can be detected in the medium, thus eliminating the possibility that the subunit is excreted and then assembled from the outside of the bacterium. Second, in experiments where individual flagellar filaments on bacteria were completely coated with antibody, the filament was still able to elongate (48). Thus, the subunit is probably not transported along the outside of flagellar filament. Third, studies of the structure of the filament suggest that there is a central cavity that may be large enough to accommodate a single flagellin subunit (31). Furthermore, the rate of elongation was observed to decrease exponentially with the increasing length of the filament (65). This could readily be explained if the rate of growth was proportional to the transit time required for the subunit to reach the tip. As the filament got longer, subunits would be added more slowly. The maximal length of the filament depends upon the culture conditions, but it generally ranges from 16–22 μm.

The normal structure is 20 nm in diameter and has a wavelength of 2.3 μm. The shape of the flagellar filament depends upon conditions in the medium and upon the primary structure of the flagellin (71) subunit. Mutants have been isolated that have a variety of forms. Straight mutants have very short wavelengths or lack the helical wave entirely, and curly mutants form tighter helical waves than normal with wavelengths of 1.1 μm (68). The arrangement of flagellin subunits in the filaments of straight mutants from *Salmonella* (99) and *E. coli* (82) has been studied by optical diffraction of high-resolution electron micrographs. The subunits are arranged in a cylinder made up of 11 longitudinal rows. The subunits can also be seen as forming a helix with just under 11 subunits in two turns. Slight changes in orientation of the flagellin subunits within this basic structure can account for the superhelical forms the filament assumes (16, 32). These different forms have been observed to result from changes in the pH of the medium. For example, when the pH was decreased (77, 111), detached filaments underwent a transition from the normal superhelical form (2.3 μm) to the curly form (1.1 μm). This involved a change in helical handedness since the normal form is a left-handed superhelix and the curly form is a right-handed helix. The change has been observed to propagate along the flagellar filament (59). These kinds of transitions may also play a role in the swimming behavior of the organism. The normal-to-curly form transition of the flagellar bundle was initially observed 20 years ago and was referred to as biplicity (105). When bacteria swim smoothly in one direction, all of their flagella come together to form a single bundle, which takes on the normal helical form. Occasionally, cells stop smooth swimming and tumble (28). Tumbling appears to be correlated with a change in the shape (from normal to curly) of the flagellar filament. More recently, it has been possible to record the shape of individual filaments with

dark field microscopy. By using this technique, the normal-to-curly transition has been correlated with tumbling activity (90). It has been suggested that this transition can be induced by the mechanical forces involved in rotating the flagellar filament and changing its direction of rotation (92).

The Hook Structure

The hook structure is located at the base of the flagellum. It is generally about 900 nm long and has a diameter slightly greater than that of the flagellar filament. It is composed primarily of a single polypeptide subunit with a molecular weight in *E. coli* (115) and *Salmonella* (76) of 42,000. The hook protein in *B. subtilis* has an apparent molecular weight of 33,000 (45). Both the hook polymer and the subunit have properties that are very different than those of flagellar filaments or flagellin. The hook is much more stable than the filament, so conditions of low pH, high concentrations of urea, organic solvents, or other denaturing agents that completely dissociate flagellar filaments have very little effect on the hook (1). This observation has been the basis for the separation of the hook from the filament. Specific antisera, against both the hook structure and the hook subunit protein, have been prepared. Antihook antiserum does not cross-react with flagellar filaments (45, 115). However, the antiserum shows a wider range of cross-reaction with other hook antigens (75). The hook structure may be more highly conserved than that of flagellin.

One of the difficulties involved in studying the hook subunit has been the availability of material. The hook represents only 1% of the total protein of the flagellar organelle. However, mutants have been found that cannot terminate hook formation and, therefore, overproduce the hook protein and form extended polyhook structures. These can be readily removed from the cell and purified. The mutations that cause the formation of polyhooks in *E. coli* (115) and *Salmonella* (103) do not affect the structure of the hook subunit. They probably affect the mechanism that regulates the termination of the hook structure. Hook subunits prepared from polyhook mutants and from wild-type flagella have the same antigenicity and show the same tryptic peptide maps (115; P. Matsumura, M. Silverman, and M. Simon, manuscript in preparation).

The structural gene for the hook subunit has been located on the *E. coli* genome (94). However, at present, there are no specific mutants that affect the antigenicity of the hook. Very little is known about the function of the hook. It is thought to act as a "universal joint" at the base of the filament and to allow the efficient transmission of rotational motion to the rest of the structure. The distal end of the hook structure acts as the initiation point for the growth of the filament (72), and the proximal end of the structure terminates in a rod inserted through the cell membranes to the basal structure (41, 45). The presence of the hook structure on the flagellar filament strongly stabilizes the structure of the filament (44). This observation may also add to an understanding of the role of the hook structure. Little is known about the mechanisms involved in regulating the length of the hook or assembling the hook. The discovery (94) of the structural gene that controls the hook protein may provide some insight into the role it plays in flagellar function and formation.

The Basal Structure

The basal structure accounts for about 1% of the mass of the organelle; however, it is the most complex part of the structure. The isolation of whole intact flagella from both *E. coli* and *B. subtilis* has allowed the initial exploration of the structure of the components of the base and their relationships to the cell membranes. *E. coli* and, in general, gram-negative organisms have four ringlike structures bound to the central rod, which ends in the hook structure (42). The outer rings are tightly bound to the lipopolysaccharide layer of the cell. The outer-most ring has been called the L-ring; the second ring has been called the P-ring and is presumed to be associated with the peptidoglycan layer. Figure 1 shows the insertion of the basal

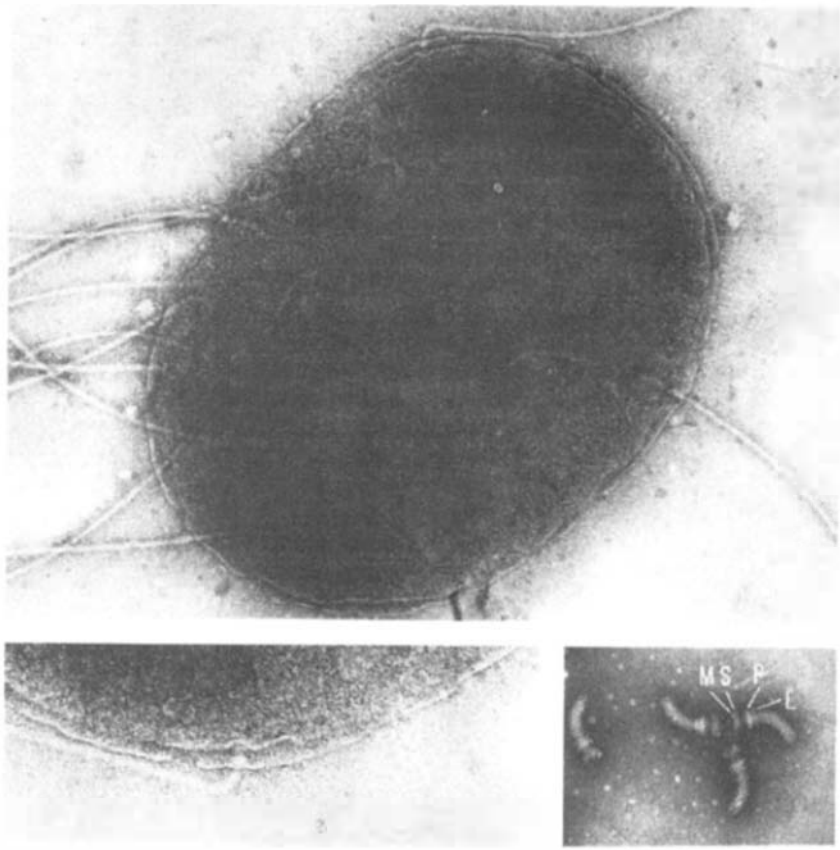

Figure 1 Insertion of the flagellar organelle of *Escherichia coli* into the cell membrane. Cells were plasmolysed to reveal attachment to inner and outer membranes (57) (*top, bottom left*). Purified isolated hook-basal structures are shown at bottom right. The apparent diameter of the hook structure is 17 nm.

complex into the membrane system and the morphology of the isolated structure. The two inner rings termed the S- and M-ring, appear to be in close contact with the plasma membrane. The M-ring is directly associated with the membrane and the S-ring is above the membrane (40, 43). In gram-positive organisms, the structure is less complex (3, 4, 45); only two rings have been found. The inner ring appears to be in contact with the plasma membrane of the cell, and the outer ring may be associated with the teichoic acid polymers on the outer surface of the cell.

The entire basal structure can be separated from the rest of the filament by using relatively mild procedures designed to dissociate the filament. The surviving hook and basal structure complex can then be separated and purified by differential centrifugation, isopycnic gradient centrifugation, and finally, by velocity gradient centrifugation (56, 57). The hook and basal structure was derived directly from bacteria by using mutants that could not make flagellin (124). These mutants make intact hook structures with no filaments (140). Membranes were isolated from the mutants, and the basal structure was released from the membranes (40) by detergent and purified. The basal complexes that were prepared by both of these procedures were dissociated, and 10 distinct polypeptide components were resolved from the dissociated structures (57). The polypeptides are listed in Table 1. They include the hook subunit with an apparent molecular weight of 42,000 and nine other polypeptides with molecular weights ranging from 60,000–9,000. The polypeptides were

Table 1 Flagellar components

Components (MW)	Location	Gene
54,000 (flagellin)	filament	*hag*
42,000	hook	*fla* region I
60,000	basal structure	*fla* region I
39,000	basal structure	*fla* region I
31,000	basal structure	*fla* region I
27,000	basal structure	*fla* region I
20,000	basal structure	not determined
18,000	basal structure	not determined
13,000	basal structure	not determined
11,000	basal structure	not determined
9,000	basal structure	not determined
31,000	inner membrane	*motA*
39,000	inner membrane	*motB*
76,000; 66,000	cytoplasm	*cheA*
12,000	cytoplasm	*cheW*
60,000; 61,000; 63,000	inner membrane	*cheM*
38,000	cytoplasm	*cheX*
28,000	cytoplasm	*cheB*
8,000	cytoplasm	*cheY*
24,000	cytoplasm	*cheZ*
64,000; 65,000	inner membrane	*cheD*

also characterized by two-dimensional polyacrylamide gel electrophoresis. Although there were clearly 10 polypeptides associated with the structure that has the morphology of the hook and basal components, there could also be other necessary structural components that were lost during the purification. For example, polypeptides with apparent molecular weights of 36,000 and 74,000 were found at early stages of the preparation but not after extensive purification (57). Furthermore, in occasional preparations, the 60,000-molecular-weight component was resolved into two bands. Thus, the structure may have as many as 13 components and as few as 10. At present, there is no functional assay for the purity of the preparation. Essentially, each of the specific polypeptides will have to be associated with the gene that regulates its structure and formation. By completely cataloguing these parts, a clearer picture of the entire structure will be obtained.

On the basis of occasional electron micrographs, it has been suggested that other structures exist that are associated with the base of the flagellum in *E. coli* (22). Evidence for these structures in other bacterial species has been obtained by electron microscopy (98). It is clear that the flagellar system in *E. coli* and *Salmonella* includes components that are localized in the cytoplasm and the cell membranes, in addition to the basal components that have been identified. These may be part of a labile structure or they may have some intermittent association with the basal structure. If such structures exist, techniques for fixing and stabilizing them will have to be developed before they can be isolated.

In recent work, the use of hybrid plasmids and phages carrying *E. coli* flagellar genes to stimulate specific synthesis of flagellar components has revealed that the synthesis of at least five of the basal complex components is controlled by genes that map in flagellar region I on the *E. coli* genome (Figure 2). The polypeptides include the hook subunit, a 60,000-molecular-weight polypeptide, a 39,000-molecular-weight subunit, and the 30,000- and 27,000-molecular-weight polypeptides (94). This approach should eventually establish all of the genes that control the structural components of the organelle. Furthermore, the isolation and characterization of the polypeptide components opens the way for partial dissociation studies to determine which of the polypeptides are concerned with the assembly of individual basal complex structures. Region I may carry most of the genes that control the structure of the basal complex. The genetic analysis of this region has not yet been completed.

THE GENETIC BASIS FOR FLAGELLAR FORMATION

Mutant Phenotypes

The isolation of mutants defective in the synthesis and function of the flagellar organelle was facilitated by the discovery of the phage chi (97). The flagellar filament acts as part of the phage receptor for this virus and similar viruses. The course of events in chi infection was inferred to involve the following steps: the attachment of the phage tail fibers to the flagellar filament; the movement of the phage down the filament to the base of the flagellum; and the injection of the phage DNA in the vicinity of the flagellar basal structure (109). An active flagellum is required for chi infection (134). This requirement may reflect the ability of the flagellum to move

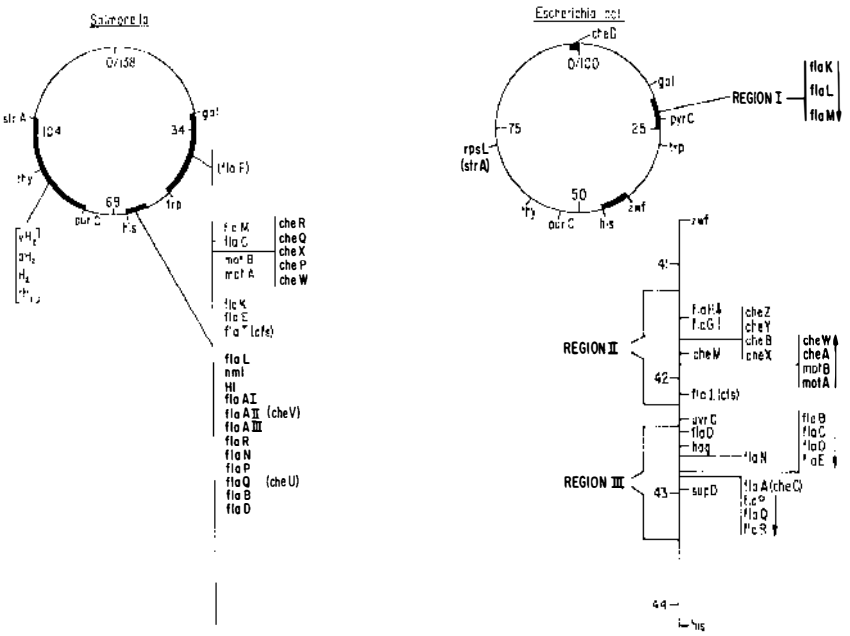

Figure 2 Distribution of flagellar genes on the genetic maps of *Salmonella* (*left*) and *Escherichia coli* (*right*).

the attached phage down the filament, or it may result from some property of a basal component necessary for phage injection. Strains of *E. coli* and *Salmonella* resistant to chi have three phenotypes: Mot⁻ strains, which have flagella that appear to be normal but that are "paralyzed" because the flagellar filaments do not rotate; Hag⁻ strains, which have flagella that are altered in configuration or antigenicity or which lack flagellar filaments entirely [these result from mutations in the structural gene for the flagellin subunit (97); in *Salmonella,* there are two genes, H1 and H2, whereas in *E. coli,* there is a single *hag* gene]; and Fla⁻ strains, which have no apparent flagellar structures, although closer scrutiny reveals partial or defective structures in some of the mutants.

Another group of mutant strains was selected by repeated propagation from the center of stabs on motility agar. In addition to Mot⁻, Fla⁻, and Hag⁻ strains, other strains that were motile but defective in chemotaxis Che⁻ were found (15). These were sensitive to chi infection and were able to swim in liquid media. However, they showed aberrant swimming behavior and did not respond to gradients of attractants or repellants. A variety of other selection procedures have been used to obtain Che⁻ mutants (20, 69, 100, 136), and their behavior has been examined extensively. They fail to show the normal change in the frequency of tumbles (abrupt changes in direction of travel) when subjected to a chemotactic stimulus (28, 100). One group of Che⁻ mutants tumbled incessantly whereas another rarely tumbled. Both classes

of Che$^-$ mutants are presumably defective in their ability to integrate input information and to transmit it to the flagellar organelle (101). Many mutants defective in components of the chemotaxis system have been isolated, including mutants in specific chemoreceptors. Some of the receptors correspond to components of systems for the transport of sugars and amino acids (6, 8, 55). Here, we are concerned with the chemotaxis mutants that directly impinge upon the flagellar organellar function, i.e. mutants defective in general chemotaxis.

Flagellar Genes in Salmonella

Transduction with phage P22 (51, 52, 66, 74, 130) and colicinogenic factor-mediated transfer (125) was used to establish the linkage relationships between flagellar genes and other loci in *Salmonella.* Complementation tests were performed by measuring the occurrence of abortive transductants, which appeared on semisolid growth media as trails of microcolonies emanating from the zone of inoculation of the transduction mixture. More recently, extensive analysis with overlapping deletions (58, 139) has established the order of many of the genes and their precise map positions in *Salmonella.* For the sake of discussion, it is worthwhile to consider the genes linked to H1 as being in two clusters so that they correspond to the homologous regions in *E. coli.* Region III would include the cistrons *flaD, flaB, flaQ, flaP, flaN, flaR, flaAIII, flaAII, flaAI,* H1, *nml, flaL.* Region II includes the cistrons *flaT, flaE, flaK,* (order uncertain) *motA, motB, cheW, cheP, cheX, cheQ, cheR, flaC,* and *flaM.* The analysis of region I in *Salmonella* is incomplete. There are probably a number of cistrons that map in this region (between *trp* and *gal*); however, they are only represented by *flaF* (66, 125). Finally, there is a region in *Salmonella* that has no homologue in *E. coli,* i.e. the phase 2 region. The precise map position of these genes has not been defined. They are between *purC* and *strA* (93, 125) and include the structural gene for the phase 2 antigen, H2, a gene that specifies the synthesis of a repressor of phase 1 (*rh1*) (54), a region that regulates phase transition (*vh2*) (60), as well as a regulator of the expression of the phase 2 gene product (aH2) (61).

Chemotaxis mutants were isolated and mapped between *motB* and *flaC,* as well as within the *flaAII* gene (36). More recently, a large number of Che$^-$ mutants in *Salmonella* were collected by using preformed vertical gradients of chemoattractants (20, 137). Complementation tests divided these mutants into nine cistrons (137). The map positions of these genes are shown in Figure 2. Two groups have not yet been mapped. At least two of the *che* mutant groups coincide with *fla* mutants, a situation also found when *che* and *fla* mutants are compared in *E. coli* (see below).

Flagellar Genes in E. coli

Genetic experimentation to define flagellar genes in *E. coli,* in contrast to the work with *Salmonella,* began with the characterization of mutants defective in chemotaxis. In addition to the Che$^-$ mutants and the Mot$^-$ mutants, Fla$^-$ mutants were also isolated, and three regions of the genome were defined (12–15) where all of these

mutations mapped. In subsequent studies, large numbers of chi-resistant mutant strains were ordered into complementation groups, by using F factors and Rec⁻ hosts (116). Further genetic analysis with strains carrying mu phage-induced mutations defined additional *fla* cistrons and revealed the transcriptional organization of the flagellar genes (117). Two- and three-factor transductional crosses with phage P1 were used to establish the relative linkage of the genes (116). Finally, recombination tests with F' factors carrying overlapping deletions were used to establish the linear order of the genes (118). These approaches and subsequent work established 11 flagella-related genes in region III (*flaR, flaQ, flaP, flaA, flaE, flaO, flaC, flaB, flaN, hag, flaD*) and 12 in region II (*flaI, motA, motB, cheA, cheW, cheM, cheX, cheB, cheY, cheZ, flaG, flaH*). The analysis in region I is continuing; thus far, at least three cistrons (*flaK, flaL, flaM*) have been defined (56). Each flagellar gene region will be considered separately, beginning with region III (see Figure 2).

FlaA, flaP, flaQ, and *flaR* are all cotranscribed. Mutants belonging to the *flaA* complementation groups have two phenotypes: some lack flagella entirely whereas others are motile but not chemotactic. It has been suggested (117) that the *flaA* gene product has dual functions, as a structural component of the organelle as well as a part of the chemotactic pathway. A similar argument holds for the genes in region III in *Salmonella* that show both phenotypes (137). *FlaB, flaC, flaO,* and *flaE* also form a cotranscribed unit. Mutations in *flaE* lead to a distinctive phenotype. The strains have long polyhook-like structures with occasional flagellar filaments attached to the distal end. The hook subunit protein extracted from polyhooks and from hook structures on isolated flagella are identical. Deletions of *flaE* or mu-induced mutations mapping in *flaE* also result in polyhooks. This data has been taken to mean that *flaE* is involved in regulating the length of the hook rather than in specifying the structure of the hook subunit (115). A gene with a similar function, *flaR,* has been characterized in *Salmonella* (103). The function of *flaB, flaC,* and *flaO* is not known.

The *hag* gene controls the structure of flagellin and maps between *flaN* and *flaD.* Mutations that eliminate filament formation result in cells that still have the flagellar basal structures and hooks (124, 140). Some mutations that map in the *flaD* gene also appear to have hooklike structures, but not all mutations in *flaD* have the hook (M. Hilmen and M. Simon, unpublished results). The formation of flagellar filaments in many gram-negative bacteria is temperature sensitive, so cells grown at 42°C do not make flagella (95, 106). When the temperature is decreased to 33°C flagella synthesis resumes. Mutants can be isolated that are insensitive to temperature and, thus, that form flagella and swim even at 42°C. Some of these mutants appear to map in or near the *flaD* gene (M. Silverman and M. Simon, unpublished results). The characterization of flagellar mutants in region III has been repeated by using entirely different techniques (81). Lambda was inserted near the *supD* gene, and then a series of deletions were prepared that removed the genes in region III that controlled flagellar synthesis. Complementation and mapping of flagellar mutants by using these strains and a variety of other mutants agreed, on the whole, with earlier reports; there was one exception, the suggestion of a new complementation group between *hag* and *flaD.*

Ultimately, the genes have to be defined in terms of both their complementation activity and their products. The recent introduction of molecular cloning techniques has allowed this kind of analysis to be applied to the flagellar-related genes in region II. The gene products were identified by using two different systems. (*a*) Bacteriophage lambda was used as a vehicle, and Eco R1 restriction endonuclease fragments carrying flagellar genes were inserted into the vehicle in place of a dispensible region. These hybrids could then be used to infect ultraviolet-irradiated hosts that were lysogenic for lambda. Ultraviolet irradiation eliminated host DNA expression and the resident lambda repressor eliminated lambda gene expression so that the major incorporation of radioactivity was into polypeptides coded for by the inserted DNA. Deletions of the hybrid lambda generally resulted in the elimination of specific portions of the inserted piece. By using the deletions, it became possible to correlate the presence of a gene product with a specific genetic complementation activity. Finally, the *flaI* gene product appeared to be necessary for the synthesis of many of the other flagellar gene products (120). In cells that carried a *flaI* mutation, flagellar genes carried on lambda were not expressed. This allowed a further test for the specificity of a putative flagellar gene product. It should be produced in FlaI$^+$ host cells but not an FlaI$^-$ cells (112). (*b*) Colicinogenic factor E1 was used as the cloning vehicle, and sheared fragments of the *E. coli* genome were used as the inserted piece. Clarke & Carbon (34) used the enzyme terminal transferase to elongate the fragments and the vehicle with either a short polyadenylic acid chain or a polythymidylic acid chain (34). These "tails" were used to form hybrids between the Col El and the randomly sheared DNA fragments from *E. coli.* After transformation, a large number of individual clones were picked, and the colony bank was shown to contain representatives of all of the regions of the *E. coli* genome, including the flagellar genes. The hybrid plasmids carrying flagellar genes were transferred to a strain of *E. coli* that produced minicells (which lack a nucleus but retain the capacity to synthesize proteins). The hybrid plasmid segregated in to the minicells. Thus, if the minicells were purified and their expression was followed, they were found to synthesize the polypeptides directed by genes on the hybrid plasmid (107). Restriction enzymes could be used to prepare deletions of the plasmid. Furthermore, since the initial colony bank was made from fragments of DNA with random end points, different plasmids carried different subsets of genes, and again the presence or absence of a specific polypeptide could be correlated with the residual complementation activity of the plasmid in genetic tests.

In addition to identifying gene products, these techniques have also helped to clarify the genetics in region II. Initially, the results of complementation tests were interpreted in terms of the minimum number of cistrons. It was suggested that *mot* could be a single cistron with two parts showing intracistronic complementation (12). The *cheA* and *cheB* loci were also defined as single genes containing subgroups that showed intracistronic complementation (13, 102). It is now clear that the gene formerly referred to as *cheA* is made up of two genes, *cheA* and *cheW* (123). *Mot* is composed of two cistrons, *motA* and *motB,* and *motA, motB, cheA,* and *cheW* form a cotranscribed unit that has been referred to as the Mocha operon (114, 122, 123). The gene product formed by *motA* was a 31,000-molecular-weight polypep-

tide, and the product of the *motB* gene had an apparent molecular weight of 39,000. The *cheA* gene directed the synthesis of two polypeptides with apparent molecular weights of 66,000 and 76,000. The evidence that both gene products resulted from the *cheA* gene is twofold: physical measurements of the length of the DNA between *motA* and *cheW* indicated that it was not long enough to accommodate the synthesis of two contiguous polypeptides of 76,000 and 66,000 molecular weights; peptide map analysis of the two polypeptides indicated that they have most of their peptides in common. The *cheW* gene product has an apparent molecular weight of 12,000 (122, 123).

Studies of the polypeptides synthesized by deleted hybrid phages indicated that there was a region adjacent to the *cheW* gene responsible for the synthesis of a group of polypeptides that appeared on sodium dodecyl sulfate-acrylamide gel electrophoresis as three bands. This group of bands, called triplet, had apparent molecular weights corresponding to 63,000, 61,000, and 60,000. The region responsible for the synthesis of this group of bands has been called *cheM* (113). The multiple bands have properties on polyacrylamide gel electrophoresis that are similar to those of other polypeptides called MCP (methyl-accepting chemotaxis protein). MCP has been identified as a membrane protein that was methylated reversibly and its methylation was related to chemotaxis (84). It has been suggested that MCP is the same as triplet and that the *cheM* gene is responsible for the synthesis of these polypeptides. However, analysis of the genetic basis of the formation of MCP indicated (M. Silverman and M. Simon, manuscript in preparation) that these polypeptides were the products of two different chemotaxis genes, *cheM* and *cheD*. When strains that carried deletions in the *cheM* gene were constructed, they were found to be capable of chemotaxis toward some attractants, e.g. serine, but not toward others, e.g. aspartate. Their phenotype was that of previously described (95a) aspartate taxis mutants. Furthermore, these mutants no longer formed all of the MCP polypeptides; the lower-molecular-weight bands (60,000, 61,000) were missing. However, they were able to form methylated proteins with higher apparent molecular weights (greater than 63,000). In experiments with wild-type cells, the lower-molecular-weight MCP polypeptides were found to be preferentially methylated when the cells were stimulated with aspartate. When serine was used as attractant, the higher-molecular-weight components of MCP were preferentially methylated. The null phenotype of the chemotaxis gene *cheD*, which maps near *thr* (Figure 2), was lack of serine taxis (100), and the product of the *cheD* gene was shown to correspond to a polypeptide with an approximate molecular weight of 64,000 (M. Silverman and M. Simon, manuscript in preparation). Mutants in the *cheD* gene no longer methylated the higher-molecular-weight MCP components but were able to form the lower-molecular-weight MCP polypeptides. The double mutant *cheM, cheD* was found to be unresponsive to attractants and repellents. It formed no detectable MCP polypeptides with molecular weights in the 60,000–65,000 range. Thus, the *cheD* and *cheM* genes account for the synthesis of the MCP polypeptides (approximately 60,000- to 65,000-molecular-weight polypeptides).

Adjacent to the *cheM* gene is the *cheB* complex. This is composed of four genes, *cheX, cheB, cheY,* and *cheZ*: the product of the *cheX* gene had a molecular weight

of 28,000; the *cheB* gene product had a molecular weight of 38,000, whereas *cheY*, which is adjacent to *cheB*, made a polypeptide with a molecular weight of 8000; and *cheZ* was responsible for the synthesis of a 24,000-molecular-weight polypeptide. All four of these genes may be cotranscribed, beginning with *cheX* and reading through *cheZ* (123).

There are at least three other flagellar-related genes in this region, *flaI*, *flaG*, and *flaH*. *FlaI* has associated with it a region called *cfs*. This appears to be a regulatory region that makes the synthesis of *flaI* dependent upon the presence of cAMP. The *flaI* product was clearly involved in the positive control of the entire flagellar gene complex (120). However, this polypeptide and the polypeptides corresponding to the *flaG* and *flaH* genes have not been identified. They may be synthesized at levels much lower than the level of synthesis of the *mot* and *che* gene products. Although the products of *flaG* and *flaH* are not known, they may function to influence the association of the basal structure with the outer membrane (79).

The techniques involving molecular cloning are also being applied to regions I and III in *E. coli*. It appears that many of the genes responsible for polypeptides that make up the hook and the basal structures map in region I. Experiments involving protein synthesis directed by specific plasmids segregated into minicells indicated that the 60,000-molecular-weight polypeptide, the hook subunit (42,000), and the 39,000- as well as the 30,000- and 27,000-molecular-weight subunit components of the basal structure were all synthesized when plasmids carrying region I are used (94). Initially, only three genes, *flaK*, *flaL*, and *flaM*, that formed a cotranscribed unit were described (56). However, more recent complementation tests suggest that there are more genes in this region. Thus far, at least six have been identified (Y. Komeda, M. Silverman, and M. Simon, unpublished results).

The polypeptides synthesized in ultraviolet-irradiated cells infected with deleted hybrid phage were able to function. Mot$^-$ or Che$^-$ host cells acquired the missing activity after infection with the phage (114, 122). In these cells, the gene products must be able to be inserted into structures and membranes so that they can function properly. The localization of the polypeptides was followed. The *cheD*, *cheM*, *motA*, and *motB* gene products are localized exclusively in the inner membrane of the cell. The products of the *cheA*, *cheW*, *cheX*, *cheB*, *cheY*, and *cheZ* genes seem to be in the cytoplasm, although it would be difficult to eliminate the possibility that they have a labile or transient association with the inner membrane (H. Ridgeway, M. Silverman, and M. Simon, manuscript in preparation). The hook, basal body complex, and flagellar filament are usually isolated with the outer membrane fraction of the cell (56). Thus, the products of the genes that direct flagellar structure and function are found in almost all of the compartments of the cell (Table 1).

We can expect that all of the products of the flagellar genes will be identified in the next few years. The gene products from region II, particularly the *mot* and *che* gene products, are synthesized in relatively large amounts. The products of many of the *fla* genes may be synthesized at lower levels. However, there are a variety of ways in which the levels of these gene products can be increased so that they may be identified. Once the gene products and their localization is known, it should be possible to determine their specific function in flagellar assembly and activity.

Comparison of Flagellar Genes in E. coli and Salmonella

It is clear from a comparison of the *E. coli* and *Salmonella* genetic maps (Figure 2) that there is a great deal of homology in the distribution of flagellar genes. However, it was not easy to make a gene by gene correspondence. Thus, for example, *flaR* in *Salmonella* and *flaE* in *E. coli* are functionally equivalent and yet are not congruent with respect to the *fla* genes on either side of them. Furthermore, *Salmonella* appeared to have gene functions that were not found in *E. coli.* For example, *nm1,* the gene responsible for the methylation of flagellin, was present in *Salmonella* but not in *E. coli* (83, 129). Cross-species complementation tests between *Salmonella* and *E. coli fla* functions have been done with a number of the genes, and there is usually no difficulty in demonstrating complementation. An F' carrying *che* genes from *E. coli* complemented *che* defects in *Salmonella* (137). In two cases where *fla* genes were functionally equivalent with respect to complementation activity, their map order was reversed: *flaC* in *Salmonella* corresponded to *flaH* in *E. coli* and *flaM* in *Salmonella* corresponded to *flaG* (35a). These kinds of changes in gene positions may be very interesting in terms of the evolution of the *E. coli* and *Salmonella* genomes.

REGULATION OF FLAGELLAR SYNTHESIS

The bacterial flagellar structure is complex; it requires more than 25 specific gene products for its assembly and function. These are required in varying amounts, ranging from a minimum of 1 polypeptide per organelle to about 10,000 (flagellin). The organelle is assembled and integrated into the cell membrane system, and the distribution and number of flagella per cell appears to be regulated. However, very little is known about the coordination of flagellar gene activity, i.e. does the orderly assembly of the organelle require a specific sequence of gene expression? At present, the answer can only be a tentative yes, since our information about regulation is fragmentary. Most of the observations suggesting regulation of parts of the flagella forming pathway involve the regulation of flagellin synthesis. As information about the other gene products becomes available, the mechanisms that regulate their formation and interaction will become clearer.

Phase Variation in Salmonella

Most *Salmonella* species have been shown to possess two distinct flagellar antigens (flagellin subunit) that differ markedly from each other (9). A given bacterium generally expresses only one flagellar antigen (phase) at any time. The transition from the expression of one phase to the other occurs at a fixed frequency that ranges from 10^{-3}–10^{-5} per bacterium per generation. The frequency of transition in one direction is generally different than the frequency in the reverse direction (93, 128). Lederberg & Iino (89) showed that two loci, H1 and H2 (Figure 2), were the structural genes for phase 1 and phase 2 flagellin. They also showed that the phase expressed in the bacterium was determined by the "state" of the phase 2 gene or by some closely linked phase-determining factor. Thus, in one state, H2 was ex-

pressed and H1 repressed, whereas in the other state, H2 was not expressed and H1 was expressed. Enomoto & Stocker (53) have recently reviewed the properties of the other genetic elements involved in regulating phase transition. *rh1* is a gene thought to control the formation of a repressor substance that can act through the cytoplasm (in *trans*) to repress the synthesis of the H1 gene product. The expression of *rh1* appears to be coordinate with H2 gene expression (54, 104). Another locus, *ah2*, is defined by mutations that prevent the expression of H2 and *rh1*. Mutations in *ah2* only affect the genes that are *cis* to the mutation. Thus, *ah2* could be the promotor for the *H2* and *rh1* genes. Another *cis* acting element is *vh2*. The wild-type allele $vh2^+$ presumably allows phase variation at frequencies of 10^{-3}–10^{-5} per bacterium per generation. The $vh2^-$ allele can have two possible phenotypes. One phenotype is $H2^{on}$, in which only H2 is expressed and the frequency of transition to H1 gene expression is very low (10^{-6}–10^{-7} per bacterium per generation). In the other phenotype, $H2^{off}$, the *vh2* mutation fixes expression so that only the H1 gene is expressed (54, 60). The following facts suggest that the phase switch involves a change at the DNA level: *vh2* only acts *cis* to the H2 gene, thus it must affect a region of DNA contiguous with H2; the state of the gene can be transduced, and since the transducing phage carries the donors DNA, the state of the gene must be a function of that DNA; and no H2 mRNA can be found when H2 is off, suggesting that regulation is at the transcriptional level (131). On the other hand, it was observed that, when the H2 gene, which was not expressed in *Salmonella* because of the presence of $vh2^-$, was transferred to *E. coli*, it was expressed and showed phase transition. When it was transferred back to *Salmonella*, it again showed only low levels of phase transition (53). This observation suggests that, although the primary switch involves a change at the DNA level, the regulation of the frequency of switching (*vh2*) may involve other cytoplasmic factors.

There have been a variety of models proposed to explain the mechanism of phase variation. These were reviewed by Iino (63). Recently, evidence has developed for a model that involves the inversion of a segment of DNA adjacent to the H2 gene. The *Salmonella* H2 gene (phase 2) was cloned from an Eco R1 endonuclease digest of *Salmonella* DNA and from randomly sheared fragments of *Salmonella* DNA. When these DNA fragments were treated with endonuclease, heat denatured, and reannealed, a small region corresponding to a bubble was observed by electron microscopy (Figure 3). Furthermore, by using various restriction enzymes and retransformation with restricted plasmids, the DNA region coding for H2 formation was mapped to the segment of DNA adjoining the apparent anomaly in the DNA. The DNA was also inserted into a lambda bacteriophage vehicle (charon 1), and the expression of H2 in individual clones of lambda phage was examined. The lambda was used to transduce the H2 gene. Two kinds of lambdas were found in all single plaques: high-level transducers presumed to correspond to phase 2-on, and low-level transducers thought to represent phage particles carrying phase 2-off. When DNA from each of the populations was denatured and reannealed with itself, low levels of bubbles were found (2% for phase 2-off and 12% for phase 2-on). When they were mixed and heteroduplexed, a large fraction of the molecules showed the apparent inversion structure (34%). These experiments were interpreted as indicat-

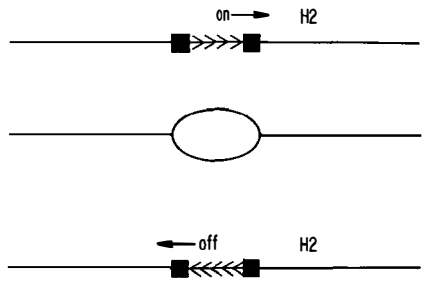

Figure 3 A model for phase variation. An inversion of the sequence between the dark blocks could result in a change in the orientation of a promotor contained within the region or it could allow read-through from a promotor outside the region.

ing that the apparent bubble structure is the result of a heteroduplex of phase 2-off and phase 2-on DNA. This suggested that phase variation resulted from an inversion in a region that controls the initiation of H2 gene transcription (142). In one orientation transcription occurs, whereas in the other it does not (see Figure 3). Molecules associated by a duplex region that corresponded to the bubble with nonhomologous single-stranded ends were observed among the heteroduplexes. These findings give strong support to the inversion hypothesis. Testable hypotheses to explain phase variation at the molecular level and the material to do the appropriate experiments now exist. There are a number of intriguing mysteries that can be addressed in this system. For example, what is the role of the *vh2* element and how does it control the rate of phase transition? Why are the frequencies of transition from phase 2-on to phase 2-off different in opposite directions? What kind of mechanism is involved in the transition itself, i.e. does it require the *rec* system of the host cell or is there an independent enzyme system for phase variation? The answers to these questions may also give some insight into the extent to which the mechanism represents a general kind of regulatory system found in both prokaryotic and eukaryotic organisms.

Catabolite Repression of Flagella Formation

Synthesis of the flagellar organelle in *E. coli* is repressed by growth in glucose and a variety of catabolites (7). The repression could be reversed by the addition of cAMP (46). Furthermore, *E. coli* and *Salmonella* strains defective in adenylcyclase (*cya*) or in the cAMP receptor protein (*crp*) were unable to synthesize that flagellar organelle (141). All of these observations suggested that cAMP exercised its regulation of flagellar formation through the binding of a cAMP-CRP complex to the flagellar genes to activate their expression. To define the site of regulation, mutants able to synthesize flagella were selected in a parent strain that was Crp^-. A number of these mutants were mapped. They defined a new locus designated *cfs* (constitutive flagellar synthesis), which mapped adjacent to or as part of the *flaI* gene in *E. coli*. For *cfs* to function, even when it was on an exogenotic fragment, it was necessary that the adjacent *flaI* gene were intact and functional. The *cfs* mutation had pleiotropic effects: it allowed flagellar synthesis in Cya^- and Crp^- strains and

in cells grown under catabolite repressing conditions; it increased the number of flagella per cell by two- to threefold; it allowed some synthesis of flagella when the cells were grown at 42°C; and it allowed the intracellular synthesis of hook subunit and *hag* gene fragments in Cya^- and Crp^- strains (120).

In *Salmonella,* a mutation *flaT* similar to *cfs* was described. Although catabolites have very little effect on flagellar synthesis in *Salmonella,* H1- and H2-specific RNA were not present in Cya^- strains unless cAMP was added or the *flaT* mutation was present (80). All of these results have been interpreted to mean that *cfs* defines a site at which a CRP-cAMP complex acts to activate the expression of the *flaI* gene.

The *flaI* gene product was essential for the expression of a large variety of flagellar genes. In experiments with cloned flagellar genes on hybrid lambda in ultraviolet-irradiated cells, the synthesis of the *mot, che,* and *hag* gene products required the presence of the *flaI* gene product (112–114, 122, 123). Thus, *flaI* appeared to have an "executive" role in the management of flagellar regulation. It may function to induce the expression of all of the flagellar gene groups simultaneously, or it may have a sequential effect, i.e. *flaI* could stimulate the synthesis of one group of flagellar genes and the product of these genes could in a sequential manner induce the expression of another group.

Flagellar formation in gram-negative cells is generally temperature sensitive (106). A series of strains able to form flagella when grown at high temperatures (42°C) were isolated. Mutations in these strains were found to map at two loci in or near the *flaI* and the *flaD* genes, suggesting that an interaction between the *flaI* and *flaD* product might be necessary for temperature sensitivity (M. Silverman and M. Simon, unpublished results). The interaction of the *flaI* gene product with other *fla* genes could be necessary for the orderly assembly of the components of the organelle.

Regulation of Flagellin Synthesis

A number of attempts have been made to find intracellular pools of flagellin subunits in a variety of *hag* and *fla* mutants, as well as in the wild-type cell. *E. coli* flagellin pools were not found except in some *hag* amber mutants of *E. coli* where an immunologically active fragment has been detected (120). These results suggested that when effective *hag* subunits are synthesized they are assembled into the growing flagellar structure almost immediately. It has been observed that, when there was a temporary derepression of phase 1 flagellin synthesis in *Salmonella* that were in phase 2, the newly synthesized material appeared as a block of phase 1 flagellin on the tips of the growing phase 2 filaments (70). On the other hand, when both kinds of flagellins were synthesized simultaneously, they formed totally mixed sections of flagellar filament (119). Thus, if there were a pool of subunits, it was very small and could not sustain filament assembly for more than 2 or 3 min. The absence of pools of missense mutant subunits suggested that they are either degraded or can effectively repress their own synthesis (120).

The presence of intact hook structures on the *hag,* H1 or H2 mutants suggests that the flagellin subunit is not necessary for the synthesis of the other structural proteins (124, 140). There is evidence, however, indicating that the presence of other

fla gene products is necessary to get *hag, mot,* and *che* gene expression. Most *fla* mutants do not make flagellin or the intracellular flagellin amber mutant polypeptide fragment (120). In *Salmonella,* 11 different *fla* mutants were tested and none of them had detectable levels of mRNA for flagellin (132). This effect was not restricted to flagellin. In many *fla* mutants, only low levels of the MCP products were found (84). Furthermore, when a *flaB* mutant was used as host for protein synthesis directed by hybrid lambda, the *mot, che,* and *hag* genes were not expressed (M. Silverman and M. Simon, unpublished results). These observations support the idea that there may be an hierarchical order of *fla* gene regulation. Thus, for example, the *flaI* gene product may be required for the expression of a number of first-tier operons, and some of the products of these genes could act to induce the expression of second-tier genes. The precise scheme of things may come clear from further studies of the expression of cloned genes in specific backgrounds.

FLAGELLAR FUNCTION

Investigation of the structure and genetics of the flagellar organelle ultimately should provide an explanation in molecular terms of the actual function of the organelle. Flagellar function can be divided into several questions. How does the flagellum propel the cell? How is this motion orchestrated to provide purposeful movement? What is the source of energy for flagellar movement? How are chemotactic signals processed to direct flagellar movement?

Flagellar filaments propel the cell by rotating. The helical filament is driven by a rotary motor at the base, part of which is the basal structure embedded in the cell membrane. Arguments for a rotary motor have been made from existing evidence (27). Conclusive evidence for this type of motion was provided by tethering experiments that measured the rotation resulting from the torque generated by the flagellar organelle. It was shown that when mutants with only polyhook structures (Hag^-, $FlaE^-$) were tethered to glass slides with antibody to *E. coli* hooks, the cell body rotated. This rotation was also demonstrated with cells with straight filaments. When these cells were free, they were non-motile, but when tethered, the cells spun at 10–20 revolutions per sec. Rotation could be clockwise or counterclockwise, and it was suggested that the ability to modulate the direction of rotation might be the basis for the mechanism of chemotaxis (121). The dynamic properties of flagellar motors have been extensively analyzed by using tethering experiments (23, 26).

When the paths of cells of *E. coli* swimming in an isotropic environment were measured, the motion was of two types: straight movement (runs) terminated by occasional abrupt changes in direction (twiddles, tumbles). The net result was a three-dimensional random walk. In spacial gradients of attractant (i.e. serine, aspartate), cells swimming up the gradient tumbled less, whereas cells swimming down the gradient tumbled at the same frequency as in an isotropic solution. *E. coli,* therefore, biased its random walk in a favorable direction by suppressing tumbles when movement was in that direction (28). With *Salmonella,* paths of movement were measured after rapid mixing in an apparatus, which allowed the formation of temporal gradients of attractant. A sudden decrease in attractant concentration resulted in an increased, if short lived, tumbling response, and a sudden increase in

attractant concentration resulted in a long suppression of tumbling; swimming was only in long, smooth runs. The response to attractants was very similar to that of *E. coli,* and in addition, it was obvious that cells must sense attractant concentrations with time. This implied the existence of a memory of the stimuli sensed. Cells swimming in a spacial gradient sense differences in concentrations of attractant with respect to time as they move through space (91). The response of *E. coli* to temporal gradients was measured with similar results (30). Attractants and repellants had opposite effects, and the response depended upon the time rate of change of the fractional amount of chemoreceptor bound (37, 38, 96, 135). For a review of the detailed mechanism of chemotaxis, see Adler (4), Berg (25), Parkinson (101), and Koshland et al (85, 86).

Using tethered *E. coli* cells to infer the direction of filament rotation, it was shown that the direction of flagellar rotation correlated with chemotactic behavior (88). Wild-type cells stimulated with attractant rotated their filaments counterclockwise, and those stimulated by repellant rotated their filaments clockwise. Che$^-$ mutants with the ever-tumbling phenotype (uncoordinated) rotated their filaments in the clockwise direction, and Che$^-$ mutants, which swam smoothly, rotated their filaments in the counterclockwise direction. Thus, smooth swimming correlated with counterclockwise rotation and tumbling correlated with clockwise rotation. A swimming response was determined by the direction of filament rotation. Explanation of why counterclockwise rotation gave smooth swimming and clockwise rotation gave tumbly swimming has been based on the properties of filament interaction (90, 92).

Motility in eukaryotes has been shown to require ATP (108), but ATPase activity has not been demonstrated in isolated flagellar structures (43, 51). Recent isolation of mutants in *E. coli* and *Salmonella* blocked in steps in oxidative phosphorylation has allowed the definition of the source of energy for flagellar movement. By using these mutants and uncouplers of oxidative phosphorylation, it was shown that an intermediate of oxidative phosphorylation (i.e. proton motive force), not ATP, was required for bacterial motility (87, 134). This was true for clockwise or counterclockwise rotation (87), although in one report (21), ATP-depleted cells showed only counterclockwise rotation. Significantly, ATP was required for chemotaxis (21, 87). This may reflect a requirement for ATP in the formation of S-adenosylmethionine (see below). Certain components of ion flux have been shown to correlate with the application of chemotactic stimuli and to be missing in Mot$^-$ mutants (133). Since the products of the *mot* and *che* genes have been identified and their localization in the cell has been determined (113, 114, 122, 123), an examination of their properties as ion gates or regulators of ion gates will be interesting.

The phenotypes of generally nonchemotactic mutants (Che$^-$) suggested that the *che* gene products acted in the transduction of sensory information from the chemoreceptors to the flagellar motor. It has been suggested that some *che* gene products associate to act as a "tumble generator," whereas other *che* gene products integrate information from chemoreceptors and influence the activity of the tumble generator (101). A general model for sensory systems designed to account for the memory and the adaptive properties, i.e. the adjustment of the system to ranges of input signal, was proposed by Delbrück and Reichardt (39a). They postulated a

bifurcation of input information so that an integrative process accumulated the signal while a second process instantaneously monitored the environment. A comparison between these information pools accounted for the responsive properties of the system. Several decision-making models have been proposed for chemotaxis (29, 91), involving two process mechanisms, i.e. comparison between a signal reservoir and an adaptation reservoir (29), or comparison between a fast and slow reaction, which is itself influenced by signal input (91).

Biochemical approaches to determining the nature of sensory information and sensory transduction have focused on the L-methionine requirement for bacterial chemotaxis (5, 19, 127). Methionine or probably S-adenosyl-L-methionine (10, 11, 21) was necessary for chemotaxis or, more specifically, for the generation of tumbles. The requirement for ATP would reflect the necessity for the activation of S-adenosyl-L-methionine, which could act as a methyl donor (21, 87). Consistent with these expectations, the methylation of a protein, MCP (methyl-accepting chemotaxis protein), was discovered in the inner membrane of *E. coli* (84). The methylation of MCP responded to chemotactic stimuli (84). MCP was found to be the product of two genes, *cheD* and *cheM* (113, 123; M. Silverman and M. Simon, manuscript in preparation). The *cheM* gene product was involved in the responses to a group of chemoeffectors including aspartate, and the *cheD* gene product was involved in response to a group of effectors including serine. A further gene product, *cheZ,* was methylated; however, its methylation was different than that of the *cheM* or *cheD* products. In *E. coli,* mutations in the *cheX* and *cheW* genes lead to a marked decrease in MCP methylation. In *Salmonella,* the product of a *che* gene has been shown to correspond to an enzyme that methylates MCP (127a). It is possible that methylation of the membrane-bound MCP could correspond to the adaptive process postulated in the models for chemotaxis, whereas the methylation of *cheZ* could be the process that is instantaneously monitoring the sensory input. The other *che* genes would be involved in translating the sensory input into methylation events. All of the pieces of the chemotaxis puzzle have not been put in place, but the outlines of a solution are emerging.

The function of the flagellar organelle is to provide the cell with motility and chemotaxis. The mechanisms involved in the processes are complex. It is not surprising, therefore, to find that the structure as well as the genetics and regulation of flagellar assembly are complex. The system has already provided interesting and novel examples of mechanisms that control gene expression, protein assembly, energy transduction into motion, and the processing of sensory information. As the nature of the components of the organelle and their interactions become clear, it should be possible to understand these mechanisms at the molecular level and to determine the extent to which they are generally applicable in other systems.

NOTE ADDED IN PROOF This review covers material available to us in April, 1977. Subsequently, we became aware of several manuscripts dealing with the genetics and function of bacterial flagella: Iino, T. 1977. *Ann. Rev. Genet.* In press; Parkinson, J. S. 1977. *Ann. Rev. Genet.* In press; Springer, M. S., Goy, M. F., Reader, R. W., Adler, J. 1977. *Proc. Natl. Acad. Sci. USA* In press.

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